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HIV-1 group P infection: towards a dead-end infection?

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Running head: new insights on HIV-1/P

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ABSTRACT

Objectives: HIV/1 group P (HIV-1/P) is the last HIV/1 group discovered and to date, comprises only two strains. To obtain new insights into this divergent group, we screened for new infections by developingspecific tools, and analysed phenotypic and genotypic propertiesof theprototypic strain RBF168. In addition, the follow-up of the unique patient monitoredso far, has raised the knowledge of the natural history of this infection and its therapeutic management.

Design/Methods: We developed anHIV-1/Pspecific sero-molecular strategy and screenedover 29,498specimen samples. Infectivity and evolution of the gag-30 position, considered as marker of adaptation to human, were explored by successive passages of RBF168 strain onto human PBMCs. Natural history and immuno-virological responses to combined antiretroviral therapy (cART) were analysed based on CD4 counts and plasmatic viral load evolution.

Results: No new infection was detected. Infectivity of RBF168 was found lower, relative to other main HIV groups and the conservative methionine found in the gag-30 position revealed a lack of adaptation to human. The follow-up of the patient during the five-year ART-free period, showed a relative stability of CD4 cell countwith a mean of 326 mm^{-3}. Initiation of cART led to rapid RNA undetectability with a significant increase of CD4, reaching 687 mm^{-3} after 8 years.

Conclusions: Our resultsshowed that HIV-1/P strains remain extremely rare and could be less adapted and pathogenic than other HIV strains. These data lead to the hypothesis that HIV-1/P infection could evolve towards, or even already correspond to, a dead-end infection.

Key words: HIV genetic diversity ; group P ; adaptation ; immuno-virological response to cART

INTRODUCTION

Human Immunodeficiency Virus type 1 (HIV-1) is characterized by high genetic diversity, classified into 4 groups - M to P [1, 2]; each group results from a separate cross species transmission to humans of simian immunodeficiency virus (SIV) found in chimpanzees (SIVcpz) or gorillas (SIVgor) [3, 4].

HIV-1 group P (HIV-1/P), the last group identified, is derived from gorillas [5, 6] and represented by only two cases to date. The prototypic strain, RBF168, was reported in a Cameroonian woman living in France [6], while the second strain, U14788, was identified in retrospective serological screening on specimens collected in Cameroon [7].

The scarcity of group P infection may be linked to a relatively recent emergence in humans, since the time of the most recent common ancestor (tMRCA) of HIV-1/P was estimated, based on only two sequences, between the 1950s and 1990s [8]. Another hypothesis is that HIV-1/P has distinct virological properties from the other groups, especially HIV-1/M, leading to a limited spread. It has been shown that residue replacements of methionine or leucine found at the gag-30 position in SIVcpz/SIVgor, by lysine or arginine in the inferred HIV-1 ancestral sequences of group M, N and O strains, represent a host-specific adaptation marker [9, 10]. For group P, the RBF168 strain harbours methionine, supporting a shared property with simian strains, whereas the second strain, U14788, harbours lysine [7].

Concerning *in vivo* features, only limited data from patient RBF168 have been reported [6]. Data on immuno-virological response to combined antiretroviral-treatment (cART) were not available, while these variants are phylogenetically close to HIV-1/O, harbouring natural polymorphism impacting therapeutic management [11].

In order to obtain new insights into HIV-1/P, we screened for new infections, and analysed phenotypic and genotypic properties of the prototypic strain RBF168. In addition, the follow-up of the unique HIV-1/P positive patient monitored so far, gave unique data on the natural history, and on the immuno-virological responses to cART.

METHODS

We first defined and validated specific tools for HIV-1/P detection. We adapted a serological immunoassay, initially designed to discriminate between HIV-1 groups M, N and O, HIV-2 and major SIV lineages [12], by including a group P specific antigen, selected among four candidate synthetic oligopeptides (supplementary methods, http://links.lww.com/QAD/B242). To confirm group P reactivity, we developed a specific nested RT-PCR, based on the amplification of a fragment of 1,110 pb in the gp120 region (supplementary methods, http://links.lww.com/QAD/B242).

We then screened for new HIV-1/P infections among 1,083 samples from collections, for which previous results using a serological immunoassay without a P specific antigen were inconclusive; they corresponded to:

- 609 serum/plasma samples received by our laboratory for exploration of atypical reactivities at diagnosis time or during the patient's follow-up.
- 405 serum/plasma samples from HIV collections from Cameroon and Central African Republic, constituted in the 90's.
- 69 simian samples

All samples with reactivity (OD) $\geq$ 0.500 were first subjected to the group P specific RT-PCR. Samples not amplified with the specific RT-PCR were subjected to a nested-RT-PCR (Lentivirus PCR) that amplified a 650 pb fragment of *pol* of HIVs and SIVs (supplementary methods,

http://links.lww.com/QAD/B242). Prospective screening was also performed on 28,415 samples, diagnosed as HIV positive between March 2013 and June 2015 by the Centre Pasteur of Cameroun (CPC), using all the HIV-1 and HIV-2 antigens of the serological immunoassay, as previously described [13].

We then proceeded to phenotypic and genotypic characterization of the prototypic strain RBF168. The infectivity of the RBF168 strain was analysed after six serial passages in human PBMCs by calculating the TCID50 (tissue culture infective dose 50%) as previously described [14], and compared to those of seven primary isolates representative of HIV genetic diversity (supplementary methods, http://links.lww.com/QAD/B242). To determine the residue evolution at gag-30 position during the six passages, we amplified a 493 pb fragment of *gag* using an in-house RT-PCR (supplementary methods, http://links.lww.com/QAD/B242). Natural genetic polymorphism of the cART targets : protease (PR), reverse transcriptase (RT), integrase (IN), and gp41, was also determined on viral RNA from three plasma samples collected before treatment initiation (supplementary methods, http://links.lww.com/QAD/B242), and compared to that of the second P strain U14788; mutations were subjected to genotypic resistance interpretation using the last version (v27) of the ANRS resistance algorithm (http://www.hivfrenchresistance.org/).

Lastly, patient RBF168 was followed up from March 2004 to March 2017; monitoring of CD4 cell count was performed by Beckman Coulter flow cytometer systems (Beckman, Brea, California) and of plasma viral load (pVL) by the Real*Time* HIV-1 Viral Load assay (Abbott, Des Plaines, IL) [6, 15].

RESULTS

Validation of specific tools led to selection of the candidate peptide CP684 for serological screening, and to definition of the analytical sensitivity of the group P specific RT-PCR as close to 2 Log cp/mL, with a specificity of 100% (supplementary results, http://links.lww.com/QAD/B242).

Using peptide CP684, 992 of the 1083 (91.6%) samples from the collections were found negative (median OD at 0.029), including all the simian samples. Seventy-three (6.7%) gave equivocal results (0.1=<OD<0.5), with median OD at 0.146. Eighteen samples corresponding to 14 patients (1.6%) were reactive with OD >=0.5 (median at 1.189), six samples being highly reactive from 1.518 to 2.438. Group P specific RT-PCR performed on samples of these 14 patients gave negative results ; the non-specific Lentivirus RT-PCR identified HIV-1/M variants (CRF02_AG, n=4 and CRF11-like, n=1), HIV-1/O (n=6), and one dual HIV-1/M+O (data not shown) ; two samples were not amplified. Finally, no group P strain was found among the 28,415 HIV positive samples diagnosed by the CPC, which were reactive to HIV-1/M (99%), HIV-1/O or HIV-1/M+O (0.9%), HIV-2 (0.08%) infections, and HIV-1/N (1 case).

Infectivity testing of the six successive amplifications showed an extremely low median at 261 TCID50/mL [149; 447], relative to the strains belonging to the other type or groups, especially HIV-1/M with a mean TCID50/mL at 250,625 (n=4), while HIV-2 and HIV-1/O showed TCID50/mL at 1,171 (n=1) and 22,627 (n=2), respectively. Genotypic characterisation of the gag-30 position in each of the six successive amplifications revealed no evolution with conservation of methionine.

Concerning the cART targets, the natural polymorphism of RBF168 was similar between the three sequences before treatment. Based on the ANRS algorithm used to predict genotypic resistance of HIV-1/M viruses, the highest number of mutations was detected in the PR with 11 resistance associated mutations, conferring natural (or possible) resistance to atazanavir, tipranavir and saquinavir (table 1). The presence of T69S, A98G, K103R and the atypical mutation V179E in the RT, had no genotypic impact (table 1). The IN harboured no resistance mutation; but the L44M found in the gp41 led to resistance to enfuvirtide. The genotypic profile of strain U14788 was similar to RBF168, leading to the same interpretation (table 1), except for the IN, with presence of a T97A mutation leading to genotypic resistance to elvitegravir.

Monitoring of patient RBF168 started in March 2004 at HIV diagnosis; she did not receive cART until December 2008, according to the French guidelines at that time. During this period, the mean pVL was at 4.7 Log cp/mL and the mean CD4 count at 326 mm^{-3}, ranging from 260 (June 2004) to 430 (December 2005) (Figure 1a). In February 2009, cART was initiated with tenofovir+emtricitabine+lopinavir/ritonavir, but switched after 2 months to abacavir+lamivudine+lopinavir/ritonavir, due to tenofovir adverse effects. This allowed to achieve undetectability (<1.6 Log cp/mL) at M3 (Figure 1b), maintained until the last monitoring in March 2017 (Figure 1b). During cART, the mean CD4 cell count was at 648 mm^{-3}, with the last point at 687 mm^{-3} in March 2017.

DISCUSSION

Only two viral sequences and scarce data on HIV-1/P infection were available until now; thus, all new data are needed to improve our knowledge of this group and to assess its potential for transmission and pathogenicity.

We screened for new infections using a sero-molecular strategy for group P specific detection, as previously done for other HIV/SIV variants [12, 13, 16]. After validation of specific tools, we tested a large number of samples from collections and prospective diagnosis. Despite a wide panel of simian samples or samples with HIV atypical reactivities (profiles similar to that of RBF168 at diagnosis), we failed to detect a new HIV-1/P strain. The same is true in older collections from central Africa and in the thousands of HIV positive samples, diagnosed currently in Cameroon. This confirms that HIV-1 group P infection is extremely rare [2, 7].

Reasons for this limited spread are likely multifactorial. In order to better estimate the emergence of this group, we performed evolutionary analysis using the two HIV-1/P sequences previously analysed [8], and three additional RBF168 sequences (supplementary methods and results, http://links.lww.com/QAD/B242). This analysis indicates that HIV-1/P strains share a tMRCA dating back to the 1960s. Thus, it would appear that HIV-1/P emerged more recently than strains M and O [8], and possibly around the same time period as group HIV-1/N [17]. Another factor could be specific viral properties; weak counteracting of human cell restricting factors was already demonstrated for tetherin [8]. Focusing on infectivity, our work showed that the serial isolates of RBF168 were consistently and reproducibly less infective, in particular relative to HIV-1/O (the closest phylogenetic group) and HIV-2 (the lowest pathogenic HIV known so far). When searching for residue evolution in gag-30 position, we failed to identify a switch from methionine to arginine (harboured by the second P strain, U14788), or lysine (found in other HIV-1 variants). However, this evolution probably requires further passages. This apparent lack of adaptation of RBF168 to human hosts contrasts with the high replication level *in vivo* (mean pVL at 4.7 Log cp/mL). In this context, it is crucial to screen for other viral determinants of adaptation.

The follow-up of this single patient during the first five-year ART-free period, showed she remained symptom-free, with a relative stability of the CD4 cell count, despite high viral replication; this appears similar to the situation encountered in non-pathogenic SIV infections harbouring a high level of replication in their symptom-free hosts [18]. The singular natural polymorphism of the cART targets (distinct to that of the closest phylogenetic group O), did not prevent determination of an efficient therapy, that led to virological success during an 8-year follow-up, with a significant increase of CD4 of 600 between nadir and zenith; this suggests no difficulty for therapeutic management of this infection.

In conclusion, we have provided new insights into the last described divergent group. We have shown that HIV-1/P strains are extremely rare, suggesting that this variant expanded in a limited manner after its recent emergence in the human population, and that subsequently numbers have declined. Phenotypic characteristics of the prototypic strain, associated with data on the natural course of RBF168 infection, suggest that HIV-1/P could be less adapted and less pathogenic than the other HIV-1 strains, accounting for its lack of success in the human population. These observations have led to the hypothesis that HIV-1/P infection could evolve towards or even already correspond to a dead-end infection.

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EAG and JCP conceived and managed the study. EAG, FDO, ML, VL, PAN performed the experiments. DLR and FF performed computational analyses. PM and FS provided sample collections. EAG, DLR and JCP wrote the paper. All the authors were involved at all stages of manuscript development.

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Table 1. Natural polymorphism of RBF168 and U14788 strains in the Protease, Reverse Transcriptase, Integrase and gp41.

Region	Type of mutation	Mutations at resistance positions*		
		Specific to RBF168	Shared by the two strains	Specific to U14788
PR	RAM	I15V	L10V, V11I, L33V, M36L, Q58E, D60E, I62V, H69R, A71V, L89I	
	Atypical mutation		K20C, L63K,	
	Interpretation of mutations on drug resistance	R: saquinavir	R: atazanavir	I: saquinavir
RT	RAM		T69S, A98G, K103R	
	Atypical mutation		V179E, L210Y	K101Q, E138D
	Interpretation of mutations on drug resistance		No resistance	No resistance
IN	RAM			T97A
	Interpretation of mutations on drug efficacy			R:elvitegravir
gp41	RAM		L44M	
	Interpretation of mutations on drugs resistance		R: enfuvirtide	

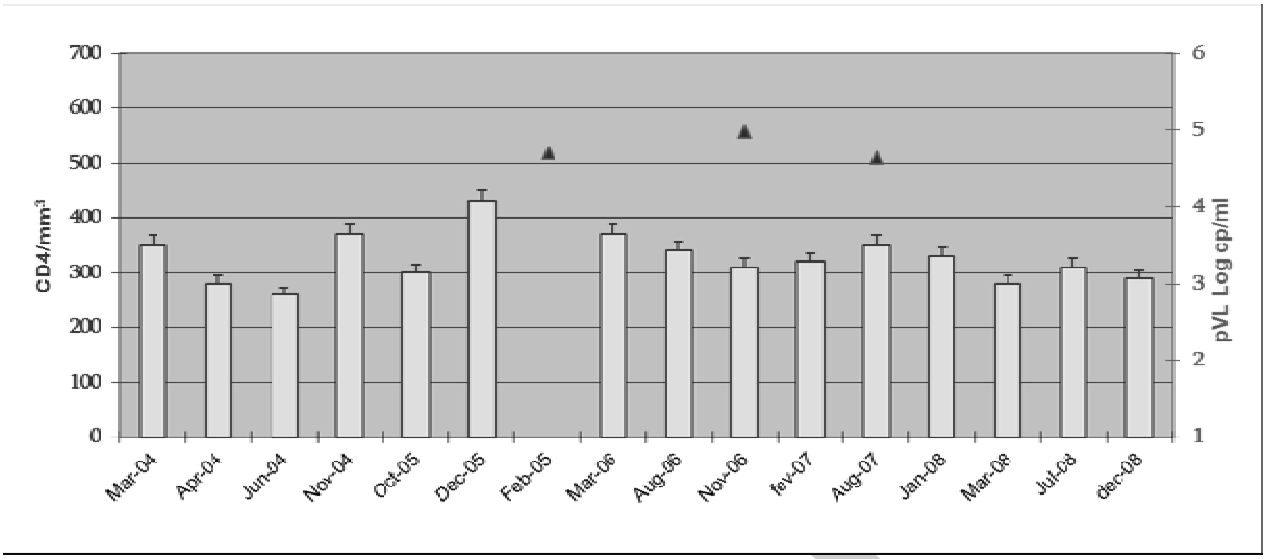
RAM: Resistance Associated Mutation, PR: Protease, RT: Reverse Transcriptase IN: Integrase

Atypical mutation: mutation not described as associated with resistance, but present at resistance position.

*Mutations found at positions associated with resistance according to the ANRS HIV-1 genotypic drug resistance interpretation algorithm 2017 v27.

I: Intermediate, R:Resistant, drugs not mentioned are interpreted as susceptible.

a)



b)

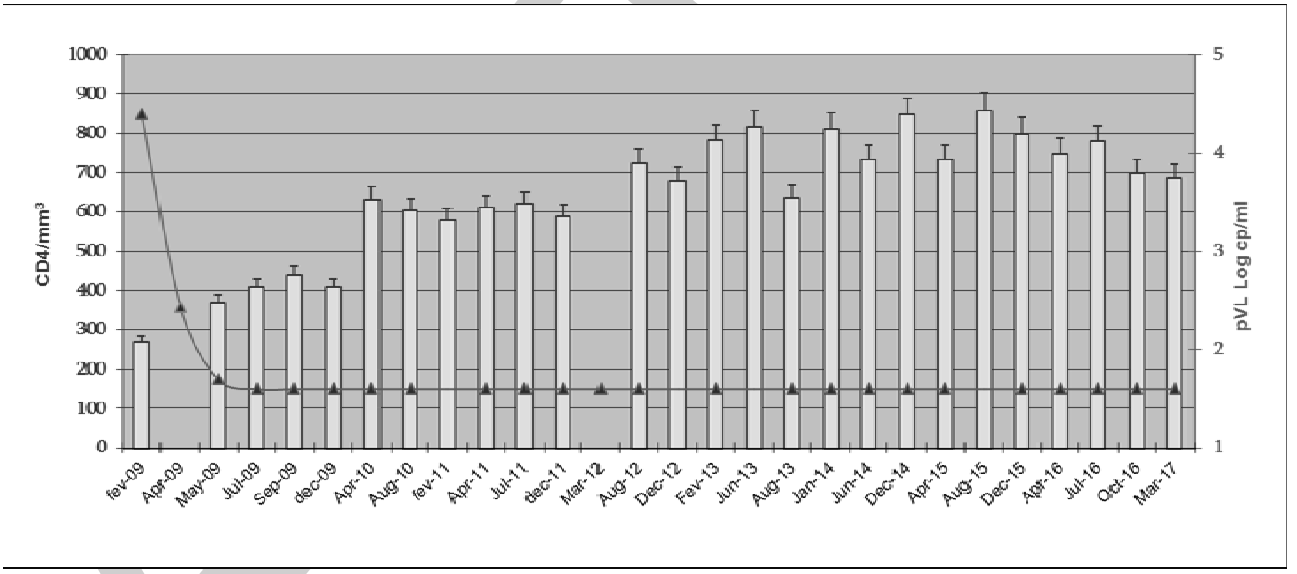


Figure 1. Biological monitoring of patient RBF168. CD4 positive cell count (bar chart) and plasmatic viral load (pVL ▲) are represented before (a) and after (b) treatment initiation. Undetectability is mentioned at 1.6 log cp/ml corresponding to the quantification threshold of the Real*Time* HIV-1 Viral Load assay.