

Association of Effective Control of HIV-1 with Strong CTL Selection Pressure and a Highly Mutated Nef in a HIV-1-Infected Patient.

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What are the conclusions from the STEP trial ?

(= the failed preventive vaccine trial using an AD5-vector to induce HIV-1-specific T-cells)

1. There will never be a HIV-1 vaccine ?

2. We have to learn more about the correlates of protection to make better vaccines !



Viruses of the Immune System
Friedrich-Alexander-Universität Erlangen-Nürnberg
in Cooperation with Harvard Medical School
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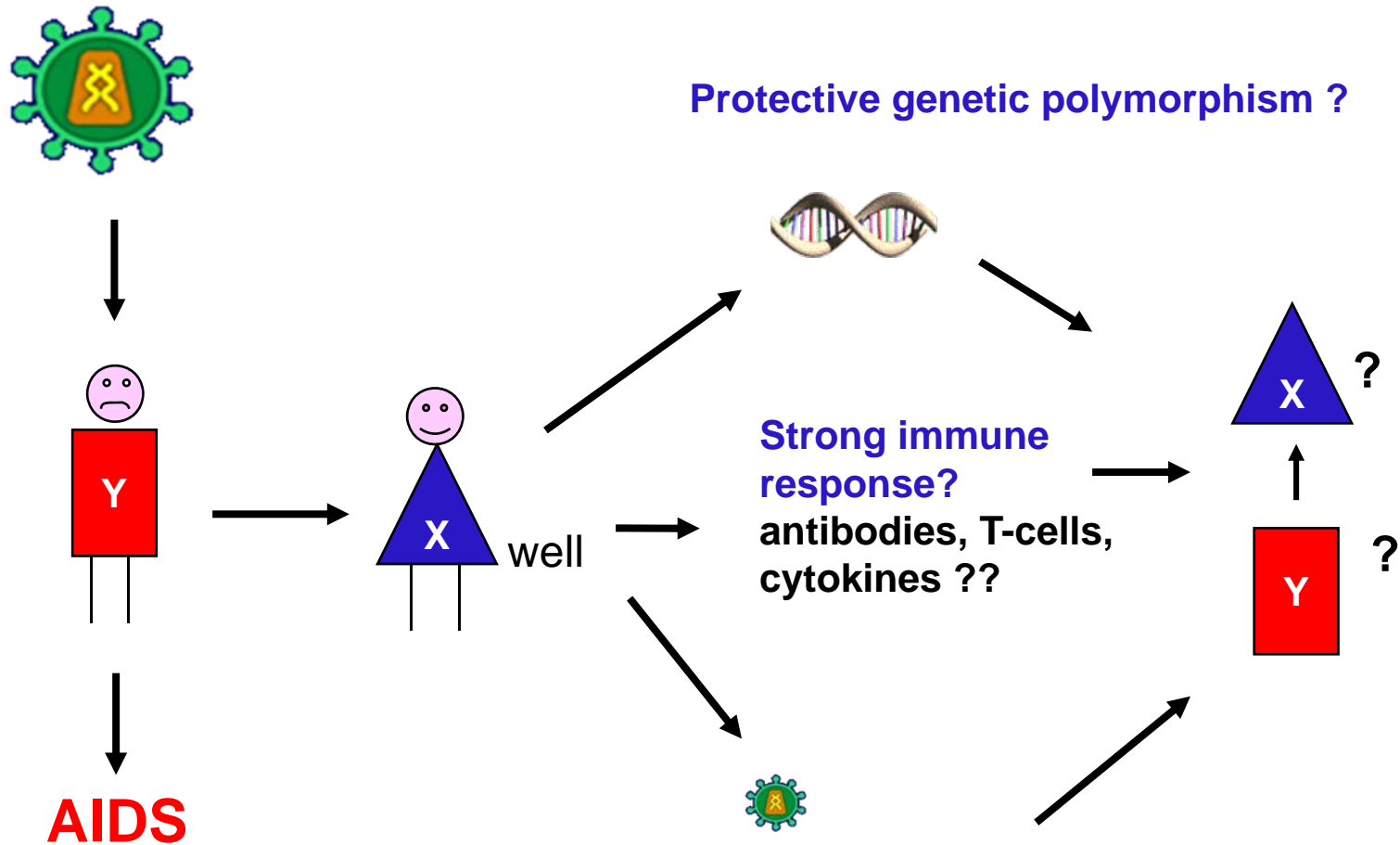
Discordant outcome in a couple infected by the same HIV-1 strain

Patient:	X (female)	Y (male)
First diagnosis:	2006 *	2006 *
Time of infection	between 1993 and 2006	presumably 1993
Clinical symptoms	none	pancytopenia, BK-virus cystitis
CD4 (cells/ μ l):	623 (687 2009) *	26
CD4/CD8:	1.3 (0.7)	0.1
Viral load (cop/ml):	< 50 (590 2009) *	240.000

* No therapy

- Heterosexual couple: female (X) was infected by her partner (Y)
- while male progressed to AIDS, female shows perfect control of viremia and normal CD4 counts

Correlates of Protection?



Less virulent viral strain ?

Attenuation of HIV-1
by immune selection ?

Virological Analyses of HIV-1 in Patient X.



- Initial viral load < 50 copies /ml (after 38 months 590 copies/ml)
- Detection of HIV-1 proviral DNA in PBMC
- Co-culture of CD8 depleted PBMC from **patient X** / **patient Y** with uninfected CD8 depleted PHA-blasts

p24 ELISA day 14: **X 3500pg/ml**; **Y: 1029pg/ml**

(PBMC from 2 healthy donors could be infected with supernatant from co-culture)

➔ **=> viruses from X do replicate and are infectious**

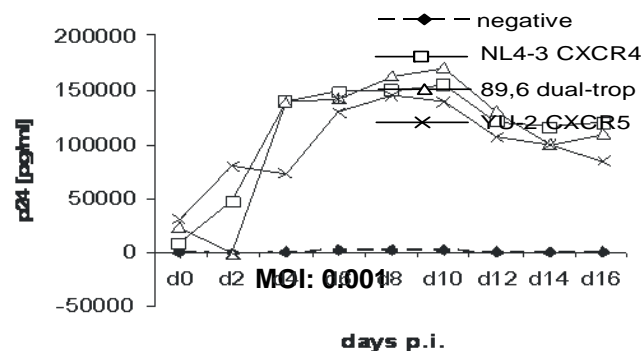
this argues against defects in replication capacity as a reason for low viremia

Are genetic Host factors the reason for low HIV-1 viral load ?

- **CCR5:** normal expression, no d32-Deletion
- **Apobec 3G:** no evidence for APOBEC-induced G to A Hypermethylation
 - » Sequencing of Apobec 3G (Q279E mutation, no influence on activity)
 - » Analysis of mutation pattern
 - » Analysis of mutations of viruses passaged through CD4-cells from X

- **Infection of CD8-depleted PBMC by various HIV-1 strains**

- 89.6: CXCR4/ CCR5 (dual-trop)
- NL43: CXCR4 (T)
- YU2: CXCR5 (M)
- All strains infect PBMC from patient X and replicate well
- This argues against a genetic polymorphism inhibiting viral replication

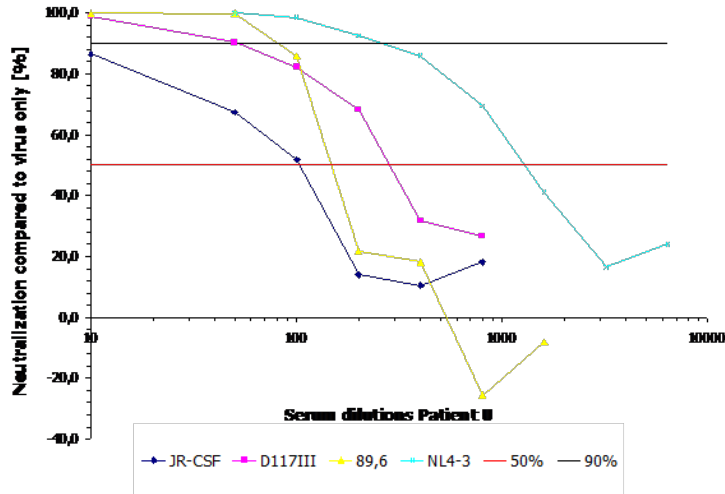


- **Analysis of antiviral cytokines (Levy Factor)**

- No inhibition of NL4-3 by supernatants from PHA-stimulated PBMC from X

- **HLA type:** no long-term non-progressor alleles

Cooperation Sascha Antoni, Frankfurt: Neutralizing capacity of Serum Patient X

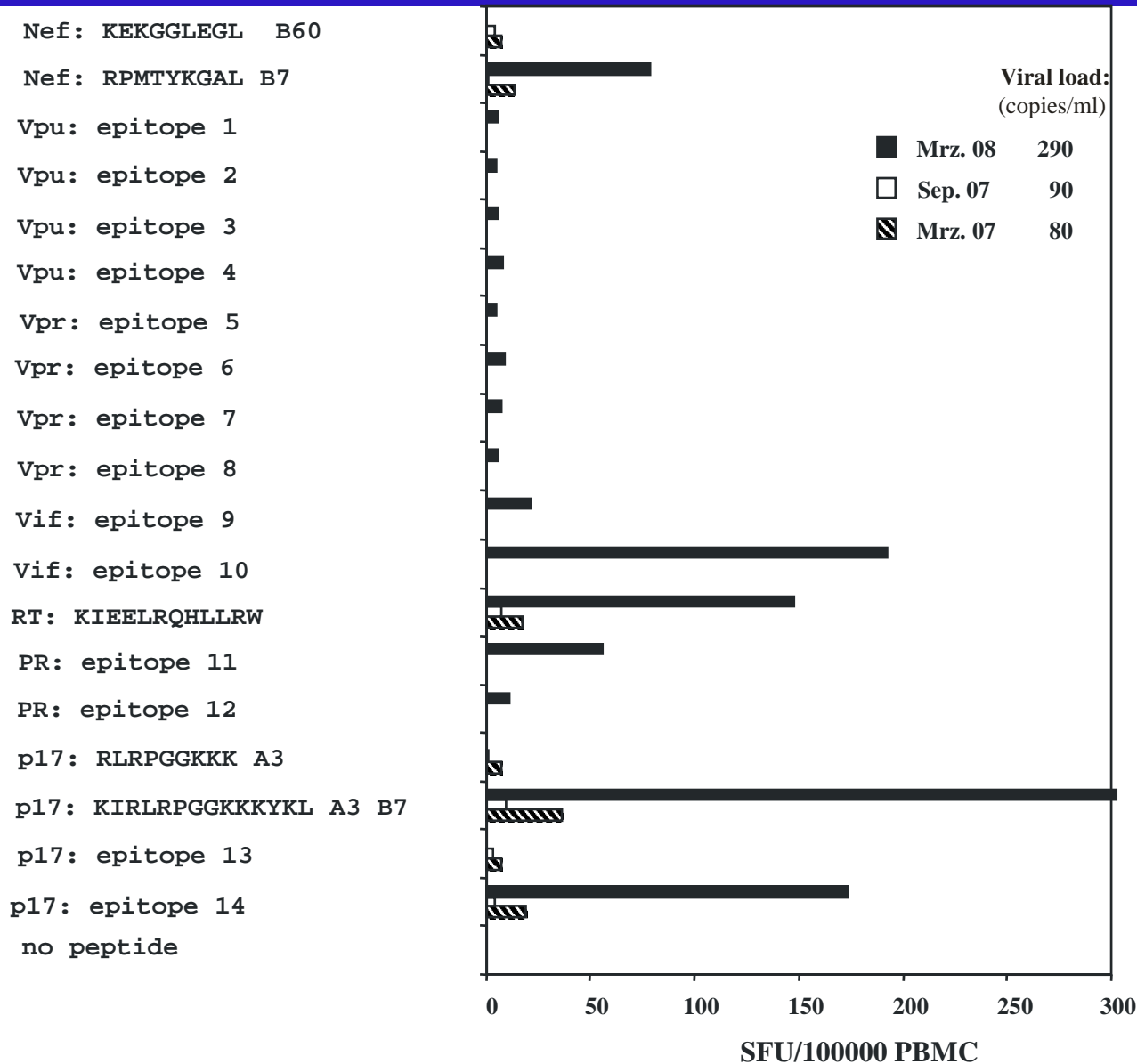


Mean neutralization 90 %	JR-CSF (CCR5)	D117III (CCR5)	89.6 (dual-trop)	NL4-3 (CXCR4)
Long Term Non Progressors	1:27	1:225	1:66	1:674
Progressors	1:13	1:46	1:43	1:209
Patient X	1:<10	1:32	1:66	1:219

[Humbert et al., 2007 Eur.J.Immunol]

➔ Patient X: Good but no extraordinary neutralizing antibody response

Strong CTL responses detected by g-IFN-ELISPOT



Patient X: **301 peptides**
 (Nef, Gag, Pol, Vif, Vpu, Vpr, Tat, Rev, gp120)

In addition
CD4 responses against Nef

Functional Analysis of Nef alleles from patients X and R: Jan Schmökel and Frank Kirchhoff

No Effects of U's Nef allele on

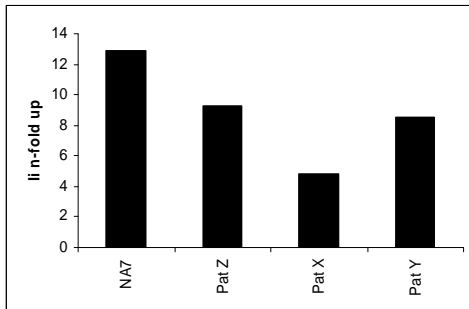
Downregulation of MHC-I, CD3, CD28 and CXCR4

Expression of CD69 and CD25

Induction of apoptosis in PBMC

Lower upregulation of Li (Invariant Light Chain of HLA class II: CD74) by Nef from U in comparison to X, another patient with AIDS: Z and HIV-1 NA7

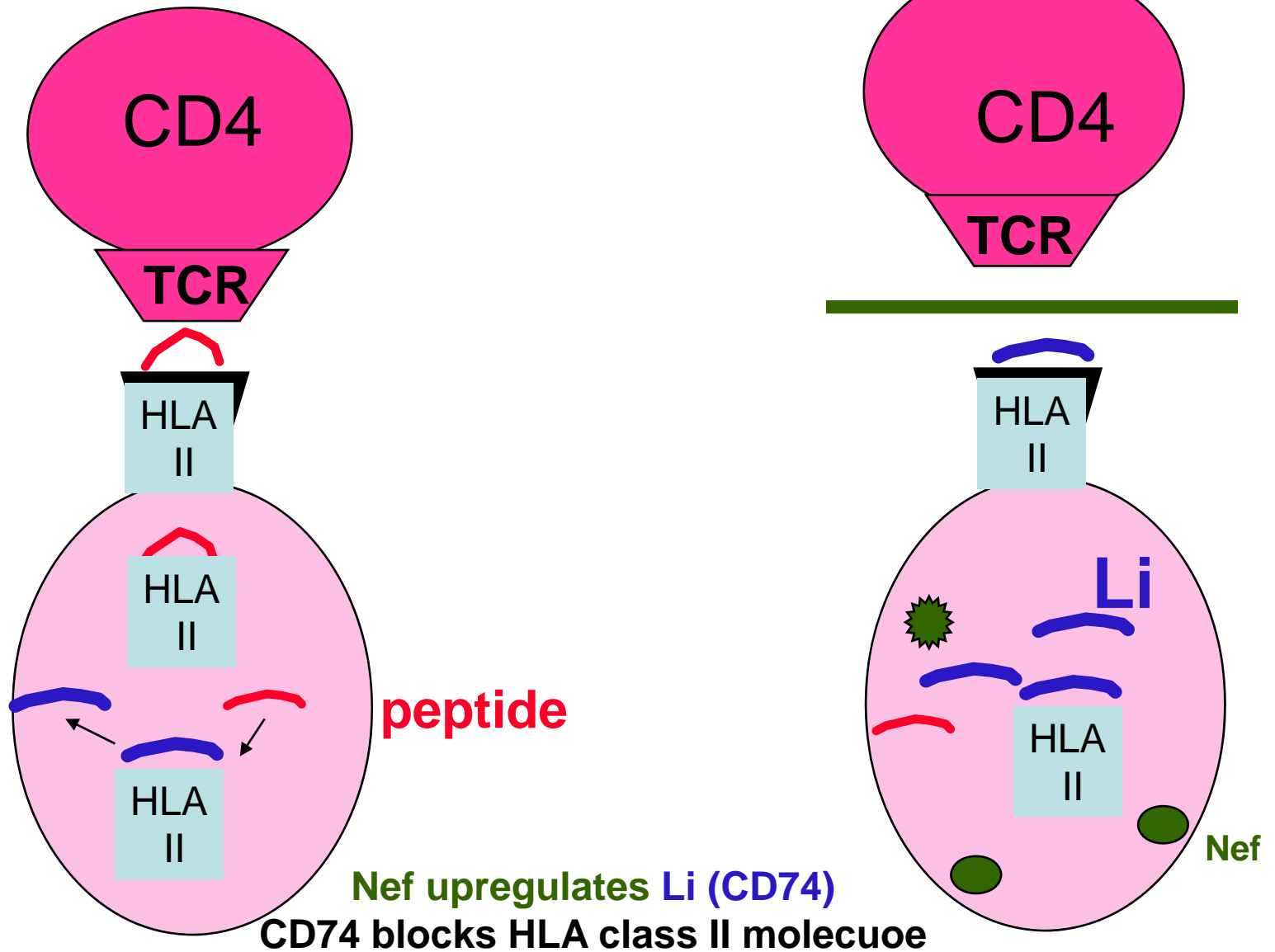
Li (CD74) upregulation in THP, 3dpi



Upregulation of CD74 by nef blocks presentation of peptides by HLA class II,

Inhibition of presentation of epitopes to CD4+ T-cells

Inhibition of HIV-1-specific CD4-response !



Dendritic cell, Monocyte, B-cell

Summary

- Low viremia not due to defects in viral replication capacity or polymorphic host genes
- Good but no extraordinary neutralizing antibody response
- Strong CTL response
- mutated viruses especially in Nef, with deletion in Nef
- Good evidence that mutations and the Nef deletion was selected by CTL
- Lower upregulation of CD74 could attenuate immune escape functions
- **This indicates an important role of CD74 mediated inhibition of HIV-1-specific CD4 responses**
- **Our data support the concept of CTL-mediated control of HIV-1, at least in subgroups of patients.**

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