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G3BP1 restricts HIV-1 replication in macrophages and T-cells by sequestering viral RNA

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ABSTRACT

HIV-1 exploits the cellular machinery for replication and therefore several interactions with cellular factors take place, some of which are yet unknown. We identified GTPase-activating protein-(SH3 domain)-binding protein 1 (G3BP1) as a cellular factor that restricts HIV-1, by analyzing transcriptome profiles of *in vitro*-cytokine-activated macrophages that are non-permissive to HIV-1 replication. Silencing of G3BP1 by RNA interference resulted in increased HIV-1 replication in primary T-cells and macrophages, but did not affect replication of other retroviruses. G3BP1 specifically interacted with HIV-1 RNA in the cytoplasm, suggesting that it sequesters viral transcripts, thus preventing translation or packaging. G3BP1 was highly expressed in resting naïve or memory T-cells from healthy donors and HIV-1 infected patients, but significantly lower in IL-2-activated T-cells. These results strongly suggest that G3BP1 captures HIV-1 RNA transcripts and thereby restricts mRNA translation, viral protein production and virus particle formation.

Introduction

Antiretroviral therapy has dramatically improved the clinical outcome of HIV-1 infection. However, total eradication of HIV-1 cannot be entirely achieved, due to the persistence of viral reservoirs that allows for continuous residual viral replication and dissemination of the infection, while avoiding triggering of immune responses. Indeed, plasma viral load in HIV-1 infected patients can very rapidly rebound after stopping therapy (Chun et al., 1999; Harrigan et al., 1999; Palmer et al., 2008). Macrophages become highly susceptible to HIV-1 infection after they differentiate from monocytic precursors (Rich et al., 1992; Schuitemaker et al., 1992; Sonza et al., 1996). HIV-1 infected macrophages found in the tissues of HIV-1 infected patients (Koenig et al., 1986) allow for residual viral replication at sites such as the gut-associated lymphoid tissue and the brain, even during antiretroviral therapy (Chun et al., 2008; Eisele and Siliciano, 2012; Lambotte et al., 2005). Thus, macrophages play a crucial role in the maintenance and dissemination of the infection. Their importance is further underscored by studies showing that they are highly resistant to the cytopathic effects of viral replication, and are able to render resting T-cells permissive for HIV-1 infection (Ancuta et al., 2006; Swingler et al., 2003). HIV-1 can only replicate in activated proliferating T-cells, where the proviral genome is integrated into the host genome. *In vivo,* these cells can go into a resting memory state, and become latent viral reservoirs (Finzi et al., 1997). These cells persist during long term therapy and are able to efficiently reinitiate virus production upon stopping therapy (Chun et al., 1997; Finzi et al., 1997; Wong et al., 1997).

The phenotype and function of macrophages are greatly determined by their activation by cytokines while differentiating in the tissues. We and others have previously shown that cytokine-mediated activation of macrophages results in inhibition of HIV-1 replication at different stages of the replication cycle: in IL-4 activated macrophages, HIV-1 is inhibited at the level of reverse transcription, whereas IFNγ+TNFα and IL-10 stimulation leads to restriction of later stages in the replication cycle (Cassetta et al., 2013; Cassol et al., 2009; Cobos Jiménez et al., 2012). Several new cellular factors have been identified to restrict early steps of HIV-1 replication upon type 1 interferon stimulation of

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macrophages, such as MX2 (Goujonet al., 2013), IFI16 (Jakobsen et al., 2013) and SAMHD1 (Hrecka et al., 2011; Laguette et al., 2011; Lahouassaet al., 2012), or MCPIP1 in activated CD4$^+$ T cells (Liu et al., 2013). There is however a lack of knowledge regarding cellular factors that affect post-integration steps and thereby allow infected cells that harbor an integrated provirus to persist as viral reservoirs and remain undetected by the immune system.

Therefore, we aimed to identify cellular factors that could restrict HIV-1 replication in primary cells. For this purpose, we compared gene transcriptome profiles of permissive macrophages with those of non-permissive/ IFNγ+TNFα activated

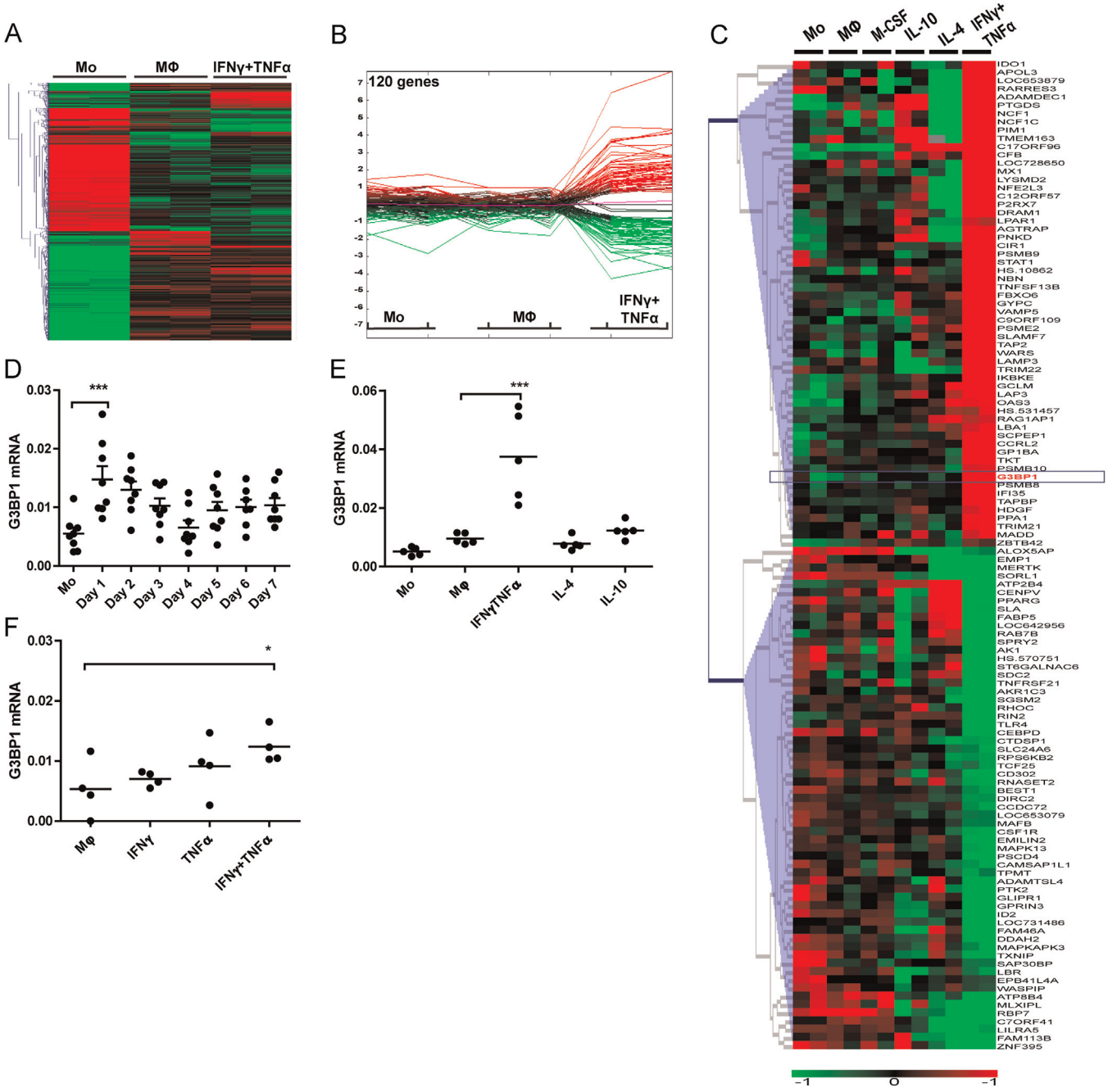

Fig. 1. IFNγ+TNFα stimulation of macrophages induces G3BP1 expression. (A) Hierarchical clustering of genes significantly induced during maturation of monocytes (Mo) into macrophages (MΦ) for 5 days and stimulation with IFNγ+TNFα (50 U/ml and 12.5 ng/ml) for 5 days, using average median-centered values from 2 donors; (B) Genes that were regulated only by stimulation with IFNγ+TNFα were selected by a Pavlidis Template Matching (PMT) analysis, using median-centered values; (C) Hierarchical clustering of expression of the PMT selected genes in monocytes (Mo), unstimulated macrophages (MΦ), or cells stimulated for 5 days with M-CSF (50 ng/ml), IL-4 (50 ng/ml) or IL-10 (50 ng/ml), using median centered values. The location of G3BP1 is indicated on the right side; (D) Expression of G3BP1 relative to a housekeeping gene, in monocytes and macrophages cultured for 1 to 7 days without cytokine stimulation, and isolated from 5 donors; (E) Expression of G3BP1 relative to a housekeeping gene, in monocytes, and unstimulated macrophages (MΦ) or stimulated with IFNγ+TNFα (50 U/ml and 12.5 ng/ml), IL-4 (50 ng/ml) and IL10 (50 nl/ml) for 5 days. (F) Expression of G3BP1 relative to a housekeeping gene, in unstimulated macrophages (MΦ) or stimulated with IFNγ (50 U/ml), TNFα (12.5 ng/ml) or IFNγ+TNFα (50 U/ml and 12.5 ng/ml). Significant differences in the expression levels are indicated by asterisks (One-way ANOVA and Bonferroni's Multiple Comparison Test, $p<0.05^*$, $p<0.01^{**}$, $p<0.001^{***}$).