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Activation of Toll-like receptor 3 inhibits HIV infection of human iPSC-derived microglia

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Abstract

As a key immune cell in the brain, microglia are essential for protecting the central nerve system (CNS) from viral infections, including HIV. Microglia possess functional Toll-like receptor 3 (TLR3), a key viral sensor for activating interferon (IFN) signaling pathway-mediated antiviral immunity. We therefore studied effect of poly (I:C), a synthetic ligand of TLR3, on activation of the intracellular innate immunity against HIV in human iPSC-derived microglia (iMg). We found that poly (I:C) treatment of iMg effectively inhibits HIV infection/replication at both mRNA and protein levels. Investigations of the mechanisms revealed that TLR3 activation of iMg by poly (I:C) induced the expression of both type I and type III IFNs. Comparing with untreated cells, the poly (I:C)-treated iMg expressed significantly higher levels of IFN-stimulated genes (ISGs) with known anti-HIV activities (ISG15, MxB, Viperin, MxA and OAS-1). In addition, TLR3 activation elicited the expression of the HIV entry coreceptor CCR5 ligands (CC chemokines) in iMg. Furthermore, the transcriptional profile analysis showed that poly(I:C)-treated cells had the upregulated IFN signaling genes (ISG15, ISG20, IFITM1, IFITM2, IFITM3, IFITM10, APOBEC3A, OAS-2, MxA and MxB) and the increased CC chemokine signaling genes (CCL1, CCL2, CCL3, CCL4, and CCL15). These observations indicate that TLR3 is a potential therapy target for activating the intracellular innate immunity against HIV infection/replication

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Author Contributions

WZH, PW, and WHH, contributed to the conception and design of the study. PW, JBL, FZM, QHX and XW conducted the experiments. PW, JBL, XW and WZH analysed the data and wrote the manuscript. LL performed RNA sequencing bioinformatics analysis. JZ and WHH reviewed and revised the manuscript. All authors read and approved the submitted version of this manuscript.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The invention disclosure filing submitted.

in human microglial cells. Therefore, further studies with animal models and clinical specimens are necessary to determine the role of TLR3 activation-driven antiviral response in control and elimination of HIV in infected host cells.

Keywords

Poly (I:C); TLR3; Interferon; iPSC-derived Microglia; HIV

Introduction

Microglia are the primary resident immune cells in the central nervous system (CNS) and play a key role in the antiviral response to viral infections including HIV [1]. However, microglia also serve as the major target cells for both productive and latent HIV infection in the CNS [2, 3]. Importantly, infected microglia produce the inflammatory cytokines and the neurotoxins which contribute to HIV-associated neurocognitive disorder [4]. Therefore, it is of importance to study the role of the intracellular innate immunity in control of HIV replication in microglia. However, studies with primary human microglia have been hampered by limited brain tissue availability, particularly from healthy individuals. Recently, the establishment of human induced pluripotent stem cells (iPSC)-derived microglia (iMg) culture [5, 6] provides a great opportunity to study HIV infection of microglia. Studies have shown that human iMg are like primary human microglia by morphology, gene expression, and cytokine release profile [7]. Importantly, these cells are distinct from other tissue macrophages as they display a profile of neuronal-co-culture-specific expression and inflammatory response [7]. Human iMg model has been successfully utilized to study the neurological diseases, such as Alzheimer's disease [8], Parkinson's disease [9], amyotrophic lateral sclerosis and frontotemporal dementia [10]. In addition, it was reported that iMg could be infected by Zika and dengue virus infections [11]. Recently, several laboratories demonstrated that like primary human microphages, iMg are highly susceptible to HIV infection [12–14].

As a key immune cell in the CNS, microglia express functional pattern-recognition receptors (PRRs), the dynamic sensors of pathogen invasion, which can recognize pathogen invasion and activate interferon (IFN)-mediated antiviral innate immunity in the CNS [15, 16]. Among PRRs, Toll-like receptor 3 (TLR3) in conjunction with TLR7 and TLR9 constitutes an efficient system to monitor viral infections. TLR3 recognizes viral double-stranded RNA [17] while TLR7 and TLR9 can be triggered by single-stranded RNA and cytosine phosphate guanine DNA, respectively [18–21]. Although HIV is a single-strained RNA virus, it can form double-stranded RNAs during its replication, which can be recognized by TLR3 [22, 23]. TLR3 activation can induce the production of both type I and type III IFNs as well as IFN-stimulated genes (ISGs). In CNS HIV infection, released viral RNA from either outside the blood brain barrier or HIV-killed cells could activate the TLR signaling pathway in microglial cells, eliciting the antiviral response in the brain. A study showed that TLR3 is an innate antiviral immune receptor in primary human fetal microglial cells which could be activated by its ligand and induce IFN regulatory factor 3 (IRF3)-dependent anti-HIV effect [24]. We previously reported that TLR3 activation significantly inhibited

HIV and SIV infection/replication in primary human and macaque macrophages through producing the multiple intracellular viral restriction factors [23, 25, 26]. In the present study, we investigated the role of TLR3-IFN signaling activation in control of HIV replication in human iMg. We studied whether iMg possess immunologically functional TLR3, activation of which by Poly (I:C) can induce the antiviral cellular factors against HIV. In addition, we examined the mechanisms for TLR3 activation-driven HIV inhibition in iMg.

Materials and Methods

Generation of microglia from human induced pluripotent stem cells (iPSC)

We obtained three human iPSC derived $CD41^+$ $CD235^+$ common myeloid progenitor (CMP) lines from the Human Pluripotent Stem Cell Core at the Children's Hospital of Philadelphia, University of Pennsylvania. We then generated microglial cells from these iPSC-derived CMPs following the protocol [27]. In brief, the iPSC lines were cultivated and differentiated into $CD41^+$ $CD235^+$ common myeloid progenitors (CMPs). The progenitors were then plated at 56,000 cells/well in a 96-well CellBIND plate (Corning, New York, NY, USA) and cultured in human iPSC-derived microglia medium (RPMI 1640 medium, HyClone laboratories, South Logan, UT, USA) with 10% FBS (HyClone laboratories, South Logan, UT, USA), recombinant human interleukin-34 (IL-34) (100 ng/mL, R&D Systems, Minneapolis, MN, USA), and CSF-1 recombinant human protein (25 ng/mL, Thermo Fisher Scientific, Waltham, MA, USA). These factors are necessary for microglia development *in vivo*. In addition, we added recombinant human transforming growth factor β1 (TGF-β1, Peprotech, Part of Thermo Fisher Scientific, Waltham, MA, USA) in the cultures for microglia gene expression. Half of cultures were replaced with fresh medium every 2 days for 11 days during which CMPs differentiated into human microglia.

Poly (I:C) treatment and HIV infection

Human iPSC-microglia (iMg) were incubated with or without poly (I:C) (0.5 or 1 μg/ml) for 12 h before HIV infection. The cells were then infected with HIV Jago strain (20 ng/ml of p24 protein) for 24 h at 37°C in the presence or absence of poly (I:C). The cells were washed three times with DMEM to remove input viruses, and fresh medium without poly (I:C) was added to the cultures. The final wash was tested for HIV reverse transcriptase (RT) activity and shown to be free of residual viruses. Full medium exchanges occur every 3 days for 15 days, and culture supernatant were harvested for HIV RT activity which was measured based on the modified method [28].

Immunofluorescence and confocal image analyses

Antibodies used for immunofluorescence are listed as follows: Rabbit anti-CX3CR1 (Abcam, Cambridge, UK, 1:500); Rabbit anti-TMEM119 (Abcam, Cambridge, UK, 1:200); Rabbit anti-IBA1 (FUJIFILM Wako Chemicals, Richmond, VA, USA 1:500); Mouse anti-HIV-1 p24 (Abcam, Cambridge, UK, 1:500); Goat anti-mouse IgG (H+L) and Alexa Fluor 488 (Thermo Fisher Scientific, Waltham, MA, USA, 1:500). Because TMEM119 antibody staining requires antigen retrieval, cells were heated to 100 °C for 4 min in 10 mM sodium citrate first and then incubated for 15 min at room temperature prior to antibody block [14]. Human iMg were washed twice with PBS and fixed in 4% PFA (Santa Cruz Biotechnology.

Dallas, TX, USA) for 15 min at room temperature. After fixation, cells were washed 3 times in PBS for 5 min at room temperature before being stored in PBS at 4°C. The fixed cells were blocked with 5% BSA and 0.1% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) for 60 min at room temperature. The primary and secondary antibodies were diluted in blocking buffer (0.3% Triton-X 100) and cells were incubated with the primary antibodies overnight at 4°C. Cells were then washed 3 times and 5 min each with PBS-T (0.1% Tween20, Sigma-Aldrich, St. Louis, MO, USA). Secondary antibodies were incubated with the cells in the dark for 60 min at room temperature. After being washed, the cells were then mounted with Prolong gold antifade (Life Technologies, Carlsbad, CA, USA). Images were taken with a Nikon eclipse N1 scope equipped with LED-based epifluorescence (Nikon Instruments Inc, Melville, NY, USA).

Real-Time qPCR

Total cellular RNAs were extracted from iMg with Tri-reagent (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instruction and were subjected to reverse transcription reaction using the random primer, dNTPs, AMV reverse transcriptase and RNase inhibitor (Promega Co., Madison, WI, USA) to generate complementary DNA (cDNA), which was then used as a template for real-time PCR with Powerup SYBR Green Master Mix (Applied Biosystems, Carlsbad, CA, USA) as previously described [20]. The mRNA level of GAPDH was used as an endogenous reference to normalize the quantities of the target mRNAs. The sequences of oligonucleotide primers for tested mRNAs are available upon request.

ELISA for IFN-α/β and CC chemokines

Cell-free culture supernatant were collected from iMg cultures treated with or without poly(I:C) for 24 h. ELISA for analysis of IFN-α and IFN-β proteins were performed as described in the protocol provided by the manufacturers (PBL Biomedical Laboratories, Piscataway, NJ, USA). The CC chemokines (MIP-1α/β and RANTES) proteins were analyzed by ELISA kits from R&D Systems Inc (Minneapolis, MN, USA). The plate was read on a microplate reader (ELX800; Bio-Tek Instruments, Inc., Winooski, VT, USA).

Western Blot

Cells were lysed with RIPA buffer (Sigma-Aldrich, St. Louis, MO, USA) supplemented with a protease (100 x, Thermo Fisher Scientific, Waltham, MA, USA). Proteins from the culture supernatant were extracted by the TCA/acetone precipitation method. Equal amounts of proteins, along with protein molecular weight, were separated on 4 to 12% Bis-Tris gels (Invitrogen, Grand Island, NY, USA) and transferred to an Immun-Blot® PVDF membrane (Bio-Rad, Hercules, CA, USA). The blots were blocked with 5% non-fat milk for 2 h at room temperature and then incubated with primary antibodies overnight at 4°C. The blots were washed in PBS with 0.1% Tween 20 (PBST) four times and then incubated with HRP-conjugated second antibodies for 2 h at room temperature. The blots were developed with SuperSignal™ West Pico Chemiluminescent Substrate (Thermo Fisher Scientific, Waltham, MA, USA). The blots were then exposed to an iBright 1500 imaging analyzer (Invitrogen, Carlsbad, CA, USA).

Bulk RNA sequencing (RNA-Seq) and RNA-Seq data analysis

iPSC-derived microglia treated were with or without poly(I:C) (1μg/ml) for 12 h and collected for total RNA extraction with AllPrep DNA/RNA/Protein Mini kit (Qiagen, Germantown, MD USA). Total RNA samples (300-800 ng/sample) were sent to Novogen Corporation Inc. For quality control, cDNA library preparation, and sequencing with specific configurations. Sequencing data quality was checked using FastQC software.

The analysis was conducted in statistical computing environment R, using the Bioconductor suite of packages and RStudio (version 1.4.1717). To describe briefly, after read mapping with Kallisto, TxImport was used to read kallisto outputs into the R environment. Annotation data from Biomart was used to collapse data from transcript-level to gene-level. Filtering was carried out to remove lowly expressed genes. Genes with less than 1 count per million (CPM) in at least 3 or more samples were filtered out. This reduced the number of normalized genes from 30124 to 12321. Differentially expressed genes (DEG) between the Poly(I:C) treated and control cells were identified using a criteria of fold change (FC) was >0.5 greater than or equal to 1.5 and a p-value less than 0.05 (FC >1.5 and a p-value <0.05). Gene ontology (GO) and pathway enrichment analysis was performed using ToppGene and Enrichr.

Statistical Analysis

Data were expressed as means ± standard deviations (SD) from at least three independent experiments. For comparison of the mean of two groups (treated versus untreated with Poly (I:C), statistical significance was measured by the student's *t*-test. If there were more than two groups, a one-way analysis of variance was used. All statistical analysis was performed using GraphPad Prism 7.0 Software (GraphPad Software Inc., San Diego, CA). Statistical significance was defined as $p < 0.05$, $p < 0.01$.

Results

iMg express specific microglia markers and HIV entry receptors

We first examined whether iMg express the specific human microglia markers. The same numbers of cells (56,000 cells/well) seeded in a 96 well plate before HIV infection were shown in Fig. S1. The cells expressed all three specific microglia markers (IBA1, CX3CR1, and TMEM119). In addition, these cells showed the positive staining for the HIV entry receptors, CD4 and CCR5 (Fig. 1).

TLR3 activation inhibits HIV infection of human iMg

We infected iMg with a CSF-derived HIV R5-tropic strain (Jago) at the dose of 20 ng p24/ml and examined several parameters of HIV infectivity. As shown in Fig. 2A, HIV-infected iMg cultures exhibited the multinucleated giant-cells and HIV capsid protein p24 positive cells. In contrast, uninfected cultures had little multinucleated cells and no positive staining for HIV p24 protein. In addition, we measured the expression of HIV gag gene mRNA and viral RT activity in the culture supernatant at different time points after infection. As demonstrated in Fig. 2, there was a steady increase of HIV gag mRNA

expression (Fig. 2B) and RT activity (Fig. 2C) in culture supernatant collected during the course of infection.

To evaluate the effect of TLR3 activation on HIV infection of iMg, the cells were treated with or without poly (I:C) before HIV infection with Jago strain. As shown in Fig. 3, pretreatment of iMg with poly (I:C) for 12 h significantly protected cells from HIV infection and the inhibitory effect of poly (I:C) on HIV was dose-dependent and last for the course of HIV infection (15 days). Morphologically, HIV-infected iMg cultures without poly (I:C) treatment demonstrated characteristic giant syncytium formation (Fig. 3C), whereas poly (I:C)-treated cells failed to develop HIV-induced giant syncytia (Fig. 3C).

Induction of IFNs and ISGs by TLR3 activation of iMg

To study the molecular mechanism(s) by which TLR3 activation could inhibit HIV infection of iMg, we first examined whether iMg express endogenous IFN-α/β, λ1 which can be induced by poly (I:C) treatment. We found that poly (I:C) treatment of iMg dose-dependently induced the expression of IFN-α/β, λ1 at both mRNA (Fig. 4A) and protein (Fig. 4B) levels. We then studied whether poly (I:C) can elicit the expression of the cellular HIV restriction factors, including ISG15, MxB, Viperin, MxA and OAS-1 which are known to be regulated by both type I and type III IFNs [29, 30]. As shown in Fig. 5, poly (I:C) treatment of iMg selectively induced the expression of ISG15, MxB, Viperin, MxA, and OAS-1 at both mRNA (Fig. 5A) and protein (Fig. 5B) levels in a dose-dependent manner (Fig. 5).

Induction of CC chemokines by TLR3 activation of iMg

As the natural ligands for HIV entry coreceptor CCR5, the CC chemokines (RANTES, MIP-1α and MIP-1β) are known to be the HIV suppressive factors for virus entry [31]. We therefore investigated whether TLR3 activation has an impact on the expression of HIV entry coreceptor, MIP-1α, MIP-1β and RANTES in iMg. As demonstrated in Figure S2, poly(I:C) treatment has little effects on the expression of the HIV entry receptors (CD4, CCR5 and CXCR4) in iMg. In contrast, poly (I:C) treatment of iMg dose-dependently increased the mRNA levels of these chemokines (Fig. 6A). In addition, poly (I:C)-treated iMg produced higher levels of MIP1-α, MIP-β and RANTES proteins than untreated cells (Fig. 6B).

Poly (I:C) alters the iMg cellular transcriptome

To further understand the effect of poly(I:C) on genes involved in observed antiviral immunity, we performed the bulk RNA sequencing to analyze the transcriptional profiles of poly(I:C)-treated iMg as compared with untreated cells. As shown in Supplementary Table 1 and Fig. 7A, we analyzed 12321 transcripts, with 246 up- and 95 downregulated transcripts in Poly(I:C)-treated cells compared to untreated ones. Ontology enrichment analysis showed that these differentially regulated genes involved in the biological processes associated with the response to virus, specifically defense response to virus, viral process, and regulation of viral life cycle (Fig. 7B). In addition, the genes in cytokine-mediated signaling pathway were upregulated (Fig. 7 and 8).

To further identified candidate genes related to anti-HIV activities in poly(I:C)-treated iMg cells, we studied transcripts of different signaling pathways. As shown in Fig. 8A, compared to the untreated cells, poly(I:C) upregulated innate immune response- and cytokine-mediated signaling pathway. We focus on upregulated genes associated with Interferons, Inflammatory cytokines such as TNF-α. As shown in Fig. 8B, poly(I:C) condition was enriched with genes involved with innate immune response of both type I and II IFNs. The antiviral genes such as IFITM1/3, ISG15, MxA, MxB, IFIT1/3, OASL, OAS2, and BST2 are upregulated. In addition, there were increase of the chemokine-mediated signaling genes (CCl2, CCL5, CXCL1, IL-1α/β, and IL-18) and the genes relevant to viral defense in iMg treated with poly(I:C).

Discussion

Because of a well-controlled environment and limited pathogen-specific immune responses in the brain, microglial cells are poor antigen-presenting cells and lack MHC class I/II proteins [32]. Therefore, the brain may rely heavily on microglia to produce innate immune factors such as IFNs and ISGs for control of viral infections including HIV [33]. IFNs function as autocrine or paracrine factors on cells expressing their receptor. IFNs released from producing cells can bind to their corresponding receptors and JAK/STAT signaling pathway, leading to STAT1/STAT2 phosphorylation and transcription of the antiviral ISGs (Fig. 8, and 9). Therefore, this study aims at the role of the intracellular innate immunity in control of HIV replication in human microglia. Because of difficulty and limited availability to obtain highly purified primary human microglia, we used human iMg to examine whether activation of TLR3, a key viral sensor, can induce intracellular anti-HIV innate immunity. A recent study with a comprehensive comparison of human microglial culture models showed that iMg is one of more representative microglial culture models for HIV research [34]. We demonstrated that iMg could be productively infected by HIV. This finding supports the studies by others showing that human iMg are readily infected by HIV [21–23]. Importantly, we observed that TLR3 activation by a dsRNA analogue poly (I:C) could effectively and dose-dependently inhibit HIV infection of iMg (Fig. 3), which was evidenced by the observations that HIV gag gene expression was significantly inhibited (>90%) by poly (I:C) in the 15-day course of infection (Fig. 3A and B) and that there was little HIV-induced giant syncytia in poly (I:C)-treated iMg (Fig. 3C). Mechanistically, we found that activation of TLR3 triggers the activation of IFN signaling pathway and induced production of IFNs (Fig. 4), which are known as the first line of the TLR3 activation-mediated antiviral response because they are critical for the eliciting down-stream antiviral activities. It is likely that IFNs induced by poly (I:C) treatment can be released from iMg and bind to IFN receptors on the cell membrane and activate the JAK/STAT pathway, which leads to the induction of anti-HIV ISGs (Fig. 8 and 9).

We showed that poly (I:C)-treated iMg expressed significantly higher levels of ISGs (ISG15, MxB, Viperin, MxA, and OAS-1) than untreated control cultures (Fig. 5). These ISGs are the intracellular viral restriction factors involved in type I/III IFN-dependent anti-HIV activities. They can inhibit HIV by interfering with several stages of the viral life cycle (Fig. 9), including assembling, releasing, and inhibiting the capsid-dependent nuclear import of subviral complexes [35]. For example, ISG15 has a crucial role in the IFN-mediated

inhibition of late stages of HIV assembly and release [35]; Viperin is able to limit viral egress of HIV through the plasma membrane rafts [36, 37]; MxB is a HIV post-entry inhibitor that can abolish capsid-dependent nuclear import of subviral complexes [38]; MxA (also known as Mx1) is a well-known inhibitor of many RNA and DNA viruses, including HIV [39, 40]; 2' −5' -oligoadenylate synthetase (OAS) is activated by dsRNA and IFNs to produce 2' −5' oligoadenylates, the activators of RNase L which degrades viral and cellular RNAs, thus restricting HIV infection [41, 42]. In addition to induction of the anti-HIV ISGs, TLR3 activation of iMg also elicited the production of the CC chemokines (RANTES, MIP-1α and MIP-1β), the ligands for HIV entry coreceptor CCR5. These CC chemokines are well known to be the HIV entry suppressive factors [31].

To further examine the effects of poly(I:C) treatment on HIV infection of iMg, we examined the transcriptional profiles of poly(I:C)-treated iMg and observed that there are many upregulated transcripts related to viral response and regulation of host cellular pathways (Figs. 7 and 8). The results of the gene upregulation in IFN signaling pathway such as ISG15, ISG20, IFITM1, IFITM2, IFITM3, IFITM10, APOBEC3A, OAS-2, MxA and MxB supports the findings at cellular level. In addition, the increase of the chemokine signaling genes including CCL1, CCL2, CCL3, CCL4, and CCL15 (Fig. 8) is in line with our observation that poly IC induce CC chemokine expression in iMg, which contribute to HIV inhibition in iMg. These data provide a sound molecular mechanism for TLR3 activation-mediated anti-HIV effect in iMg.

Taken together, we have demonstrated that TLR3 activation of iMg by poly (I:C) can effectively suppresses HIV infection through eliciting the intracellular antiviral factors that blocked viral replication cycle at different levels. Although additional mechanism(s) might also be involved, the induction of type I/III IFNs, the IFN-dependent antiviral ISGs and the ligands for HIV entry coreceptors should account for much of the TLR3 activation-driven HIV inhibition in iMg. These observations suggest the possibility that TLR3 activation can enhance and restore host cell immunity suppressed by HIV [15]. Because activating the intracellular innate immunity can elicit type I and III IFN production as well as multiple IFN-inducible antiviral gene expression, it is unlikely for HIV to develop resistance. Clinically, the therapeutic TLR agonists have been developed and used for the treatment of infectious diseases [43]. Studies [44–50] have demonstrated that the agonists for TLR3, TLR7 and TLR9 have treatment potential for viral infections including HIV. Therefore, the findings of this study support necessity of further investigations on developing a TLR3 agonist-based immunotherapy for HIV infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability statement

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

References

1. Guillemin GJ; Brew BJ, Microglia, macrophages, perivascular macrophages, and pericytes: a review of function and identification. J Leukoc Biol 2004, 75, (3), 388–97. [PubMed: 14612429]
2. Garden GA, Microglia in human immunodeficiency virus-associated neurodegeneration. Glia 2002, 40, (2), 240–251. [PubMed: 12379911]
3. Williams KC; Hickey WF, Central nervous system damage, monocytes and macrophages, and neurological disorders in AIDS. Annu Rev Neurosci 2002, 25, 537–62. [PubMed: 12052920]
4. Borrajo A; Spuch C; Penedo MA; Olivares JM; Agis-Balboa RC, Important role of microglia in HIV-1 associated neurocognitive disorders and the molecular pathways implicated in its pathogenesis. Ann Med 2021, 53, (1), 43–69. [PubMed: 32841065]
5. Muffat J; Li Y; Yuan B; Mitalipova M; Omer A; Corcoran S; Bakiasi G; Tsai LH; Aubourg P; Ransohoff RM; Jaenisch R, Efficient derivation of microglia-like cells from human pluripotent stem cells. Nat Med 2016, 22, (11), 1358–1367. [PubMed: 27668937]
6. Pandya H; Shen MJ; Ichikawa DM; Sedlock AB; Choi Y; Johnson KR; Kim G; Brown MA; Elkahloun AG; Maric D; Sweeney CL; Gossa S; Malech HL; McGavern DB; Park JK, Differentiation of human and murine induced pluripotent stem cells to microglia-like cells. Nat Neurosci 2017, 20, (5), 753-+. [PubMed: 28253233]
7. Douvaras P; Sun B; Wang M; Kruglikov I; Lallos G; Zimmer M; Terrenoire C; Zhang B; Gandy S; Schadt E; Freytes DO; Noggle S; Fossati V, Directed Differentiation of Human Pluripotent Stem Cells to Microglia. Stem Cell Reports 2017, 8, (6), 1516–1524. [PubMed: 28528700]
8. Xu M; Zhang L; Liu G; Jiang N; Zhou WX; Zhang YX, Pathological Changes in Alzheimer's Disease Analyzed Using Induced Pluripotent Stem Cell-Derived Human Microglia-Like Cells. J Alzheimers Dis 2019, 67, (1), 357–368. [PubMed: 30562902]
9. Li H; Jiang H; Zhang B; Feng J, Modeling Parkinson's Disease Using Patient-specific Induced Pluripotent Stem Cells. J Parkinsons Dis 2018, 8, (4), 479–493. [PubMed: 30149462]
10. Centeno EGZ; Cimarosti H; Bithell A, 2D versus 3D human induced pluripotent stem cell-derived cultures for neurodegenerative disease modelling. Mol Neurodegener 2018, 13, (1), 27. [PubMed: 29788997]
11. Muffat J; Li Y; Omer A; Durbin A; Bosch I; Bakiasi G; Richards E; Meyer A; Gehrke L; Jaenisch R, Human induced pluripotent stem cell-derived glial cells and neural progenitors display divergent responses to Zika and dengue infections. Proc Natl Acad Sci U S A 2018, 115, (27), 7117–7122. [PubMed: 29915057]
12. Akiyama H; Jalloh S; Park S; Lei M; Mostoslavsky G; Gummuluru S, Expression of HIV-1 Intron-Containing RNA in Microglia Induces Inflammatory Responses. J Virol 2020.
13. Rai MA; Hammonds J; Pujato M; Mayhew C; Roskin K; Spearman P, Comparative analysis of human microglial models for studies of HIV replication and pathogenesis. Retrovirology 2020, 17, (1), 35. [PubMed: 33213476]
14. Ryan SK; Gonzalez MV; Garifallou JP; Bennett FC; Williams KS; Sotuyo NP; Mironets E; Cook K; Hakonarson H; Anderson SA; Jordan-Sciutto KL, Neuroinflammation and EIF2 Signaling Persist despite Antiretroviral Treatment in an hiPSC Tri-culture Model of HIV Infection. Stem Cell Reports 2020, 14, (4), 703–716. [PubMed: 32220329]
15. Liu H; Zhou RH; Liu Y; Guo L; Wang X; Hu WH; Ho WZ, HIV infection suppresses TLR3 activation-mediated antiviral immunity in microglia and macrophages. Immunology 2020, 160, (3), 269–279. [PubMed: 32053234]
16. Olson JK; Miller SD, Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. J Immunol 2004, 173, (6), 3916–24. [PubMed: 15356140]

17. Tissari J; Siren J; Meri S; Julkunen I; Matikainen S, IFN-alpha enhances TLR3-mediated antiviral cytokine expression in human endothelial and epithelial cells by up-regulating TLR3 expression. J Immunol 2005, 174, (7), 4289–94. [PubMed: 15778392]

18. Cen P; Ye L; Su QJ; Wang X; Li JL; Lin XQ; Liang H; Ho WZ, Methamphetamine inhibits Toll-like receptor 9-mediated anti-HIV activity in macrophages. AIDS Res Hum Retroviruses 2013, 29, (8), 1129–37. [PubMed: 23751096]

19. Guo L; Xu XQ; Zhou L; Zhou RH; Wang X; Li JL; Liu JB; Liu H; Zhang B; Ho WZ, Human Intestinal Epithelial Cells Release Antiviral Factors That Inhibit HIV Infection of Macrophages. Front Immunol 2018, 9, 247. [PubMed: 29515574]

20. Meng FZ; Liu JBA; Wang X; Wang P; Hu WH; Hou W; Ho WZ, TLR7 Activation of Macrophages by Imiquimod Inhibits HIV Infection through Modulation of Viral Entry Cellular Factors. Biology-Basel 2021, 10, (7).

21. Xu XQ; Guo L; Wang X; Liu Y; Liu H; Zhou RH; Gu J; Liu JB; Xu P; Zhou L; Ho WZ, Human Cervical Epithelial Cells Release Antiviral Factors and Inhibit HIV Replication in Macrophages. J Innate Immun 2019, 11, (1), 29–40. [PubMed: 30032138]

22. Brule F; Marquet R; Rong L; Wainberg MA; Roques BP; Le Grice SF; Ehresmann B; Ehresmann C, Structural and functional properties of the HIV-1 RNA-tRNA(Lys)3 primer complex annealed by the nucleocapsid protein: comparison with the heat-annealed complex. RNA (New York, N.Y.) 2002, 8, (1), 8–15. [PubMed: 11873759]

23. Zhou Y; Wang X; Liu M; Hu Q; Song L; Ye L; Zhou D; Ho W, A critical function of toll-like receptor-3 in the induction of anti-human immunodeficiency virus activities in macrophages. Immunology 2010, 131, (1), 40–9. [PubMed: 20636339]

24. Suh HS; Zhao ML; Choi N; Belbin TJ; Brosnan CF; Lee SC, TLR3 and TLR4 are innate antiviral immune receptors in human microglia: role of IRF3 in modulating antiviral and inflammatory response in the CNS. Virology 2009, 392, (2), 246–59. [PubMed: 19646728]

25. Sang M; Liu JB; Dai M; Wu JG; Ho WZ, Toll-like receptor 3 signaling inhibits simian immunodeficiency virus replication in macrophages from rhesus macaques. Antiviral Res 2014, 112, 103–12. [PubMed: 25453343]

26. Dai M; Wang X; Li JL; Zhou Y; Sang M; Liu JB; Wu JG; Ho WZ, Activation of TLR3/interferon signaling pathway by bluetongue virus results in HIV inhibition in macrophages. Faseb J 2015, 29, (12), 4978–88. [PubMed: 26296370]

27. Ryan SK; Jordan-Sciutto KL; Anderson SA, Protocol for Tri-culture of hiPSC-Derived Neurons, Astrocytes, and Microglia. STAR Protocols 2020, 100190. [PubMed: 33377084]

28. Ho WZ; Lioy J; Song L; Cutilli JR; Polin RA; Douglas SD, Infection of cord blood monocyte-derived macrophages with human immunodeficiency virus type 1. J Virol 1992, 66, (1), 573–9. [PubMed: 1727500]

29. Liu MQ; Zhou DJ; Wang X; Zhou W; Ye L; Li JL; Wang YZ; Ho WZ, IFN-lambda3 inhibits HIV infection of macrophages through the JAK-STAT pathway. PLoS One 2012, 7, (4), e35902. [PubMed: 22558263]

30. Wang YZ; Li JL; Wang X; Zhou Y; Zhang T; Ho WZ, Comparison of Antiviral Activity of Lambda-Interferons Against HIV Replication in Macrophages. J Interf Cytok Res 2015, 35, (3), 213–221.

31. Proost P; Schols D, [Role of chemokines in the HIV infection process]. Verh K Acad Geneeskd Belg 2002, 64, (6), 403–20. [PubMed: 12649932]

32. Chastain EM; Duncan DS; Rodgers JM; Miller SD, The role of antigen presenting cells in multiple sclerosis. Biochim Biophys Acta 2011, 1812, (2), 265–74. [PubMed: 20637861]

33. Joseph J, Eradication of HIV-1 from CNS reservoirs: current strategies and future priorities. J Neurovirol 2015, 21, (3), 219–21. [PubMed: 25939608]

34. Gumbs SBH; Kubler R; Gharu L; Schipper PJ; Borst AL; Snijders G; Ormel PR; van Berlekom AB; Wensing AMJ; de Witte LD; Nijhuis M, Human microglial models to study HIV infection and neuropathogenesis: a literature overview and comparative analyses. J Neurovirol 2022, 28, (1), 64–91. [PubMed: 35138593]

35. Okumura A; Lu G; Pitha-Rowe I; Pitha PM, Innate antiviral response targets HIV-1 release by the induction of ubiquitin-like protein ISG15. Proc Natl Acad Sci U S A 2006, 103, (5), 1440–5. [PubMed: 16434471]

36. Helbig KJ; Beard MR, The role of viperin in the innate antiviral response. J Mol Biol 2014, 426, (6), 1210–9. [PubMed: 24157441]

37. Nasr N; Maddocks S; Turville SG; Harman AN; Woolger N; Helbig KJ; Wilkinson J; Bye CR; Wright TK; Rambukwelle D; Donaghy H; Beard MR; Cunningham AL, HIV-1 infection of human macrophages directly induces viperin which inhibits viral production. Blood 2012, 120, (4), 778–88. [PubMed: 22677126]

38. Liu Z; Pan Q; Ding S; Qian J; Xu F; Zhou J; Cen S; Guo F; Liang C, The interferon-inducible MxB protein inhibits HIV-1 infection. Cell Host Microbe 2013, 1v4, (4), 398–410.

39. Sadler AJ; Williams BR, Interferon-inducible antiviral effectors. Nat Rev Immunol 2008, 8, (7), 559–68. [PubMed: 18575461]

40. Gurney KB; Colantonio AD; Blom B; Spits H; Uittenbogaart CH, Endogenous IFN-alpha production by plasmacytoid dendritic cells exerts an antiviral effect on thymic HIV-1 infection. J Immunol 2004, 173, (12), 7269–76. [PubMed: 15585849]

41. Fagone P; Nunnari G; Lazzara F; Longo A; Cambria D; Distefano G; Palumbo M; Nicoletti F; Malaguarnera L; Di Rosa M, Induction of OAS gene family in HIV monocyte infected patients with high and low viral load. Antiviral Res 2016, 131, 66–73. [PubMed: 27107898]

42. Martinand C; Montavon C; Salehzada T; Silhol M; Lebleu B; Bisbal C, RNase L inhibitor is induced during human immunodeficiency virus type 1 infection and down regulates the 2–5A/RNase L pathway in human T cells. J Virol 1999, 73, (1), 290–6. [PubMed: 9847332]

43. Farooq M; Batool M; Kim MS; Choi S, Toll-Like Receptors as a Therapeutic Target in the Era of Immunotherapies. Front Cell Dev Biol 2021, 9, 756315. [PubMed: 34671606]

44. Cheng L; Wang Q; Li G; Banga R; Ma J; Yu H; Yasui F; Zhang Z; Pantaleo G; Perreau M; Zurawski S; Zurawski G; Levy Y; Su L, TLR3 agonist and CD40-targeting vaccination induces immune responses and reduces HIV-1 reservoirs. J Clin Invest 2018, 128, (10), 4387–4396. [PubMed: 30148455]

45. Martinsen JT; Gunst JD; Hojen JF; Tolstrup M; Sogaard OS, The Use of Toll-Like Receptor Agonists in HIV-1 Cure Strategies. Front Immunol 2020, 11, 1112. [PubMed: 32595636]

46. Riddler SA; Para M; Benson CA; Mills A; Ramgopal M; DeJesus E; Brinson C; Cyktor J; Jacobs J; Koontz D; Mellors JW; Laird GM; Wrin T; Patel H; Guo S; Wallin J; Boice J; Zhang L; Humeniuk R; Begley R; German P; Graham H; Geleziunas R; Brainard DM; SenGupta D, Vesatolimod, a Toll-like Receptor 7 Agonist, Induces Immune Activation in Virally Suppressed Adults Living With Human Immunodeficiency Virus-1. Clin Infect Dis 2021, 72, (11), e815–e824. [PubMed: 33043969]

47. Saxena M; Sabado RL; La Mar M; Mohri H; Salazar AM; Dong H; Correa Da Rosa J; Markowitz M; Bhardwaj N; Miller E, Poly-ICLC, a TLR3 Agonist, Induces Transient Innate Immune Responses in Patients With Treated HIV-Infection: A Randomized Double-Blinded Placebo Controlled Trial. Front Immunol 2019, 10, 725. [PubMed: 31024557]

48. SenGupta D; Brinson C; DeJesus E; Mills A; Shalit P; Guo S; Cai Y; Wallin JJ; Zhang L; Humeniuk R; Begley R; Geleziunas R; Mellors J; Wrin T; Jones N; Milush J; Ferre AL; Shacklett BL; Laird GM; Moldt B; Vendrame E; Brainard DM; Ramgopal M; Deeks SG, The TLR7 agonist vesatolimod induced a modest delay in viral rebound in HIV controllers after cessation of antiretroviral therapy. Sci Transl Med 2021, 13, (599).

49. Vibholm L; Schleimann MH; Hojen JF; Benfield T; Offersen R; Rasmussen K; Olesen R; Dige A; Agnholt J; Grau J; Buzon M; Wittig B; Lichterfeld M; Petersen AM; Deng X; Abdel-Mohsen M; Pillai SK; Rutsaert S; Trypsteen W; De Spiegelaere W; Vandekerchove L; Ostergaard L; Rasmussen TA; Denton PW; Tolstrup M; Sogaard OS, Short-Course Toll-Like Receptor 9 Agonist Treatment Impacts Innate Immunity and Plasma Viremia in Individuals With Human Immunodeficiency Virus Infection. Clin Infect Dis 2017, 64, (12), 1686–1695. [PubMed: 28329286]

50. Vibholm LK; Konrad CV; Schleimann MH; Frattari G; Winckelmann A; Klastrup V; Jensen NM; Jensen SS; Schmidt M; Wittig B; Zuwala K; Mack K; Olesen R; Hua S; Lichterfeld M; Ostergaard

L; Denton PW; Tolstrup M; Sogaard OS, Effects of 24-week Toll-like receptor 9 agonist treatment in HIV type 1+ individuals. AIDS 2019, 33, (8), 1315–1325. [PubMed: 30932955]

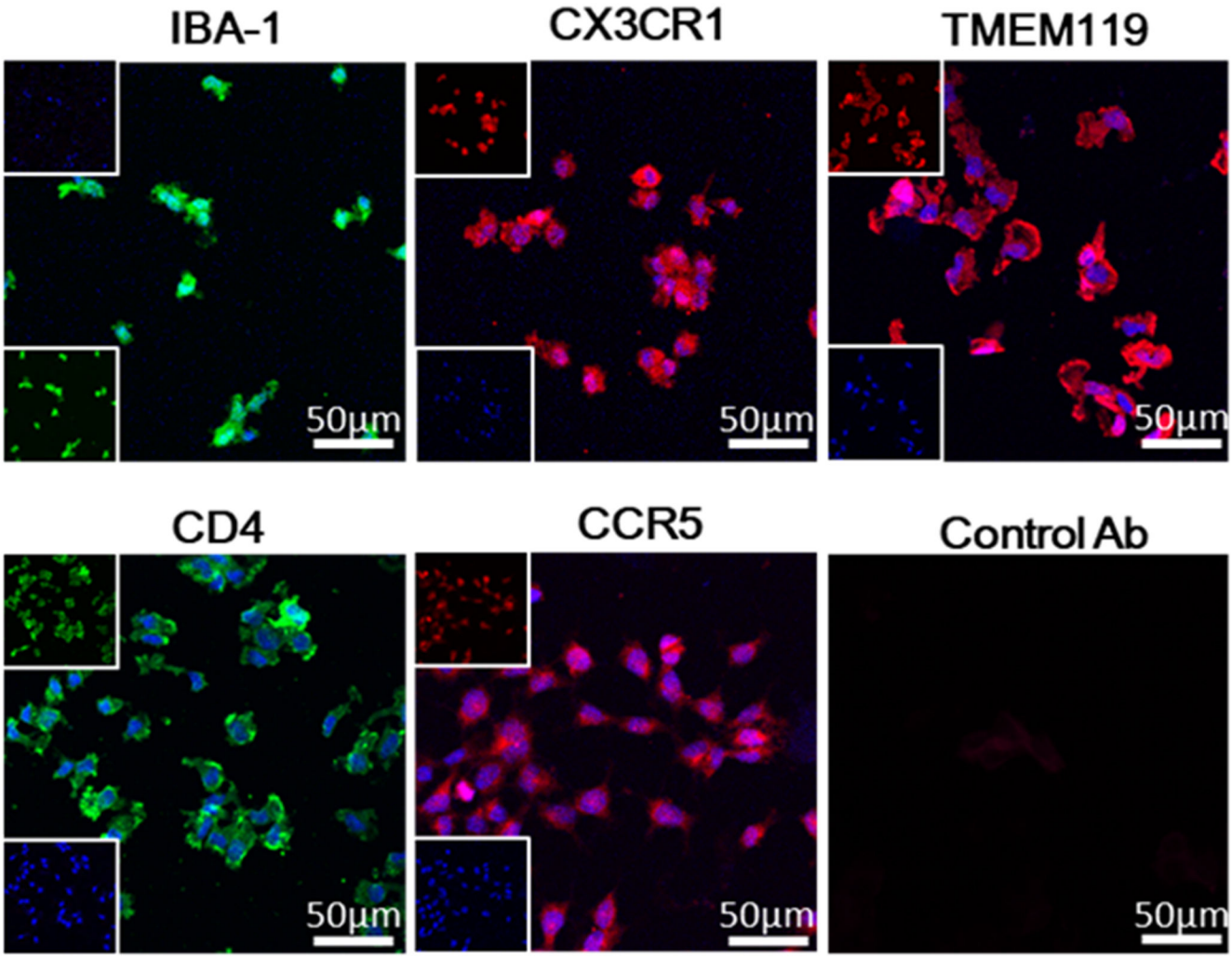


Figure 1. Immunostaining for microglia specific markers and HIV entry receptors.

Eleven day-cultured iPSC-derived microglia were fixed in 4% paraformaldehyde and stained for microglia specific markers or HIV infection receptors as indicated: IBA-1(green), CX3CR1(red), TMEM119 (red), CD4 (green), and CCR5 (red). Nuclei were stained with Hoechst. Scale bar represents 50 μm.

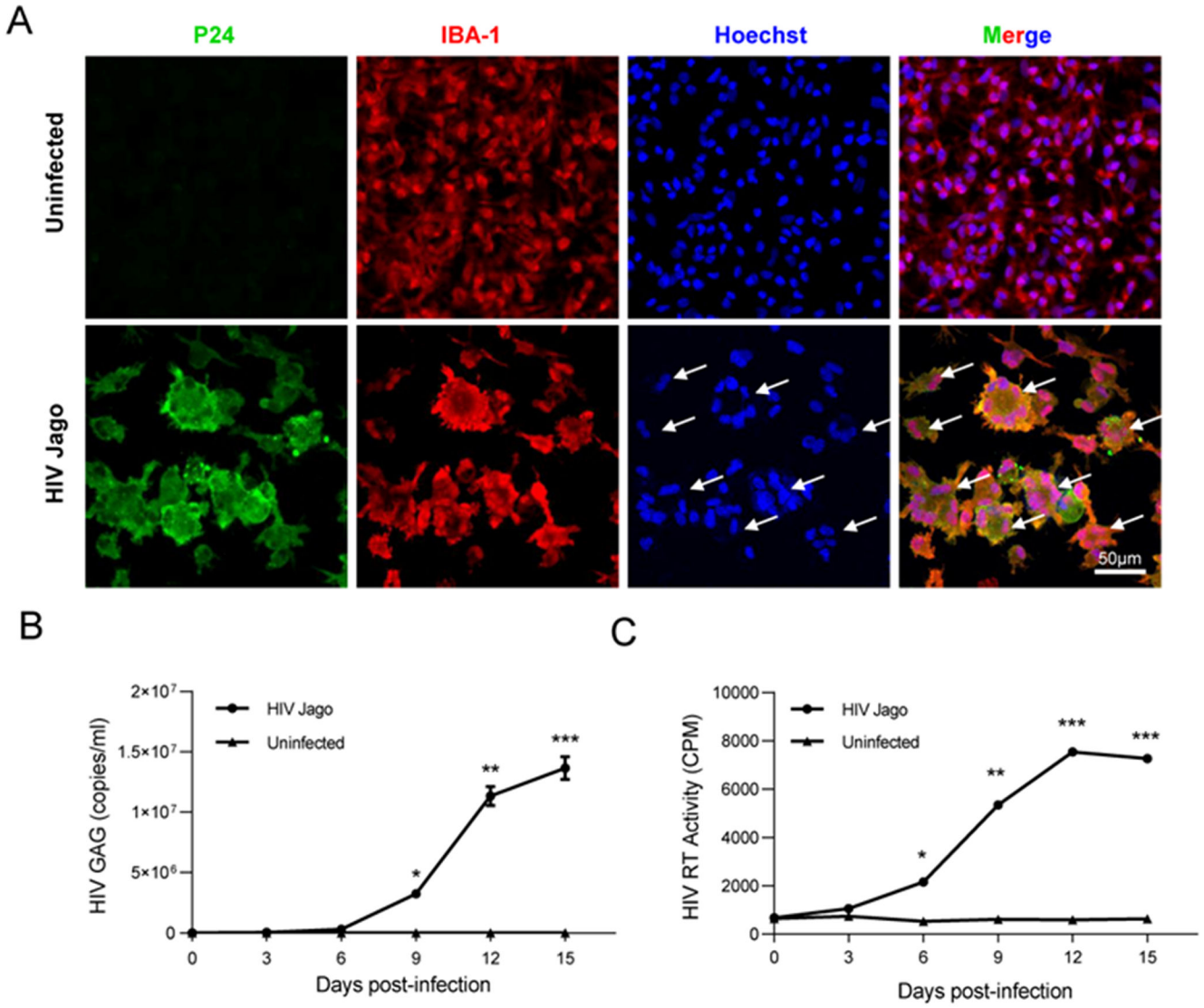


Figure 2. HIV-induced syncytium formation in human iPSC-derived microglia.

(**A**) The morphology of uninfected and HIV Jago infected human iPSC-derived microglia were observed. Human iPSC-derived microglia at 15 day-post-infection were washed and fixed in 4% paraformaldehyde and treated with 0.5% triton X-100. HIV p24 and human iPSC-derived microglia specific markers IBA-1 were stained, nuclei were stained with Hoechst and observed through fluorescence microscope. The arrows indicate HIV-induced giant syncytium formation in human iPSC-derived microglia. (**B-C**) Time course of HIV GAG gene expression and HIV reverse transcriptase activity in HIV infection of iPSC-derived microglia. Human iPSC-derived microglia infected with HIV JAGO and total RNA extracted from cell free supernatant was then subjected to RT-PCR for HIV gag gene (**B**), and reverse transcriptase activity test (**C**), uninfected as a negative control (*p <0.05, **p < 0.01, ***p < 0.001; error bars represent SD.

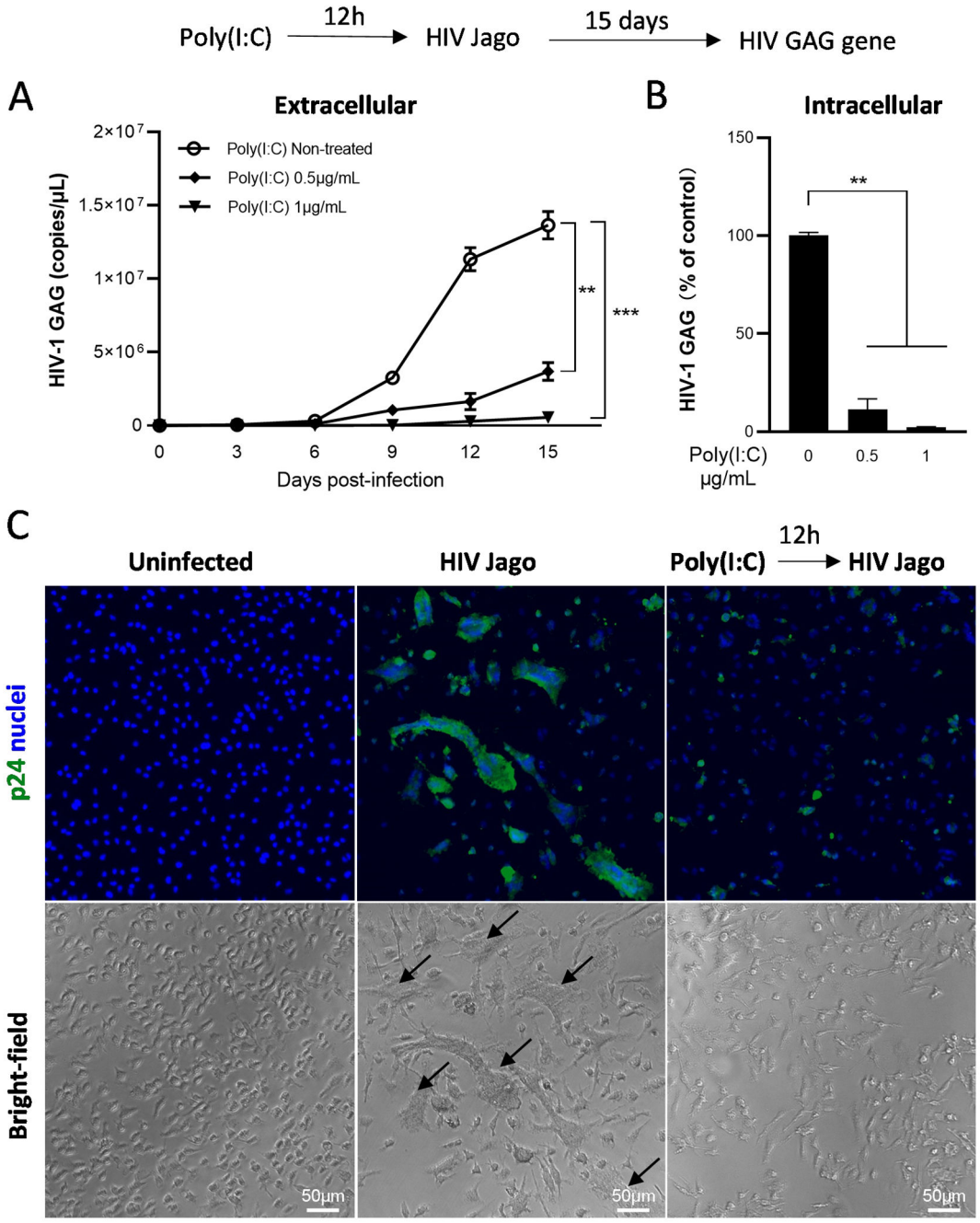


Figure 3. TLR3 activation inhibits HIV infection of human iPSC-derived microglia.

(**A-B**) HIV gag gene RNA expression. Human iPSC-derived microglia were treated with or without poly (I:C) at the indicated concentrations for 12 h, then infected with HIV JAGO. (**A**) Total RNA extracted from cell free culture supernatant. (**B**) Total cellular RNA extracted from iMg at day 15 post infection for HIV GAG gene RNA expression. (**C**) The morphology of uninfected cells comparing with HIV Jago-infected cells pretreated with or without ploy(I:C) (1μg/mL) was photographed by a confocal microscope on day 15 post-infection. Cells were stained to identify nuclei (Hoechst, blue) and HIV infection (p24,

green). The black arrows indicate HIV-induced giant syncytium formation in p24 positive human iPSC-derived microglia. Scale bar represents 50μm. The results shown are the mean ± SD of triplicate wells, representing three independent experiments (**$p < 0.01$; ***$p < 0.001$).

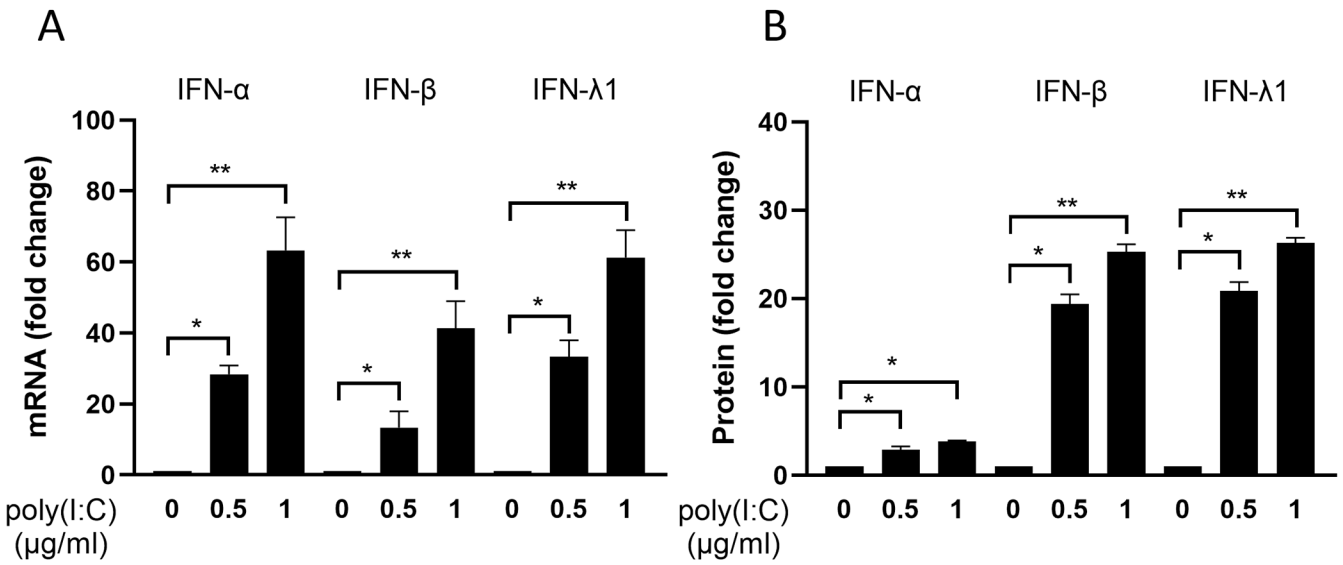


Figure 4. TLR3 activation induces IFN expression in iPSC-derived microglia.

(**A**) Human iPSC-derived microglia were treated with or without poly (I:C) at the indicated concentrations for 12 h. Total RNA extracted from cells was then subjected to the RT-PCR for the mRNA expression of IFNs (IFN-α/β, λ1). (**B**) Human iPSC-derived microglia were treated with or without poly (I:C) at the indicated concentrations for 24 h. Supernatants collected from cultures were then assayed to measure the protein level of IFN-α/β, λ1. The data are expressed as IFN levels relative (fold) to the control (without poly (I:C) treatment, which is defined as 1). The results shown are the mean ± SD of triplicate wells, representing three independent experiments (*$p < 0.05$; **$p < 0.01$).

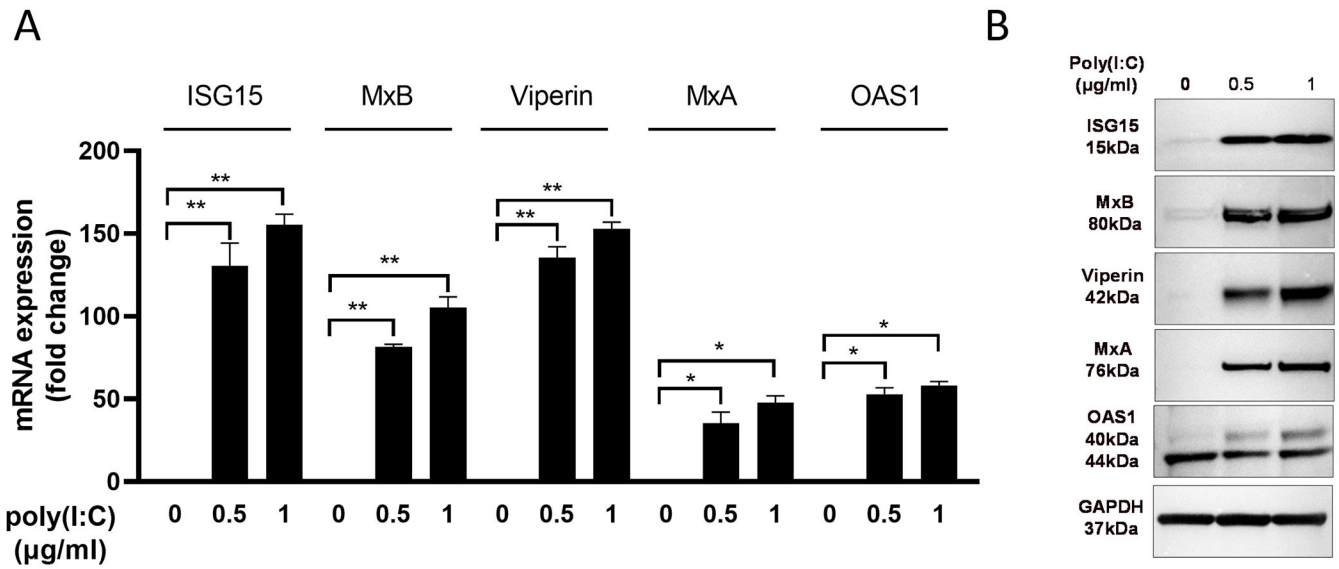


Figure 5. TLR3 activation induces the antiviral ISGs.

(**A**) Human iPSC-derived microglia were treated with poly (I:C) at the indicated concentrations for 12 h. Total RNA extracted from cells was subjected to the RT-PCR for the mRNA the antiviral levels of ISGs (ISG15, MxB, Viperin, MxA, and OAS-1). The data are expressed as the ISG mRNA levels relative (fold) to the control (without poly (I:C) treatment, which is defined as 1). The results shown in A are the mean ± SD of triplicate wells, representing three independent experiments (*$p < 0.05$; **$p < 0.01$). (**B**) Human iPSC-derived microglia were treated with or without poly (I:C) at the indicated concentrations for 24 h and proteins extracted from the cells were subjected to western blotting.

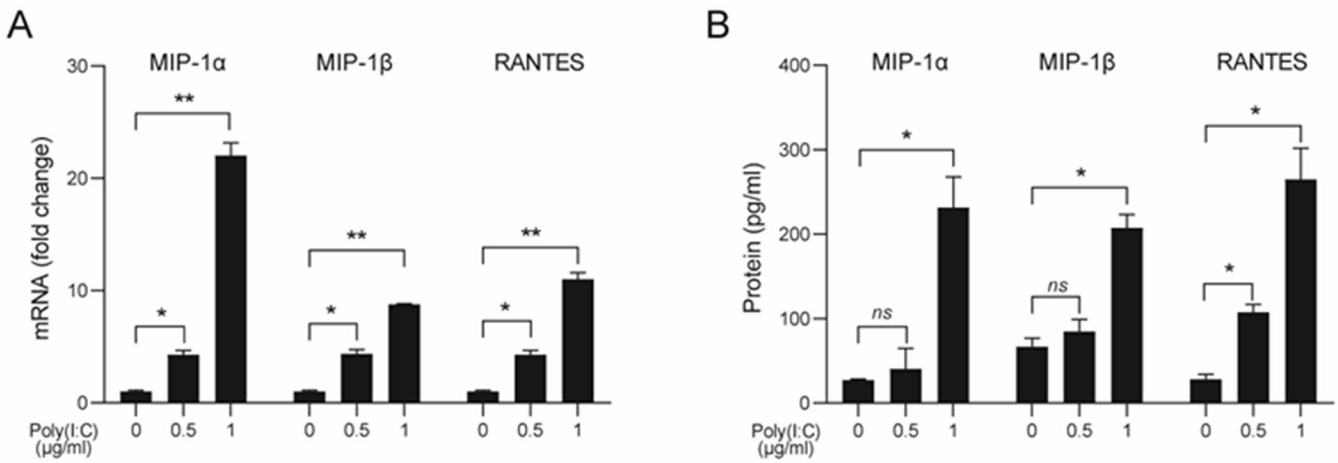


Figure 6. TLR3 activation up-regulates CC chemokines.

Human iPSC-derived microglia (iMg) were treated with poly (I:C) at the indicated concentrations for 24 hours. A. Total RNA extracted from cells was subjected to the RT-PCR for CC chemokines (MIP-1α, MIP-1β, and RANTES). The data are expressed as the CC chemokine mRNA levels relative (fold) to the control (without poly (I:C) treatment, which is defined as 1). The results shown are the mean ± SD of triplicate wells, representing three independent experiments (** $p < 0.01$; * $p < 0.05$). B. proteins from the cells supernatant were subjected to ELISA analysis, The results shown are the mean ± SD of triplicate wells, representing three independent experiments (* $p < 0.05$; ** $p < 0.01$).

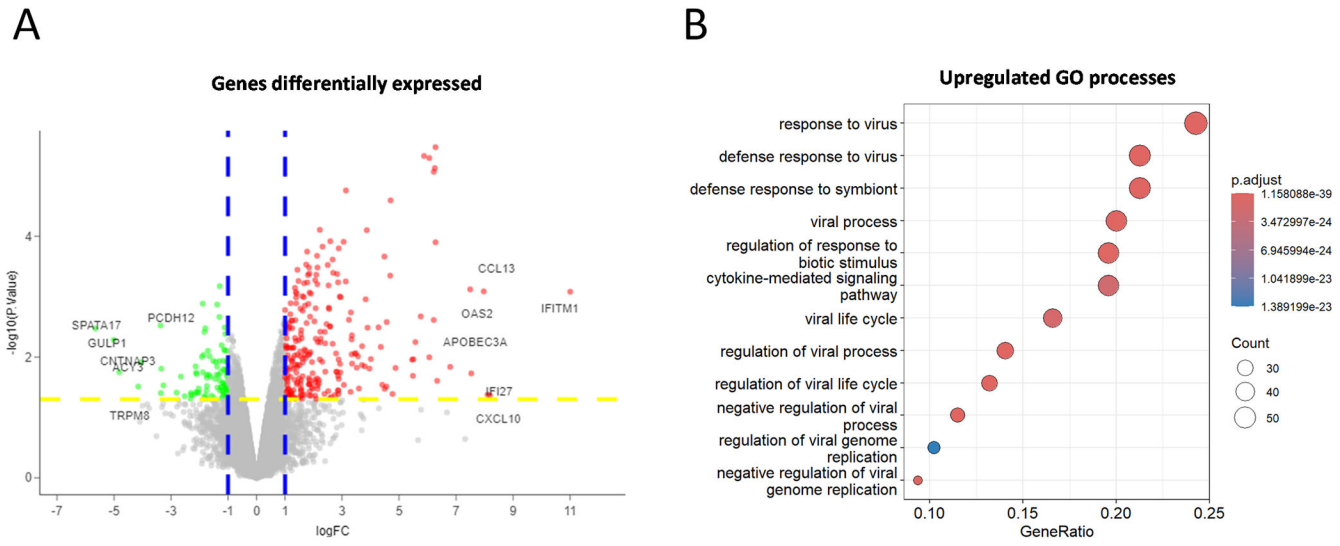
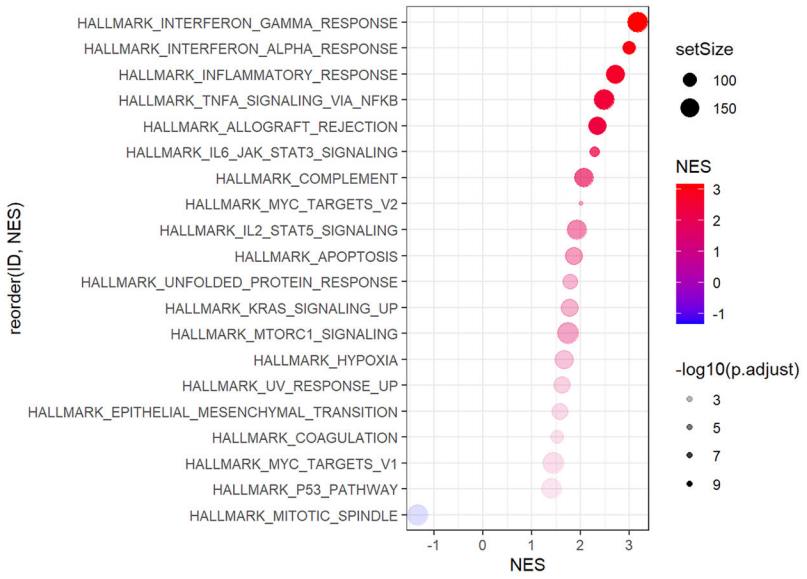


Figure 7. Differential gene expression in iMg following Poly(I:C) exposure.

(A) Volcano plot of statistical significance analysis of genes that are upregulated (red) or downregulated (green) in the iMg in the presence/absence of poly(I:C). (B) Dot plots showing enriched biological process associated with upregulated gene ontology. Circle size represents the number of genes in each GO category and the color indicates the false discovery rate associated with each GO category.

A



B

Gene	Poly I:C vs control (logFC)
Type I interferons	
IFITM1	11.0
CXCL10	8.2
IFI27	8.1
OASL	6.8
MxA	6.3
LY6E	6.3
IFITM3	6.2
SAMD9L	6.1
ISG15	6.1
IFIT3	5.8
Inflammatory	
TNFAIP6	5.5
CCL5	4.0
LAMP3	3.5
CCL2	3.3
EBI3	3.0
EIF2AK2	2.9
TNFRSF9	2.8
FFAR2	2.6
IL1A	2.3
IL18R1	2.3

Gene	Poly I:C vs control (logFC)
Type II interferons	
OAS2	7.5
IFIT1	6.2
SAMD9L	6.1
OAS3	5.9
USP18	5.6
MxB	4.5
LGALS3BP	3.9
DDX60	3.8
ISG20	3.7
BST2	1.7
TNF-α	
IFIT2	5.5
IL1B	3.4
PTX3	3.0
SLC2A6	2.9
IFIH1	2.9
CD80	2.8
TNFAIP3	2.1
CXCL1	2.0
ZC3H12A	1.9
RELB	1.8

Figure 8. Transcriptomic profiling of iMg in the presence/absence of poly(I:C).

(A) Functional enrichment analysis of transcript from different pathways. Y-axis indicate top enrichment and X-axis indicate significance level of enrichment, Blue and red circle size indicate expression pattern of enriched terms. (B) Top10 significantly differentially expressed genes involved in antiviral response pathways: Type I interferons (IFN-α), Type II interferons (IFN-γ), Inflammatory cytokines and Tumor necrosis factor alpha (TNF-α). LogFC, log fold change.

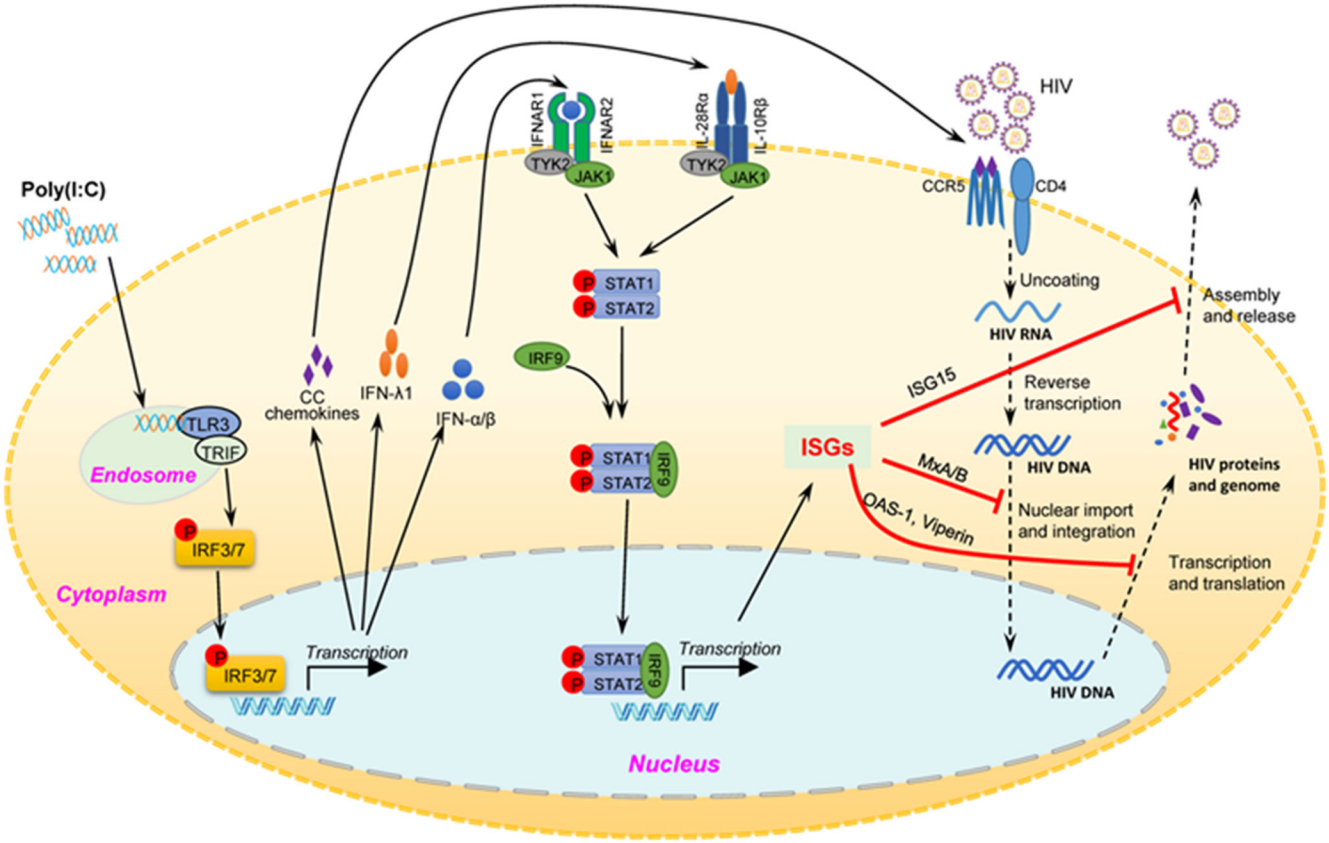


Figure 9. Schema of TLR3 activation-driven intracellular antiviral immunity against HIV in iMg.

Poly(I:C) treatment of iMg activates the TLR3 in the endosome, which facilitates the phosphorylation and translocation of IFN regulatory factors 3 and 7 (IRF3 and IRF7), resulting in the transcription of IFN-α, IFN-β, IFN-λ1, and the CC chemokines. The induced IFN-α, IFN-β and IFN-λ1 are released from cells and then bind to their corresponding receptors on the cell surface, which triggers JAK/STAT pathway, leading to STAT1/STAT2 phosphorylation and transcription of the anti-HIV IFN stimulated genes (ISGs: ISG15, MxA, MxB, OAS-1, and Viperin). These antiviral ISGs inhibit HIV at different steps of the viral replication cycle as indicated. In addition, CC chemokines (MIP-1α, MIP-1β, and RANTES) released from cells can bind to HIV entry co-receptor CCR5 on the cell membrane and block HIV entry.