Modulation of gene expression in CD4+ T lymphocytes following in vitro HIV infection: a comparison between human and chimpanzee

Bénédicte Puissant-Lubrano^{1,2} · Pol-André Apoil^{1,2} · Arnaud Gleizes¹ ·
Lionel Forestier³ · Raymond Julien³ · Peter Winterton⁴ · Christophe Pasquier⁵ ·
Jacques Izopet⁵ · Antoine Blancher^{1,2}

Received: 5 December 2014 / Accepted: 12 May 2015 / Published online: 6 June 2015
© Indian Virological Society 2015

Abstract Chimpanzees are susceptible to experimental infection by human deficiency virus (HIV)-1, but unlike humans, they exceptionally develop an immunodeficiency syndrome after HIV-1 inoculation. To explore the difference between human and chimpanzee, we analyzed the expression of 1547 genes of various functions in human or chimpanzee CD4+ lymphoblasts inoculated in vitro with HIV-1. We observed that, 1 day after HIV inoculation, fifty-eight genes were up-regulated in lymphoblasts of the three humans while their expression remained unchanged in lymphoblasts of the three chimpanzees. One gene is involved in adhesion of HIV (catenin- α), three in the

immune response (semaphorin 4D, placental growth factor, IL-6), three in apoptosis (deleted in colorectal carcinoma, caspase 9 and FOXO1A). No difference between species was revealed for the expression of 373 genes related to glycosylation pathways. The in vitro human/chimpanzee comparison reveals new candidate genes up-regulated after inoculation with HIV-1 only in human lymphoblasts and which could be related to the higher sensitivity of human to HIV-induced AIDS.

Keywords Human · Non-human primates · Array · qPCR · Immune response · CD4+ T lymphocytes

Electronic supplementary material The online version of this article (doi:10.1007/s13337-015-0252-1) contains supplementary material, which is available to authorized users.

Pol-André Apoil and Arnaud Gleizes have contributed equally to this work.

✉ Antoine Blancher
blancher.antoine@neuf.fr; blancher.a@chu-toulouse.fr

- ¹ Laboratoire d'Immunogénétique Moléculaire (EA3034), Faculté de médecine Toulouse- Rangueil, Université Paul Sabatier, Bâtiment A2, 133 Route de Narbonne, 31062 Toulouse Cedex 04, France
- ² Laboratoire d'Immunologie, CHU de Toulouse, Hôpital Rangueil, 1 Avenue Jean Poulhès, TSA 50032, 31059 Toulouse Cedex 9, France
- ³ Institut des Sciences de la Vie et de la Santé, Université de Limoges (EA3 176), Limoges, France
- ⁴ Université de Toulouse-Paul Sabatier, 118 route de Narbonne, 31062 Toulouse Cedex 9, France
- ⁵ Laboratoire de Virologie, CHU de Toulouse, Hôpital Purpan, TSA 40031, 31059 Toulouse Cedex 9, France

Introduction

The HIV-1 (human immunodeficiency virus) pandemic originated from inter-species transfer of simian immunodeficiency virus_{cpz} (SIV_{cpz}) from chimpanzees to humans [10]. Until recently, it was thought that chimpanzees, like other African non-human primates naturally infected with SIV, remained healthy during SIV infection [27]. However, it has been reported increased mortality and acquired immunodeficiency syndrome (AIDS)-like immunopathology in wild chimpanzees infected by SIV_{cpz}, although the SIV_{cpz} seems to be less pathogenic for chimpanzees than HIV-1 in humans [17]. Chimpanzees are also susceptible to experimental infection by HIV-1, but unlike humans, they exceptionally develop AIDS [27, 18]. Out of more than 200 chimpanzees experimentally inoculated with various human HIV strains, only six developed AIDS due to a recombinant virus derived from two human HIV-1 strains [25]. The six animals had a progressive decrease of blood CD4 T cells and an increased lymphocyte apoptosis was described. Apart from this unique recombinant strain, no

other human HIV-1 strain led to AIDS in chimpanzees. The resistance of chimpanzee to develop AIDS is associated to the resistance of their T cells to HIV-1-induced apoptosis [11], the absence of chronic immune activation [11, 12] and a low level of CCR5 on the CD4 T cells [26]. Moreover, chimpanzees experimentally infected with HIV-1 mount both humoral and cellular immune responses capable of controlling viremia for a long time [12, 31]. Low in vitro replication of HIV-1 in the chimpanzee peripheral blood mononuclear cells (PBMCs) [24] and the resistance of monocytes/macrophages of chimpanzees to HIV-1 infection [32] have also been reported. The latter point correlates with the absence of CCR5 monocyto-tropic HIV-1 strains in chimpanzees [32]. More recently, it was shown that HIV-1-infected chimpanzees did not display the inversion V δ 2/V δ 1 of $\gamma\delta$ T-cells observed in infected humans [14]. Other African non-human primates than chimpanzee are naturally infected by species-specific SIV viruses but these animals remain free of AIDS and the various mechanisms of resistance have been reported (for review see [34]). Although numerous non-human primate models are available, chimpanzee, the primate phylogenetically the closest to humans, remains an exceptional model of non-progressive infection by HIV and/or SIVcpz.

The work presented here was aimed to identify genes whose expression is modulated differently in human and chimpanzee CD4+ enriched T cells after their in vitro inoculation by HIV-1. We hypothesize that such genes might be involved in the resistance of chimpanzees to the disease and/or the sensitivity of humans to the disease.

Materials and methods

Samples and culture

PBMCs from 3 healthy blood donors (Etablissement Français du Sang Pyrénées-Méditerranée, France) and from 3 chimpanzees (Laboratory for Experimental Medicine and Surgery in Primates, New York, USA) were thawed. Cell viability was assessed by Trypan blue exclusion and was above 90 % for chimpanzee PBMCs and above 95 % for human PBMCs. Cells were depleted of CD8+ cells (CD8 MicroBeads human, Myltenyi Biotech, Paris, France) and CD8-depleted cells were activated in the presence of PHA 5 μ g/ml (Fisher Scientific, Illkirch, France) for 24 h. Cells were then cultured in the presence of hIL-2 (20U/ml, Roche, Meylan, France) for 2 weeks. This culture was necessary to expand the chimpanzee cells.

The percentage of CD3+CD4+ T cells among the cells after 15 days of culture was >96 % for humans and >95 %

for chimpanzees as assessed by flow cytometry analysis (Facs Calibur, BD-Biosciences, Le Pont-de-Claix, France).

HIV-1 inoculation

Activated cells enriched in CD4+ T lymphocytes from the three individuals of each species were inoculated (or not in control culture experiments performed in parallel) with the CXCR4-tropic HIV-1 LAI-strain (i.e. T-tropic strain, 0.01 multiplicity of infection). After 1 h, the cells were washed and cultured in the presence of hIL-2. They were collected 24 h after inoculation and total RNA was extracted (RNeasy Mini kit, Qiagen, Courtaboeuf, France). The quality and quantity of RNA were assessed with the Agilent 2100 Bioanalyzer (Agilent Technologies). Cell infection and HIV replication were assessed by quantification of HIV-1-RNA in supernatant and in total cellular RNA by real-time PCR [40].

Analysis of gene expression

The expression of 1174 genes was analyzed after hybridization on “Atlas cDNA Expression Array—Human 1.2” (Clontech). The membrane was exposed to a low-energy phosphorimage screen (Amersham Biosciences) for 5 days, image acquisition was performed on a Storm 860 and analysis was performed with ImageQuant 5.2 software (Molecular Dynamics). Data were analyzed with the web service BioPlot (<http://biopuce.insa-toulouse.fr/>; Genopole-Toulouse, Toulouse, France). The intensity of each spot was corrected by subtracting the background. For normalization, the intensity of each spot was divided by the mean intensity of the nine housekeeping genes. The relative expression of genes in infected cells was assessed by comparison to matched uninfected cells. The relative expression expressed in log was converted into a linear change. The final data set consisted of genes that had at least a twofold linear change and a $p < 0.05$ from the BioPlot analysis (Student's t test).

The expression of 373 glycosylation-related genes was assessed with a custom-designed TLDA (TaqMan Low Density Array Card, Applied Biosystems, Foster City, USA) (see Supplementary Table 1). Each target assay consisted of a forward primer, a reverse primer and a TaqMan MGB probe (6-FAM dye-labelled). Real-time RT-PCR amplifications were run on an ABI Prism[®] 7900HT Sequence Detection System (Applied Biosystems). The comparative CT method of relative quantification with *18S* and *BTAFL* as normalisers compared to the CT value of the target gene (Δ CT) was used. The relative quantification between HIV-infected samples versus non-infected samples was then determined according to the $2^{-\Delta\Delta$ CT method.

For real over- or under-expression, an arbitrary limit was fixed at 2.

Results

The level of intracellular HIV-1 RNA increased exponentially after inoculation of the CD4⁺ T lymphoblasts derived from all three humans and all three chimpanzees, although the rate of infection was slower in chimpanzees than in humans (Fig. 1), as previously reported [24]. A parallel increase of HIV-1 RNA was observed in the respective supernatants. These results confirmed that HIV-1 actively replicated in CD4⁺ lymphoblasts from the three humans and the three chimpanzees.

Modulation of gene expression in human CD4⁺ lymphoblasts

In experiments with both human and chimpanzee lymphoblasts, we compared the gene expression levels in HIV-inoculated CD4⁺ lymphoblasts to those observed in the not inoculated control cells. The pairwise comparisons with the respective controls were performed at three times after inoculation (30 min, 24 and 72 h post-inoculation). At 30 min and 72 h after inoculation, the differences observed between inoculated cells and controls were not statistically significant. The following analysis will concern only changes observed at 24 h p.i. As for the three humans, comparison of HIV-1-inoculated CD4⁺ T lymphoblasts and non-inoculated controls revealed that no gene (for one out of the three individuals) or a few (seven genes or eight genes, respectively, for the two remaining individuals) were under-expressed 24 h after inoculation. No genes were under-expressed in common in the two latter individuals. In contrast, in these three individuals, 491 genes,

Fig. 2 Euler diagram of the overexpressed genes in the three humans. The Euler diagram was not presented for the three chimpanzees because no gene was overexpressed in the three chimpanzees

232 genes and 261 genes were over-expressed in lymphocytes inoculated with HIV-1, among which 58 were significantly over-expressed ($p < 0.05$) in all three individuals. The Euler diagram shows the intersection of the over-expressed genes between the three humans (Fig. 2).

The Table 1 details the 15 genes the most over-expressed 24 h after HIV-1 inoculation. We observed an increased expression of the CD4 gene which occurs earlier than the down-regulation of CD4 previously reported by others upon HIV infection (1 day compared to 4–10 days after infection in other studies) [35]. We can hypothesize that a 2-stage phenomenon occurred in CD4 T cells: an increase of CD4 mRNA followed by a decrease. The CD19 mRNA increase observed here seems not compatible with the low proportion of B lymphocytes in the enriched CD4⁺ T lymphoblast suspensions we have used and could reflect

Fig. 1 HIV-1 mRNA was quantified in infected cells in three individuals of each species at different times after infection: (a) human 1 (diamonds), human 2 (squares), human 3 (triangles) and (b) chimpanzee 1 (diamonds), chimpanzee 2 (squares) and chimpanzee 3 (triangles)

Table 1 Genes whose expression was significantly increased 24 h after HIV-1 inoculation of CD4+ T lymphoblasts obtained from 3 humans

Acronym names	Gene names	Relative expression (AU) at 24 h p.i. (mean $\pm$ SD)	ROB	BO	BA	Function
CTNNA1	Catenin (cadherin-associated protein), alpha 1 (102kD)	12.1 $\pm$ 14	28.24	6.19	2.01	Cellular adhesion/HIV-1 receptor
SEMA4D	Sema 4D	8.4 $\pm$ 8.7	18.36	4.83	2.12	Immune activation
DCC	Deleted in colorectal carcinoma	7.8 $\pm$ 4.8	11.73	9.24	2.36	Apoptosis
ID3	Inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	7.3 $\pm$ 4.7	7.90	11.65	2.34	transcription
ABL2	v-abl Abelson murine leukemia viral oncogene homolog 2	7.3 $\pm$ 4.3	8.46	10.93	2.48	oncogene
PGF	Placental growth factor, vascular endothelial growth factor-related protein	6.7 $\pm$ 3.8	10.47	6.80	2.94	Immune activation
HOXD3	Homeo box D3	6.3 $\pm$ 5.8	3.83	12.91	2.17	Transcription
PRKMR1	Protein kinase, cAMP-dependent, regulatory, type I, beta	6.1 $\pm$ 5.3	4.10	12.07	2.04	Signalling pathway
GAD2	Glutamate decarboxylase 2 (pancreatic islets and brain, 65kD)	5.6 $\pm$ 3.9	4.55	9.95	2.28	No function related with infection
CD4	CD4	5.2 $\pm$ 2.7	6.57	6.90	2.12	Cell surface antigen/HIV-1 receptor
FOXO1A	Forkhead box O1A (rhabdomyosarcoma)	5.1 $\pm$ 2.5	7.14	5.90	2.34	Transcription
CASP9	Caspase 9, apoptosis-related cysteine protease	4.7 $\pm$ 3.1	3.66	8.20	2.27	Apoptosis
MMP14	Matrix metalloproteinase 14 (membrane-inserted)	4.7 $\pm$ 2.8	3.99	7.76	2.30	No function related with infection
IL6	Interleukin 6 (interferon, beta 2)	4.5 $\pm$ 2	4.83	6.33	2.33	Inflammation
CD19	CD19	4.1 $\pm$ 1.8	6.75	8.55	2.10	Cell surface antigen

Detail of the 15 genes most highly over-expressed in HIV-1 inoculated CD4+ from the 3 humans studied. Data are expressed as the mean $\pm$ standard error of the relative expression (arbitrary units) of values obtained from infected cells from three humans relative to those uninfected. The relative expressions of the genes of each of the three humans are also detailed

an inappropriate expression of CD19 by some CD4 T cells after HIV inoculation.

Some of the 15 up-regulated genes are related to immune activation and inflammation. The SEMA4D gene encodes a membrane protein constitutively expressed by T and B lymphocytes and dendritic cells [21], which expression increases upon cell activation. PGF is an angiogenic growth factor involved in the inflammatory response [33]. These two proteins share a common receptor, neuropilin-1 which plays a role on T cell and dendritic cell interactions [36]. Interleukin-6 is a pro-inflammatory cytokine, which is involved in T cell activation and which augments the susceptibility of monocyte-derived macrophages to infection with T-cell tropic HIV-1 strains [43].

We also found that some transcription factors were increased after HIV-1 infection: the inhibitor factor “inhibitor of DNA binding 3”, homeo box D3 and forkhead box O1A (FOXO1A). FOXO proteins are known to decrease cell survival and proliferation by regulating TRAIL expression

[23]. Although in our study, TRAIL expression was increased in only 1 of the 3 humans studied (2.3-fold increase) and not modulated in the 2 others, we can hypothesize that a TRAIL increase may have occurred later after HIV infection, secondarily to the FOXO1A increase, as reported in immature DC 4 days after infection [19]. Two other genes involved in cell death were simultaneously increased in CD4 T cells from humans but not in chimpanzee cells: deleted in colorectal carcinoma (DCC) [9] and caspase 9. Alpha-catenin is a protein involved in cell adhesion and interacts specifically with viral gp41 [20]. Two genes displayed no implicit relationship with HIV infection: GAD2 (glutamate decarboxylase 2, OMIM 138275) and MMP14 (matrix metalloproteinase 14, OMIM 600754).

The remaining 43 over-expressed genes belong to various functional clusters: oncogenes/tumor suppressors, adaptors/receptor-associated proteins, transcription activators/repressors, protein kinases, facilitated diffusion proteins and cell receptors (see Supplementary Table 2).

Modulation of gene expression in chimpanzee CD4+ lymphoblasts

The HIV-1 inoculation of chimpanzee CD4+ enriched T lymphocytes modulated the expression of a much smaller number of genes than in their human counterparts: 6, 18 and 71 genes were under-expressed and 26, 21 and 4 genes were over-expressed respectively in the three chimpanzees (see above for comparison with human results). No gene was underexpressed or overexpressed in the three chimpanzees.

Among the 58 genes up-regulated in the lymphoblasts of the three humans after HIV-1 infection, only one, the apolipoprotein E gene, was up-regulated in the lymphoblasts of one of the three chimpanzees. Remarkably, the HIV-1 infection level observed with lymphoblasts of the latter animal was the highest among the three chimpanzees tested, and was comparable to those observed with human lymphocytes (as assessed by quantification of viral RNA per cell).

Modulation of glycosylation-related genes

Because the Clontech macro-array set encompasses only a few genes involved in glycosylation, we decided to enlarge our study to 373 genes related to glycosylation by the TLDA set quantitative PCR technique. We used a set of primers which were adapted to amplification of human cDNA amplification (for the list of the genes see the Supplementary Table 2), and most of these primers were also adapted to chimpanzee cDNA amplification. However, eight chimpanzee cDNA were not amplified or detected because of the presence of 1–2 mismatches in either one of the primers or the probe (see Supplementary Table 3). Among the genes studied by TLDA set, 96 had no detectable expression in the human CD4+ T lymphoblasts (inoculated or not with HIV-1) and 104 (including 96 same as in human and the 8 genes with mismatch) had no detectable expression in the chimpanzee CD4+ T lymphoblasts. Thus, the TLDA revealed the expression of 277 genes and 269 genes in human or chimpanzee lymphoblasts, respectively. The expression of these genes was not significantly modified 24 h after inoculation with HIV-1 ($n = 3$ for each species). Nine genes were explored by both the Clontech macro-array and the TLDA set and the two techniques revealed no modulation of expression of these genes by HIV-inoculation. Four genes (mannosyl glycoprotein beta 1,2-*N* acetylglucosaminyl-transferase (MGAT1), mannosidase beta A lysosomal-like (MANBAL), GM2activator (GM2A) and neuraminidase 3 (Neu3)) were under-expressed in HIV-inoculated human CD4 cells lymphoblasts (2 out of the 3 individuals studied) but these genes were not modulated by HIV inoculation in chimpanzees (all three individuals).

Discussion

We analyzed here, for the first time, the differential expression of 1174 genes of various functions (including genes related to cell adhesion, immune activation, inflammation, transcription, apoptosis, cell surface antigens, oncogenes, signalling pathways...) and 373 glycosylation-related genes, in human and chimpanzee enriched CD4+ T lymphoblasts after their *in vitro* inoculation by HIV-1. We chose to analyze lymphoblasts derived from peripheral blood CD4 T lymphocytes because they are closer to the natural virus targets than continuous cell lines. The simultaneous macro-array analysis of 3 individuals of each species and the selection of differentially modulated genes (Student's *t* test $p < 0.05$ and change >twofold) revealed genes of interest not previously reported.

The number of the genes showing expression modulated by HIV-1 inoculation in CD4 T cells was much lower in chimpanzees than in humans, as previously observed in HIV-1-infected immature DC from these two species [19]. We chose the Clontech technology because it displays oligonucleotide probes of at least 200 base-pairs long (mean 250 bp). As the average nucleotide difference between human and chimpanzee is around 1 % in the coding regions, the quality of hybridization of cDNA of both species with the probes can be considered equivalent. In addition, for all genes with lower expression in the chimpanzee CD4 uninfected lymphoblasts compared to their human counterparts, we checked that the differences between human and chimp sequences were not greater than average (data not shown). We thus validated our comparison of the modulation of gene expression between human and chimpanzee lymphoblasts.

We observed that after HIV-1 infection, several genes were up-regulated in CD4 T lymphoblasts from the three humans but not in those from chimpanzees. The exception was apolipoprotein E gene which expression was increased in the chimpanzee displaying the highest *in vitro* infection level. Apart from its role in lipid transport, Apo E has immunoregulatory properties [28]. Previous studies reported an association between ApoE polymorphism and HIV-1 disease progression as well as with the level of *in vitro* HIV-1 infection of CCR5-expressing cell lines [7]. The 15 genes the most over-expressed 24 h after HIV-1 inoculation are related to cell adhesion, immune activation, inflammation, transcription, apoptosis, cell surface antigens, oncogenes and signalling pathways. One interesting gene is the one encoding the alpha-catenin which interacts specifically with viral gp41 and may favour HIV-1 infection. The over-expression of SEMA4D and PGF genes could indicate either an increase of interaction between T cells and DC which could favour HIV-1 transmission from

DC to T cells or an immune activation of human CD4 T cells. Interestingly, Interleukin-6 has some similar effects and is involved in T cell activation and augments the susceptibility of monocyte-derived macrophages to infection with T-cell tropic HIV-1 strains [43]. The HIV-induced increased expression of Sema4D, PGF and IL-6 in human cells is not observed in chimpanzee cells. This could be related to the absence of chronic immune activation reported in infected chimpanzees.

As previously reported by others, we also found that some transcription factors and genes involved in cell death were increased after HIV-1 inoculation of human cells. Among them, the transcription factor FOXO1A is known to decrease cell survival and proliferation by regulating TRAIL expression [23]. In accordance with our results, another pro-apoptotic member of the FOXO family (FOXO3) was shown to be increased in PBMCs from HIV-1 infected patients under efficient antiretroviral therapy but not in long-term non progressors, compared to healthy controls [22]. The decreased expression of FOXO1 after in vitro HIV-1 infection was also reported, the discordance with our observation is probably due to the use of quiescent CD4 T cells 7 days p.i. [37]. DCC and caspase 9 are also involved in cell death. Interestingly, it was previously shown that DCC drives the activation of caspase 3 through caspase 9 [9]. Altogether, our results are in agreement with the increased susceptibility to apoptosis of HIV-1-infected human cells compared to their chimpanzee counterparts.

Glycosylation plays an important role in HIV infection (see review in [41]), we thus specifically analyzed the modulation of glycosylation-related gene expression after in vitro HIV inoculation of activated CD4+ lymphocytes. Indeed, the glycosylation of the gp120 envelope protein of HIV influences its interaction with its cellular receptors [29] and the recognition of HIV by immune effectors [5]. As HIV does not encode for glycosyltransferase, glycosylation of the HIV proteins depends on the repertoire of glycosyltransferases of the host-cell which varies with the host genotype and with the cell differentiation and cell activation status. Moreover, infection of the cell by viruses can modify the expression of genes related to glycosylation, as it was reported for HIV and the ectopic expression of glycosaccharide antigens (Lewis Y and A) in T cell lines or PBMC [1, 2].

We observed that, both in humans and chimpanzees, HIV-1 infection did not strongly modulate the expression of glycosylation-related genes in CD4 T lymphoblasts. However, after HIV inoculation, four genes (MGAT-1, GM2A, NEU3 and MANBAL) were under-expressed in CD4 lymphoblasts from 2 humans out of the 3. The genes MGAT-1 and GM2A were previously involved in HIV replication and previous works suggest that their hypo-expression could be associated with a high rate of virus

replication in human cells [6, 44]. Conversely, the under-expression of the mannosidase MANBAL and of the sialidase NEU3 could be associated with the production of viruses with low infectivity [15, 4]. The fucosyltransferase genes explored here (FUT1, FUT5, FUT8, FUT10 and FUT11) were not modulated in the two species studied. Therefore, the fucosyltransferase responsible for the expression of Le^Y in T cells of HIV infected patients [1] remains to be identified. The viral control of host cell glycosylation as a viral pathogenic factor was previously reported with HTLV-1 which induces expression of sialyl Le^X in leukemic T cells [13]. Our study did not reveal a similar process during in vitro HIV infection.

Overall, our study reveals 58 genes which are modulated after HIV-inoculation of human lymphoblasts but not chimpanzee ones. To our knowledge, these genes have not been reported by previous studies that have analyzed the genes modulated by HIV inoculation. Most of these studies have used CD4 T cell lines or human PBMCs [39, 42, 38]. Among the genes previously reported, some were present on the Clontech membrane that we used, such as CD71, thymosin-beta 10 and prothymosin-alpha. For these genes, we did not find a significant modulation after HIV-infection. Type-1 IFN-stimulated genes, including TRAIL and IRF-7 (interferon regulatory factor), were shown to be increased after HIV-1 infection [19, 3], while IRF-1 was decreased [42]. We found that the expression of TRAIL was increased in one individual and the expression of IRF-7 was increased in 2 of the 3 humans studied while IRF-1 was not modulated in humans. Of note, TRAIL and IRF-7 were not modulated in the chimpanzees studied. Only one study analyzed the effect of HIV-1 infection on human primary cells enriched in CD4 T lymphocytes and reported an impact on apoptosis, RNA metabolism, DNA repair and cell cycle and particularly on p53 tumor suppressor [16]. We observed no modulation of p53 gene expression in HIV-infected CD4 T cells from chimpanzee and a slight increased of p53 gene expression in HIV-infected CD4 T cells from the three humans (mean relative expression 1.75; range 1.6–1.9) which was lower than the one reported by Imbeault and colleagues, possibly due to different experimental conditions. A more recent study has compared gene expression in progressors and non progressors using T cells from HIV-1-infected humans and SIV-infected non-human primates [30]. This study reveals that the genes of caspase 1 precursor, LAG3, CD38 and TNF ligand superfamily member 13B were upregulated in progressors while SOCS 1 gene expression is increased in non progressors.

Thus, the overlap between genes described in the studies quoted above is quite low. These inter-study discrepancies could result from the variety of cells used as models (in vitro infected CD4 T cell lines, human PBMC or human

CD4 T cells as well as ex vivo T cells from HIV-infected patients), the heterogeneity between HIV strains used and the diversity of techniques used to analyze gene expression (arrays from Clontech or Affymetrix, genome wide study). The slight overlaps between different genome-wide studies have been described in a meta-analysis of the host cell genes linked to HIV replication [8]. In addition, our study as well the others can only reveal some of the complexity of the problem. Indeed, (i) there is no strict correlation between the expression of mRNA and protein expression, (ii) the epistatic interaction can ricochet successive bring up profiles over-(or under-) expression of genes not directly related with the original cause, (iii) the interaction networks are so complex that the system is partly stochastic: at one point we see this but another everything changes, the dynamic aspect is very important. However, as others, we think that macroarrays can help elucidating interaction between HIV-1, target cells and uninfected cells at a molecular level.

The human-chimpanzee comparison allowed us to identify new candidate genes potentially involved in HIV infection (catenin- α), HIV-induced apoptosis (DCC, caspase 9 and FOXO1A) and immune response (semaphorin 4D, placental growth factor, IL-6) which could be related to the different susceptibilities of the two species to HIV-1 infection. Although further studies are required to confirm the potential role of these proteins in HIV infection, this work presents promising new data about HIV and host cell interaction.

Acknowledgments This study was supported by the Ministère Français de la Recherche (Contract EA3034) and Association de Recherche sur le SIDA (A.R.S., Toulouse, France). We thank Christine Bousquet and Christine Taureau for their excellent technical assistance.

References

- Adachi M, Hayami M, Kashiwagi N, Mizuta T, Ohta Y, Gill MJ, et al. Expression of Ley antigen in human immunodeficiency virus-infected human T cell lines and in peripheral lymphocytes of patients with acquired immune deficiency syndrome (AIDS) and AIDS-related complex (ARC). *J Exp Med*. 1988;167:323–31.
- Arendrup M, Hansen JE, Clausen H, Nielsen C, Mathiesen LR, Nielsen JO. Antibody to histo-blood group A antigen neutralizes HIV produced by lymphocytes from blood group A donors but not from blood group B or O donors. *Aids*. 1991;5:441–4.
- Audige A, Urošević M, Schlaepfer E, Walker R, Powell D, Hallenberger S, et al. Anti-HIV state but not apoptosis depends on IFN signature in CD4+ T cells. *J Immunol*. 2006;177:6227–37.
- Balzarini J. The $\alpha(1,2)$ -mannosidase I inhibitor 1-deoxymannojirimycin potentiates the antiviral activity of carbohydrate-binding agents against wild-type and mutant HIV-1 strains containing glycan deletions in gp120. *FEBS Lett*. 2007;581:2060–4.
- Botarelli P, Houlden BA, Haigwood NL, Servis C, Montagna D, Abrignani S. N-glycosylation of HIV-gp120 may constrain recognition by T lymphocytes. *J Immunol*. 1991;147:3128–32.
- Brass AL, Dykxhoorn DM, Benita Y, Yan N, Engelman A, Xavier RJ, et al. Identification of host proteins required for HIV infection through a functional genomic screen. *Science*. 2008;319:921–6.
- Burt TD, Agan BK, Marconi VC, He W, Kulkarni H, Mold JE, Cavrois M, Huang Y, Mahley RW, Dolan MJ, McCune JM, Ahuja SK. Apolipoprotein (apo) E4 enhances HIV-1 cell entry in vitro, and the APOE epsilon4/epsilon4 genotype accelerates HIV disease progression. *Proc Natl Acad Sci U S A*. 2008;105:8718–23.
- Bushman FD, Malani N, Fernandes J, D'Orso I, Cagney G, Diamond TL, et al. Host cell factors in HIV replication: meta-analysis of genome-wide studies. *PLoS Pathog*. 2009;5:e1000437.
- Forcet C, Ye X, Granger L, Corset V, Shin H, Bredesen DE, et al. The dependence receptor DCC (deleted in colorectal cancer) defines an alternative mechanism for caspase activation. *Proc Natl Acad Sci U S A*. 2001;98:3416–21.
- Gao F, Bailes E, Robertson DL, Chen Y, Rodenburg CM, Michael SF, et al. Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes. *Nature*. 1999;397:436–41.
- Gougeon ML, Lecoœur H, Boudet F, Ledru E, Marzabal S, Boullier S, et al. Lack of chronic immune activation in HIV-infected chimpanzees correlates with the resistance of T cells to Fas/Apo-1 (CD95)-induced apoptosis and preservation of a T helper 1 phenotype. *J Immunol*. 1997;158:2964–76.
- Heeney J, Jonker R, Koornstra W, Dubbes R, Niphuis H, Di Rienzo AM, et al. The resistance of HIV-infected chimpanzees to progression to AIDS correlates with absence of HIV-related T-cell dysfunction. *J Med Primatol*. 1993;22:194–200.
- Hiraiwa N, Hiraiwa M, Kannagi R. Human T-cell leukemia virus-1 encoded Tax protein transactivates $\alpha 1 \rightarrow 3$ fucosyltransferase Fuc-T VII, which synthesizes sialyl Lewis X, a selectin ligand expressed on adult T-cell leukemia cells. *Biochem Biophys Res Commun*. 1997;231:183–6.
- Hodara VL, Parodi LM, Chavez D, Smith LM, Lanford R, Giavedoni LD. Characterization of $\gamma\delta$ T cells in naïve and HIV-infected chimpanzees and their responses to T-cell activators *in vitro*. *J Med Primatol*. 2014;43:258–71.
- Hu H, Shioda T, Moriya C, Xin X, Hasan MK, Miyake K, et al. Infectivities of human and other primate lentiviruses are activated by desialylation of the virion surface. *J Virol*. 1996;70:7462–70.
- Imbeault M, Ouellet M, Tremblay MJ. Microarray study reveals that HIV-1 induces rapid type-I interferon-dependent p53 mRNA up-regulation in human primary CD4+ T cells. *Retrovirology*. 2009;6:5.
- Keele BF, Jones JH, Terio KA, Estes JD, Rudicell RS, Wilson ML, et al. Increased mortality and AIDS-like immunopathology in wild chimpanzees infected with SIVcpz. *Nature*. 2009;460:515–9.
- Kestens L, Vingerhoets J, Peeters M, Vanham G, Vereecken C, Penne G, et al. Phenotypic and functional parameters of cellular immunity in a chimpanzee with a naturally acquired simian immunodeficiency virus infection. *J Infect Dis*. 1995;172:957–63.
- Kim N, Dabrowska A, Jenner RG, Aldovini A. Human and simian immunodeficiency virus-mediated upregulation of the apoptotic factor TRAIL occurs in antigen-presenting cells from AIDS-susceptible but not from AIDS-resistant species. *J Virol*. 2007;81:7584–97.
- Kim JT, Kim EM, Lee KH, Choi JE, Jhun BH, Kim JW. Leucine zipper domain of HIV-1 gp41 interacted specifically with α -catenin. *Biochem Biophys Res Commun*. 2002;291:1239–44.
- Kumanogoh A, Kikutani H. Immune semaphorins: a new area of semaphorin research. *J Cell Sci*. 2003;116:3463–70.

22. Lucque MC, Santos CC, Mairena EC, Wilkinson P, Boucher G, Segurado AC, Fonseca LA, Sabino E, Kalil JE, Cunha-Neto E. Gene expression profile in long-term non progressor HIV infected patients: in search of potential resistance factors. *Mol Immunol*. 2014;62:63–70.
23. Modur V, Nagarajan R, Evers BM, Milbrandt J. FOXO proteins regulate tumor necrosis factor-related apoptosis inducing ligand expression. Implications for PTEN mutation in prostate cancer. *J Biol Chem*. 2002;277:47928–37.
24. Ondoa P, Davis D, Kestens L, Vereecken C, Garcia Ribas S, Fransen K, et al. In vitro susceptibility to infection with SIVcpz and HIV-1 is lower in chimpanzee than in human peripheral blood mononuclear cells. *J Med Virol*. 2002;67:301–11.
25. O'Neil SP, Novembre FJ, Hill AB, Suwyn C, Hart CE, Evans-Strickfaden T, et al. Progressive infection in a subset of HIV-1-positive chimpanzees. *J Infect Dis*. 2000;182:1051–62.
26. Pandrea I, Apetrei C, Gordon S, Barbercheck J, Dufour J, Bohm R, et al. Paucity of CD4+CCR5+ T cells is a typical feature of natural SIV hosts. *Blood*. 2007;109:1069–76.
27. Peeters M, Janssens W, Vanden Haesevelde M, Fransen K, Willems B, Heyndrickx L, et al. Virologic and serologic characteristics of a natural chimpanzee lentivirus infection. *Virology*. 1995;211:312–5.
28. Pepe MG, Curtiss LK. Apolipoprotein E is a biologically active constituent of the normal immunoregulatory lipoprotein, LDL-In. *J Immunol*. 1986;136:3716–23.
29. Pollakis G, Kang S, Kliphuis A, Chalaby MI, Goudsmit J, Paxton WA. N-linked glycosylation of the HIV type-1 gp120 envelope glycoprotein as a major determinant of CCR5 and CXCR4 coreceptor utilization. *J Biol Chem*. 2001;276:13433–41.
30. Rotger M, Dalmau J, Rauch A, McLaren P, Bosinger SE, Martinez R, et al. Comparative transcriptomics of extreme phenotypes of human HIV-1 infection and SIV infection in sooty mangabey and rhesus macaque. *J Clin Invest*. 2011;121:2391–400.
31. Rutjens E, Balla-Jhaghoorsingh S, Verschoor E, Bogers W, Koopman G, Heeney J. Lentivirus infections and mechanisms of disease resistance in chimpanzees. *Front Biosci*. 2003;8:d1134–45.
32. Schuitemaker H, Meynard L, Kootstra NA, Dubbes R, Otto SA, Tersmette M, et al. Lack of T cell dysfunction and programmed cell death in human immunodeficiency virus type 1-infected chimpanzees correlates with absence of monocytotropic variants. *J Infect Dis*. 1993;168:1140–7.
33. Selvaraj SK, Giri RK, Perelman N, Johnson C, Malik P, Kalra VK. Mechanism of monocyte activation and expression of proinflammatory cytochemokines by placenta growth factor. *Blood*. 2003;102:1515–24.
34. Silvestri G, Paiardini M, Pandrea I, Lederman MM, Sodora DL. Understanding the benign nature of SIV infection in natural hosts. *J Clin Invest*. 2007;117:3148–54.
35. Stevenson M, Zhang XH, Volsky DJ. Downregulation of cell surface molecules during noncytopathic infection of T cells with human immunodeficiency virus. *J Virol*. 1987;61:3741–8.
36. Tordjman R, Lepelletier Y, Lemarchandel V, Cambot M, Gaulard P, Hermine O, et al. A neuronal receptor, neuropilin-1, is essential for the initiation of the primary immune response. *Nat Immunol*. 2002;3:477–82.
37. Trinite B, Chan CN, Lee CS, Mahajan S, Luo Y, Muesing MA, Folkvord JM, Pham M, Connick E, Levy DN. Suppression of Foxo1 activity and down-modulation of CD62L (L-selectin) in HIV-1 infected resting CD4 T cells. *PLoS One*. 2014;9(10):e110719.
38. Vahey MT, Nau ME, Jagodzinski LL, Yalley-Ogunro J, Taubman M, Michael NL, et al. Impact of viral infection on the gene expression profiles of proliferating normal human peripheral blood mononuclear cells infected with HIV type 1 RF. *AIDS Res Hum Retrovir*. 2002;18:179–92.
39. van 't Wout AB, Lehrman GK, Mikheeva SA, O'Keeffe GC, Katze MG, Bumgarner RE, et al. Cellular gene expression upon human immunodeficiency virus type 1 infection of CD4(+)-T-cell lines. *J Virol*. 2003;77:1392–402.
40. Viard JP, Burgard M, Hubert JB, Aaron L, Rabian C, Pertuiset N, et al. Impact of 5 years of maximally successful highly active antiretroviral therapy on CD4 cell count and HIV-1 DNA level. *Aids*. 2004;18:45–9.
41. Vigerust DJ, Shepherd VL. Virus glycosylation: role in virulence and immune interactions. *Trends Microbiol*. 2007;15:211–8.
42. Wen W, Chen S, Cao Y, Zhu Y, Yamamoto Y. HIV-1 infection initiates changes in the expression of a wide array of genes in U937 promonocytes and HUT78 T cells. *Virus Res*. 2005;113:26–35.
43. Zaitseva M, Lee S, Lapham C, Taffs R, King L, Romantseva T, et al. Interferon gamma and interleukin 6 modulate the susceptibility of macrophages to human immunodeficiency virus type 1 infection. *Blood*. 2000;96:3109–17.
44. Zhou H, Xu M, Huang Q, Gates AT, Zhang XD, Castle JC, et al. Genome-scale RNAi screen for host factors required for HIV replication. *Cell Host Microbe*. 2008;4:495–504.