Neutralizing Antibodies in Humans Infected with Zoonotic Simian Foamy Viruses

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Background

Simian foamy viruses (SFVs) are widespread and highly prevalent in many nonhuman primate (NHP) species. They are easily isolated from the saliva and oropharyngeal secretions of infected NHPs. Penetrating bite wounds are therefore a potential route of transmission to humans, where they cause life-long persistent infections.

From an immunological point of view, human zoonotic infection with SFVs corresponds to the efficient control of a retroviral infection in the new host after interspecies transmission: viral propagation is successfully restricted in the human population.

Aim

We aimed to study neutralizing antibodies in the plasma of SFV-infected people living in rural areas of South Cameroon.

Study population

Field studies were performed on adult populations living in villages and settlements across rural areas of the rainforest in Cameroon. We included only individuals who declared having had a direct contact with NHP body fluids, in most cases bites (**Figure 1**). SFV infection was defined by a clearly positive Gag doublet on western-blot and the amplification, by PCR, of the *integrase* gene and/or LTR DNA fragments. The origin of the SFV was identified by phylogenetic analysis of the sequence of the *integrase* gene.



Figure 1

Scars and lesions caused by NHPs bites (reproduced from Betsem et al. 2011).

Neutralization assay

Serial dilutions of plasma samples from 46 SFV-infected individuals and seven uninfected subjects were incubated with SFV isolates. Residual viral infectivity was quantified with an indicator cell line expressing the beta-galactosidase gene under the control of the LTR from the prototypic strain, SFVcpzPFV (**Figure 2**).

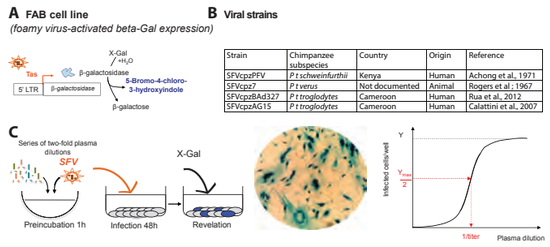


Figure 2

A. Principe of FAB indicator cell line described by Yu et al., 1993. B. Four strains from the chimpanzee clade were used. C. Schematic representation of the neutralization assay Neutralizing antibody titers were defined as the inverse of the plasma dilution required to reduce viral infectivity by half.

Neutralization of primary strains by autologous plasma

Six individuals were infected with a SFV strain from chimpanzee subspecies *Pan troglodytes troglodytes*. Replication competent viruses, SFVcpzBAD327 and SFVcpzAG15, isolated from the peripheral blood of two of them were neutralized by autologous plasma with titers of 1/238 and 1/960 (Figure 3), and by two of the four heterologous plasmas.

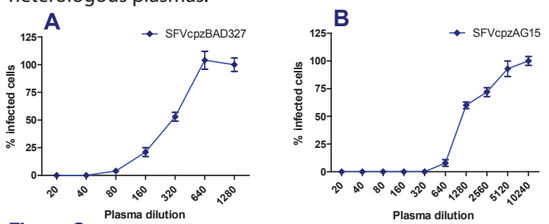


Figure 3

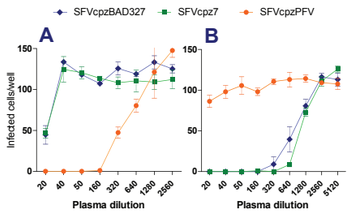
Neutralizing activity of plasmas against autologous primary SFV strains. The number of infected cells in each well is expressed as the percentage of infected cells observed at the highest plasma dilution. Means and SD of 3 replicates are shown as a function of plasma dilution.

Plasma from individuals infected with SFV of chimpanzee and gorilla origins neutralized viruses from the two chimpanzee' serogroups

Two of the six individuals infected with a SFV strain of chimpanzee origin lacked neutralizing activity against primary SFV strains. The absence of neutralizing activity may be due to infection with a strain of a distinct serogroup, as two such serogroups have been described in animal studies. We used lab-adapted viruses (SFVcpzPFV for serogroup 6 and SFVcpz7 for serogroup 7, Bieniaz et al., 1995) in neutralization assays. Those individuals infected with a SFVcpz strain, but whose plasma did not recognize the primary strains neutralized SFVcpzPFV but not SFVcpz7. Conversely, individuals neutralizing the primary strains neutralized SFVcpz7 but not the SFVcpzPFV (**Figure 3**).

Figure 3

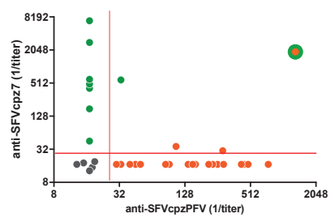
Neutralizing activity of plasma against SFVcpzBAD327, SFVcpzPFV and SFVcpz7. Number of infected cells per wells (mean ± SD) are presented as a function of plasma dilution. Results for two individuals are presented (A: Bad326, B: Pyl106).



Of 35 individuals infected with SFV of gorilla origin, 21 neutralized the SFVcpzPFV strain, eigth neutralized the SFVcpz7 strain, and five showed no neutralizing activity (**Figure 4**). Plasma from one individual neutralized both strains; Two *pol* sequences were amplified from the buffy-coat of this individual. Plasma from seven uninfected individuals and five individuals infected with SFV from monkeys did not neutralize any SFVcpz strain (not shown).

Figure 4

Plasma from 35 individuals infected with a SFV strain of gorilla origin were tested against SFVcpzPFV and SFVcpz7. Orange dots represent samples neutralizing the SFVcpzPFV strain, green dots those neutralizing the SFVcpz7 strain and grey dots those with no neutralizing activity.



Primary and lab-adapted strains have similar antigenic properties and similar susceptibility to neutralization

Neutralizing titers for reactive samples ranged from 1/30 to 1/2060. The titers of neutralizing antibodies against the two primary strains were strongly correlated with the titers of neutralizing antibodies against SFVcpz7 (Spearman's rho=0.998, P<0.0001 and rho=0.957 P<0.0001, respectively, **Figure 5A-B**). Thus, both SFVcpzBAD327 and SFVcpzAG15 belong to serogroup 7.

The primary strain SFVcpzBAD327 and the lab-adapted strains SFVcpz7 and SFVcpzPFV had similar susceptibility to neutralizing antibodies from infected individuals: the neutralizing titers of the reactive plasmas were not significantly different (Kruskal-Wallis's *P*=0.22, **Figure 5C**).

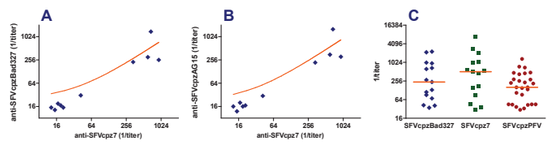


Figure 5

Plasma from 10 individuals was tested against the two primary strains and SFVcpz7. Neutralizing titers against SFVcpzBAD327 (A) and SFVcpzAG15 (B) are presented as a function of neutralizing titers against SFVcpz7. (C) All plasma were tested for their neutralizing activity against SFVcpzBAD327, SFVcpz7 and SFVcpzPFV and neutralizing titers of reactive plasmas were compared.

Conclusions

Individuals infected with SFV from chimpanzees and gorillas generated neutralizing antibodies.

Cross-recognition of SFV from chimpanzees by plasma primed against viruses from gorillas was frequent, despite Env sequence homology being only 77-80 %.

The conservation of the neutralizing epitopes may account for the efficient immune control of these retroviruses. We are currently mapping of these epitopes.

Acknowledgments

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