

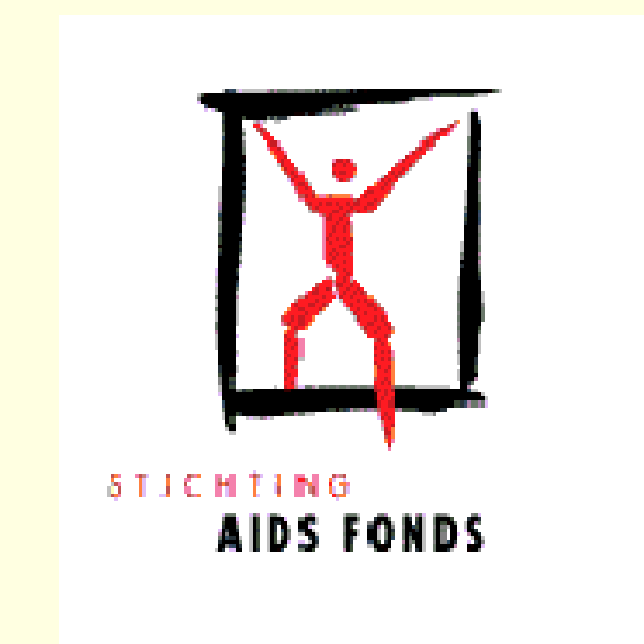
In vivo evolution of X4 HIV-1 variants in the natural course of infection coincides with reduced sensitivity to CXCR4 antagonists

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Introduction

- Syncytium inducing, CXCR4-utilizing HIV-1 variants develop in appr. half of the infected individuals in the course of infection. These variants are associated with an enhanced decline of CD4+ T cells.
 - Previously we showed that early after their emergence in vivo, CXCR4-utilizing variants evolve from a R5X4 phenotype (able to use both CCR5 and CXCR4) into a CXCR4-restricted phenotype (X4), concurrent with an ongoing genetic evolution of the gp120 V3 region (Van Rij et al, JCI 2000).
 - Antagonists to chemokine receptor CXCR4 are potent inhibitors of entry of CXCR4-utilizing HIV-1 variants and are considered for use as therapeutic drugs.

Aim of this study

- To study the sensitivity of evolving X4 HIV-1 variants to inhibition with CXCR4-antagonists.

Viruses and compounds

- Biological virus clones from two participants of the Amsterdam Cohort on HIV-1 infection and AIDS (ACH039 and ACH208).
 - From each patient, two R5X4 HIV-1 clones isolated early after the emergence of CXCR4-utilizing variants in vivo, and two X4 variants isolated three to four years later were analysed (table 1).
 - All clones from patient ACH039 additionally used CCR3.
 - Sensitivity to entry inhibitors was tested on the MT2 T cell line, PBMC from a healthy donor who is homozygous for CCR5 Δ 32 (Δ/Δ), and pooled PBMC from two healthy donors who are homozygous for the normal non-deleted CCR5 gene (+/+).
 - Entry inhibitors that were used: CXCR4 antagonists AMD3100 (Schols et al, J Exp Med 1997) and T22 (Murakami, J Exp Med 1997); a panel of CXCR4-directed monoclonal antibodies (mAbs); a pool of MIP-1 α , MIP-1 β and RANTES at a 1:1:1-ratio, and the gp41-derived C34 peptide.

Table 1. Biological characteristics and IC₅₀ values of biological CXCR4-utilizing HIV-1 clones used in this study.

Patient	Clone	Time after seroconversion (months)	Time after emergence of X4 variants (months)	Coreceptor usage ^a	AMD3100 (ng/ml)		T22 (ng/ml)		β -chem (ng/ml)	C34 (nM)	
					MT2	PBL +/-	PBL Δ/Δ	PBL +/-			PBL Δ/Δ
ACH039	20B10	18.2	2.1	R3R5X4	4.0	21.6	8.4	808.0	223.2	>2000	15.5
	20C6	18.2	2.1	R3R5X4	7.4	12.6	7.6	345.4	215.5	>2000	21.2
	X1C4	51.2	35	R3X4	533.2	150.8	54.8	1095.8	494.5	>2000	3.3
	X1H4	51.2	35	R3X4	328.5	184.6	33.0	1456.7	703.4	>2000	17.4
ACH208	12F4	16.5	2.5	R5X4	13.7	7.5	5.7	212.9	62.0	>2000	NT
	13B1	20.4	6.4	R5X4	29.2	23.0	7.8	456.7	220.2	>2000	9.1
	X1A1	61.3	47.3	X4	>2000	>2000	644.6	1686.4	1057.4	>2000	4.2
	X1B1	61.3	47.3	X4	>2000	342.6	38.2	1202.5	542.6	>2000	5.4

PBL Δ/Δ , PBMC from CCR5 Δ homozygous donor; PBL +/-, PBMC from CCR5 wildtype homozygous donor; NT, not tested
^a Coreceptor usage was defined as detectable p24 production after inoculation of U87 cells transfected with CCR3, CCR5 or CXCR4, data available from previous study (van Rij et al, JCI 2000).

Results

1. Decreasing sensitivity to CXCR4 antagonists in MT2 cell line

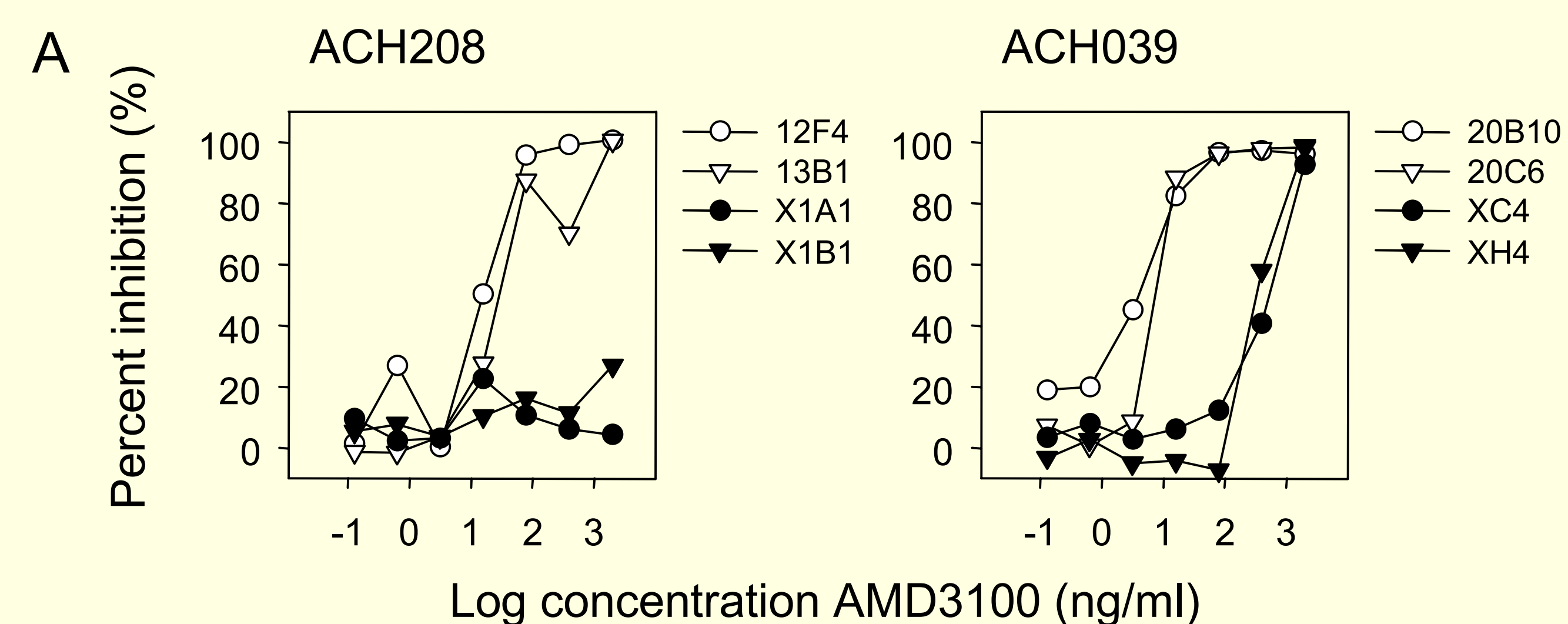
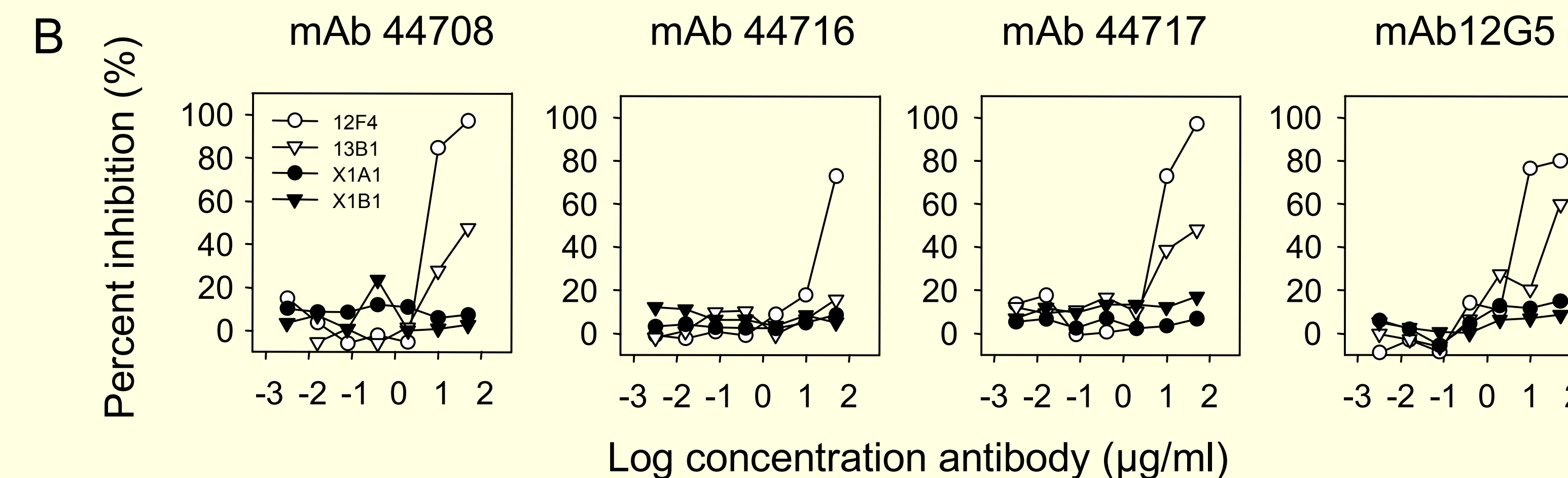


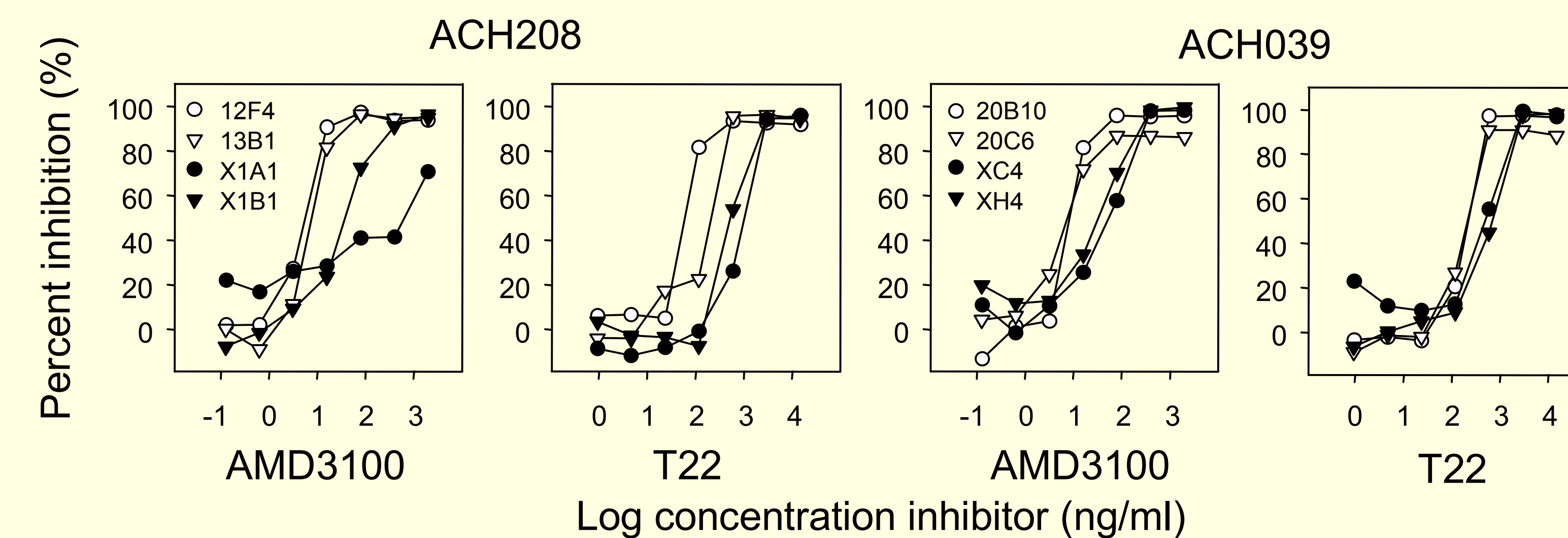
Fig. 1. Sensitivity of early R3R5X4 / R5X4 and late R3X4 / X4 HIV-1 virus clones to inhibition with **A.** AMD3100, and **B.** a panel of CXCR4-specific monoclonal antibodies. Percent inhibition relative to infections in the absence of inhibitor was calculated. Experiments were performed in triplo and average values are depicted. Open symbols indicate early R5X4 or R3R5X4 clones, filled symbols indicate late R3X4 or X4 clones.

Results

1. Decreasing sensitivity to CXCR4 antagonists in MT2 cell line



2. Decreasing sensitivity to CXCR4 antagonists in CCR5 Δ 32/ Δ 32 PBMC



Conclusions

> Early R5X4 clones are more sensitive to inhibition with AMD3100 than late X4 clones from both patients.
 For IC₅₀ values, see table 1.
 > Early R5X4 clones from ACH208 were inhibited by a panel of CXCR4-mAbs, whereas late X4 were not inhibited, even at the highest concentrations used (50 µg/ml).
 > None of the virus clones from ACH039 were inhibited by these antibodies.

Fig. 2. Sensitivity of early R3R5X4 / R5X4 and late R3X4 / X4 HIV-1 virus clones to inhibition with AMD3100 and T22 in CCR5 Δ/Δ PBMC, that lack CCR5 expression. Experiments and symbols as described under Fig. 1.

Conclusions

> Early R5X4 clones are more sensitive to inhibition with AMD3100 and T22 than late X4 clones from both patients in primary PBMC.

3. Decreasing sensitivity to CXCR4 antagonists and inability of R5X4 HIV-1 to use CCR5 in CCR5 +/- PBMC

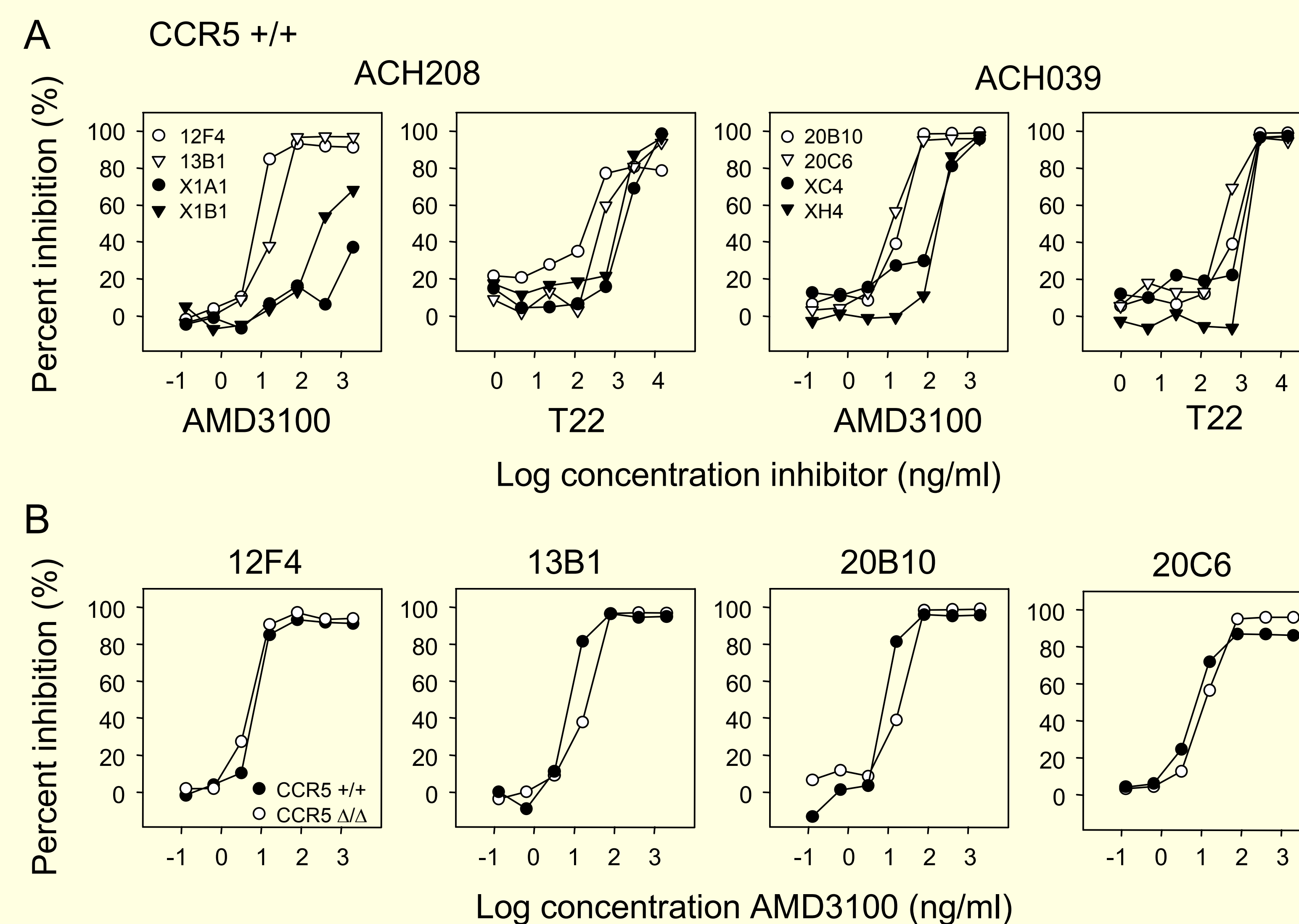


Fig. 3 A. Sensitivity of early R3R5X4 / R5X4 and late R3X4 / X4 HIV-1 virus clones to inhibition with AMD3100 and T22 in CCR5 +/- PBMC, that do express CCR5. Experiments and symbols as described under Fig. 1.

B. Comparison between neutralisation of R5X4 HIV-1 variants by AMD3100 on CCR5 +/- and CCR5 Δ 32/ Δ 32 PBMC. Open symbols, neutralisation on CCR5 Δ/Δ PBMC; filled symbols, neutralisation on +/- PBMC.

Conclusions

> Early R5X4 clones are more sensitive to inhibition with AMD3100 and T22 than late X4 clones from both patients in primary PBMC.
 > Complete inhibition of R5X4 variants on CCR5 +/- PBMC by CXCR4 specific antagonists.
 > Identical neutralisation profile of R5X4 variants on CCR5 Δ/Δ PBMC and on CCR5 +/- PBMC.

4. No inhibition of early R5X4 and late X4 variants by β -chemokine mix; No difference in sensitivity to C34-peptide (see table 1)

Conclusions

- Ongoing evolution of X4 variants in the natural course of infection coincides with reduced sensitivity to a panel of CXCR4-antagonists.
 No effect of C34-peptide, which acts downstream of coreceptor binding
 - Increased affinity for CXCR4?
 - IC₅₀ values of late X4 variants similar to in vitro generated 'AMD3100-resistant' derivative of NL4-3 (Schols et al, J Virol 1998).
- Discrepancy between coreceptor usage in cell lines and in primary PBMC: R5X4 variants were unable to use CCR5 in vivo.
 - Coreceptor usage in cell lines should be confirmed on relevant cell types, using specific antagonists.