E2 e

HIV-1 Super-infection :
AE subtype supplanted by B subtype

S. Jost, M. C. Bernard, L. Kaiser, S. Yerly, B. Hirschel, L. Goh*, and L. Perrin

Division of Infectious Diseases, University of Geneva, Switzerland, *project leader Quest, GlaxoSmithKline, Greenford road, London, United Kingdom

Abstract E2e

HIV-1 Super-infection : AE subtype supplanted by B subtype

S. Jost, M. C. Bernard, L. Kaiser, S. Yerly, B. Hirschel, L. Goh*, and L. Perrin
Division of Infectious Diseases, University of Geneva, Switzerland, *project leader Quest, GlaxoSmithKline, Greenford road, London, United Kingdom.

Background

New HIV-1 circulating recombinant forms are increasingly reported. This suggest that co-infection occurs in vivo.

Case history

A 38 year-old male with an acute retroviral syndrome (ARS) following previous multiple unprotected sexual contacts with male partners was enrolled in the Quest trial (AZT, 3TC, Abacavir, Amprenavir for 25 months initiated within x weeks after infection, with from 19 to 25 months, vaccination by Alvac vCP 1452 and stop of all treatment at month 25). Viremia declined from >10^6 HIV-1 RNA copies/ml (c/mL) and remained < 200 c/mL while on HAART. One months after treatment interruption, viremia rebounded to 80'000 c/mL then declined to 20'000 c/mL and raised again, two weeks later, at 200'000 c/mL (second rebound), to finally fluctuate between 200'000-400'000 c/mL for 4 months before HAART re-initiation.

Methods and Results

Protease (Pr), reverse transcriptase (RT) gag and C2V3 gene sequencing documented an initial infection by subtype AE during the ARS whereas subtype B rapidly replaced AE at the time of the second rebound. To discriminate between co-infection and super-infection we set up a subtype specific PCR (end Pr-proximal third of RT) using subtype specific primers for AE and B designed according to patient's sequences. The subtype specific PCR confirmed

1/ the absence of B subtype in both plasma and proviral DNA before the second viremia rebound

2/ the emergence, during the second rebound and later on of B subtype as the majority subtype in both DNA and plasma.

The C2V3 sequences of the B subtype was related to B Brazilian strains. This correlates with a Brazilian trip of the patient with several unprotected sexual contacts 3 weeks before B emergence. In *in vitro* cultures B subtype primary isolate had a much higher replicative capacity than AE subtype.

Conclusions

This is the first documented case of HIV-1 supe-rinfection. Super-infection has implications for the ever increasing HIV-1 genetic diversity, public health and vaccines development.

Background

- The detection of an ever growing number of circulating recombinant forms (CRFs) of HIV-1 indicates that genetic recombination occurs in vivo in dually infected cells (1;2). HIV-1 co-infection with 2 circulating strains has been detected, in a transversal study, in few cases living in community of high HIV-1 endemicity (3). Co-infection might result from exposure to a second virus either during the window period at the time of the initial infection or in patients with established HIV-1 infection. This is called a super-infection.
- It is thought that HIV-1 super-infection is a rare event (1-6) and is prevented by previous viral exposure through a phenomenon called super-infection immunity (5). However, HIV-1 super-infection has been induced in chimpanzees (7). In this animal model and in super-infection induced with the simian immunodeficiency virus (SIV) in macaques, the second infection produces a slower immune deterioration and more efficient control of viremia as compared to the initial infection (7-9).
- These observations support the development of vaccines based onattenuated HIV-1/SIV that could modify the clinical course of subsequent retroviral infections (10).
- We here report a case of HIV-1 super-infection.

Case Report

- November 1998: a 38 year-old Caucasian male with multiple unknown male partners presented with an acute retroviral syndrome. Anti-HIV-1 antibodies undetectable, p24 Ag > 100 pg/mL, plasma HIV-1 RNA: 805.000 c/mL and the CD4 cell count 684/μl.
- Sequences of HIV-1 reverse transcriptase (RT) and protease (Pr): no drug resistance mutations and HIV-1 AE subtype.
- The patient, enrolled in the Quest trial, was treated with zidovudine, lamivudine, abacavir and amprenavir for 27 months. From month 21 to 27 he was vaccinated with Alvac vCP1452 (Figure 1).
- Following initiation of HAART: rapid decline of HIV-1 RNA within 6 weeks to 1000 c/mL. Treatment was next interrupted for 6 weeks due to hepatic toxicity, (acute hepatitis B). Upon reintroduction of HAART, rapid viremia decrease to < 50 c/mL.
- HAART was stopped in January 2001. In February the patient's viremia rose to 18.000 c/mL, then to 80.000 c/mL and within 2 weeks, declined to 21.000 c/mL (first rebound). A rapid increase of viremia occurred 2 weeks later in April (second rebound), then viremia fluctuated between 200.000 and 400.000 c/mL for 4 months.

Sequencing of RT, Pr, p17 and C2V3 revealed a subtype B during the second rebound. Symptoms were mild (transient fatigue and fever) and the patient declined reintroduction of HAART. The patient's history revealed several unprotected sexual contacts in Brazil 3 weeks before the second viremia rebound. Serological data and quantification of hepatitis C virus RNA (Fig.1) documented an acute HCV infection of genotype 3a around March 2001.

* Patient HLA was A 01, A 02 and B 51, B 37 . There were 17 described T cell epitopes corresponding to the HLA typing of the patient of which 13 are included in the Alvac vCP 1452: 4 of them within the C2V3, gag p17 and RT (SYFPEITHI data base), <u>in 3 of the 4 identified T cell epitopes there was one or several mutations in the B virus as compared to ALVAC vCP 1452 sequences.</u>

Methods

HIV-1 and HCV RNA were quantitated by Cobas Amplicor Monitor.

Purification of plasma RNA, proviral DNA, population sequencing
RT, Pr, *gag* p17, *env* C2V3 regions and analysis of sequences were performed as reported: Yerly et al. J Infect Dis 2001;184(3):369 and Ibidem
AIDS 2001;15(17):2287.

Subtype specific PCR: Primers were selected according to patient's AE and B Pr and RT sequences: sense primer (2289-2312 number in reference to HXB2)
AE: 5'ACAGTAAAAATAGGAGGACAG and
B: 5'ATAAAGGTAGGGGGCAATTAAAG;
antisense primer: AE (2786-2806): 5'TCCTGAGTTCTTTTATTGAGC and B (2687-2712): 5'GATTTTCAGGCCCAATTTTTGAAATT. The amplification consisted of 40 amplification cycles: 20 sec at 95°C, 30 sec at 65°C followed by 30 sec at 72°C. Successive plasmas were amplified by both primer pairs. Twenty µl of the amplicons, spotted on nylon membranes were revealed with Digoxigenin end-labeled probe (2431-2451: 5'ATTAAAGCCAGGAATGGATGG) (Detection Starter kit II reagents).

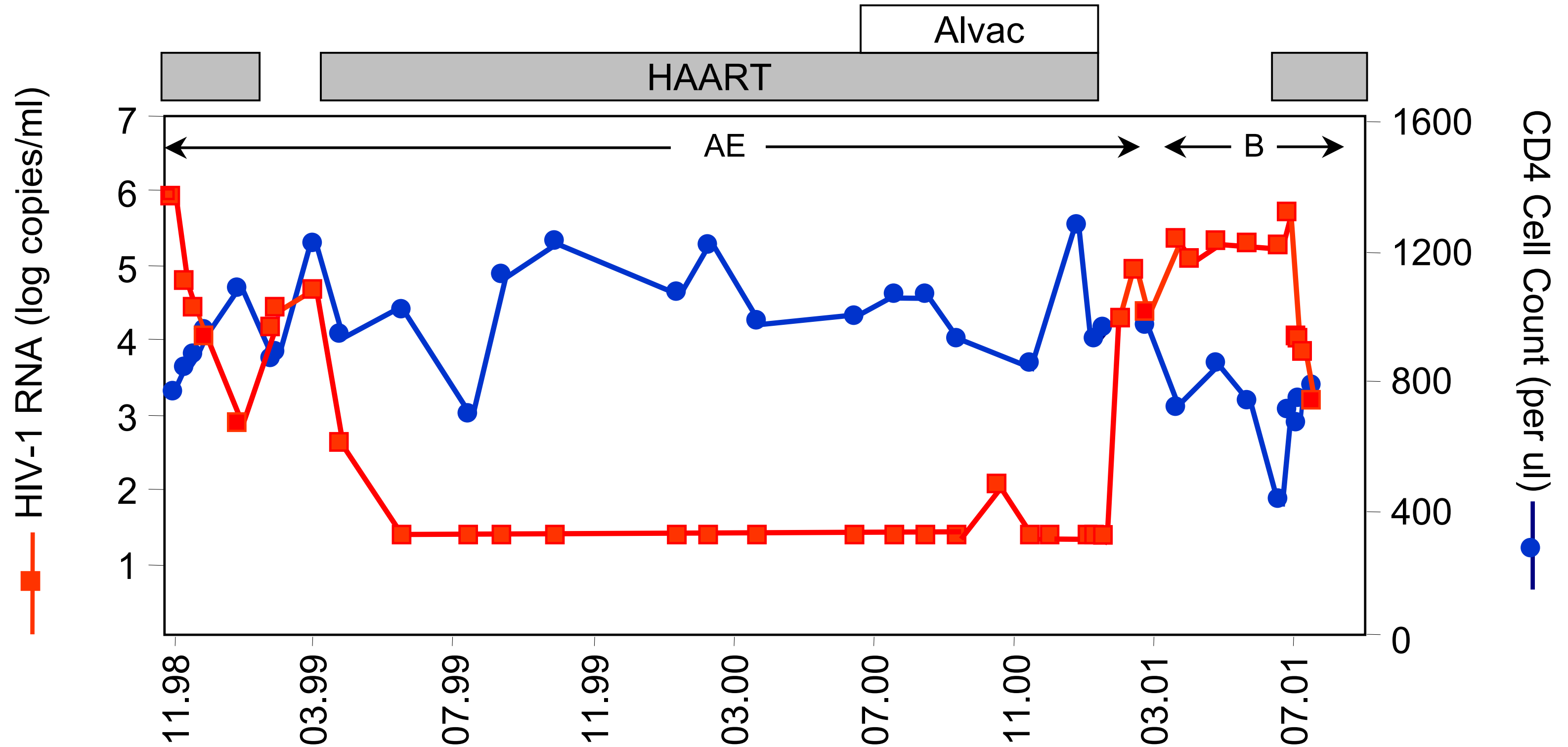
HIV-1 proviral DNA PCR

PCR was performed on DNA purified from peripheral blood mononuclear cells (PBMC) by nested PCR. The first PCR consisted of 40 amplification cycles: 20 sec at 95°C, 30 sec at 55°C followed by 30 sec at 72°C using sense and antisense primers (1627-1655, 2791-2812 HXB2). Five percent of the amplicons were used in the nested PCR using subtype specific primers asabove. Twenty µl of the amplicons were electrophoresed on 2% agarose gel before ethidium bromide staining.

Isolation and growth of AE and B subtypes in vitro

Stored PBMC collected in November 98 and April 01 were depleted in CD8 T cells, co-cultivated with phytohemaglutinin stimulated CD8-depleted PBMC from a healthy blood donor. Twenty and 100 pg of p24 Ag of AE and B culture supernatants, collected during the exponential viral growth, were inoculated to new CD8-depleted PBMC cultures from the same blood donor in duplicate after control of HIV-1 subtype by RT sequencing. Viral replication was monitored through the quantification of p24 Ag released in culture supernatants.

Patient 6992 from Quest with an acute retroviral syndrome in November 98



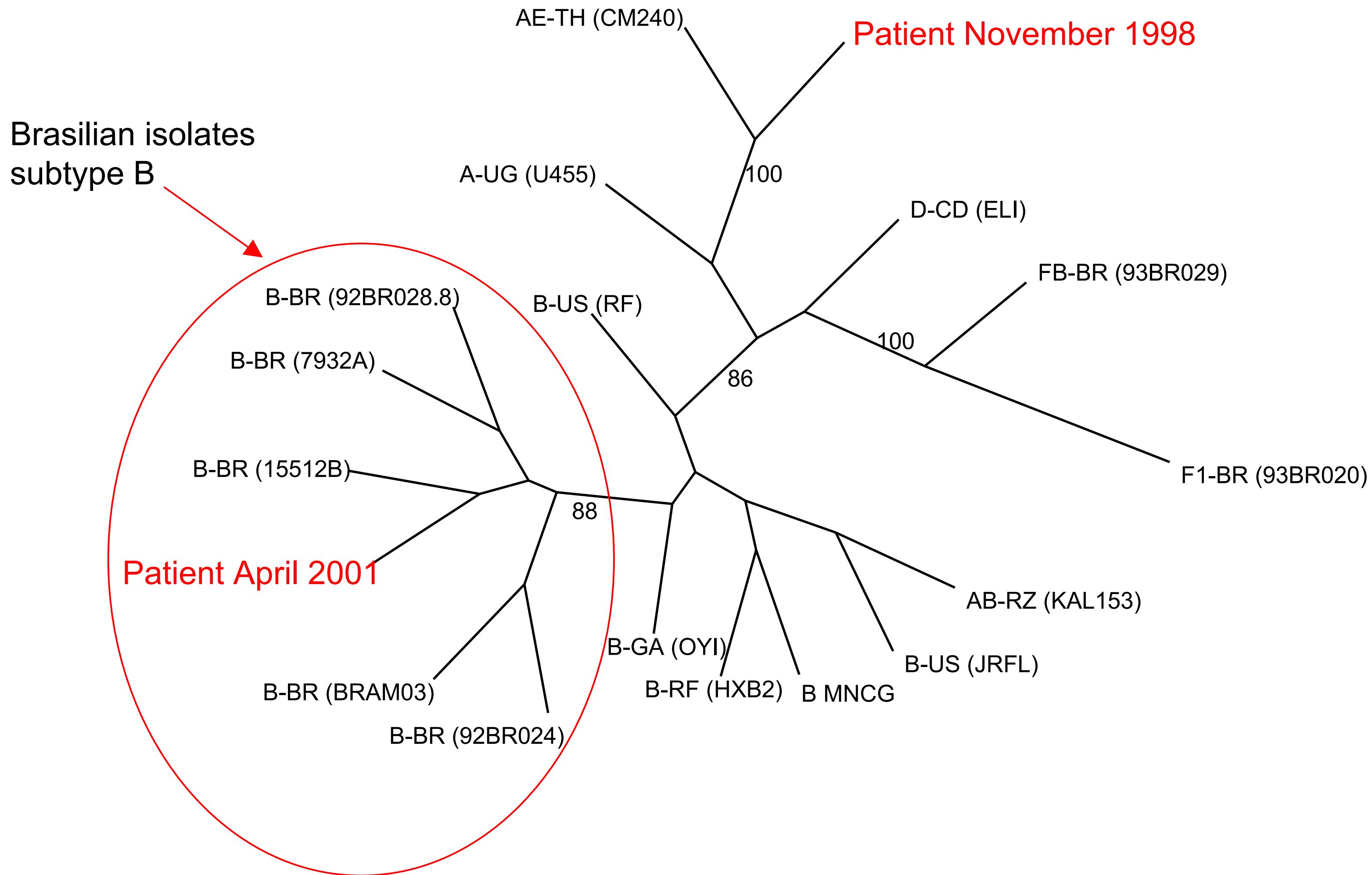
HBs Ag + - -

Anti-HBs - + +

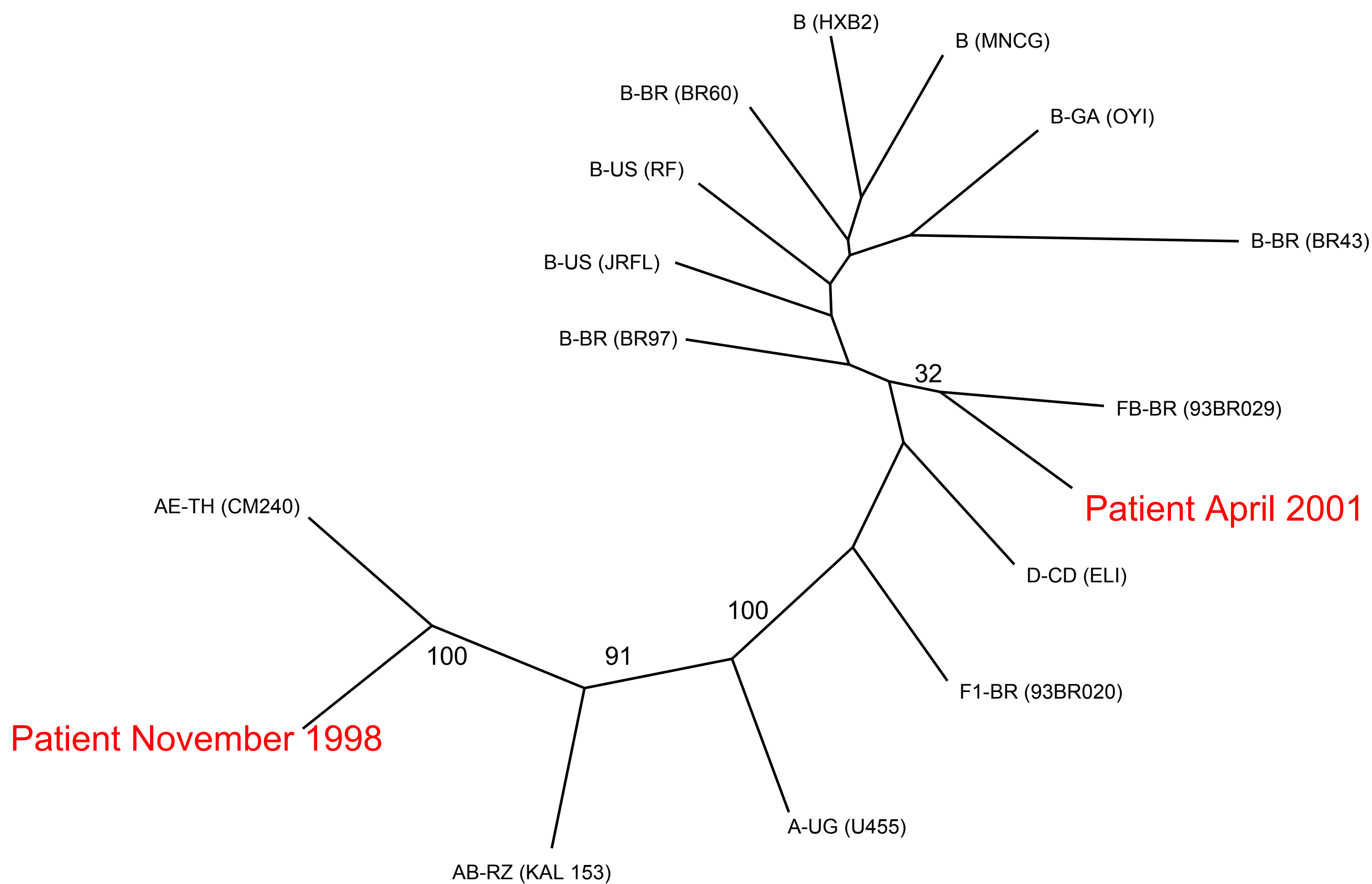
HCV RNA - - + + +

HCV Ab - - - - +

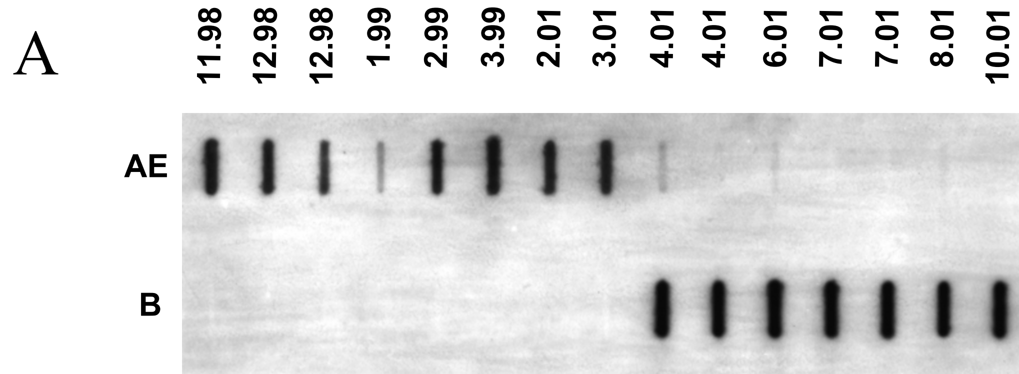
Phylogenetic tree C2V3

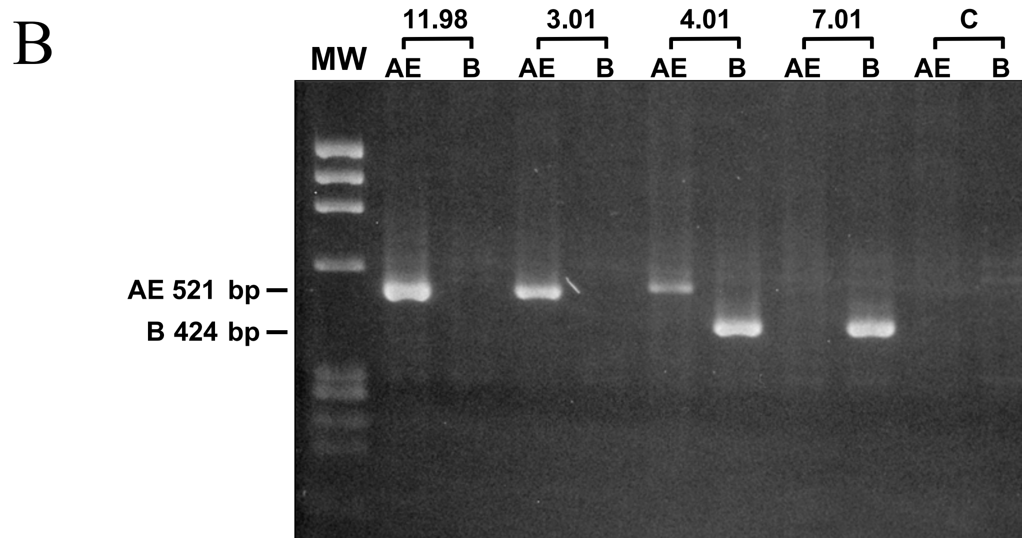


Phylogenetic tree P17



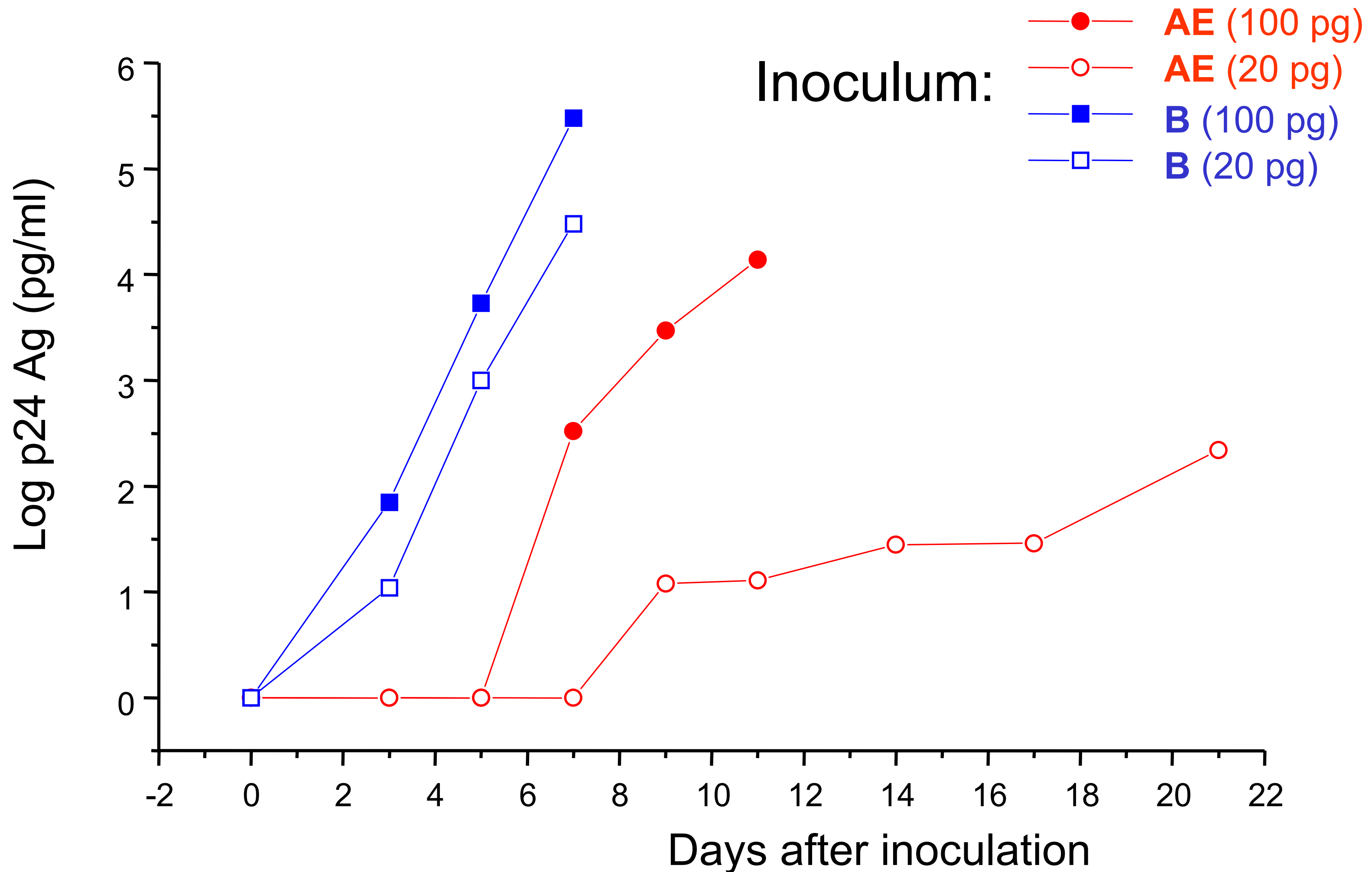
HIV-1 subtype specific PCR in plasma RNA and on PBMC DNA collected at various time points





Each sample amplified in parallel by AE and B specific primers

Replicative capacity of AE and B subtype assessed by quantification of P24 Ag released in supernatants of CD8 depleted PBMC cultures inoculated by known amount of p24 Ag of AE and B (second passage)



Discussion / Conclusion

- This is the first documented case of HIV-1 super-infection. Our patient had a typical HIV-1 seroconversion in November 98 due to AE subtype.
  Two years later during a treatment interruption and following multiple sexual exposures in Brazil, he had a B subtype super-infection. B subtype rapidly overgrew AE subtype in vivo and in vitro. Three of the 4 four T cell epitopes identified within the available B sequences presented mutations as compared to the antigens derived from B subtypes MN and LAI expressed by Alvac vCP 1452 (SYFPEITHI data base).

- Co-infection at the time of acute infection and during the following 2 years was excluded. Indeed, we failed to detect B subtype in both plasma RNA and proviral DNA up to the second viremia rebound using subtype specific PCR.

- The biological course following HIV-1 B subtype super-infection was characterized by the persistence of high viremia and a loss of 300 CD4 cells/μl within the 4 months following the emergence of B subtype. This indicates that the B super-infection produced a rapid disease progression. These data contrast with those observed in experimental super-infections in monkeys which were not performed with wild type isolates from the corresponding monkey species (7-9). Our data show that in our patient crossclade protection was not achieved by natural infection, suggesting that attenuated HIV-1 might not either induce systemically crossclade protection in humans. The frequency of super-infection may be underestimated due to difficulties to detect it within the context of rapid diversification of the virus within individual patient.

In conclusion, our data indicate that natural infection does not necessarily induce crossclade protection and support the close monitoring of circulating HIV-1 strains in the context of vaccine development. In a public health context, the present data also support the recommendations for safe sexual practices in HIV-1 infected couples.

References :

1. Korber BG. Br Med Bull 2001;58:19-42.

2. http://hiv-web.lanl.gov/CRFs/CRFs.html.

3. Kampinga Gaetal. J. Virology 1997;227(1):63-76.

4. Zhu T et al. J Virol 1995;69(2):1324-7.

5. Malim MH, Emerman M. Cell 2001;104(4):469-72.

6. Angel JB et al abstract of the 7th Conference.

7.Shibata R et al. J Virol 1996;70(7):4361.

8. Stephens EB et al. Virology 1997;234(2):328-39.

9. Stahl-Hennig C et al. J Virol 1996;77:2969-81.

10. Johnson RP, Desrosiers RC. Curr Opin Immunol 1998;10(4):436-43.