Differentiation stimuli strongly impact the ability of macrophages to support HIV-1 replication due to SAMHD1 restriction pathway

#204

IrsiCaixa Institut de Recerca de la Sida

Ester Ballana[1], Roger Badia[1], Eva Riveira-Muñoz[1], Alba Ruiz[1], Javier Torres-Torrenteras[2], Bonaventura Clotet[1], Eduardo Pauls[1], Ramón Martí[2] and José A. Esté[1]

[1] IrsiCaixa, Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona; [2] Group of Mitochondrial Pathology and Neuromuscular Disorders,Vall d'Hebron Institut de Recerca, Barcelona

E-mail:eballana@irsicaixa.es
Phone: 34 934656374
Fax: 34 934653968

ABSTRACT

BACKGROUND: Macrophages are a highly heterogenic cell population with cellular properties strongly influenced by the factors present during differentiation of the macrophage from the precursor cell, the monocyte. Indeed, monocytes are refractory to HIV infection and only become susceptible to infection after differentiation into macrophages, due to deactivation of the restriction factor SAMHD1 by phosphorylation. We have shown that SAMHD1 deactivation is controlled by cyclin-dependent kinases (CDK), which in turn, control cell activation and proliferation. Here, we have used human primary monocyte-derived macrophages (MDMs) differentiated through different sets of conditions to evaluate macrophage heterogeneity on cell activation and proliferation, differential gene and protein expression patterns determining susceptibility to HIV-1 infection and the effect of drugs blocking either HIV replication or cell activation.

METHODOLOGY: CD14+ human monocytes were incubated with M-CSF and GM-CSF for 4 days to generate M-CSF (M-MDM) or GM-CSF-induced monocyte-derived macrophages (GM-MDM). Expression of cell surface antigens and cell activation and proliferation markers were evaluated by flow cytometry. Susceptibility to HIV-1 infection was examined by flow cytometry after infection with a VSV-pseudotyped NL4-3 GFP-expressing virus in the presence or absence of drugs either targeting HIV RT or inhibiting cell activation and/or proliferation. SAMHD1 restriction was bypassed by treatment with VLP-containing HIV-2 Vpx. Gene expression was assessed by quantitative PCR. Protein expression and phosphorylation were analyzed by immunoblotting. dNTP intracellular levels were determined using a polymerase-based method.

RESULTS: MDMs displayed different morphological characteristics dependent on the differentiation conditions, but no significant differences in cell surface expression of HIV receptor and co-receptors were observed. M-MDM were roughly 10-fold more susceptible to HIV-1 infection than GM-MDM, which correlated with higher dNTP levels in M-MDM compared to GM-MDM (from 3 to 80 fold difference depending on the nucleotide assessed, being dCTP the lowest and dTTP the highest). Although no differences were found in SAMHD1 expression, a change in SAMHD1 activation was observed, consequence of different cell activation after M-CSF or GM-CSF differention. Degradation of SAMHD1 by HIV-2 Vpx increased HIV replication in both cases, minimizing the original differences in susceptibility. In concordance, SAMHD1 degradation led to an increase in dNTP intracellular levels under both conditions.

CONCLUSIONS: Our results demonstrate that differentiation strongly impacts the ability of the MDMs to support HIV-1 replication due to differences in the restriction factor SAMHD1, consequence of cell activation differences. Our results emphasize that the choice of macrophage model system is important for evaluation of innate immune responses in general, including virus-cell interactions during HIV-1 infection.

M-CSF AND GM-CSF DERIVED MACROPHAGES SHOW DIFFERENT CELL PROLIFERATION PATTERNS

A.

	Cell antigen (% ± SD)									
	CD14	CD86	CD4	CCR5	CXCR4	CD40	CD69	CD25	CD28	Ki67
M-CSF	92 ± 4	75 ± 12	96 ± 3	26 ± 12	16 ± 6	nd	nd	nd	92 ± 5	7,1 ± 5,3
GM-CSF	82 ± 10	58 ± 15	78 ± 16	35 ± 10	21 ± 8	nd	nd	nd	75 ± 12	0,5 ± 0,4

nd, not detected

B.

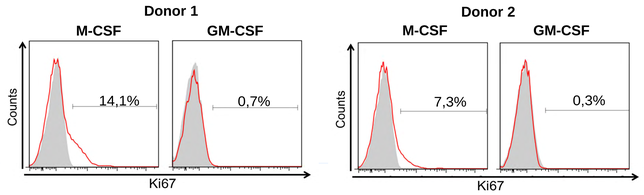


Figure 1. Expression of cell antigens in M-CSF and GM-CSF differentiated macrophages. (A) Summary of cell antigens expression. Markers of macrophage differentiation, HIV-1 receptor and coreceptors and cell activation and proliferation markers were evaluated by flow cytometry. The table shows mean ± SD of at least 3 different donors. The more apparent difference was observed for cell proliferation marker ki67. **(B) Evaluation of cell proliferation by Ki67 staining.** Histograms of two representative donors showing Ki67 staining in M-CSF and GM-CSF derived macrophages. Percentage of positive cells is shown in each case.

DIFFERENCES IN HIV-1 INFECTION SUSCEPTIBILITY ARE MINIMIZED AFTER Vpx-MEDIATED DEGRADATION OF SAMHD1

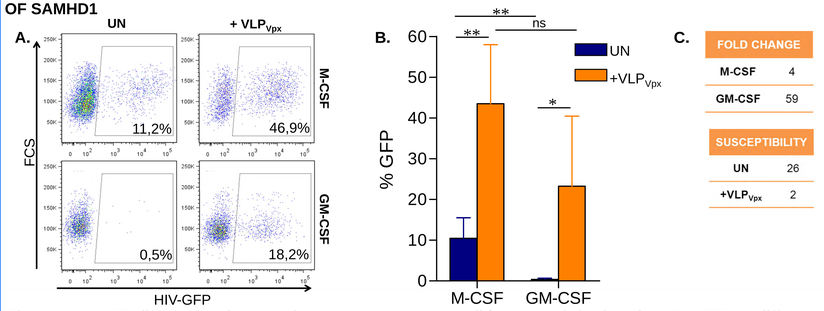


Figure 2. M-CSF differentiated macrophages are more susceptible to HIV infection than GM-CSF, a difference minimized after SAMHD1 degradation. (A) Dot plots of infected M-MDM (upper panels) or GM-MDM (lower panels) untreated (left) or after treatment (right) with VLP_{Vpx}. A representative donor is shown. **(B)** Percentage replication in M-MDM and GM-MDM untreated (blue bars) or transduced with VLP_{Vpx} (orange bars). Mean ± SD of 4 different donors performed in triplicate is shown. **(C)** Mean susceptibility and fold change ± VLP_{Vpx}. UN; untreated, ns; not significant, * p<0.05; ** p<0.005

DRUG SENSITIVITY PROFILE IN M-CSF AND GM-CSF MDM: ANTIVIRAL PROPERTIES OF CDK4/6 INHIBITOR PALBOCICLIB ARE LOST IN THE ABSENCE OF SAMHD1

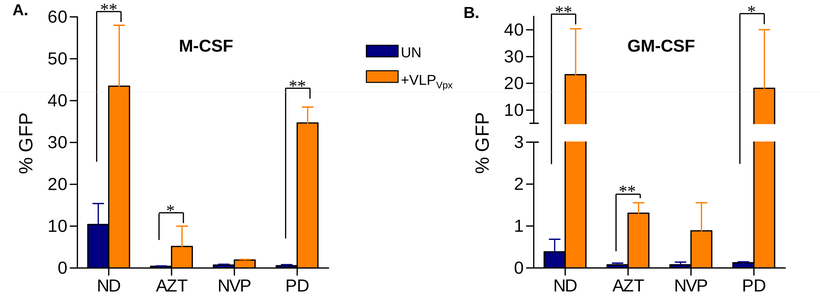


Figure 3. M-CSF and GM-CSF differentiated macrophages are equally sensitive to drugs. Percentage replication in M-MDM **(A)** and GM-MDM **(B)** untreated (blue bars) or transduced with VLP_{Vpx} (orange bars). Antiviral effect of Zidovudine (AZT, 1 μM), Nevirapine (NVP, 5 μM) and Palbociclib (PD, 4 μM) was evaluated. Antiviral potency was decreased with AZT and completely lost with PD after degradation of SAMHD1 with VLP_{Vpx}. Mean ± SD of at least 3 different donors performed in triplicate is shown. UN; untreated, ns; not significant* p<0.05; ** p<0.005

INTRACELLULAR dNTP LEVELS ARE HIGHER IN M-CSF MDM COMPARED TO GM-CSF

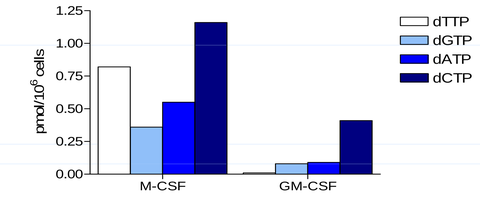


Figure 4. Intracellular dNTP levels in M-CSF or GM-CSF differentiated MDM. Intracellular dNTPs were extracted from M-CSF (left) or GM-CSF (right) differentiated MDM and dNTP content was determined using a polymerase-based method. Results from a representative donor are depicted.

IMPACT OF DIFFERENTIATION STIMULI ON SAMHD1-RELATED GENES EXPRESSION

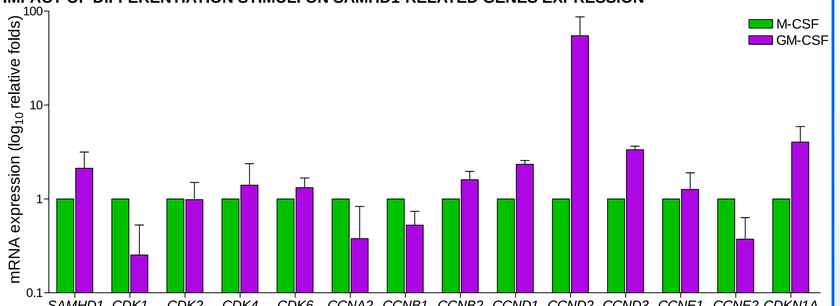


Figure 5. Gene expression of SAMHD1 restriction pathway genes. mRNA of SAMHD1, CDK1, 2, 4 and 6, the corresponding cyclins (A2, B1, B2, D1, D2, D3, E1 and E2) and CDK2 inhibitor p21 (CDKN1A) was quantified in samples of M-CSF (green bars) and GM-CSF (purple bars) macrophages. Data is normalized to M-CSF relative expression. Mean ± SD of 3 independent donors is shown.

PHOSPHORYLATION OF SAMHD1 IS INCREASED IN M-CSF MDM COMPARED TO GM-CSF, CONCOMITANT TO A DISTINCT REGULATION OF ITS EFFECTOR PROTEINS

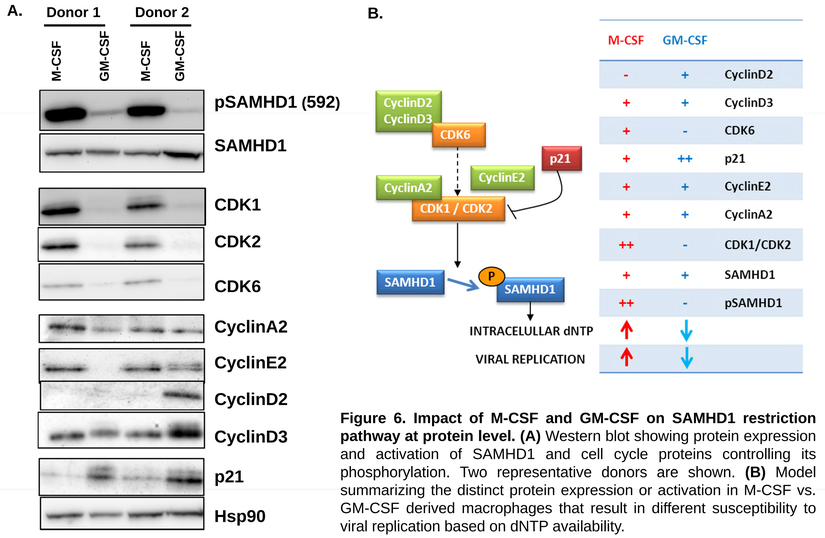


Figure 6. Impact of M-CSF and GM-CSF on SAMHD1 restriction pathway at protein level. (A) Western blot showing protein expression and activation of SAMHD1 and cell cycle proteins controlling its phosphorylation. Two representative donors are shown. **(B)** Model summarizing the distinct protein expression or activation in M-CSF vs. GM-CSF derived macrophages that result in different susceptibility to viral replication based on dNTP availability.

DISCUSSION

- SAMHD1 restriction pathway determines the ability to support HIV-1 replication in different *in vitro* macrophage models.
- M-CSF derived macrophages show higher degree of cell proliferation, concomitant to higher expression of CDK responsible of cell cycle entry, therefore leading to inactivation of SAMHD1 by phosphorylation, higher dNTP and higher replication rates.