Strong Ifitm1 Expression In Cd4 T Cells In Hiv Controllers Is Correlated With Immune Activation

Etienne Canoui, Nicolas Noël, Camille Lécuroux, Faroudy Boufassa, Asier Sáez-Cirión, Christine Bourgeois, Olivier Lambotte, Anrs Co21 Codex Group

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Etienne Canoui[1, *], Nicolas Noël[1,2,3,4 *], Camille Lécuroux[1,4], Faroudy Boufassa[2,5,6], Asier Sáez-Cirión[7], Christine Bourgeois[1,2,4], Olivier Lambotte[1, 2, 3,4 §] and the ANRS CO21 CODEX study group

[1]: INSERM U1184, Immunologie des infections virales et autoimmunité, Le Kremlin-Bicêtre, France

[2]: Université Paris Sud, Le Kremlin-Bicêtre, France

[3]: Assistance Publique – Hôpitaux de Paris, Service de Médecine Interne et Immunologie Clinique, Groupe Hospitalier Universitaire Paris Sud, Hôpital Bicêtre, Le Kremlin-Bicêtre, France

[4]: CEA, DSV/iMETI, Division of Immuno-Virology, IDMIT, France

[5]: INSERM U1018, Centre de recherche en Epidémiologie et Santé des Populations, Le Kremlin-Bicêtre, France

[6]: Assistance Publique – Hôpitaux de Paris, Département d'épidémiologie, Groupe Hospitalier Universitaire Paris Sud, Hôpital Bicêtre, Le Kremlin-Bicêtre, France

[7]: Institut Pasteur, Unité HIV inflammation et persistance, Paris, France

*: co-first authors, equal contribution

§: Corresponding author

Pr Olivier Lambotte, Service de Médecine Interne et Immunologie Clinique

CHU Bicêtre ; 78, rue du Général Leclerc, 94275 Le Kremlin-Bicêtre CEDEX, FRANCE

Phone: +33 145 212 783, Fax: +33 145 212 733

olivier.lambotte@aphp.fr

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INTRODUCTION

During HIV infection, the Interferon (IFN)-stimulated genes (ISGs) have been associated with HIV pathophysiology by restricting HIV replication [1], or enhancing the immune activation [2,3]. The expression levels of ISGs depend on their function, as well as their regulation mechanisms and the viral progression [4,5]. Indeed, most ISGs are upregulated in CD4 and CD8 T cells from viremic untreated patients [5–7], and tend to diminish on antiretroviral therapy (ART). However, whereas monocytes are important in HIV pathogenesis [8], few studies have focused on ISGs regulation in monocytes from HIV-infected patients [9].

Little is known regarding the expression of ISGs in the rare patients who spontaneously control HIV replication (HIV controller patients (HICs)). In PBMCs from HICs, *ifit1* and *mxA* seem overexpressed as compared with HDs and ART treated patients [10]. We have recently showed that plasma IP10 levels are higher in HICs than HDs and associated with low CD4 T cell counts [11]. Other studies reported that some ISGs known to restrict HIV, such as *samhd1, schlaffen 11*, *apobec3c* and *apobec3d* might be overexpressed in CD4 T cells or PBMCs from HICs [12–14].

Here, we studied the expression of four ISGs in three sorted cell subtypes (CD4 and CD8 T lymphocytes, and monocytes). We compared their expressions between HICs, HIV-1 infected ART-treated (ARTs), viremic treatment-naïve patients (VIRs), and healthy donors (HD). The expression of these ISGs according to the levels of T cell- and monocyte activation was analyzed. A strong *ifitm1* expression in CD4 T cells in HIV controllers was correlated with immune activation.

PATIENTS AND METHODS

HICs (n=11) were enrolled in the ANRS CO21 CODEX cohort [11] with the following definition: known HIV diagnosis for at least 5 years prior to enrolment, with HIV VL < 400 copies/mL in the last five consecutive measurements. ART-treated patients (n=8) and VIRs (n=8) were defined as previously reported [11]. HIV – negative healthy donors (HDs) (n=8) were recruited as controls. All patients gave their informed consent for this study, in accordance with the Helsinki declaration.

Freshly collected PBMCs were stained with the following conjugated monoclonal antibodies: PE-CD14, PerCP Cy5.5-CD8, APC-CD3, and APC-H7-CD4, before the FACS-based sorting procedure on BD ARIA (BD Biosciences^{TM}). CD4+ T cells, CD8+ T cells and monocytes (CD3-CD14+) were sorted and immediately lysed in RNeasy Lysis Buffer (QIAGEN*) and cryopreserved at -80°C. RNA isolation and reverse transcription were performed according to the manufacturers' protocols (RNeasy mini kit, Qiagen^{TM}, and enhanced avian RT-PCR Kit, SIGMA^{TM}, respectively). qRT-PCR analysis was performed using a SyBR Green RT PCR method (LightCycler system, Roche^{TM}). All results were normalized for *gapdh* expression.

Levels of soluble CD14 (sCD14) were determined by ELISA (R&D Systems, Minneapolis, Minnesota, USA), and the surface expression of HLA-DR and CD38 on T cells was analyzed by flow cytometry, as described previously [11].

For statistical analyses, continuous variables were compared using the non-parametric Kruskall-Wallis test followed by Dunn's test for multiple analysis. Categorical variables were compared using chi² test, and Spearman's coefficient was used for correlation analyses. The threshold for statistical significance was set to *p*

less than 0.05. Data were stored and analysed using PRISM software (version 5, GraphPad software, La Jolla, California, USA).

RESULTS

The characteristics of the study population are summarized in the **Supplemental Table 1**. There was no difference in terms of age and gender between each group of patients.

Ifitm1 expression is elevated in CD4 T cells from HICs

The analysis of ISGs expression in the 4 groups and the 3 cell subtypes is depicted in **Figure 1**. In VIRs, the expression of *ifitm1* in monocytes, *pkr* in all cell types, *mxA* in CD4 and CD8 T cells, and *ifit1* in CD4 were higher than in ARTs, suggesting a strong role of the virus in ISGs induction. Similar results were seen when comparing ISGs expression between VIRs and HICs, except for *mxA* in all cell types and *ifit1* in CD8 T cells and monocytes, for which the expression were similar in the two groups.

Importantly, we found that the expression of *ifitm1* expression in CD4 T cells from HICs (p=0.01) and ARTs (p=0.001) was similar to VIRs and higher than HDs. In contrast, the expression of ifitm1 in CD8 T cells and monocytes and those of the other genes in the three cell subtypes were similar in HICs and in HDs.

The expression of most ISGs is correlated positively with HIV viral load

In view of these observations, we hypothesized that the expression of most ISGs was associated with the HIV viral load (VL). Indeed, pooling the data of all HIV infected patients, the HIV VL was significantly correlated with the expression of *pkr* in CD4 T cells, CD8 T cells, and monocytes, as well as *mxA* in CD4 and CD8 T cells, and with *ifit1* in CD4 T cells. Of note, similar results were obtained when classifying the HIV VL into categorical variables (i.e., detectable or undetectable). Also, when

considering all HIV infected patients, the CD4 T cell count was negatively correlated with the expression of *pkr* in CD4 T cells and monocytes (with a strong trend for CD8 T cells), and *ifit1* only in CD4 T cells, but not with *mxA* expression.

Interestingly, the expression of *ifitm1* was associated with the HIV VL in monocytes only, but was negatively correlated with CD4 count in every cell subtypes. These results suggested that *ifitm1* expression could depend on other conditions than the amount of virus itself, especially in T cells.

***ifitm1* expression is associated with immune activation**

Thus, we lastly investigated the relationships between ISGs expression and immune activation. When all HIV-1 infected patients were considered, positive correlations were observed between *ifitm1* expression in each cellular subtype and the corresponding activation markers (*i.e.* % of HLA-DR+CD38+ CD4+ T cells (r= 0.4, *p*= 0.03) and CD8+ T cells (r= 0.4, *p*= 0.04), and sCD14 for monocyte activation (r=0.48, *p*= 0.02)) (**Supplemental Figure 1a**). Such relationships were not observed with the other ISGs, except for *ifit1* in CD4 T cells. Moreover, the correlation between *ifitm1* expression in CD4 T cells and the frequency of circulating HLADR+CD38+ CD4+ T cells was still observed when HICs were analyzed separately (r=0.8, *p*= 0.003) (**Supplemental Figure 1b**).

DISCUSSION

We selected 4 ISGs with distinct production mechanisms and reported their expression in three cell subsets in four groups of individuals. *pkr* and *ifit1* expressions depend on the engagement of type I IFN, but can be also directly stimulated by HIV RNA or particles [15,16]. *mxA* seems to be directly correlated with type I IFN, with a strong link with HIV viral load [17]. Conversely, *ifitm1* expression depends on type I, but also type II IFN, with strong links to the proinflammatory milieu [16]. Consequently, viremic patients had higher levels of *pkr* in each cell subtypes, *mxA* in CD4 and CD8 T cells, and *ifit1* in CD4 T cells than the other groups. The correlation between these ISGs and the HIV viral load support the role of viral particles in their production [5–7,18].

The most striking point was the finding that in HICs' CD4 T cells, *ifitm1* was overexpressed compared with HDs, whereas no differences were found between HICs and HD for the other ISGs. This observation extends previous results from gene expression profiling of CD4 T cells from HICs but had never been quantified by RT-PCR in sorted cells [13,14]. *ifitm1* has been recently described as part of a HIV-1 restriction factor family [19,20]. Moreover, it has been suggested that *ifitm1* might be induced by IL-6 or other acute-phases cytokines [16]. This is consistent with our observations that *ifitm1* was globally linked with immune activation but barely with the viral load, the correlation being still present only when HICs are studied.

It seems important to separately analyze the expression of ISGs in purified CD4 T cells and monocytes, since the role of monocytes in the pathophysiology of HIV and inflammation is increasing. The analysis of the ex vivo purified monocytic compartiment has seldom been reported in the field of ISG expression. Indeed, we observed that the expression profile of most ISGs was different between CD4 T cells

and monocytes, suggesting that the individualization of these cell types might better decipher the mechanisms of ISGs regulation in the setting of HIV infection.

Our results show that the expression profile of ISGs in HIV infected patients varies according to the cell type, the disease status and the gene considered, with multiple regulation mechanisms beyond type I/II Interferons. Importantly, the gene expression profiles in monocytes should be distinguished from that in CD4 T cells. In HICs, *ifitm1* seems to have a unique expression profile and is associated with immune CD4 T cell activation. Its putative role in HIV pathogenesis and antiviral control deserves further investigations.

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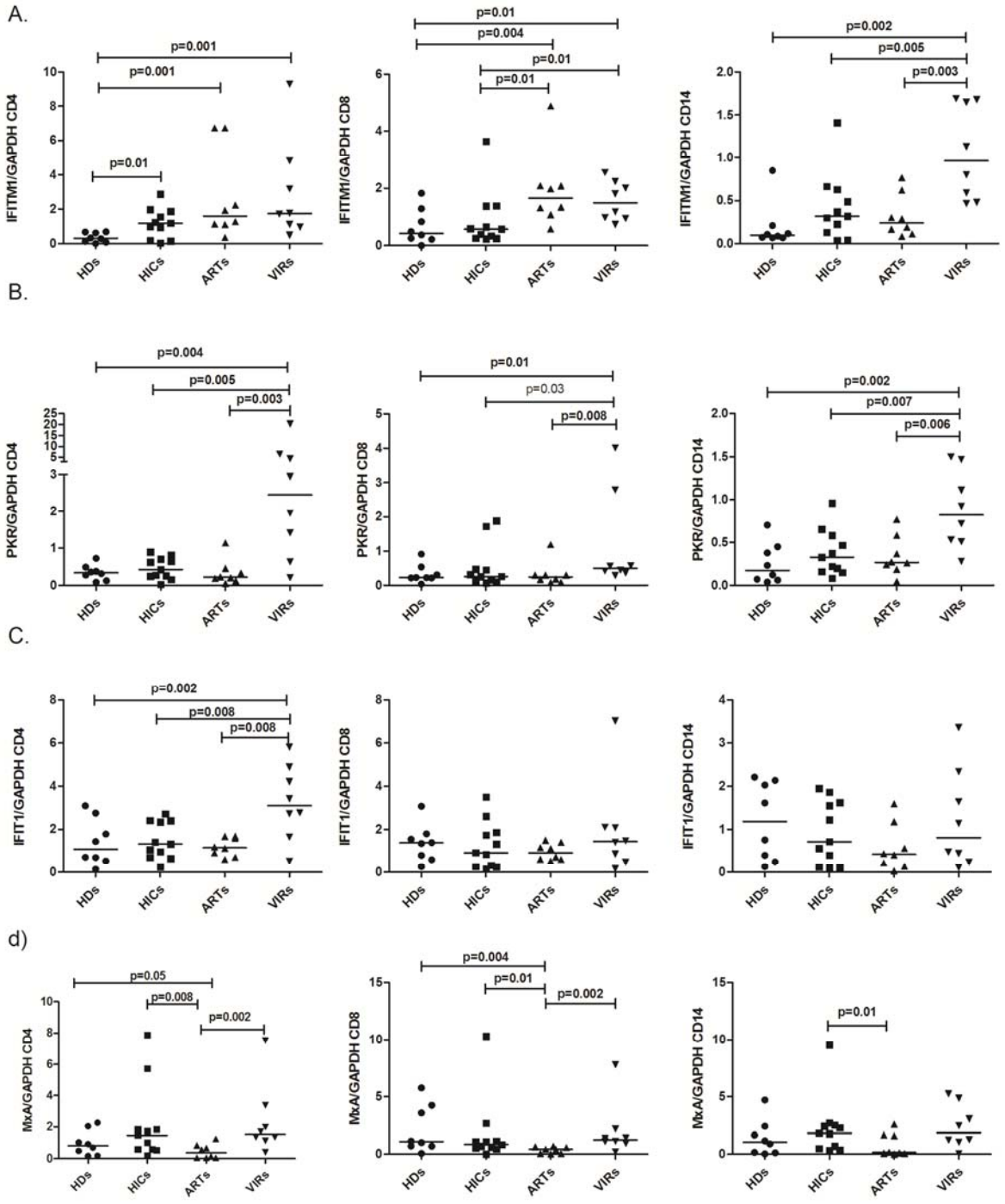
FIGURE LEGENDS

Fig. 1. Expression of ISGs between patient groups and cell types. (a) IFN-induced transmembrane protein 1 (IFITM1). (b) RNA-activated Protein Kinase (PKR). (c) IFN-induced protein with tetratricopeptide repeats (IFIT1). (d) Myxoma virus Resitance protein A (MxA). HDs, healthy donors; HICs, HIV controllers; ARTs, aviremic ART-treated patients; VIRs, viremic treatment naïve patients.

FIGURE

Figure 1.

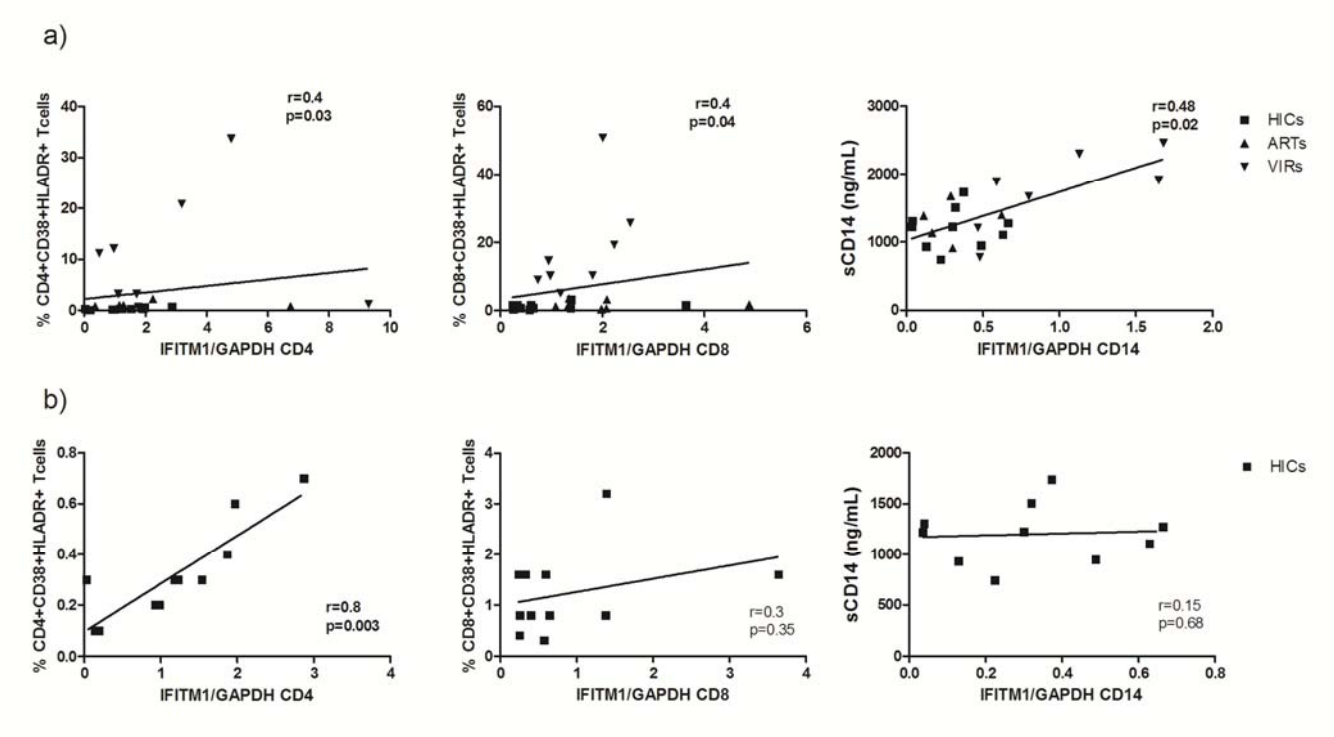


SUPPLEMENTAL MATERIAL

FIGURE LEGEND

Supplemental Figure 1. Relationships with the *ifitm1* expression and immune activation marker (HLA-DR and CD38 for CD4 and CD8 T cells and level of sCD14 for the monocytes) (a) Considering all HIV-1 infected patients. (b) Considering HICs separately. IFITM1, IFN-induced transmembrane protein 1; HICs, HIV controllers; ARTs, aviremic ART-treated patients; VIRs, viremic treatment naïve patients.

Supplemental Figure 1



SUPPLEMENTAL FIGURES/ TABLES

Supplemental Table 1. Characteristics of study participants

1 **Supplemental Table 1**

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Characteristics	HDs (n=8)	HICs (n=11)	ARTs (n=8)	VIRs (n=8)	p
Age (years)	46 [43.75-48.5]	46.5 [42.25-50.75]	47 [43-53]	37 [32-54]	0.6
Male, *n*(%)	3 (37)	4 (36)	4 (50)	3 (37)	0.7
CD4+ T-cell count at enrollment (cells/µL)	-	875 [768-1001]	467 [430-550]	100 [46-184]	<0.01
Lowest CD4+ T-cell count since HIV diagnosis (nadir) (cells/µL)	-	523 [464.5-637]	285 [272-330]	100 [46-184]	<0.01
Plasma HIV RNA VL at enrollment (log10copies/ml)	-	1.6 [<1.6-1.6]	<1.6 [<1.6-<1.6]	4.9 [4.4-5.35]	<0.01
HCV coinfection, *n*(%)	0 (0)	0 (0)	0 (0)	1 (14)	0.3
HBV coninfection, *n*(%)	0 (0)	0 (0)	0 (0)	2 (28)	0.06
Active opportunistic infection at enrollment, n (%)	-	0	0	5 (62)	<0.01
Results are shown as median (IQR) or *n* (%). HDs, healthy donors; HICs, HIV controllers; ARTs, aviremic ART-treated patients; VIRs, viremic treatment naïve patients ; VL, viral load					

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