Nicotiana Produced Broadly Neutralizing Monoclonal Antibodies as a Microbicide Strategy

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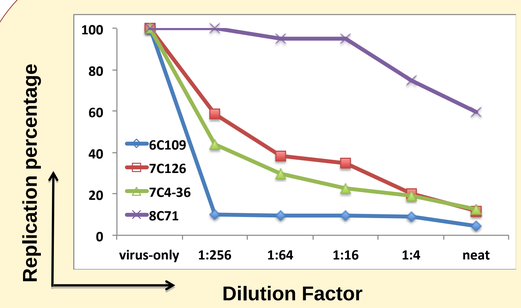
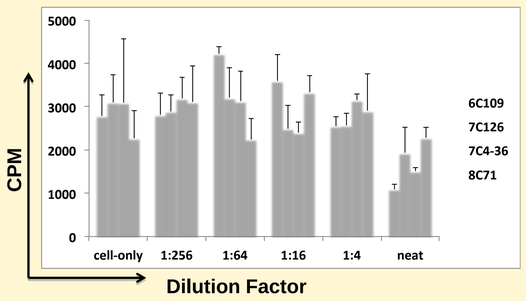
Background

In the absence of an effective vaccine to prevent the transmission of HIV, alternative prevention methods need to be explored to slow the progression of the epidemics. While condoms have provided good protection from transmission, their use is not necessarily acceptable in select communities and therefore the development of a female controlled efficacious microbicide remains a top priority. Such goal has however been a challenge for HIV as most common approaches have failed or promoted transmission. Our team has taken a different strategy proposing the use of recombinant human monoclonal antibodies (Mabs) broadly neutralizing HIV produced in nicotiana as a cost effective approach.

Methods of Study:

We have studied the HIV neutralizing efficacy of nicotiana produced Mabs VRC01-N and 4E10-N in vitro against panels of HIV and SHIVs. We then tested the pharmacokinetic and distribution of VRC01-N and 4E10-N in the vaginal environment of cynomolgus macaques following administration in 1.5% hydroxyethyl cellulose gel (HEC). Sequential collections of vaginal fluid were performed using TearFlo strips from 5 vaginal sites at 0.5, 4, 24 and 72 hour and cervicovaginal lavages (CVL) at 4, 24 and 72 hour time points. Cynomolgus were chosen due to their relative abundance and the fact that similar to humans and unlike rhesus macaques, they reproductive cycle is continuous instead of seasonal.



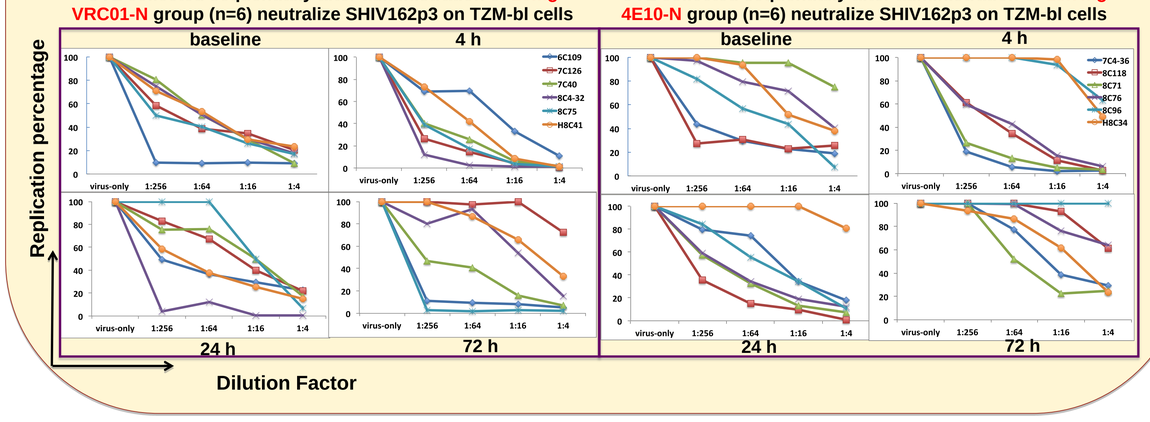


CVL was tested for neutralization in the vaginal vault. Of interest was the finding that baseline CVL and HEC vehicle CVL showed inhibition of SHIV162p3 at lower dilutions suggesting the presence of yet to be defined antiviral molecules.

Serial dilution of baseline CVL was incubated with TZM-bl cells for 28 hours, followed by addition of ^{3}H overnight and evaluation of cell turnover. The TZM-bl cells viability was not affected at CVL dilutions 1:4 or beyond.

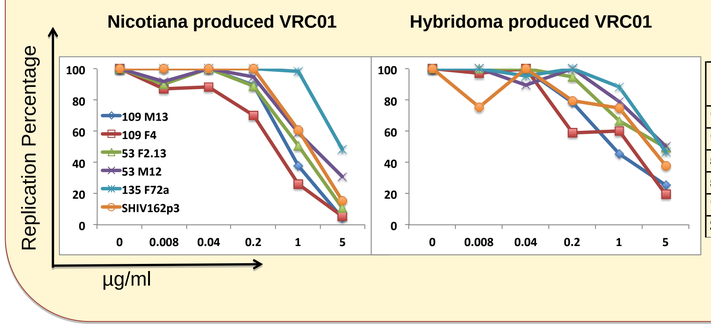
CVL collected sequentially after administration of 20mg VRC01-N group (n=6) neutralize SHIV162p3 on TZM-bl cells

CVL collected sequentially after administration of 18.7mg 4E10-N group (n=6) neutralize SHIV162p3 on TZM-bl cells



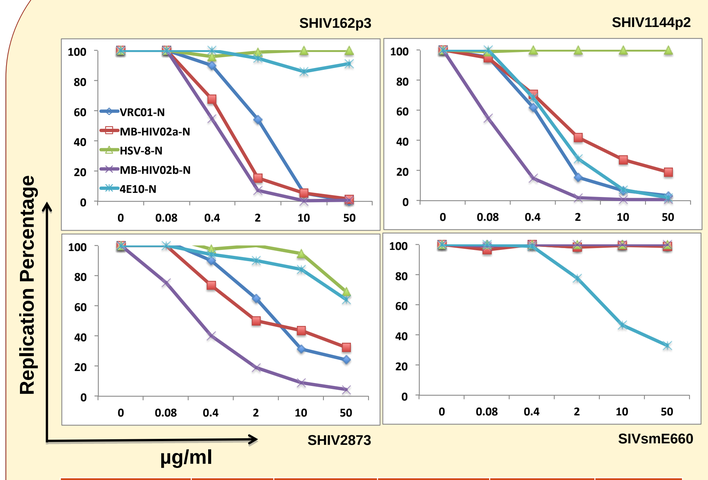
CVL collected at 4 and 24 hours post Mab administration showed potent neutralization of SHIV162p3. At 72 hours the neutralization activity of CVL was decrease in particular for 4E10-N.

Comparison of Nicotiana vs Hybridoma produced VRC01 for neutralization of SHIV162p3 and a panel of HIV-1 Subtype C viruses on TZM-BL cells



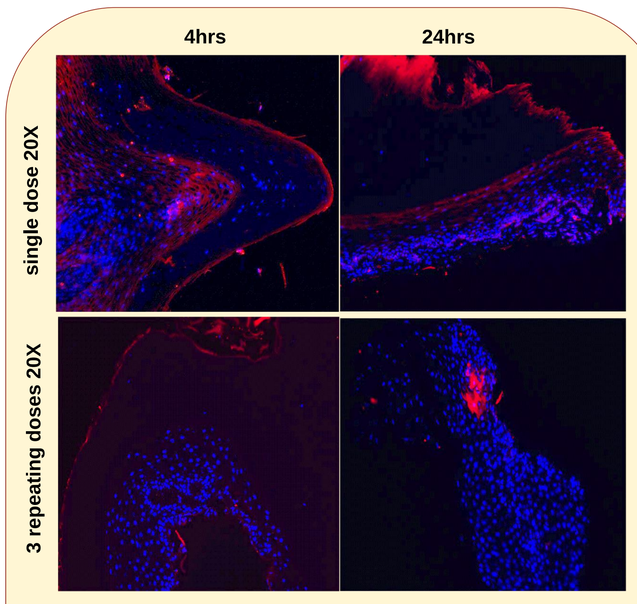
	IC50	
	Nicotiana VRC01-N	Hybridoma VRC01
109 M13	0.445	0.442
109 F4	0.296	0.773
53 F2.13	0.465	4.937
53 M12	1.060	>5
135 F72a	4.895	4.612
SHIV162p3	0.972	1.109

Comparison of the neutralizing activity of nicotiana and hybridoma produced VRC01 showed similar neutralization potency against a panel of primary HIV clade C isolates from Africa and SHIV162p3 (clade B).

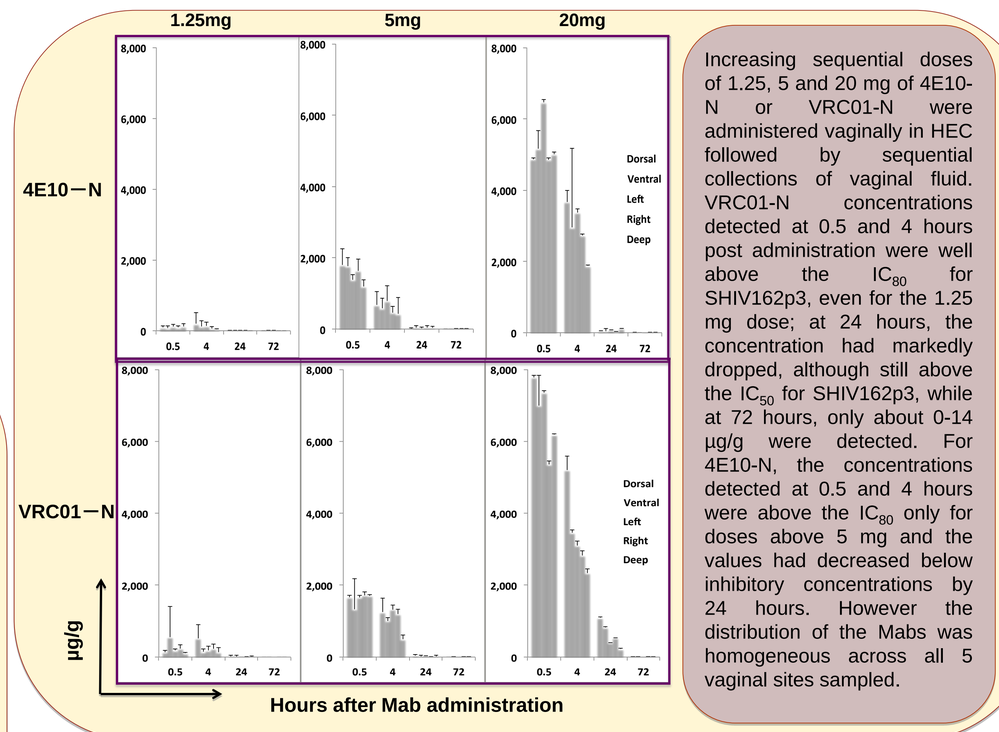


IC50 (µg/ml)	4E10-N	VRC01-N	MB-HIV02a-N	MB-HIV02b-N	HSV8-N
SHIV162p3 (Clade B)	>125	1.688	1.104	0.862	---
SHIV KNH1144p2 (Clade A)	0.965	0.860	1.721	0.147	---
SHIV2873 (Clade C Tier2)	>125	5.561	3.534	0.398	>50
SIVsmE660	12.424	---	---	---	---

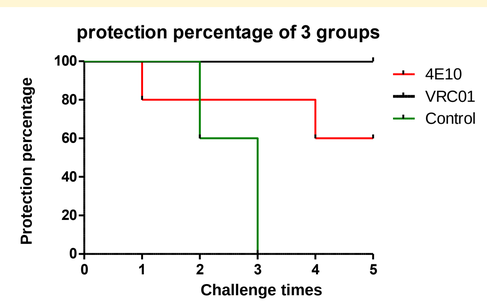
Note ---- VRC01-N, MB-HIV02a-N and MB-HIV02b-N are nicotiana-produced monoclonal antibodies bind to CD4 binding site of HIV; HSV8-N is nicotiana-produced monoclonal antibody which against Herpes Simplex Virus 8



Administration of Cy3 labeled Mab in HEC either as a single or repeated daily dose showed that the Mab was restricted to the superficial layers of the stratum corneum with limited penetration by 4 hours and patchy localization by 24 hours, suggesting limited coating of the vaginal epithelium with protective Mabs beyond a few hours.



Increasing sequential doses of 1.25, 5 and 20 mg of 4E10-N or VRC01-N were administered vaginally in HEC followed by sequential collections of vaginal fluid. VRC01-N concentrations detected at 0.5 and 4 hours post administration were well above the IC_{80} for SHIV162p3, even for the 1.25 mg dose; at 24 hours, the concentration had markedly dropped, although still above the IC_{50} for SHIV162p3, while at 72 hours, only about 0-14 µg/g were detected. For 4E10-N, the concentrations detected at 0.5 and 4 hours were above the IC_{80} only for doses above 5 mg and the values had decreased below inhibitory concentrations by 24 hours. However the distribution of the Mabs was homogeneous across all 5 vaginal sites sampled.



Cynomolgus macaques (n=15) were divided into 3 groups (n=5), administered either 4E10, VRC01 or control HEC. 30 minutes later, the macaques were exposed to SHIV162p3 vaginally. After 3 challenges, all control animals were infected; while none of the VRC01 (20 mg) monkey were infected. In contrast, 2 of 5 monkeys administered 18.7mg 4E10 became infected. Additional challenges against decreasing Mab doses are being tested.

The neutralization potency of nicotiana produced 4E10 and VRC01 was tested against clade A, B and C SHIVs in TZM-bl cells, showing IC_{50} of 4E10 generally >20 fold higher than the IC_{50} of VRC01 for identical isolates

Conclusion:

We submit that cynomolgus macaques constitute a good model to study not only the pharmacokinetic but also the efficacy of HIV broadly neutralizing Mab base microbicides.

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Results:

- **Nicotiana produced Mabs neutralization potency are comparable to hybridoma produced Mabs**
- **4E10-N and VRC01-N are broadly neutralizing antibodies which can neutralize different strains of SHIV. While 4E10-N has a broader spectrum, its efficacy is markedly lower than the neutralization potency of VRC01-N**
- **At 4 hours after administration, the Mab concentration is still high enough to neutralize virus**
- **Innate immune molecule may exist in cynomolgus vagina able to inhibit virus**
- **Vaginal biopsies show limited penetration of Mab into the vaginal tissue, which was not enhanced by repeated administration**
- **VRC01-N alone is fully protective at high dose from vaginal SHIV162p3 challenge, while the efficacy of 4E10-N appears limited**