Journal of Medical Virology

WILEY

JOURNAL OF MEDICAL VIROLOGY

RESEARCH ARTICLE OPEN ACCESS

Monkeypox Virus Subverts the Inflammatory Response of Macrophages at the Maternal-Fetal Interface

Jonatane Andrieu[1] | Margaux Valade[2] | Nathalie Wurtz[2] | Marion Lebideau[2] | Florence Bretelle[2,3] | Bernard La Scola[2] | Jean-Louis Mège[1,4] | Soraya Mezouar[1,5]

[1]Aix-Marseille Univ, Centre National de la Recherche Scientifique, Établissement Français du Sang, Anthropologie bio-culturelle, Droit, Éthique et Santé, Marseille, France | [2]Aix-Marseille Univ, Institut Recherche Développement, Assistance Publique – Hôpitaux de Marseille, Microbe, Evolution, Phylogeny Infection, Marseille, France | [3]Department of Gynecology-Obstetric, La Conception Hospital, Marseille, France | [4]Department of Immunology, Timone Hospital, Marseille, France | [5]Faculty of Medical and Paramedical Sciences, Aix-Marseille University, HIPE Human Lab, Marseille, France

Correspondence: Soraya Mezouar (soraya.mezouar@univ-amu.fr)

Received: 28 March 2025 | **Revised:** 5 May 2025 | **Accepted:** 12 May 2025

Funding: The authors received no specific funding for this work.

Keywords: antiviral response | macrophage polarization profiles | macrophages | monkeypox virus | placenta | pregnancy

ABSTRACT

Monkeypox is a viral zoonosis caused by the monkeypox virus (MPXV). Although the virus was identified decades ago, human immunity to MPXV infection has not been widely characterized. During MPXV infection, macrophages are recruited at the site of infection and are thought to contribute to the spread of the virus. Cases of MPXV vertical transmission were reported in infected pregnant women to the developing fetuses *in utero* resulting to high viral burden in placenta tissue and abortion. We aim to understand the impact of MPXV infection at the foeto-maternal interface by focusing on macrophages functions. Using full-term placental explant model, macrophages were recruited at site of infection. Isolated naive macrophages are permissive to MPXV infection and secrete high levels of pro-inflammatory cytokines associated with a strong M1 polarization profile. Analysis of antiviral gene expression reveals upregulation of *IFNA* and IFN-associated genes suggesting that MPXV induces the expression of some component of antiviral response from macrophages that are unable to clear the virus. Our study shows that macrophages are permissive to MPXV that subverts inflammatory and antiviral machinery without virus clearance. Such findings contribute to better knowledge of MPXV vertical transmission pathogenesis.

1 | Introduction

Monkeypox virus (MPXV) is a *Poxviridae* virus closely related to smallpox virus, genus *Orthopoxvirus*, that was responsible of a Monkey smallpox or Monkeypox (MPX) epidemic in 2022 [1, 2]. Appearing mainly in Europe and North America, this viral zoonotic infection was detected in non-endemic regions for a virus found in the forest area of Central and West Africa. MPXV infection causes a smallpox-like illness in humans presenting as fever, headache, chills, exhaustion, swollen lymph nodes, myalgia, sore throat, rectal pain, proctitis, and anogenital lesions and localized rashes [3–5]. MPXV is transmitted through several routes, including respiratory droplets during human-to-human transmission or by direct contact with mucocutaneous lesions of infected humans or animals [1, 6–9]. The pathogenesis and clinical manifestation are closely related to the host immune response.

Following viral infection of humans, MPXV infects and replicates in immune cells including B and T lymphocytes, monocytes, dendritic cells and macrophages [10]. In Vitro and In Vivo studies reported that monocytes represent the first target cell of MPXV [11]. These latter cells may be potential vehicles for the dissemination of the virus in the body [12].

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2025 The Author(s). *Journal of Medical Virology* published by Wiley Periodicals LLC.

At site of MPXV infection, an infiltration of dendritic cells, T lymphocytes and macrophages was reported [11]. In reported cases of human infection, increased level of cytokines was reported with variation in the type of cytokines and concentrations depending on the severity of the disease [13, 14]. The severity of the disease ranges from mild to fatal. Mild cases are mainly skin lesions, while moderate to severe cases involve systemic damage, with the risk of secondary infection in the lungs or gastrointestinal tract. The most severe cases show encephalitis, septicemia or ocular damage, leading to permanent loss of vision and even death. The severity of the disease is also influenced by patient-related factors such as age, immune status and access to quality healthcare. Severe forms and higher mortality rates are more frequent in young children, immunocompromised people and those infected with virulent clades of MPXV [14–16]. the innate immune response from MPXV infected-humans, the roles for associated immune cells are currently unknown. Nevertheless, it was reported that human macrophages are permissive to MPXV contributing to cell-cell spread [17], however little is known about the immunological and antiviral responses of phagocytes to infection.

Cases of vertical transmission were reported in infected mothers to the developing fetus in utero resulting to abortion [18–23] with the presence of a high viral load in the fetal tissue of the umbilical cord and placenta [18, 23], which suggests a tropism for placenta. The placenta is a target organ for numerous pathogens including virus responsible of vertical transmission including cytomegalovirus, zika virus, rubella virus or recently Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [24–28]. Trophoblasts, major cell type in the placenta, form barriers to viral infections in different anatomical structures of the placenta (decidua and chorionic part) [29]. Although trophoblasts are usually resistant to viral infections [30], we have previously shown that MPXV infects and replicates within them (JEG-3 cell line and healthy primary trophoblast) [31]. The decidual and chorionic parts of the placenta house many immune cells that help in the protection of the placenta from infections. Fetus-derived macrophages (Hofbauer cells) and decidual macrophages from maternal origin represent the first lines of defense during infections by presenting unique properties compared to other macrophages residing in tissues or monocytes within the systemic circulation [32–35]. Macrophages from placenta present a high phenotype plasticity as they can adapt their phenotype in response to the environment. Macrophage activation, following different stimuli, lead to M1 or M2 polarization profile for pro-inflammatory and immunoregulatory state, respectively [36–38]. During In Vitro and In Vivo viral infection, macrophages represent target cells for virus manipulating them to promote their replication and dissemination in the tissue [39, 40]. The role of macrophages at the maternal-fetal interface against MPXV infection remains unknown.

In this study, we show that macrophages are recruited at site of MPXV infection in full-term placental explant model. We report that isolated naive macrophages are permissive to MPXV infection. Upon infection, tissue-resident macrophages from placenta secrete high levels of pro-inflammatory cytokines and has a strong M1 polarization profile that is not found with monocyte-derived macrophages (MDM) as control. Analysis of antiviral gene expression reveals upregulation of *IFNA*, but not *IFNB*, gene and antiviral effectors genes showing that MPXV induces an antiviral response in macrophages that are unable to clear the virus. Our study highlights the role of macrophages at the foeto-maternal environment during the vertical transmission of MPXV.

2 | Materials and Methods

2.1 | Cell Isolation and Culture

Vero E6 cells (ATCC CRL-1586) were cultivated in minimal essential medium (MEM) supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% L-glutamine (M10 medium) (Life Technologies).

Peripheral blood mononuclear cells (PBMCs) were isolated from healthy donors (buffy coats) by density-gradient centrifugation using Ficoll (Eurobio) at $800 \times g$ for 30 min as previously described [41]. PBMCs were cryopreserved at −80°C in 90% FBS supplemented by 10% dimethyl sulfoxide in isopropanol freezing container (Sigma-Aldrich).

Monocytes were purified from PBMCs using MACS magnetic beads (Miltenyi Biotec) coated with anti-CD14 antibody and cultured in Roswell Park Memorial Institute medium (RPMI) −1640 containing 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 50 μg/mL streptomycin (Life Technologies) [42]. Monocytes were differentiated into macrophages (MDM) in RPMI-1640 containing 10% inactivated human AB-serum (MP-Biomedicals), 2 mM glutamine, 100 U/mL penicillin, and 50 μg/mL streptomycin for 3 days. After washes, cells were cultured for 4 additional days in RPMI-1640 containing 10% FBS and 2 mM glutamine. MDM purity (98.2%) was assessed by flow cytometry.

Macrophages were isolated from full-term entire placenta tissue as previously described [43, 44]. Briefly, placenta tissue was digested in Hank's Balanced Salt Solution (HBSS, Life Technologies), DNase I 2.5 mM, and 2.5% trypsin (Life Technologies), twice 40 min under agitation. Cell suspension was filtered through 100-μm pores to obtain total placental cells. Placental cells were deposited on a Ficoll cushion (Eurobio) and centrifuged at $700 \times g$ for 20 min at 4°C to collect mononuclear cells. Macrophages were isolated using magnetic beads coated with anti-CD14 antibodies. Macrophages were then cultivated in Dulbecco's Modified Eagle Medium (DMEM)-F12 supplemented with 10% FBS, 100 U/mL penicillin, and 50 μg/mL streptomycin for 24 h.

2.2 | MPXV Production and Cell Infection

The IHUMPXV8 strain was the eighth isolated from a rectal swab of a man infected during the 2022 monkeypox epidemic in Marseille. IHUMPXV8 strain was obtained after Vero E6 cells infection as previously described [31]. In some experiments, MPXV was heat-inactivated at 65°C for 3 h.

Monocyte, MDM, and macrophage at 1.10^5/well were infected with MPXV viral suspension at multiply of infection (MOI 1) of 1 for 24, 48 and 72 h using appropriate media without antibiotics.

2.3 | Placenta Explants Culture and Infection

Pieces of the entire placenta measuring approximately 1 cm^3 were removed from the area between the umbilical cord insertion and the margin of the placenta, including the maternal and fetal sides. Biopsies were rinsed with PBS, cut into approximate 4 mm^2 pieces and then transferred to six-well plates (fetal part at the bottom of the well) containing DMEM F-12, 10% FBS, 100 units/mL of penicillin and 100 μg/mL streptomycin at 37°C overnight. Explants were infected with 1×10^5 plaque forming units (PFU)/explant during 24 h at 37°C and 5% of CO_2, as previously reported [45, 46] Tissues were then harvested at different infection times (24, 48, 72 h), fixed in 4% formaldehyde for 24 h, dehydrated and embedded in paraffin. After deparaffinisation, permeabilization by 0.1% Triton X-100, treatment with Fc Blockers at 10 μg/mL (FcR blocking Reagent, Miltenyi) for a blocking step during 10 min at room temperature, 2 μm tissue sections were incubated with antibodies directed against MPXV (ACRObiosystem, 1/250) and mouse anti-CD163 (BioLegend, 1/250) for 1 h at room temperature. Alex Fluor 488-conjugated phalloidin was used to label F-actin cytoskeleton and 4′, 6-diamidino-2-phenylindole (DAPI, Thermo Fisher Scientific) for nucleus staining. After extensive washing, tissue sections were incubated with Alexa Fluor 647 anti-human and Alexa Fluor 488 anti-mouse (Life Technologies) for 1 h. Coverslips were mounted with Mowiol (F4680, Sigma). Pictures were acquired using an LSM800 Airyscan confocal microscope (Zeiss). Quantification of macrophage and MPXV presence were evaluated with ImageJ, calculating the area of each channel, as well as the intensity of each image. For each fluorescence channel, images were split and threshold using the Otsu automatic thresholding method in ImageJ (version 2.16.0). Threshold values were adjusted to include all positive signals and exclude background, typically ranging from 30 to 255 on an 8-bit scale. The threshold was applied to create a binary mask, and integrated area and density were measured using the "Analyze Particles" function, with size exclusion set to exclude noise. Background subtraction was performed before thresholding using a rolling ball radius of 30 pixels. For the colocalization quantification, the area of the yellow channel was calculated and plotted over all the MPXV and macrophage channels to obtain a colocalization percentage. The thresholds images were then analyzed using the Coloc2 plugin to calculate Pearson and Manders coefficients, applying the thresholds determined in the pre-processing step.

2.4 | Viral Replication

The infection of cells and virus replication were evaluated as follows. Viral DNA was extracted from infected cells or culture supernatants using EZ1 advanced XL Virus Mini kit v2.0 (Qiagen). Virus detection was performed using a specific program for viral qPCR presented in Table S1. The G2 gene was investigated for the MPXV detection (Table S2), and viral quantification was expressed as cycle threshold (Ct) values.

2.5 | Cell Viability

Cell viability was evaluated using the 3-[4,5-dimethylthiazol-2-yl]−2,5 diphenyl tetrazolium bromide (MTT) assay (Thermofisher). After 24, 48, 72 and 96 h of MPXV stimulation, 10 μL of MTT (5 mg/mL, Sigma-Aldrich) were added to the cell culture and incubated at 37°C for 4 h. The resulting formazan crystals were solubilized with 50 μl of dimethylsulphoxide (DMSO) for 30 min at 37°C and quantified using a Tecan infinite 200 plate reader at 570 nm (Tecan).

2.6 | Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from cells (1×10^6 cells/well) using the RNA extraction Kit (Zymo Research) and DNase I treatment as previously described [44]. The quality and quantity of the extracted RNAs were evaluated using a spectrophotometer (Nanodrop Technologies). Reverse-transcription of isolated RNA was performed using a Moloney murine leukemia virus-reverse transcriptase kit (Life Technologies) and oligo (dT) primers. The expression of polarization genes was evaluated using real time qPCR, Smart SYBR Green fast Master kit (Roche Diagnostics) and specific validated primers (Table S3). The qPCRs were performed using a CFX Touch Real-Time PCR Detection System (Bio-Rad). The results were normalized by the expression of *ACTB* housekeeping gene and are expressed as relative expression of investigated genes with $\Delta Ct = Ct_{Target} - Ct_{ACTB}$ as previously described [47].

2.7 | Transmission Electron Microscopy (TEM)

For electron microscopy, virus-infected cell cultures in microplates were processed according to Le Bideau et al. [48] Cells were fixed with glutaraldehyde (2.5%) in 0.1 M sodium cacodylate buffer. Resin embedding was microwave-assisted with a PELCO BiowavePro+ (Ted Pella Inc.), by exchanging 200 μL of the different solutions at each step. Samples were washed two times with a mixture of 0.2 M saccharose/0.1 M sodium cacodylate and post-fixed with 1% OsO4 diluted in 0.2 M potassium hexa-cyanoferrate (III)/0.1 M sodium cacodylate buffer. After two washes with distilled water, samples were gradually dehydrated by successive baths in 30%, 50%, 70%, 90%, 96%, and 100% ethanol. Substitution with Epon resin (Embed 812 mixed with NMA, DDSA, and DMP-30 hardener; Electron Microscopy Sciences) was achieved by incubations with 25%, 50%, 75% Epon resin in ethanol and incubations with 100% Epon resin. Polymerization was done in 100% fresh Epon for 72 h at 60°C. All solutions used above were 0.2 μM filtered. Resin blocks were placed in a UC7 ultramicrotome (Leica Biosystems), trimmed to pyramids, and ultrathin 100 nm sections were cut and placed on HR25 300 Mesh Copper/Rhodium grids (TAAB, Reading). Sections were contrasted according to Reynolds. Electron micrographs were obtained on a Tecnai G2 TEM (Thermo-

Fischer/FEI) operated at 200 keV equipped with a 4096 × 4096 pixels resolution Eagle camera (FEI).

2.8 | Enzyme-Linked Immunosorbent Assay (ELISA)

Monocyte, MDM, and macrophage were stimulated with virus for 24 h. TNF-α (Invitrogen), IL-1β (R&D Systems), IL-10 and IL-6 (Clinisciences) levels were quantified in cell supernatants with appropriate immunoassays according to the manufacturer's instructions. The sensitivity was 8 pg/mL for TNF-α, 0.125 pg/mL for IL-1β, 15.4 pg/mL for IL-6 and 3.9 pg/mL for IL-10. Cytokine concentrations were normalized to protein content.

2.9 | Statistical Analysis

Statistical analysis was performed using R software version 4.1.1 (R Foundation for Statistical Computing). Statistical comparisons were performed using nonparametric Mann-Whitney U test or nonparametric Kruskal-Wallis test. The Mann-Whitney U test is used to compare two independent groups, and the Kruskal-Wallis's test is an extension of the Mann-Whitney test for more than two independent groups. Without the limitations of parametric testing, nonparametric tests are employed to ensure accurate analysis. RT-PCR data including principal component analysis (PCA) and hierarchical clustering of gene expression were analyzed using the ClustVis webtool. All tests were two-sided. Differences were considered statistically significant at $p < 0.05$.

3 | Results

3.1 | MPXV Infects Macrophages at the Maternal-Fetal Interface

As fetal and placental lesions were reported following vertical transmission of MPXV in infected pregnant women [18], we investigated the local inflammatory response at the maternal-fetal interface. First, we cultivated at term-placental explants from healthy donors and infected them with MPXV. As illustrated in the Figure 1, we observed MPXV in the placenta tissue and recruitment of macrophages at the site of infection (Figure 1A). Over time, MPXV (Figure 1B) and macrophage (Figure 1C) increased suggesting that the infection of placental explants led to the recruitment of site of infection by macrophages. Interestingly, the virus burden and the number of macrophages over time were positively correlated ($R = 0.9974$, $p = 0.0458$) (Figure 1D). Also, we observed a colocalization between MPXV and macrophages, suggesting that recruited macrophages are infected (Figure 1E).

We then isolated macrophages from at term-placenta of healthy donors and infected them In Vitro with MPXV. We used MDMs and monocytes as controls. We first confirmed the infection of the different cell types by transmission electron microscopy (Figure 2A). Monocytes, monocytes-derived macrophages (MDM) and macrophages were incubated with the MPXV for 24 h. A1 we can see a monocyte with MPXV particles visible inside the cell. A2 (zoom in on image A1) we can clearly see 2 particles in vesicles (located with black arrows), one with a vesicle either forming or detaching to release the particle. A3 shows the infection of an MDM with MPXV particles. A4 (zoom in on image A3) also shows 2 particles in vesicles (localized with black arrows), one showing a possible release of the MPXV particle, and very close to mitochondria. A5 shows several MFMs with several particles inside a cell. C (zoom in on image A6) shows several particles in different vesicles (localized with black arrows), including one close to the nucleus, showing an invagination. As illustrated by confocal microscopy, MPXV was found inside macrophages as well as MDMs and monocytes (Figure 2B). Surprisingly, although macrophages were found infected, the virus load over time did not present significant differences (from 24 to 96 h, $p = 0.333$). In contrast, monocytes ($p < 0.0001$) and MDMs ($p = 0.0004$) infection increased over time (Figure 2C). Ninety-6 h postinfection, MPXV replication was moderate for all investigated cells (Figure 2D) with 7.4% for macrophage ($p = 0.0157$), 3.8% for MDM ($p = 0.0003$) and 3.05% for monocytes ($p = 0.0252$) compared to 24 h postinfection. MPXV infection impacted on cell viability (all, $p < 0.0001$) (Figure 2E). A moderate cell mortality was observed for macrophage populations in contrast to monocytes. Over time, MPXV resulted in a 42% decrease in cell viability for monocytes, compared to 27% and 23% for MDMs and macrophages, respectively.

Compared to monocytes, macrophages infection (macrophage from placenta and MDM) led to moderate MPXV replication and cell cytotoxicity. These results suggest that MPXV infects myeloid cells including macrophages.

3.2 | MPXV Induces Pro-Inflammatory Cytokine Production by Macrophages

We next assessed the ability of macrophages to produce pro-inflammatory cytokines, IL-6, TNF, IL-1β and anti-inflammatory cytokines such as IL-10 in supernatants from infected cells. Following MPXV infection of macrophages, the pro-inflammatory cytokines including IL-6 ($p < 0.0001$) and TNF ($p < 0.0001$) were increased as compared to unstimulated cells (Figure 3A). Similar results were observed in monocytes (IL-6, $p < 0.0001$; TNF, $p < 0.0001$) and in a more attenuated manner in MDMs (IL-6, $p = 0.0006$; TNF, $p = 0.0128$). Surprisingly, IL-1β level was significantly decreased in MPXV-infected macrophages ($p < 0.0001$) and monocytes ($p < 0.0001$), but not in MDMs ($p = 0.9550$). Interestingly, only macrophages exhibited a significant decrease of IL-10 ($p = 0.0078$) (Figure 3B). In contrast, MPXV led to increased secretion of IL-10 by MDMs ($p = 0.0085$). These findings demonstrate that macrophages initiate a pro-inflammatory response to MPXV infection.

3.3 | MPXV Infected-Macrophages Exhibit M1 Polarization Profile

Next, we aimed to decipher the inflammatory state of MPXV infected-macrophages by the investigation of the M1 and M2 polarization profile. The principal component analysis revealed

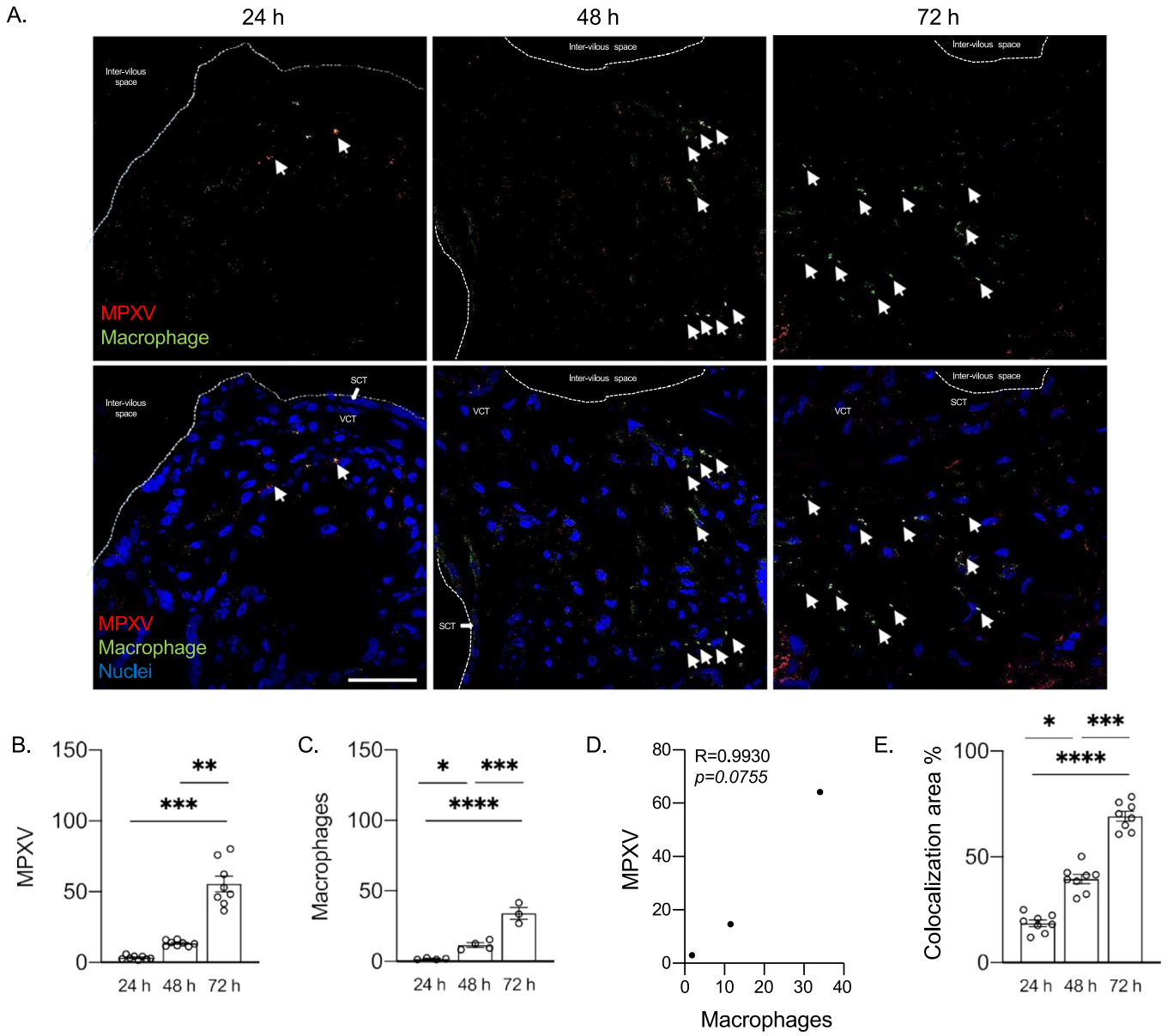

FIGURE 1 | MPXV infects macrophages. At-term placental explant from healthy woman was incubated ex vivo with MPXV with 1×10^5 plaque forming units (PFU) for 24, 48 and 72 h. (A) Representatives' illustrations of confocal microscopy of infected at-term placental explants illustrating the presence of MPXV in red, macrophages ($CD163^+$ cells) in green and nuclei in blue. White arrows illustrate infected macrophages. (B–D) The presence of (B) MPXV and (C) macrophages were monitored over time at 24-, 48- and 72-h postinfection. (C) Correlation between MPXV and macrophage fluorescent signaling was evaluated over time. (E) The presence of infected macrophages was evaluated through the quantification of the colocalization between the markers to identify MPXV and CD163.

that the response of macrophages to MPXV infection is different from MDMs (Figure 4A). Indeed, MPXV-stimulated cells were offset from all other investigated conditions. Hierarchical clustering validated this finding (Figure 4B). Interestingly, resting macrophages clustered on the same branch as cells incubated with the heat-inactivated MPXV. At the opposite, we observed the response of MDMs stimulated or not by MPXV (live and heat-inactivated). In the middle and on a separate branch, we found the cluster of macrophages stimulated by MPXV. Focusing on this cluster, we showed a clear overexpression of M1 genes, *IFNG*, *IDO1*, *CD86*, *NOS2*, *OAS1*, *CD80*, *IL6*, *TNF*, except for *IL1B* and *RSAD2*, suggesting that MPXV infection led to a M1 profile for macrophages. Compared to resting macrophages, MPXV led to significant increase of M1 genes including *IL6* ($p = 0.0104$), *TNF* ($p = 0.0477$), *NOS2* ($p = 0.0003$), *IDO1* ($p = 0.0002$), *CD80* ($p = 0.0052$), *CD86* ($p = 0.0188$) (Figure 4C). No differences were observed following the stimulation of macrophages by heat-inactivated MPXV compared to resting cells. Nevertheless, macrophages stimulated by live MPXV was associated with significant increase of *IL6* ($p = 0.0098$), *TNF* ($p = 0.0254$, *NOS2* ($p < 0.0001$), *IDO1* ($p = 0.0002$), *CD80* ($p = 0.0188$), *CD86* ($p = 0.0188$) compared to heat-inactivated MPXV, suggested that inactivated virus retained the ability of macrophages to polarize into an M1 profile. Same profile was observed for two M1 genes specific for macrophage from placenta *RSAD1* and *OAS1* (Figure S1). We observed significant

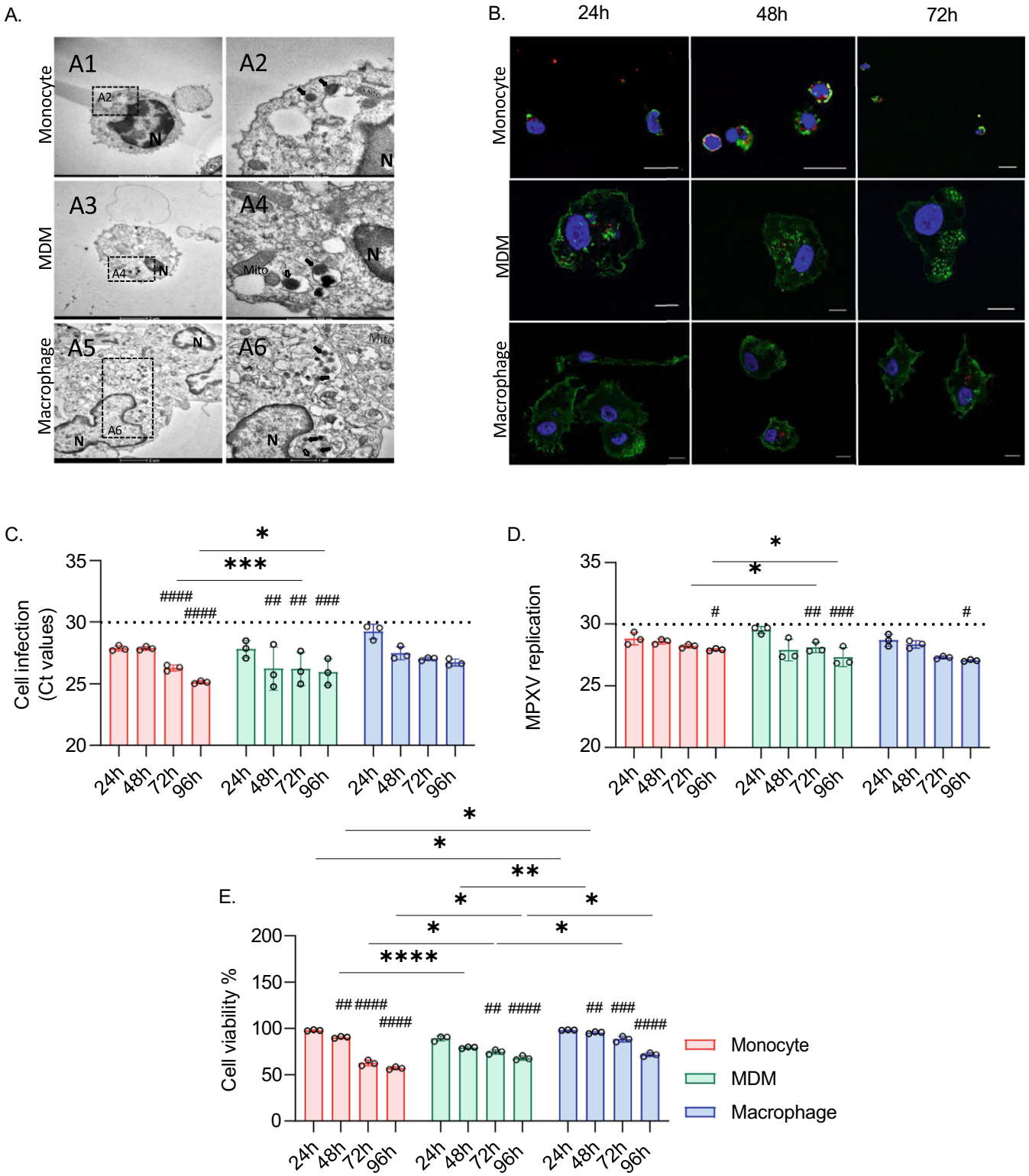

FIGURE 2 | MPXV infects and replicates in macrophages. Monocytes, monocytes-derived macrophages (MDM) and full-term naïve macrophages were incubated with the MPXV (1 multiplicity of infection) during 24, 48 and 72 h. (A) Representative's illustrations of electron microscopy of infected cells during 24 h. (B) Representatives' illustrations of confocal microscopy of infected cells with MPXV in red, F-actin in green and nuclei in blue. (C) Viral RNA was quantified at 24, 48 and 72 h in infected cells and expressed as Cycle threshold (Ct) values. Data from $n = 3$ donors were expressed as mean ± SEM. $^{**}p < 0.001$, $^{***}p < 0.0001$, $^{****}p < 0.00001$ (compared to 24 h), $^{*}p < 0.05$, $^{***}p < 0.0001$ (between investigated cells). (D) Viral RNA was quantified at 24, 48 and 72 h in supernatants from infected cells and expressed as cycle threshold (Ct). Data from $n = 3$ donors were expressed as mean ± SEM. $^{\#}p < 0.05$, $^{\#\#}p < 0.001$, $^{\#\#\#}p < 0.0001$ (compared to 24 h), $^{*}p < 0.05$ (between investigated cells). (E) Cytopathic effect was quantified at 24, 48 and 72 h and expressed as percentage of cell viability. Data from $n = 3$ donors were expressed as mean ± SEM. $^{\#}p < 0.05$, $^{\#\#}p < 0.001$, $^{\#\#\#}p < 0.0001$, $^{\#\#\#\#}p < 0.00001$ (compared to 24 h), $^{*}p < 0.05$, $^{**}p < 0.001$, $^{****}p < 0.0001$ (between investigated cells).

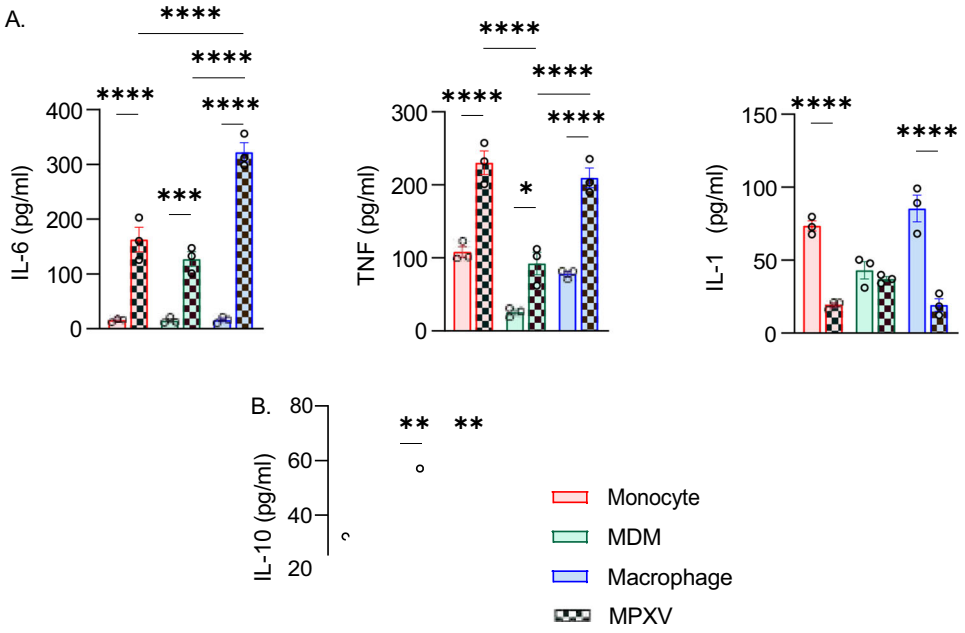

FIGURE 3 | MPXV induces pro-inflammatory cytokine production by macrophages. Monocytes, monocytes-derived macrophages (MDM) and macrophages were incubated with the MPXV (1 multiplicity of infection) during 24, 48 and 72 h. (A, B) (A) Pro- and (B) anti- inflammatory cytokine levels were determined in supernatants of infected cells by immunoassays 24 h postinfection. Data from $n = 3$ donors were expressed as mean ± SEM. $^{**}p < 0.001$, $^{****}p < 0.00001$. The sensitivity was 8 pg/ml for TNF-α, 0.125 pg/ml for IL-1β, 15.4 pg/mL for IL-6 and 3.9 pg/mL for IL-10.

decreased expression of *IL1B* transcripts by MPXV infected-macrophages ($p < 0.0001$), but not with heat-inactivated virus, concordant with decreased IL-1β secretion (Figure 4D and Figure 3A). Same observations were observed for MPXV-infected monocytes (Figure S2A and Figure 3A) Of note, no differences were observed for *IFNG* transcripts for macrophages, but MPXV led to a significant increase of *IFNG* in monocytes (Figure S2A). Focusing on M2 polarization profile, MPXV infection led only to the under-expressed of *CD206* by macrophages stimulated by the inactivated virus thus amplifying the M1 profile (Figure 4D). This suppression of the expression of anti-inflammatory genes is also observed by monocytes after infection with MPXV (Figure S2B). In contrast, MPXV infected-MDMs presented significant increase of IL-10 ($p = 0.0279$) and CD163 ($p = 0.0006$) expression. These results show that compared to MDM, macrophages respond to MPXV infection through initiation of a pro-inflammatory state characterized by an M1 polarization profile.

3.4 | MPXV Induces the Expression of Some Component of Antiviral Response of Maternal-Fetal Interface Macrophages

Finally, we investigated if infection of macrophages was associated to an antiviral response against MPXV through the evaluation of expression of several antiviral effectors and type I IFN genes. Macrophages response to MPXV infection was distinct from that observed when cells were incubated In Vitro with heat-inactivated MPXV or in resting conditions (Figure 5A). The principal component analysis and hierarchical clustering indicated that the response of macrophages to MPXV infection was also distinct from MPXV infected-MDMs suggesting a specific modulation of component of the antiviral response for the placental population (Figure 5B). We observed increased expression of *IFNA* ($p = 0.0096$) transcripts while *IFNB* and *IFNB1* were not modulated in macrophages following MPXV stimulation (Figure 5C). Interestingly, heat-inactivated MPXV significantly modulated the expression in macrophages of *IFNB* ($p < 0.0001$) and *IFNB1* ($p = 0.0008$) but in very small quantities compared to *IFNA*. Expression of *IFIT1* ($p = 0.0147$), *IFIT2* ($p = 0.0118$), *IFIT3* ($p = 0.0135$) and *IFIH1* ($p = 0.0001$) transcripts was induced in response to MPXV in macrophages (Figure 5D). No differences were observed in MDMs. In contrast, the virus induced a modulation of the antiviral response and type I IFN genes by infected-monocytes (Figure S3A, S3B). These findings show that, compared to MDMs, macrophages respond specifically to MPXV infection through a modulation of some components of the antiviral response.

4 | Discussion

Cases reported identify vertical transmissions of MPXV in infected pregnant woman to the fetus inducing fetal complications, such as diffuse skin lesions, hepatomegaly, hydrops, and signs of viral replication in several organs, and the presence of the virus at high rates within the placenta [18, 23]. Our study reports for the first time the ability of MPXV to infect and spread within placental tissue through an *ex vivo* placental explant infection model. Following the infection of the placental tissue, macrophages are largely recruited at the site of infection and that they are easily infected. It has been previously reported that other immune cells are present at the site of infection such as dendritic cells and lymphocytes (T and B) [11]. It would be interesting to investigate the recruitment of these cell

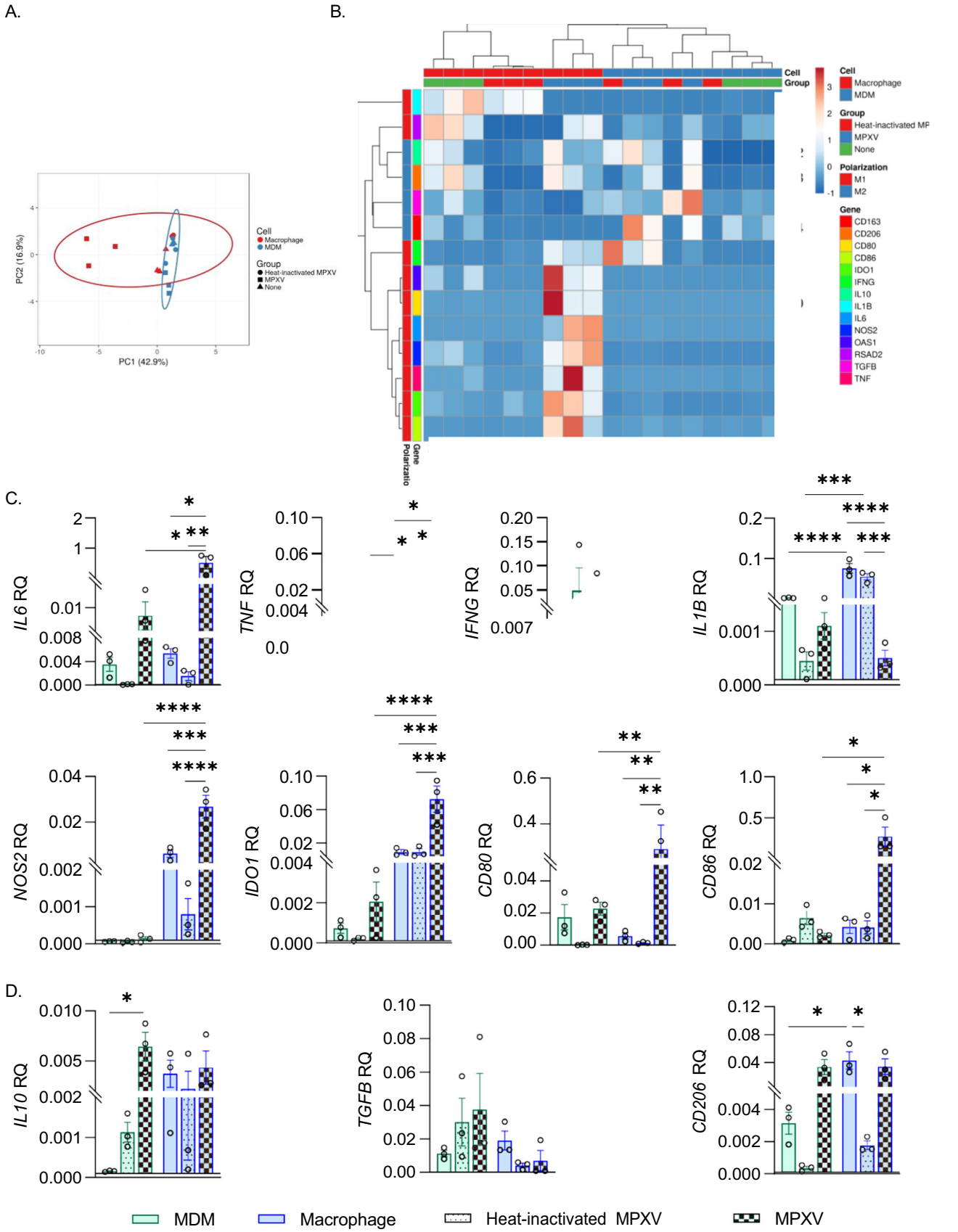

FIGURE 4 | MPXV infected-macrophages present an M1 polarization profile. Monocytes, monocytes-derived macrophages (MDM) and macrophages were incubated with the MPXV or heat-inactivated MPXV (1 multiplicity of infection) for 8 h. (A, B) Gene expression data are presented as (A) principal component analysis and (B) hierarchical clustering obtained using the ClustVis webtool. (C, D) The expression genes of (C) inflammatory and (D) immunoregulatory genes are expressed as relative quantity (RQ). Data from $n = 3$ donors were expressed as mean ± SEM. *$p < 0.05$, **$p < 0.001$, ****$p < 0.0001$, ****$p < 0.0001$.

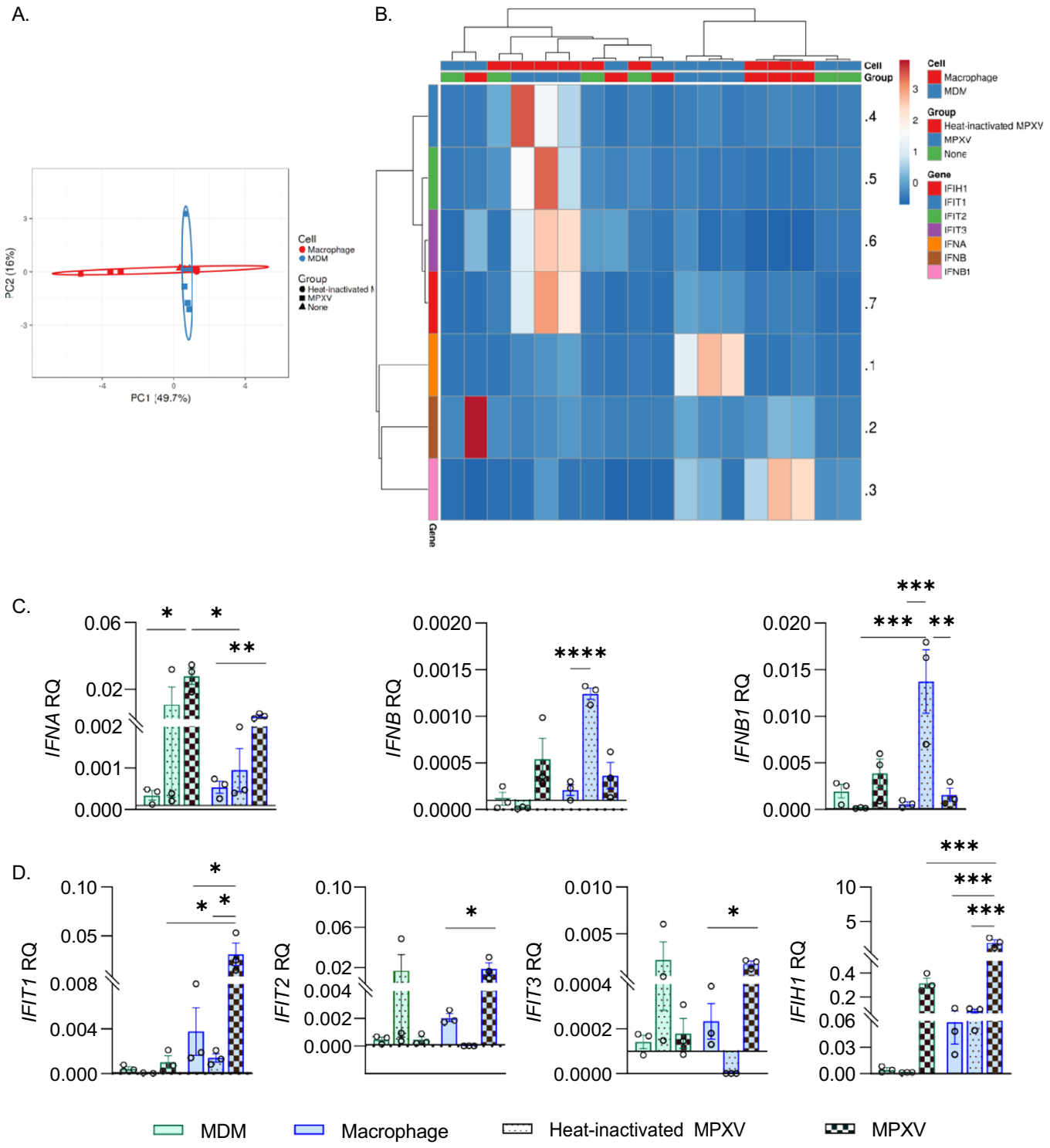

FIGURE 5 | Macrophages present an antiviral reply against MPXV infection. Monocytes, monocytes-derived macrophages (MDM) and macrophages were incubated with the MPXV or heat-inactivated MPXV (one multiplicity of infection) for 8 h. (A, B) Gene expression data are presented as (A) principal component analysis and (B) hierarchical clustering obtained using the ClustVis webtool. (C, D) The expression genes of (C) type I IFN and (D) antiviral genes are expressed as relative quantity (RQ). Data from $n = 3$ donors were expressed as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.001$, $^{***}p < 0.0001$, $^{****}p < 0.00001$.

populations and their role in controlling infection at the materno-fetal interface.

Using full-term placental explant model, macrophages were recruited at site of infection. We have previously shown that trophoblasts, the main placental cells, represent a target of MPXV in placental tissue [31]. After MPXV infection of isolated primary trophoblasts we decipher the infection and replication cycle. Placental tropism of *poxviruses* has previously been reported. The full-term trophoblasts infection by Vaccinia virus

(VAC) has been identified with a deleterious impact on the infected cell like cytopathic change, cytoplasmic condensation and cell rounding [49]. In contrast to trophoblasts [31], we show that MPXV infection induces moderate mortality of macrophages in which we observed a moderate virus replication suggesting that macrophages could constitute a niche for virus.

The polarization profile of macrophages is critical for an effective immune response against pathogen evasion. Interestingly, some pathogens can modulate the polarization profile of macrophages to facilitate their dissemination and pathogenesis. Here, we reported that MPXV lead to a clear M1 polarization of macrophages not found with MDMs. Several In Vitro and In Vivo studies of HIV-1 infected macrophages reported a M1 polarization profile with a large secretion of pro-inflammatory cytokines [50–52]. HIV-1 infection of macrophages polarized in M1 (by IFN-g or TNF-a) before infection leads to significant containment of virus replication [51, 53]. Interestingly the restimulation by IFNγ or TNF-α of M1-polarized macrophages already infected with HIV-1 lead to a quasi-silent infection inhibiting virus replication down to very low levels for several weeks [54] suggesting a state of virus quiescence within activated macrophages. This M1 polarization of HIV-1 infected macrophages is reversed to M2 polarization allowing the virus to use macrophages as a reservoir for dissemination [55, 56]. These different macrophage polarization states are related to acute and chronic forms found in HIV-infected patients [56]. The strong M1 polarization of MPXV-infected macrophages without elimination and with modest replication of the virus could suggest a control of macrophages with the pathogen profile whose mechanisms remain to be explored.

Previous studies report that *poxviruses* (VACV and modified vaccinia virus Ankara [MVA]) induce direct apoptosis in macrophages (murine and human) [57, 58] suggesting an inflammasome activation [59] mediated by caspase 1 that converts the pro-form of cytokine, IL-1β [60]. MVA-induced IL-1β production has been reported as a function of the signaling of the inflammasome activating caspase 1 [61] reinforcing the link between IL-1β and apoptosis in infected cells. Indeed, in a local infection model, MVA-activated inflammasome following macrophages infection lead to inflammation and cell death [59]. Interestingly, MPXV induces an inhibition of transcriptomic IL-1β expression and secretion by macrophages. In contrast, MPXV infection of MDM does not affect transcriptional expression and secretion of IL-1β. These data suggest that MPXV prevents apoptosis of macrophages by blocking the IL-1β pathway in macrophages to impair their apoptosis, reinforcing the hypothesis that these cells could be a niche for viral replication and dissemination as previously reported for VACV [17]. Further research is needed to investigate this hypothesis.

Our study shows a specific antiviral response of macrophages following MPXV infection. The investigation of the IFN-associated gene shows that MPXV infection induces a slight increase in the expression of these genes by the macrophages. This modulation is specific to macrophages because no differences are observed with MDM. IFNs type I are involved in the direct antiviral action of infected cells [62]. Our data indicate a MPXV impact of IFN type I genes with an increase in *IFNA* and not *IFNB*. *Poxviruses* present an ability to target this pathway via the expression of intracellular proteins such as eIF-2α homologous K3 and the double-stranded RNA-binding protein E3 [63, 64]. This virus manipulates this IFN pathway by the expression IFNα/β-binding protein (IFNα/βBP), a secreted IFN decoy receptor, which prevents interaction with cellular receptors by binding to IFNs [65–67] and protect cells from the antiviral effects of IFN. This may be related to the small decrease in viability of macrophages following MPXV infection. Finally, when macrophages are incubated with the attenuated virus, *IFNA* is decreased while *IFNB* is strongly expressed suggesting that the antiviral response mediated by type I IFN is dependent on the virulence of MPXV. Studies are needed to evaluate MPXV-mediated activation and inhibition pathways that interfere on IFN-associated genes including IFNs type I inducing a specific antiviral response as previously reported [68].

In conclusion, our study showed that MPXV infects myeloid cells including macrophages. Compared to monocytes and MDM, viral replication and cellular cytotoxicity remains moderate in macrophages suggesting that these cells could serve as a niche for the virus. Interestingly, we reported a specific pro-inflammatory state of macrophages with a modulation of some components of the antiviral response following MPXV infection. Future work will be required to determine the specificity of macrophages compared to myeloid cells during MPXV infection.

Author Contributions

Jonatane Andrieu, Margaux Valade realized experiments. Marion Lebideau, Bernard La Scola, Nathalie Wurtz provided virus strain and expertise. Florence Bretelle provided the placentas collection. Soraya Mezouar, Jean-Louis Mège supervised manuscript preparation and mentorship.

Acknowledgments

We thank Jean-Pierre Baudoin for his advice and kindness in carrying out this study. The authors received no specific funding for this work.

Ethics Statement

This prospective observational study was conducted in accordance with the Declaration of Helsinki and the French law on research involving humans. The protocol of the study was approved by an independent national review board (number 08-12).

Consent

All patients provided written informed consent. Patients were recruited at the Gynecology-Obstetrics Department of the Conception Hospital (Marseille, France). The manipulation of the MPXV strain and its nucleic acids was carried out according to ANSM (French National Agency for the Safety of Medicines and Health Products), authorization AMO-168522023.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data are available in the main text.

References

1. F. M. Lum, A. Torres-Ruesta, M. Z. Tay, et al., "Monkeypox: Disease Epidemiology, Host Immunity and Clinical Interventions," *Nature Reviews Immunology* 22, no. 10 (2022): 597–613, https://doi.org/10.1038/s41577-022-00775-4.

2. WHO Recommends New Name for Monkeypox Disease," *Neurosciences (Riyadh, Saudi Arabia)* 28, no. 1 (2023): 74–75.

3. G. Ciccarese, A. Di Biagio, B. Bruzzone, et al., "Monkeypox Outbreak in Genoa, Italy: Clinical, Laboratory, Histopathologic Features, Management, and Outcome of the Infected Patients," *Journal of Medical Virology* 95, no. 2 (2023): e28560, https://doi.org/10.1002/jmv.28560.

4. A. Carugno, M. Parietti, G. Ciccarese, et al., "Clinical-Epidemiological Aspects of the Monkeypox 2022 Epidemic: A Multicentre Study by the Italian SiDeMaST Group of Sexually Transmitted, Infectious and Tropical Diseases," *Journal of the European Academy of Dermatology and Venereology* 37, no. 11 (2023): e1277–e1279, https://doi.org/10.1111/jdv.19265.

5. F. J. Rodríguez-Cuadrado, L. Nájera, D. Suárez, et al., "Clinical, Histopathologic, Immunohistochemical, and Electron Microscopic Findings in Cutaneous Monkeypox: A Multicenter Retrospective Case Series In Spain," *Journal of the American Academy of Dermatology* 88, no. 4 (2023): 856–863, https://doi.org/10.1016/j.jaad.2022.12.027.

6. J. P. Thornhill, S. Barkati, S. Walmsley, et al., "Monkeypox Virus Infection in Humans Across 16 Countries - April-June 2022," *New England Journal of Medicine* 387, no. 8 (2022): 679–691, https://doi.org/10.1056/NEJMoa2207323.

7. E. Orviz, A. Negredo, O. Ayerdi, et al., "Monkeypox Outbreak in Madrid (Spain): Clinical and Virological Aspects," *Journal of Infection* 85, no. 4 (2022): 412–417, https://doi.org/10.1016/j.jinf.2022.07.005.

8. A. M. Vaughan, O. Cenciarelli, S. Colombe, et al., "A Large Multi-Country Outbreak of Monkeypox Across 41 Countries In the WHO European Region, 7 March to 23 August 2022," *Eurosurveillance* 27, no. 36 (2022): 2200620, https://doi.org/10.2807/1560-7917.ES.2022.27.36.2200620.

9. N. Sklenovská and M. Van Ranst, "Emergence of Monkeypox as the Most Important Orthopoxvirus Infection in Humans," *Frontiers in Public Health* 6 (2018): 241, https://doi.org/10.3389/fpubh.2018.00241.

10. A. M. McCollum and I. K. Damon, "Human Monkeypox," *Clinical Infectious Diseases* 58, no. 2 (2014): 260–267, https://doi.org/10.1093/cid/cit703.

11. E. Alakunle, U. Moens, G. Nchinda, and M. I. Okeke, "Monkeypox Virus in Nigeria: Infection Biology, Epidemiology, and Evolution," *Viruses* 12, no. 11 (2020): 1257, https://doi.org/10.3390/v12111257.

12. H. D. Hickman, G. V. Reynoso, B. F. Ngudiankama, et al., "Anatomically Restricted Synergistic Antiviral Activities of Innate and Adaptive Immune Cells in the Skin," *Cell Host & Microbe* 13, no. 2 (2013): 155–168, https://doi.org/10.1016/j.chom.2013.01.004.

13. R. F. Johnson, J. Dyall, D. R. Ragland, et al., "Comparative Analysis of Monkeypox Virus Infection of Cynomolgus Macaques by the Intravenous or Intrabronchial Inoculation Route," *Journal of Virology* 85, no. 5 (2011): 2112–2125, https://doi.org/10.1128/JVI.01931-10.

14. S. C. Johnston, J. C. Johnson, S. W. Stonier, et al., "Cytokine Modulation Correlates With Severity of Monkeypox Disease In Humans," *Journal of Clinical Virology* 63 (2015): 42–45, https://doi.org/10.1016/j.jcv.2014.12.001.

15. C. Shang, S. Shi, Q. Jiang, et al., "Clinical Manifestations and Pathogenicity of Clade IIb Monkeypox Virus in Rabbits," *Emerging Microbes & Infections* 14, no. 1 (2025): 2465309, https://doi.org/10.1080/22221751.2025.2465309.

16. S. Zhang, F. Wang, Y. Peng, et al., "Evolutionary Trajectory and Characteristics of Mpox Virus in 2023 Based on a Large-Scale Genomic Surveillance in Shenzhen, China," *Nature Communications* 15, no. 1 (2024): 7452, https://doi.org/10.1038/s41467-024-51737-4.

17. D. Byrd, N. Shepherd, J. Lan, et al., "Primary Human Macrophages Serve as Vehicles for Vaccinia Virus Replication and Dissemination," *Journal of Virology* 88, no. 12 (2014): 6819–6831, https://doi.org/10.1128/JVI.03726-13.

18. P. K. Mbala, J. W. Huggins, T. Riu-Rovira, et al., "Maternal and Fetal Outcomes Among Pregnant Women With Human Monkeypox Infection in the Democratic Republic of Congo," *The Journal of Infectious Diseases* 216, no. 7 (2017): 824–828, https://doi.org/10.1093/infdis/jix260.

19. L. Mandelbrot, C. Vauloup-Fellous, C. Huissoud, J. Ghosn, and O. Picone, "Variole du singe: propositions de conduite à tenir chez les femmes enceintes," *Gynécologie Obstétrique Fertilité & Sénologie.* 51, no. 5 (2023): 284–288, https://doi.org/10.1016/j.gofs.2023.03.002.

20. P. Fuentes-Zacarías, J. M. Murrieta-Coxca, R. N. Gutiérrez-Samudio, et al., "Pregnancy and Pandemics: Interaction of Viral Surface Proteins and Placenta Cells," *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* 1867, no. 11 (2021): 166218, https://doi.org/10.1016/j.bbadis.2021.166218.

21. P. Dashraath, M. P. Alves, D. A. Schwartz, K. Nielsen-Saines, and D. Baud, "Potential Mechanisms of Intrauterine Transmission of Monkeypox Virus," *The Lancet Microbe* 4, no. 1 (2023): e14, https://doi.org/10.1016/S2666-5247(22)00260-9.

22. D. A. Schwartz, S. Ha, P. Dashraath, D. Baud, P. R. Pittman, and K. M. Adams Waldorf, "Mpox Virus in Pregnancy, the Placenta, and Newborn," *Archives of Pathology & Laboratory Medicine* 147, no. 7 (2023): 746–757, https://doi.org/10.5858/arpa.2022-0520-SA.

23. N. K. Kisalu and J. L. Mokili, "Toward Understanding the Outcomes of Monkeypox Infection in Human Pregnancy," *The Journal of Infectious Diseases* 216, no. 7 (2017): 795–797, https://doi.org/10.1093/infdis/jix342.

24. P. S. Creisher and S. L. Klein, "Pathogenesis of Viral Infections During Pregnancy," *Clinical Microbiology Reviews* 37 (2024): e0007323, https://doi.org/10.1128/cmr.00073-23.

25. S. Mezouar, M. Katsogiannou, A. Ben Amara, F. Bretelle, and J. L. Mege, "Placental Macrophages: Origin, Heterogeneity, Function and Role in Pregnancy-Associated Infections," *Placenta* 103 (2021): 94–103, https://doi.org/10.1016/j.placenta.2020.10.017.

26. P. Abou Atmeh, S. Mezouar, and J. L. Mège, "Macrophage Polarization in Viral Infectious Diseases: Confrontation With the Reality." *Macrophages -140 Years of Their Discovery [Working Title]* (IntechOpen, 2022), https://doi.org/10.5772/intechopen.106083.

27. G. T. Tilahun, T. M. Tolasa, and G. A. Wole, "Modeling the Dynamics of Rubella Disease With Vertical Transmission," *Heliyon* 8, no. 11 (2022): e11797, https://doi.org/10.1016/j.heliyon.2022.e11797.

28. O. Guzeloglu-Kayisli, U. A. Kayisli, F. Schatz, and C. J. Lockwood, "Vertical Zika Virus Transmission at the Maternal-Fetal Interface," *Frontiers in Virology* 2 (2022): 801778, https://doi.org/10.3389/fviro.2022.801778.

29. C. J. Megli and C. B. Coyne, "Infections at the Maternal-Fetal Interface: An Overview of Pathogenesis and Defence," *Nature Reviews Microbiology* 20, no. 2 (2022): 67–82, https://doi.org/10.1038/s41579-021-00610-y.

30. E. Delorme-Axford, R. B. Donker, J. F. Mouillet, et al., "Human Placental Trophoblasts Confer Viral Resistance to Recipient Cells," *Proceedings of the National Academy of Sciences* 110, no. 29 (2013): 12048–12053, https://doi.org/10.1073/pnas.1304718110.

31. J. Andrieu, M. Valade, M. Lebideau, et al., "Pan-Microscopic Examination of Monkeypox Virus in Trophoblasts Cells Reveals New Insights Into Virions Release Through Filopodia-Like Projections," *Journal of Medical Virology* 96, no. 4 (2024): e29620, https://doi.org/10.1002/jmv.29620.

32. F. Ning, H. Liu, and G. E. Lash, "The Role of Decidual Macrophages During Normal and Pathological Pregnancy," *American Journal of*

Reproductive Immunology 75, no. 3 (2016): 298–309, https://doi.org/10.1111/aji.12477.

33. X. Jiang, M. R. Du, M. Li, and H. Wang, "Three Macrophage Subsets Are Identified in the Uterus During Early Human Pregnancy," *Cellular & Molecular Immunology* 15, no. 12 (2018): 1027–1037, https://doi.org/10.1038/s41423-018-0008-0.

34. R. Pique-Regi, R. Romero, A. L. Tarca, et al., "Single Cell Transcriptional Signatures of the Human Placenta in Term and Preterm Parturition," *eLife* 8 (2019): e52004, https://doi.org/10.7554/eLife.52004.

35. J. R. Thomas, A. Appios, X. Zhao, et al., "Phenotypic and Functional Characterization of First-Trimester Human Placental Macrophages, Hofbauer Cells," *Journal of Experimental Medicine* 218, no. 1 (2021): e20200891, https://doi.org/10.1084/jem.20200891.

36. S. Mezouar, M. Katsogiannou, A. Ben Amara, F. Bretelle, and J. L. Mege, "Placental Macrophages: Origin, Heterogeneity, Function and Role in Pregnancy-Associated Infections," *Placenta* 103 (2021): 94–103, https://doi.org/10.1016/j.placenta.2020.10.017.

37. Y. Zhang, L. Zhao, A. Wu, et al., "Abnormal M1 Polarization of Placental Macrophage Induced by IL-15/STAT5 Activation in VVC May Lead to Adverse Pregnancy Outcomes," *Microbes and Infection* 26, no. 1 (2024): 105232, https://doi.org/10.1016/j.micinf.2023.105232.

38. Y. Yao, X.-H. Xu, and L. Jin, "Macrophage Polarization in Physiological and Pathological Pregnancy," *Frontiers in Immunology* 10 (2019): 792, https://doi.org/10.3389/fimmu.2019.00792.

39. S. L. Lewis, P. J. Norris, and C. J. Holmes, "Phenotypic Characterization of Monocytes and Macrophages From CAPD Patients," *ASAIO Transactions* 36, no. 3 (1990): 575–577.

40. E. L. Johnson and R. Chakraborty, "Placental Hofbauer Cells Limit HIV-1 Replication and Potentially Offset Mother to Child Transmission (MTCT) by Induction of Immunoregulatory Cytokines," *Retrovirology* 9, no. 1 (2012): 101, https://doi.org/10.1186/1742-4690-9-101.

41. A. Boumaza, L. Gay, S. Mezouar, et al., "Monocytes and Macrophages, Targets of Severe Acute Respiratory Syndrome Coronavirus 2: The Clue for Coronavirus Disease 2019 Immunoparalysis," *The Journal of Infectious Diseases* 224, no. 3 (2021): 395–406, https://doi.org/10.1093/infdis/jiab044.

42. L. Gay, M. S. Rouviere, S. Mezouar, et al., "Vγ9Vδ2 T-Cells Are Potent Inhibitors of SARS-CoV-2 Replication and Represent Effector Phenotypes in Patients With COVID-19," *The Journal of Infectious Diseases* 229 (2024): 1759–1769, https://doi.org/10.1093/infdis/jiae169.

43. S. Mezouar, A. Ben Amara, C. Chartier, L. Gorvel, and J. L. Mege, "A Fast and Reliable Method to Isolate Human Placental Macrophages," *Current Protocols in Immunology* 125, no. 1 (2019): e77, https://doi.org/10.1002/cpim.77.

44. S. Mezouar, I. Benammar, A. Boumaza, et al., "Full-Term Human Placental Macrophages Eliminate Coxiella Burnetii Through an IFN-γ Autocrine Loop," *Frontiers in Microbiology* 10 (2019): 2434, https://doi.org/10.3389/fmicb.2019.02434.

45. N. Kupper, E. Pritz, M. Siwetz, J. Guettler, and B. Huppertz, "Placental Villous Explant Culture 2.0: Flow Culture Allows Studies Closer to the In Vivo Situation," *International Journal of Molecular Sciences* 22, no. 14 (2021): 7464, https://doi.org/10.3390/ijms22147464.

46. R. K. Miller, O. Genbacev, M. A. Turner, J. D. Aplin, I. Caniggia, and B. Huppertz, "Human Placental Explants in Culture: Approaches and Assessments," *Placenta* 26, no. 6 (2005): 439–448, https://doi.org/10.1016/j.placenta.2004.10.002.

47. P. A. Atmeh, L. Gay, A. Levasseur, et al., "Macrophages and γδ T Cells Interplay During SARS-CoV-2 Variants Infection," *Frontiers in immunology* 13 (2022): 1078741, https://doi.org/10.3389/fimmu.2022.1078741.

48. M. Le Bideau, N. Wurtz, J. P. Baudoin, and B. La Scola, "Innovative Approach to Fast Electron Microscopy Using the Example of a Culture of Virus-Infected Cells: An Application to SARS-CoV-2," *Microorganisms* 9, no. 6 (2021): 1194, https://doi.org/10.3390/microorganisms9061194.

49. N. Nørskov-Lauritsen, V. Zachar, P. M. Petersen, H. Hager, G. Aboagye-Mathiesen, and P. Ebbesen, "In Vitro Infection of Human Placental Trophoblast by Wild-Type Vaccinia Virus and Recombinant Virus Expressing HIV Envelope Glycoprotein," *Research in Virology* 143, no. 5 (1992): 321–328, https://doi.org/10.1016/s0923-2516(06)80120-2.

50. F. Porcheray, B. Samah, C. Léone, N. Dereuddre-Bosquet, and G. Gras, "Macrophage Activation and Human Immunodeficiency Virus Infection: Hiv Replication Directs Macrophages Towards a Pro-Inflammatory Phenotype While Previous Activation Modulates Macrophage Susceptibility to Infection and Viral Production," *Virology* 349, no. 1 (2006): 112–120, https://doi.org/10.1016/j.virol.2006.02.031.

51. E. Cassol, L. Cassetta, M. Alfano, and G. Poli, "Macrophage Polarization and HIV-1 Infection," *Journal of Leukocyte Biology* 87, no. 4 (2009): 599–608, https://doi.org/10.1189/jlb.1009673.

52. K. S. Burrack and T. E. Morrison, "The Role of Myeloid Cell Activation and Arginine Metabolism in the Pathogenesis of Virus-Induced Diseases," *Frontiers in Immunology* 5 (2014): 428, https://doi.org/10.3389/fimmu.2014.00428.

53. F. Graziano, E. Vicenzi, and G. Poli, "Immuno-Pharmacological Targeting of Virus-Containing Compartments in HIV-1-Infected Macrophages," *Trends in Microbiology* 24, no. 7 (2016): 558–567, https://doi.org/10.1016/j.tim.2016.02.018.

54. F. Graziano, G. Aimola, G. Forlani, et al., "Reversible Human Immunodeficiency Virus Type-1 Latency in Primary Human Monocyte-Derived Macrophages Induced by Sustained M1 Polarization," *Scientific Reports* 8, no. 1 (2018): 14249, https://doi.org/10.1038/s41598-018-32451-w.

55. G. Lugo-Villarino, C. Vérollet, I. Maridonneau-Parini, and O. Neyrolles, "Macrophage Polarization: Convergence Point Targeted by Mycobacterium Tuberculosis and HIV," *Frontiers in Immunology* 2 (2011): 43, https://doi.org/10.3389/fimmu.2011.00043.

56. T. H. Burdo, J. Walker, and K. C. Williams "Macrophage Polarization in AIDS: Dynamic Interface Between Anti-Viral and Anti-Inflammatory Macrophages During Acute and Chronic Infection," *Journal of Clinical & Cellular Immunology* 6, no. 3 (2015): 333, https://doi.org/10.4172/2155-9899.1000333.

57. L. Klaas, J. Vier, I. E. Gentle, G. Häcker, and S. Kirschnek, "Diversity of Cell Death Signaling Pathways in Macrophages Upon Infection With Modified Vaccinia Virus Ankara (MVA)," *Cell Death & Disease* 12, no. 11 (2021): 1011, https://doi.org/10.1038/s41419-021-04286-3.

58. Z. Humlová, M. Vokurka, M. Esteban, and Z. Mělková, "Vaccinia Virus Induces Apoptosis of Infected Macrophages," *Journal of General Virology* 83, no. Pt 11 (2002): 2821–2832, https://doi.org/10.1099/0022-1317-83-11-2821.

59. P. Sagoo, Z. Garcia, B. Breart, et al., "In Vivo Imaging of Inflammasome Activation Reveals a Subcapsular Macrophage Burst Response That Mobilizes Innate and Adaptive Immunity," *Nature Medicine* 22, no. 1 (2016): 64–71, https://doi.org/10.1038/nm.4016.

60. F. Liu, Q. Niu, X. Fan, et al., "Priming and Activation of Inflammasome by Canarypox Virus Vector ALVAC via the cGAS/IFI16-STING-Type I IFN Pathway and AIM2 Sensor," *The Journal of Immunology* 199, no. 9 (2017): 3293–3305, https://doi.org/10.4049/jimmunol.1700698.

61. S. Zimmerling, Z. Waibler, T. Resch, G. Sutter, and A. Schwantes, "Interleukin-1β Receptor Expressed by Modified Vaccinia Virus Ankara Interferes With Interleukin-1β Activity Produced in Various Virus-Infected Antigen-Presenting Cells," *Virology Journal* 10 (2013): 34, https://doi.org/10.1186/1743-422X-10-34.

62. C. E. Samuel, "Antiviral Actions of Interferon. Interferon-Regulated Cellular Proteins and Their Surprisingly Selective Antiviral Activities," *Virology* 183, no. 1 (1991): 1–11, https://doi.org/10.1016/0042-6822(91)90112-o.

63. M. V. Davies, O. Elroy-Stein, R. Jagus, B. Moss, and R. J. Kaufman, "The Vaccinia Virus K3L Gene Product Potentiates Translation by Inhibiting Double-Stranded-Rna-Activated Protein Kinase and Phosphorylation of the Alpha Subunit of Eukaryotic Initiation Factor 2," *Journal of Virology* 66, no. 4 (1992): 1943–1950, https://doi.org/10.1128/JVI.66.4.1943-1950.1992.

64. H. W. Chang, J. C. Watson, and B. L. Jacobs, "The E3L Gene of Vaccinia Virus Encodes An Inhibitor of the Interferon-Induced, Double-Stranded RNA-Dependent Protein Kinase," *Proceedings of the National Academy of Sciences* 89, no. 11 (1992): 4825–4829, https://doi.org/10.1073/pnas.89.11.4825.

65. A. Alcamí, J. A. Symons, and G. L. Smith, "The Vaccinia Virus Soluble Alpha/Beta Interferon (IFN) Receptor Binds to the Cell Surface and Protects Cells From the Antiviral Effects of IFN," *Journal of Virology* 74, no. 23 (2000): 11230–11239, https://doi.org/10.1128/jvi.74.23.11230-11239.2000.

66. O. R. Colamonici, P. Domanski, S. M. Sweitzer, A. Larner, and R. M. L. Buller, "Vaccinia Virus B18R Gene Encodes a Type I Interferon-Binding Protein That Blocks Interferon α Transmembrane Signaling," *Journal of Biological Chemistry* 270, no. 27 (1995): 15974–15978, https://doi.org/10.1074/jbc.270.27.15974.

67. J. A. Symons, A. Alcamí, and G. L. Smith, "Vaccinia Virus Encodes a Soluble Type I Interferon Receptor of Novel Structure and Broad Species Soecificity," *Cell* 81, no. 4 (1995): 551–560, https://doi.org/10.1016/0092-8674(95)90076-4.

68. M. M. Fernández De Marco, A. Alejo, P. Hudson, I. K. Damon, and A. Alcami, "The Highly Virulent Variola and Monkeypox Viruses Express Secreted Inhibitors of Type I Interferon," *The FASEB Journal* 24, no. 5 (2010): 1479–1488, https://doi.org/10.1096/fj.09-144733.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.