Abstract # 223
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Identification of the first nonhuman primate CD4 receptor for T/F HIV-1 isolates

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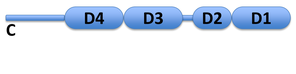
Abstract

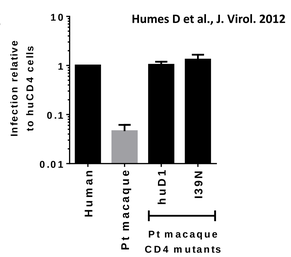
Non-human primates are resistant to infection by HIV-1 and therefore studies of viral pathogenesis often employ modified viruses referred to as chimeric SIV/HIV-1 viruses (SHIVs). Importantly, most SHIVs encode a HIV-1 envelope protein (Env) that can use macaque CD4 as an entry receptor, which is unusual among Envs from HIV-1 variants circulating in humans. Primate models that allow entry of these more relevant HIV-1 Envs are lacking. By sampling populations of eleven primate species, we identified several *CD4* alleles circulating amongst Spix's owl monkeys that support entry by HIV-1 Envs isolated from early human infections representing the major HIV-1 group M clades (A, B, C, and D). New World monkeys such as these were previously thought to be incapable of supporting HIV-1 replication due to cellular entry barriers. This result supports the hypothesis that some primate species may have been prematurely discounted as HIV-1 models and provides a novel strategy for developing new HIV-1 animal models.

Background

- Most circulating HIV-1 envelope variants obtained directly from infected individuals soon after virus acquisition do not efficiently mediate entry using the macaque CD4.
- Only select HIV-1 envelope variants, typically those derived from viruses that have been adapted in cell culture, use the macaque CD4 receptor and result in infectious chimeric SIVs encoding HIV-1 envelope (SHIVs).
- Species-specific amino acid differences in the macaque CD4 restrict replication by global circulating HIV-1 envelope variants representing viruses from recent infection.
- Species-specific differences in the functional capacity of the CD4 to mediate entry was mapped to a single amino acid difference at position 39 in the D1 domain of CD4.
  - Human CD4 bears Asparagine (Asn, N)
  - Macaque CD4 bears Isoleucine (Ile, I)

A. Schematic representation of the CD4 receptor.
The four extracellular immunoglobulin-like domains are indicated D1-D4. The intracellular tail is indicated C.





B. Effect of single residue changes in CD4 on HIV-1 replication.
HEK293T cells transiently expressing huCCR5 and indicated CD4 (pt = pig-tailed macaque, hu = human) were infected with Q23 Env-pseudotyped Q23ΔEnvGFP virions. GFP-positive cells were measured 72 hpi.

Non-synonymous SNPs in the portion of *CD4* encoding the D1 domain

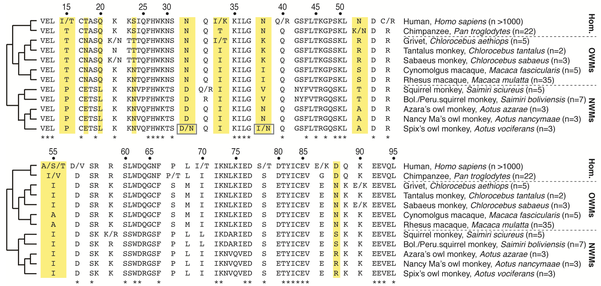


Figure 1. Amino acid alignment of the D1 domain from species included in the population study. Non-synonymous SNPs are indicated with hyphens. Species are listed according to their phylogenetic relationship, Dotted lines indicate the major clades of primates (Hom. = Hominoids, OWMs = Old world monkeys, NWMs = New world monkeys). The number of individuals analyzed from each species is shown in parenthesis. Amino acid positions highlighted in yellow were identified to be evolving under positive selection.

Some Spix's owl monkey *CD4* alleles encode receptors permissive for entry by Env of primary HIV-1 isolates

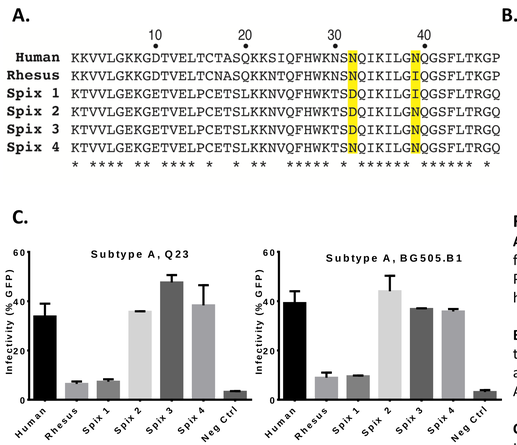


Figure 2.
A. Amino acid alignment of the D1 domain from six CD4 receptors tested for virus entry. Polymorphic sites in Spix's owl monkeys are highlighted in yellow.

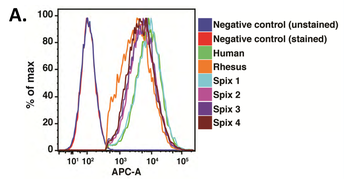
B. Expression levels of CD4s transiently co-transfected with huCCR5 into HEK293T cells as measured by flow cytometry using an APC-conjugated anti-CD4 antibody.

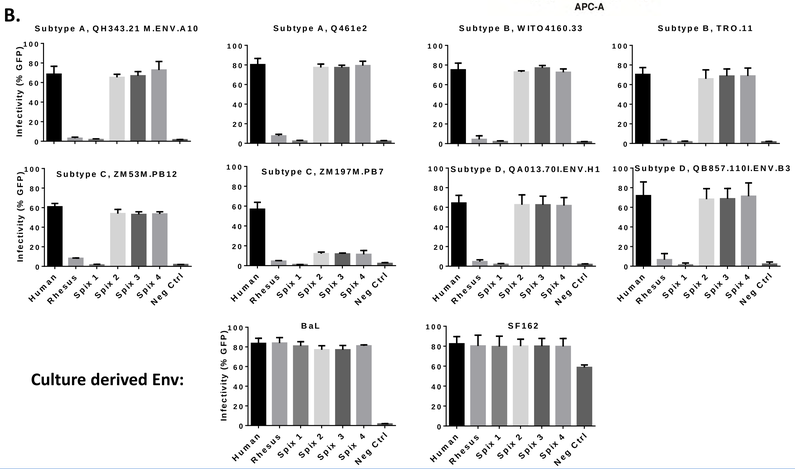
C. CD4 expressing cells were infected with the indicated Env-pseudotyped Q23ΔEnvGFP virions. GFP-positive cells were measured by flow cytometry 72 hpi. Bars represents means ± SD [*n* = 2].

Spix's owl monkey CD4 receptors are permissive for entry by the Env from the major clades of HIV-1 group M

Figure 3.
A. Expression levels of CD4s stably introduced into Cf2Th/syn CCR5 cells as measured by flow cytometry using an APC-conjugated anti-CD4 antibody.

B. CD4 expressing cells were infected with the indicated Env-pseudotyped Q23ΔEnvGFP virions. GFP-positive cells were measured by flow cytometry 72 hpi. Bars represents means ± SD [*n* = 3].





Spix's owl monkey CCR5 is not a functional coreceptor with their permissible CD4

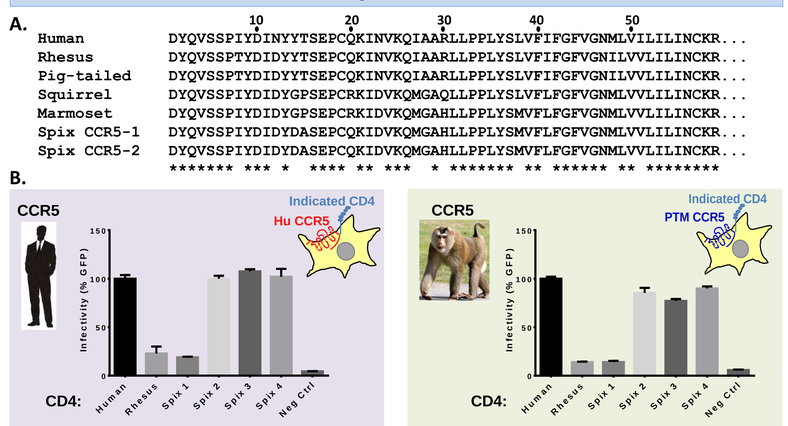


Figure 4.
A. Two *CCR5* alleles (CCR5-1 and CCR5-2) were cloned from Spix's Owl Monkey. Amino acid alignment of the first 60 residues of the CCR5 coreceptor. For comparison, CCR5 from human, two Old World monkeys (Rhesus and Pig-tailed Macaque) and two New World monkeys (Squirrel and Marmoset) were aligned.

B. HEK293T cells transiently expressing indicated CD4 and indicated CCR5 (Hu = human, PTM = pig-tailed macaque, Spix *CCR5* allele -1 & -2) were infected with Q23 Env-pseudotyped Q23ΔEnvGFP virions. GFP-positive cells were measured by flow cytometry 72 hpi.

Conclusions

- The first New World monkey CD4 compatible with entry of circulating HIV-1s is identified.
- Some Spix's owl monkey *CD4* alleles encode receptors that are functional for HIV-1 entry.
- Functional capacity of Spix's owl monkey CD4 to mediate entry, by global circulating HIV-1 envelope variants representing viruses from recent infection, maps to single amino acid difference at position 39 that is under strong positive selection.
- CCR5 from Spix's owl monkey is not a functional coreceptor when combined with their permissible CD4 for entry by Env of early, transmitted Env.
- This study provides proof of principle that there are non-human primate CD4 that can function as HIV-1 receptors. Identifying such non-human primates that also encode a functional CCR5 may allow for an improved model of HIV-1 infection.

Acknowledgements

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