Rapid Construction of HIV-1-Specific T Cell Receptor Gene Therapy Lentiviral Vectors

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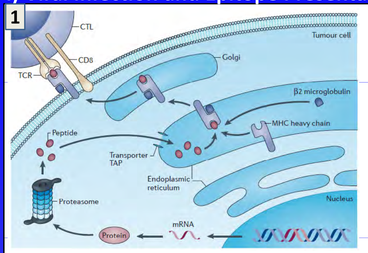
[*]Contributed equally

Abstract Number:365

Background

I) Viral Infection and Epitope Presentation on HLA

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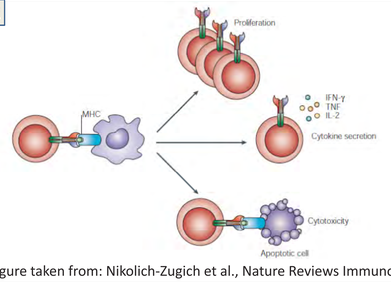


Cellular proteins, incl. viral proteins, are cut into smaller fragments by the proteasome. These peptides are transported into the ER and loaded on the HLA-class I molecule. Subsequently the pHLA complex is brought to the surface where it is recognized by the TCR of CD8+ T cells

Figure taken from: Coulie, 2014 Nature Reviews Cancer

II) Cytotoxic T Lymphocytes (CTL)

2



CTL recognize viral peptides in the context of HLA-I via their TCR. After recognition of the viral peptide, CTL start to proliferate, secrete cytokines, and kill infected cells through perforin and granzyme

Figure taken from: Nikolich-Zugich et al., Nature Reviews Immunology 2004

III) T Cell Receptor (TCR)

3

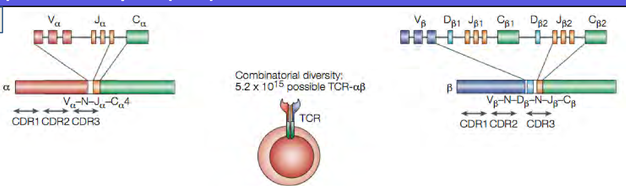


Figure taken from: Nikolich-Zugich et al., Nature Reviews Immunology 2004

TCR diversity is caused by somatic recombination, which leads to unique TCR sequences. It is estimated that after thymic selection, humans have a TCR repertoire of about $2x10^7$

IV) Each TCR has a distinct peptide recognition profile

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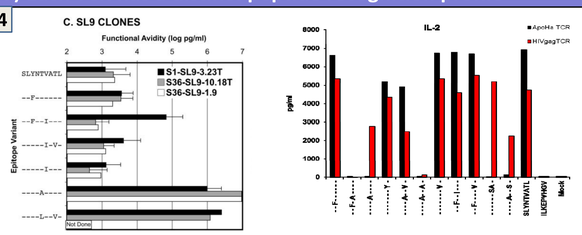


Figure taken from Bennett. 2007 Journal of Virology and unpublished data

Peptide recognition profile of two different CTL clones (left figure) and TCR reprogrammed CD8 T cells (right figure) specific for the SL9 epitope presented in HLA-A*02 is depicted. Data are showing that each TCR has a specific epitope variant recognition pattern.

V) Adoptive T cell Therapy

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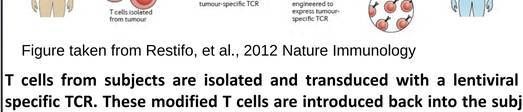
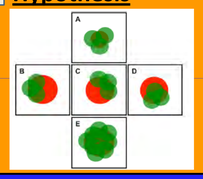


Figure taken from Restifo, et al., 2012 Nature Immunology

T cells from subjects are isolated and transduced with a lentiviral vector containing a specific TCR. These modified T cells are introduced back into the subject's system to clear virus infected.

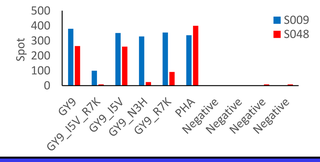
Methods and Results

6 Hypothesis



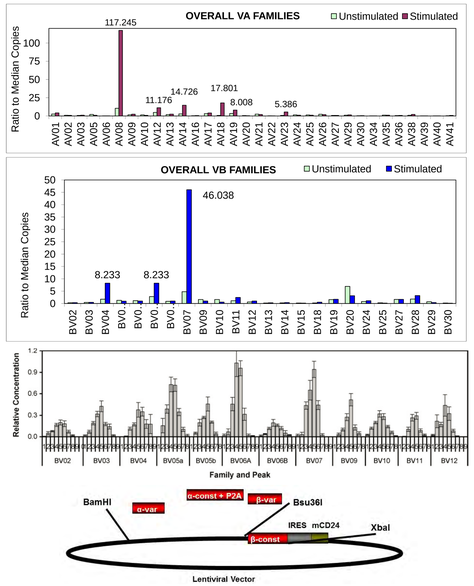
Combining TCRs against the same HIV-1 epitope from multiple people could prevent viral escape For a given epitope, the white box represents all mutants generated *in vivo*. The red circle is the subset of mutants with limited impact on viral fitness. Each green circle is the subset recognized by a single TCR. Thus escape is indicated by uncovered red: a "hole" for escape to occur. **A.** For highly conserved epitopes (red circle is small), e.g. B*27, and B*13 epitopes, escape is easily limited in most persons. **B-D.** For most epitopes, the TCR breadth in a person is insufficient to cover viable epitope variants, allowing escape. **E.** It may be possible to combine TCR from different persons to span epitope variation.

7a Screening for TCRs Recognizing the Epitope of Interest



PBMCs from two different subjects (S009 and S048) were screened by IFNγ-ELISPOT for response against the HLA-B*1501 GY9 epitope and its variants. IFNγ responses indicate the presence of CTL against the specific epitope of interest

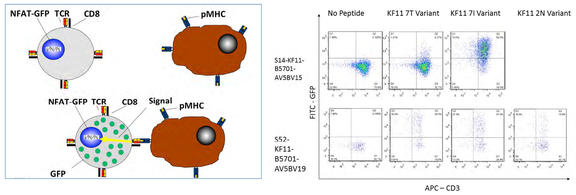
8a Identifying and Cloning of the TCRs



Identification of TCRs:

1. One set of PBMCs are stimulated for one week with peptide (stimulated) and the other one without peptide (unstimulated)
2. $CD8^+$ T cells are isolated, RNA is purified and transcribed into cDNA
3. TCR identification is done by quantitative spectratyping. A specific set of TCR primers binding in the variable domain are used to amplify the TCR α- and TCR β-chains in a real-time PCR.
4. The amounts of copies of different variable regions are compared. An increase of copies from the variable domain between unstimulated vs. stimulated $CD8^+$ T cells indicates the usage of a certain variable domain against the given epitope (a real-time PCR of subject S048 is shown. The TCR TRAV8 and TRBV7 are a match).
5. For a more in deep analysis of the TCR usage a fragment analysis is performed
6. TCR alpha and beta chains were cloned in a one-Step reaction via In-Fusion cloning into the lentiviral vector (TCR alpha variable domain, TCR alpha constant domain + P2A, TCR beta variable domain). The vector contains the codon-optimized TCR beta constant domain and a mCD24 reporter gene.

9a Functional Testing the Identified TCR in $CD8^+$ Jurkat T Cells



Lentivral vector encoding for the TCR of interest are transduced into Jurkat T cells expressing NFAT-GFP and the CD8 coreceptor. Jurkat T cells are stimulated with APCs loaded with the cognate peptide. Recognizing the cognate epitpe via the TCR leads to GFP expression (left panel). Flow data for a functional TCR is shown (right panel).

10a TCR successfully tested in $CD8^+$ Jurkat T Cells

Epitope/HLA	Subject	BV	AV
KF11/B*5701	S14	15*02	05*01
KF11/B*5701	S52	19*01	05*01
KF11/B*5701	S68	19*01	05*01
KF11/B*5701	S94	05*08	14/D4*02
WF9/B*3501	S81	02*01	02*02
KK10/B*2705	S48v3	BV7-9*03	AV8-4*03
KK10/B*2705	S48v3	BV4-3*01	AV14/DV4*02
KK10/B*2705	S76v1	BV27*01	AV14/DV4*01
KK10/B*2705	S76v1	BV20-1*01	AV14/DV4*01
KK10/B*2705	S76vcl	BV9*01	AV1-2*01
KK10/B*2705	GA	BV7-2	AV13
KK10/B*2705	GA	BV7-2	AV5
GY9/B*1501	S22v3	BV27*01	AV26-1*01
GY9/B*1501	S22v3	BV20-1*05	AV5*01
GY9/B*1501	S22v3	BV28*01	AV12-3*01
RI9/B*1302	S24vcl	BV7-9*03	AV38-2/DV8*01

Table shows the following TCR identified via the Jurkat T cell system.

- 4 TCRs against KF11/B*5701
- 1 TCR WF9/B*3501
- 7 TCRs KK10/B*2705
- 3 TCRs GY9/B*1501
- 1 TCR RI9/B*1302

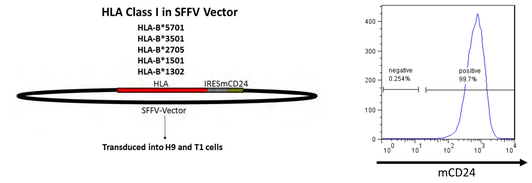


6b Screening for HLA Subtypes

HLA-B*5701 → 3 Subjects confirmed	
HLA-B*3501 → 10 Subjects confirmed	
HLA-B*2705 → 2 Subjects confirmed	
HLA-B*1501 → 9 Subjects confirmed	
HLA-B*1302 → 4 Subjects confirmed	

Immortalized B cells or PBMCs were used to screened via PCR and sequencing for the five HLA subtypes of interest (box).

7b Cloning and Transducing of HLAs into T1 and H9 cells



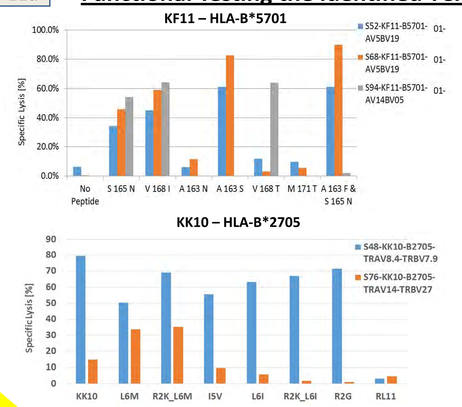
Identified HLAs (from 7a) were cloned into a lentiviral vector and transduced into the HIV-1 permissive cell lines T1 and H9 (left panel). Single cell dilution have been performed to obtain clonal populations with high expression of the HLA of interest. Indirect staining for the HLA, through the reporter gene mCD24, has been performed to verify the clonality.

8b Viral mutants of HIV-1 made to infect T1/H9 cells

HLA-B*5701	KAFSPEVIPMF	95.2%	HLA-B*3501	WASRELERF	96.9%
	KAF_PEVIPMF	0.9%		WASRELER_	0.6%
	KAFSPE_IPMF	0.6%		WA_RELERF	0.4%
	K_FSPEVIPMF	0.4%		WAS_ELERF	0.4%
	K_FSPEVIPMF	0.4%			
	KAFSPE_IPMF	0.4%			
	KAFSPEVIP_F	0.3%			
	K_F_PEVIPMF	0.3%			
HLA-B*2705	KRWIILGLNK	72.5%	HLA-B*1501	GLNKIVRMY	93.6%
	KRWIIMGLNK	10.7%		GLNKIVRMY	0.6%
	KRWIIMGLNK	3.3%		GLNKVVKMY	0.7%
	KRWIVLGLNK	1.2%		GLHKIVRMY	0.5%
	KRWIIGLNK	1%		GLNKVVRMY	0.4%
	KRWIILGLNK	0.8%		GLKKVVKMY	0.3%
	KRWIIGLNK	0.6%			
	KRWIILGLNK	0.5%			
	KRWIVMGLNK	0.2%			
HLA-B*1302	RQANFLGKI	83.5%			
	RQANFLGRI	2.7%			
	RQANFLGKV	1.84%			
	RQVNFLGKI	1.84%			
	RQANFLGKL	0.7%			
	GQANFLGKI	0.7%			
	KQANFLGKI	0.5%			
	RQANFLGRL	0.5%			
	KQANFLGRI	0.5%			

To produce virus with the epitope variant of interest, we mutated the epitope in the plasmid p83.2. To obtain NL4-3, p83-2 will be cotransfected with the vector p83-10 into T1 cells.

11a Functional Testing the Identified TCR in $CD8^+$ T cells



Preliminary data from TCR transduced into primary $CD8^+$ T cells. Upper panel epitope recognition pattern of 3 TCRs specific against KF11 presented in HLA-B*5701. Lower panel 2 TCRs specific for KK10 in HLA-B*2705. Target cells: T1 transduced with the corresponding HLA. Chromium release assay data are shown.

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