Expression of HIV-1 Intron-containing RNA in Microglia Induces Inflammatory Responses

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Abstract

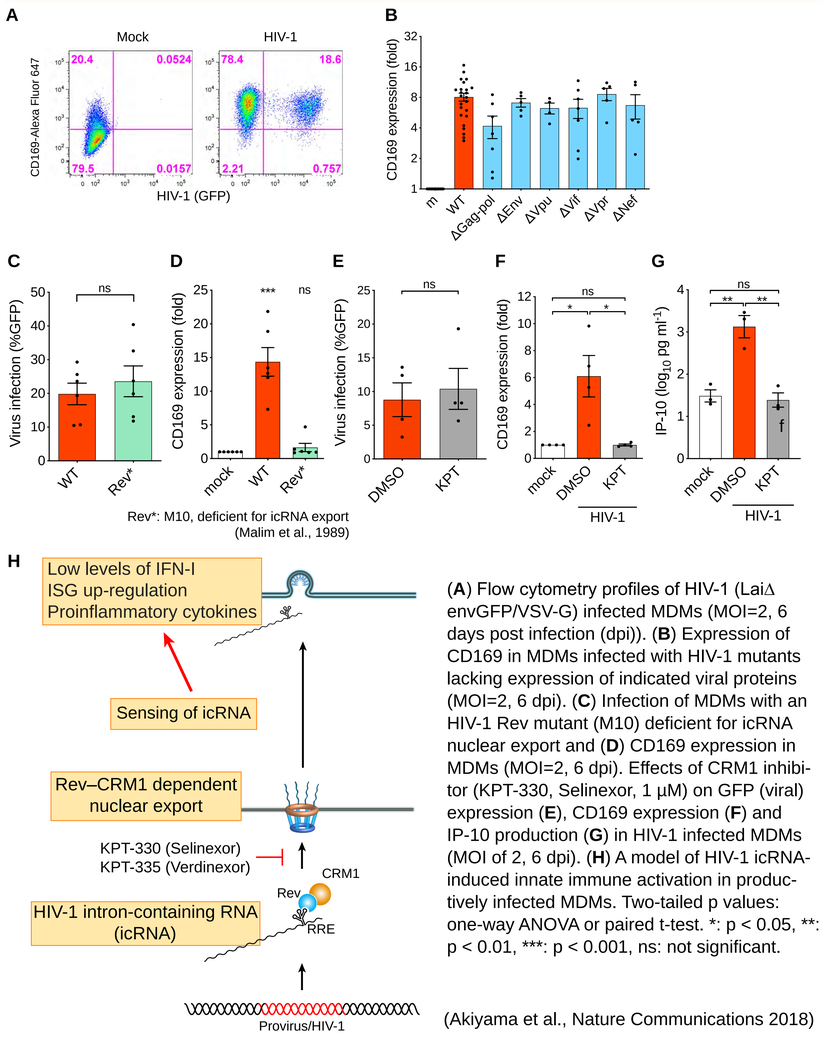
BACKGROUND: Chronic immune activation is observed in HIV-1$^+$ individuals on long-term combination antiretroviral therapy (cART) and is thought to lead to HIV-associated non-AIDS complications (HANA) such as neurocognitive impairment. We have recently reported that expression of HIV intron-containing RNA (icRNA) alone in productively infected monocyte-derived macrophages induces proinflammatory responses (PMID 30150664). Hence, in this study, we tested the hypothesis that persistent expression of HIV icRNA in microglia (MG), the brain-resident macrophage, contributes to neuroinflammation.

METHODS: Monocyte-derived microglia (MDMGs) were derived from CD14$^+$ cells purified from PBMCs. Human iPSC (induced pluripotent stem cell)-derived microglia (hiMG) were generated by co-culturing yolk-sac-derived primitive macrophages and iPSC-neurons. Expression of MG markers such as P2RY12, IBA-1 and TMEM119 was confirmed by qRT-PCR or flow cytometry. Microglia were infected with HIV-1, and extent of viral infection and induction of proinflammatory responses was determined by mRNA analysis (NanoString, qRT-PCR), flow cytometry and ELISA.

RESULTS: HIV-1 infection in MDMGs up-regulated expression of ISGs and proinflammatory cytokines such as IP-10 and MCP-1. Treatment of infected MDMGs with raltegravir or a CRM1 inhibitor that blocks Rev–CRM1-dependent nuclear export of HIV-1 icRNA, or infection of MDMGs with Rev-mutant (M10) deficient for icRNA export did not induce IP-10 expression, suggesting that nuclear export of HIV icRNA but not Rev or Tat expression is the trigger for proinflammatory responses in MDMGs. To better mimic the yolk-sac origin of MG, we generated hiMG and found that hiMGs were robustly infected with replication competent CCR5-tropic HIV-1 (YU2). Importantly, establishment of productive infection led to secretion of proinflammatory cytokines IP-10 and MCP-1, which was inhibited upon pre-treatment with raltegravir or CRM1 inhibitor. Interestingly, HIV-infected hiMGs displayed poor phagocytic activity, suggesting that HIV infection negatively impacts homeostatic functions of MG.

CONCLUSION: Collectively, our findings suggest that viral gene expression and nuclear export of HIV icRNA, even in the absence of viral spread, induces proinflammatory responses in microglia and suppresses their homeostatic functions. Since none of the current cART regimens inhibit viral RNA expression, novel strategies are needed to suppress HIV icRNA expression-induced immune activation.

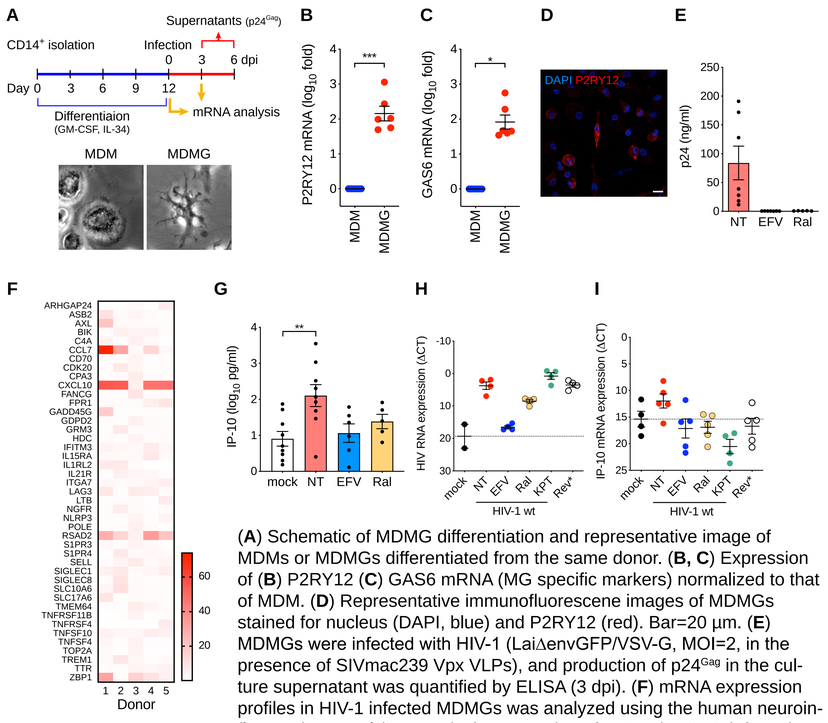
HIV intron-containing RNA (icRNA) activates innate immune responses in macrophages



(**A**) Flow cytometry profiles of HIV-1 (LaiΔ envGFP/VSV-G) infected MDMs (MOI=2, 6 days post infection (dpi)). (**B**) Expression of CD169 in MDMs infected with HIV-1 mutants lacking expression of indicated viral proteins (MOI=2, 6 dpi). (**C**) Infection of MDMs with an HIV-1 Rev mutant (M10) deficient for icRNA nuclear export and (**D**) CD169 expression in MDMs (MOI=2, 6 dpi). Effects of CRM1 inhibitor (KPT-330, Selinexor, 1 μM) on GFP (viral) expression (**E**), CD169 expression (**F**) and IP-10 production (**G**) in HIV-1 infected MDMs (MOI of 2, 6 dpi). (**H**) A model of HIV-1 icRNA-induced innate immune activation in productively infected MDMs. Two-tailed p values: one-way ANOVA or paired t-test. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ns: not significant.

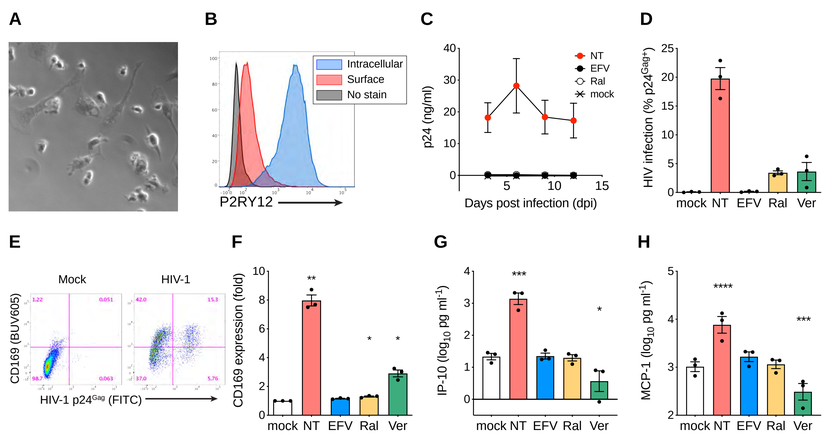
(Akiyama et al., Nature Communications 2018)

HIV-1 infection of monocyte-derived microglia (MDMG) induces pro-inflammatory cytokines



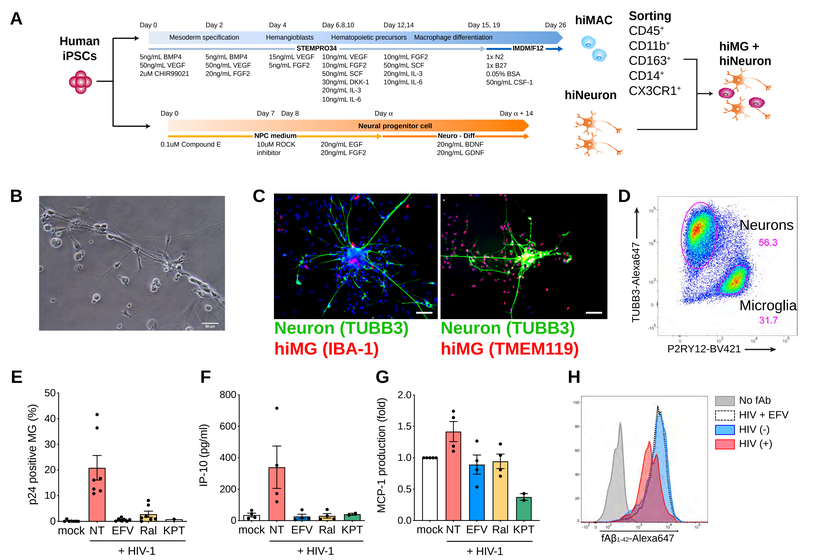
(**A**) Schematic of MDMG differentiation and representative image of MDMs or MDMGs differentiated from the same donor. (**B, C**) Expression of (**B**) P2RY12 (**C**) GAS6 mRNA (MG specific markers) normalized to that of MDM. (**D**) Representative immunofluorescene images of MDMGs stained for nucleus (DAPI, blue) and P2RY12 (red). Bar=20 μm. (**E**) MDMGs were infected with HIV-1 (LaiΔenvGFP/VSV-G, MOI=2, in the presence of SIVmac239 Vpx VLPs), and production of p24^{Gag} in the culture supernatant was quantified by ELISA (3 dpi). (**F**) mRNA expression profiles in HIV-1 infected MDMGs was analyzed using the human neuroinflammation panel (NanoString). Expression of mRNA in HIV-1-infected MDMGs was normalized to that in mock-infected MDMGs, and genes which was expressed greater than the mean+ 2xSD are shown. (**G**) Production of IP-10 in HIV-1-infected MDMGs (MOI=2, 6 dpi) measured by ELISA. Effects of CRM1 inhibitor (KPT-330) on HIV-1-infected MDMGs or infection of MDMGs with a Rev mutant deficient for icRNA nuclear export on (**H**) viral infection (fully-spliced mRNA expression, Rev-independent) or (**I**) IP-10 mRNA expression shown as ΔCT to GAPDH. Two-tailed p values: one-way ANOVA or the Wilcoxon matched-pairs signed rank test. **: $p < 0.01$. NT: no treatment, EFV: efavirenz (1 μM), Ral: raltegravir (30 μM), KPT: KPT-330 (Selinexor, 1 μM), Rev*: M10.

HIV-1 infection activates iPSC-derived microglia (iCell-MG) in a CRM1-dependent manner



(**A**) Representative phase-contrast images of iCell-MGs (Stemcell Technologies). (**B**) Representative flow cytometry profile of iCell-MGs stained for intracellular and surface P2RY12. (**C**) Replication kinetics of HIV-1 in iCell-MGs. Cells were infected with HIV-1 (Lai/YU-2env, replication competent CCR5 tropic HIV-1, MOI=1), and production of p24^{Gag} in the culture supernatant was quantified by ELISA. (**D-H**) iCell-MGs were infected with HIV-1 (Lai/YU-2env, MOI=1). (**D**) HIV-1 infection (intracellular p24^{Gag} expression) and (**E, F**) CD169 expression were analyzed by flow cytometry, and production of proinflammatory cytokines (**G**) IP-10 and (**H**) MCP-1 was measured by ELISA (6 dpi). Two-tailed p values: one-way ANOVA. *: $p < 0.05$, **: $p < 0.01$.***: $p < 0.001$, ****: $p < 0.0001$. NT: no treatment, EFV: efavirenz (1 μM), Ral: raltegravir (30 μM), Ver: KPT-335 (Verdinexor, 0.1 μM).

HIV-1 infection activates proinflammatory responses in iPSC-derived microglia (hiMG)-neuron (hiNeuron) co-culture



(**A**) Schematic of hiMG generation by co-culturing hiMAC (yolk-sac-derived primitive macrophages) and neurons from human iPSCs. (**B**) Representative phase-contrast image of hiMGs and iPSC-Neurons co-cultured for 11 days. (**C**) Representative immunofluorescene images of hiMG-iPSC-neuron co-culture stained for nucleus (DAPI, blue), neurons (tubulin beta 3: TUBB3, green) and MG markers IBA-1 or TMEM119 (red). Bar=50 μm. (**D**) Representative flow cytometry profile of hiMG-iPSC-neuron co-culture stained for neurons (TUBB3) and hiMGs (P2RY12). (**E-H**) hiMG-iPSC-neurons were infected with HIV-1 (Lai/YU-2env: replication competent CCR5 tropic HIV-1, MOI=1). (**E**) HIV-1 infection (intracellular p24^{Gag} expression) was analyzed by flow cytometry, and production of proinflammatory cytokines (**F**) IP-10 and (**G**) MCP-1 was measured by ELISA (6 dpi). (**H**) Phagocytic activity of hiMG was measured by uptake of Alexa647-labeled fibrilar amyloid beta (fAβ_{1-42}) by flow cytometry. NT: no-treatment (DMSO), EFV: efavirenz (1 μM), Ral: raltegravir (30 μM), KPT: KPT-330 (Selinexor, 1 μM).

Conclusions

- MDMGs, iCell-MG, and hiMGs are useful tools to study HIV-1 infection of CNS-resident microglia
- iPSC-derived microglia are highly susceptible to HIV-1 infection
- Expression of HIV-1 icRNA in microglia
  - results in innate immune activation
  - impairs homeostatic function
  - contributes to chronic neuroinflammation in HIV$^+$ individuals on cART?
- Considering lifelong self-renewal capacity of microglia, infection of microglia with HIV-1 and persistent transcription of HIV icRNA even without infectious virus production in the tissue might contribute to chronic inflammation
- Strategies to attenuate HIV icRNA expression are needed for suppression of inflammation-induced HANA

Acknowledgements

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