

Human Recombinant IgG₁ anti-Tat Antibodies Inhibit HIV-1 Viral Replication *in vitro*

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Conclusions

- Antibodies to conserved epitopes in the Tat molecule have been demonstrated to be able to inhibit replication of HIV *in vitro* at sub-nanomolar concentrations.
- Resistance to the inhibitory antibodies did not develop over 20 passages.
- Virus with developed resistance to Fuzeon retained sensitivity to the anti-Tat antibody.
- Antibodies to conserved Tat epitopes have a potential to be developed into a new class of therapeutics useful for controlling HIV-replication and development of AIDS

Abstract

Background: Tat has a central role in HIV replication and levels of antibodies to Tat in HIV-1-infected subjects correlate with lower virus loads and non-progression. Tat-directed immunotherapy might, thus, be useful in controlling HIV infection. Antibody blockade of circulating Tat would create no selective pressure so that resistant strains are unlikely to develop after treatment. Two conserved epitopes, aa 4 to 10 and aa 41 to 50 of the HIV-1 Tat protein have been identified as suitable as targets for therapeutic antibodies. Fully human IgG₁ antibodies recognising each epitope have been derived from the n-CoDeR scFv phage display library and we now show them to inhibit viral replication *in vitro* in Jurkat cells.

Methods: Monoclonal antibodies were selected from the scFv library using peptides covering the epitope variations. ELISA and Biacore were used to analyze binding characteristics. Jurkat cells were infected with HIV-1_{IIIIB} and the anti-Tat antibodies were titrated for suppression of viral replication. Viral levels were estimated by p24 levels 7 days post-infection. To investigate resistance to anti-Tat, Jurkat cells were infected with HIV-1_{IIIIB} and passaged weekly over 20 weeks in the presence of anti-Tat. p24 levels were determined weekly. IC₅₀ was determined on viral stocks obtained after 0, 10, 15 and 20 passages.

Results: Anti-Tat antibodies bound their target with K_Ds around 10⁻⁸ to 10⁻¹² M, as measured by surface plasmon resonance. In HIV-infected Jurkat cells viral replication or HIV-1 p24 expression was inhibited dose dependently with IC₅₀ values of 0.2 to 0.6 µg/mL. IC₅₀ values from 20-week passages show no reduction in viral sensitivity to anti-Tat suppression.

Conclusions: Anti-Tat may be a potential therapy for HIV-1 infection, with little likelihood of the development of drug resistance.

Introduction

Tat exerts its central role in HIV-replication through both intracellular and extracellular activities. Intracellularly Tat binds to the TAR region of the HIV LTR and facilitates transcription of the viral genome. Tat is also exported from the infected cell and influences neighbouring cells in several ways leading to an increased susceptibility for HIV infection and subsequent enhanced HIV replication. Tat is also immunosuppressive contributing to a defective immune response against HIV, thereby facilitating HIV spread further. Clinically, antibodies to Tat have been implicated in the control of HIV-replication and spread, since a state of non-progression correlates to high levels of anti-Tat antibodies and viral load is conversely related to anti-Tat antibody levels.

Previously, two highly conserved epitopes in the Tat molecule have been identified. Analysis of more than 1600 Tat sequences from a variety of isolates from Caucasian, Asian and African subgroups have demonstrated that a very limited sequence variability covers more than 90 % of the identified sequences. The existence of conserved sequences in these epitopes together with the fact that there is no direct link between the genotype of a potentially mutated Tat and the genotype of a HIV particle in a neighbouring cell whose replication is aided by the potentially mutated Tat suggests that resistance to a Tat specific drug with the ability to control HIV replication is less likely to occur. A therapeutic drug able to inhibit viral replication and with low propensity to allow development of resistance would be of great value in the treatment of HIV infected individuals.

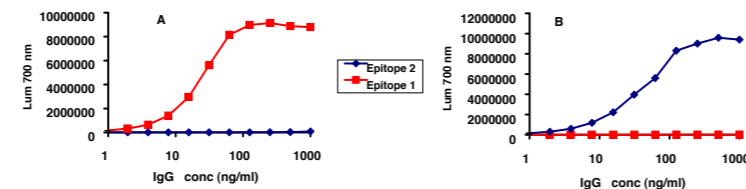
Utilising the n-CoDeR phage display, fully human antibody fragment library we have generated antibodies specific for the two conserved epitopes that bind to the most frequent sequence variants. Analysis of the antibodies has demonstrated their specificity and ability to inhibit replication of HIV with different Tat sequences *in vitro* in a dose dependent and reproducible manner. Importantly, no evidence of development of resistance to the antibodies could be detected over 20 passages *in vitro*. The data obtained support fully human anti-Tat antibodies directed to conserved epitopes and with their long plasma half-life as an interesting possibility to suppress HIV-replication and control viral spread hindering development of AIDS.

Results

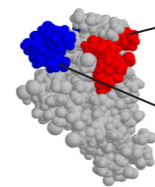
1. High affinity human antibodies against conserved epitopes in Tat

High affinity human IgG₁ antibodies specific for the conserved Tat epitopes were developed from the n-CoDeR library after three rounds of selection, subsequent screening and transfer to the full length antibody format. The antibodies were tested for binding to peptides covering the epitopes in an ELISA format and using Biacore to estimate dissociation constants to identified epitope sequence variants covering more than 90% of the known variants.

Binding of the Epitope 1 specific E1-4-E9 (A) and the Epitope 2 specific E2-28-F3 (B) to Epitope 1 and Epitope 2 peptides



NMR model (from Bayer et al. (1995) JMB 247, 529-535) depicting the two conserved epitopes in Tat Epitope 1, aa 4-10 (red) and Epitope 2, aa 41-50 (blue)

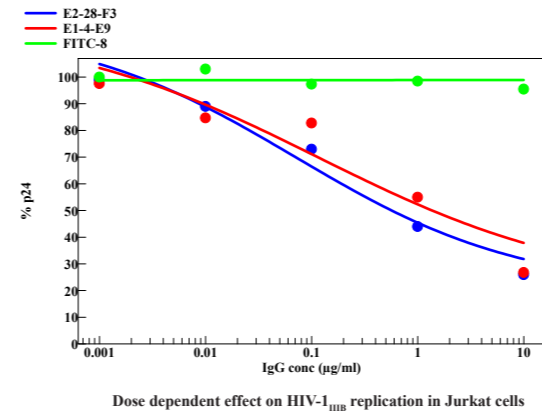


Anti-Tat MAb	Sequence variability	K _D (M)
E1-4-E9	⁴ VDPRL ¹⁰ E ¹⁰	9.5 × 10 ⁻¹²
	⁴ VDPKLE ¹⁰	1.0 × 10 ⁻¹¹
	⁴ VDPSE ¹⁰ LE ¹⁰	1.4 × 10 ⁻¹¹
	⁴ VDPNLE ¹⁰	1.4 × 10 ⁻¹¹
	⁴ VDPRLD ¹⁰	1.2 × 10 ⁻¹¹
	⁴ VDPKLD ¹⁰	9.0 × 10 ⁻¹²
E2-28-F3	⁴ VDPNLD ¹⁰	4.3 × 10 ⁻¹¹
	⁴ VDPSELD ¹⁰	6.9 × 10 ⁻¹²
	⁴ KALGISYGRK ²⁰	1.2 × 10 ⁻⁸
	⁴ KGLGISYGRK ²⁰	1.3 × 10 ⁻⁸

Affinities of the Tat-specific antibodies for variant Tat-epitope 1 and 2 demonstrating high affinities for all variants

2. Anti-Tat antibodies inhibit HIV-replication *in vitro*

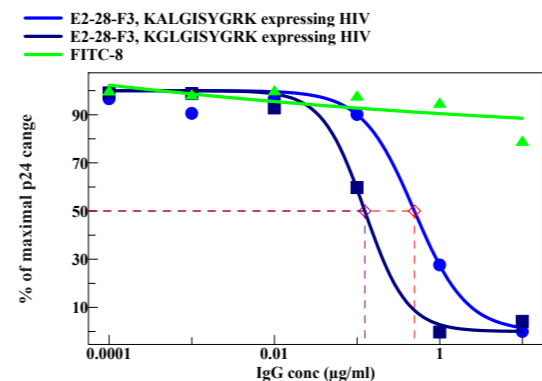
The ability of the antibodies to inhibit viral replication was tested *in vitro* using the Jurkat T cell line infected with HIV-1_{IIIIB}. The antibodies were found to inhibit viral replication as assessed by p24 levels in the tissue culture supernatants in a dose dependent manner. Maximal inhibition was reached at 10 µg/mL and IC₅₀ values are calculated as 50% of maximum inhibition. Despite the lower affinity the antibody to Epitope 2 had a somewhat lower IC₅₀ (0.2 µg/mL) as compared to the antibody directed against Epitope 1 (0.3 µg/mL). This probably indicates that epitope in or around aa 41-50 has a more important biological function in relation to viral spread and replication. A human control antibody of the same isotype as the anti-Tat antibodies and directed to FITC was without effect. Similar results were obtained using human PBMC.



	IC ₅₀ (µg/ml)
E2-28-F3	0.16
E1-4-E9	0.32

3. Similar inhibition of recombinant virus differing in Epitope 2 sequence

The E2-28-F3 antibody bound to peptides expressing an A to G variation in position 42 equally well. Together these two variants are represented in more than 90 % of all isolates of the more than 1600 found in databases and analysed. Replication *in vitro* of recombinant viruses comprising either of the two sequence variants were inhibited similarly with the anti-Tat antibody demonstrating that variant Tat produced from cells in culture could be targeted equally well by the antibody.



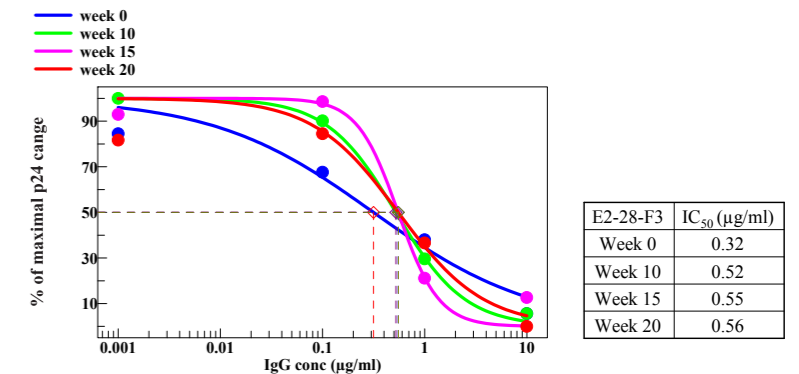
E2-28-F3	IC ₅₀ (µg/ml)
KALGISYGRK	0.49
KGLGIYGRK	0.12

4. No selection of resistant variants after 20 passages under the pressure of anti-Tat

The fact that the targeted epitopes are conserved in functional Tat, together with the mode of action of extracellular Tat, produced by one infected cell and acting on other neighbouring cells, suggest that resistance towards an antibody with anti-viral effects and binding to such an epitope should not easily develop.

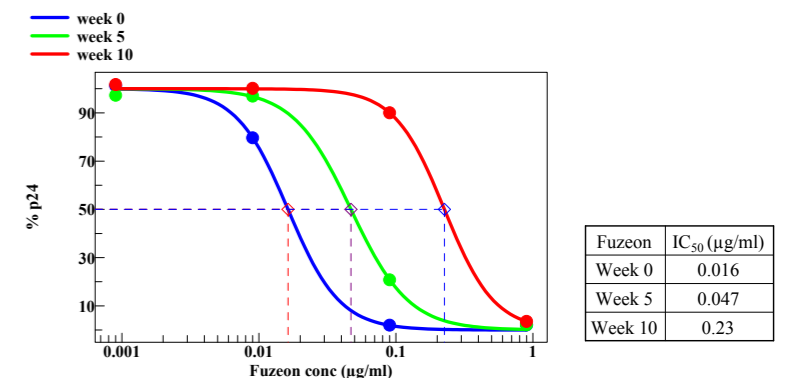
Jurkat cells were infected with HIV-1_{IIIIB} and cultivated under the pressure of increasing amounts of the E2-28-F3 antibody for 20 passages. Virus replication was inhibited to a similar extent over all passages and no change in IC₅₀ could be discerned comparing virus at the onset of the culture period with that at the end of it.

NO RESISTANCE OVER 20 WEEKS



E2-28-F3	IC ₅₀ (µg/ml)
Week 0	0.32
Week 10	0.52
Week 15	0.55
Week 20	0.56

Resistance to a control substance (Fuzeon) developed as early as after five passages and was marked after ten passages.



Fuzeon	IC ₅₀ (µg/ml)
Week 0	0.016
Week 5	0.047
Week 10	0.23

Viruses that had developed resistance to Fuzeon retained their sensitivity to the anti-Tat antibody since they showed the same IC₅₀ as the wild type virus (not shown).