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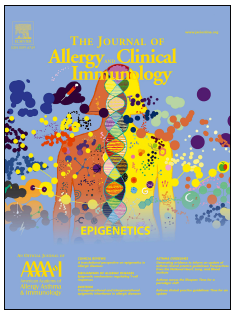

Virus-specific T cell clonotypes may contribute to drug hypersensitivity reactions via heterologous immunity

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Virus-specific T cell clonotypes may contribute to drug hypersensitivity reactions via heterologous immunity

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The authors report no conflict of interest.

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The data that support the findings of this study are available from the corresponding author upon request.

Capsule summary

We provide proof-of-principle evidence that pathogen-specific memory T cells can recognise drug altered peptide repertoire presented on autologous HLA. Results presented here may have major implications for the aetiology, prevention and management of drug HSR.

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To the editor:

Drug hypersensitivity (DHR) is a common immune mediated reaction which can be associated with severe illness including rash, hepatitis, DRESS/SJS and occasionally death. How sensitization occurs and the underlying immunological mechanism by which a drug can induce an immune response is unclear. One proposed theory is that of heterologous immunity whereby virus-specific T cells may cross-react against drug altered peptide repertoire presented on autologous HLA molecules. We provide proof-of-principle *in vitro* evidence that pre-existing virus-specific memory T cell clonotypes can recognise drug altered peptide repertoire presented on autologous HLA. Here, we show that that a human HIV Gag TW10/HLA-B57-specific T cell clone recognizes autologous HLA-B57, but only in the presence of abacavir. Results presented here are also the first to suggest that HIV-specific memory T cells may themselves participate in abacavir induced HSR via heterologous immunity.

Abacavir administration is associated with drug induced hypersensitivity reactions in HIV patients expressing the HLA-B*57:01 allele (1). It has been suggested that pre-existing naïve and memory T cells may contribute to this abacavir induced HLA-B57 reactivity (2). However, the contribution of virus-specific and in particular HIV-specific T cells to the systemic drug hypersensitivity reaction is unknown. We hypothesized that the abacavir-modified HLA-peptide repertoire (3) would induce *de-novo* self HLA-B57 specific recognition from pre-existing HIV-specific CD8 memory T cells.

Multiple HIV-specific TW10/HLA-B57 and KF11/HLA-B57 restricted CD8 T cell clones were generated from 4 different HIV seropositive, abacavir treatment naïve, individuals using single cell sorting based on HIV peptide/HLA tetrameric complex staining as previously described (4-5). A total of 16 unique TW10/B57 and 10 unique KF11/B57 restricted T cell clones were generated from the 4 different HLA-B*57:01 expressing patients (Supplementary table 1). To confirm clonality of the HIV-specific T-cell clones expression of the Vβ chain of the T cell receptors was determined

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using the TCR Vβ staining kit (Beckman Coulter) and by next generation sequencing (454 Roche), enabling the identification of alpha chain (V and J) and beta chain (V, D and J) CDR3 regions, and was performed using primer sequences described in Han et al (6).

To investigate the ability of the HIV-specific CD8 memory T cells to mediate abacavir induced self-reactivity, the HLA-B*57:01 restricted HIV GagTW10/HLA-B57 and KF11/HLA-B57 specific T cell clones were co-cultured with K562 cell lines expressing a single HLA-B*57:01 molecule (single antigen cell lines [SALs]) in the presence of abacavir or medium only. All clones were also tested against Single Antigen Lines expressing 27 other frequently occurring individual HLA-A and –B molecules (Supplementary table 2). In all experiments the cognate HIV peptide served as positive control. Cytokine assay and CD137 upregulation were used as readout, as previously described (4).

The Gag TW10/B*57:01 and KF11/B*57:01 restricted HIV-specific memory T-cell clones from abacavir treatment naïve individuals were different based on their different origin, cognate antigen or TCR TRBV usage. A HIV Gag TW10/HLA-B57 specific CD8 memory T cell (clone 457 from patient 2)(figure 1 and supplementary table 3) with TRBV10-3 usage recognised autologous HLA-B*57:01 but only in the presence of abacavir. Abacavir induced self-reactivity was associated with IFNγ production and CD137 upregulation by the HIV-specific memory T cell clone (Figure 2). Another TW10/HLA-B57 restricted T cell from the same patient, with the same TRBV usage gave identical crossreactivity. This provides evidence that pre-existing HIV-specific memory CD8 T cells can recognise abacavir altered peptide repertoire presented on autologous HLA-B57 molecule, in an abacavir treatment naïve individual. The abacavir induced autologous HLA-B57 reactivity by the HIV-specific T cell clone was specific to the HIV target peptide/HLA restriction <u>and</u> TCR TRBV usage (figure 1c and supplementary table 3).

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In this report, we provide proof-of-principle evidence that pre-existing virus-specific memory T cells can recognise a drug altered peptide repertoire presented on an autologous HLA molecule, a phenomenon termed heterologous immunity. These results suggest that drug induced peptide repertoire changes can directly activate pre-existing virus-specific CD8 memory T cells. Our in-vitro system is extremely informative as the HLA transfected target cell (K562 cell) expressed only the autologous HLA-B*57:01 molecule and the responder cell is a virus-specific memory CD8 T cell clonotype with known cognate antigen and TCR CDR3 sequence (figure 1c and supplementary table 3).

Virus-specific T cell responses are exquisitely peptide and self-HLA restricted. However, we and others have previously shown that many virus-specific memory T cells break the law of HLA restriction and directly recognise foreign HLA from unrelated (allogeneic) individuals in a peptide-specific manner (4-5,7). Specific allo-HLA crossreactivity has been shown for EBV, CMV, VZV, influenza A and HIV virus-specific T cells, and the crossreactivity is mediated by the same TCR.

We have now shown that virus-specific T cell clonotypes can potentially cross-react against a drug altered peptide repertoire presented on an autologous (self)-HLA molecule. Reactivity in our in-vitro model occurred in the absence of CD4 T cells, APCs and co-stimulatory cells, suggesting that the activation status of pre-existing memory CD8 T cells may indeed enable them to directly recognise drug altered peptide repertoire on autologous HLA. Furthermore, the migration patterns and tissue residence of memory CD8 T cells may make them uniquely positioned to mediate drug hypersensitivity reactions, as virus-specific T_{EM} and T_{RM} are preferentially located in epithelium of skin, intestinal tract and liver (8), which are primary sites of DHR pathology (supplementary figure 1). For example, Mueller and colleagues showed that in-vivo activated herpes simplex virus type I-specific CD8 T cells migrate to the skin where they interact with antigen presenting cells (8). We have confirmed that our HIV-specific T cell clones express a memory phenotype (CD45RA

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negative; data not shown). While we have only detected this phenomenon in a single HIV-specific T cell clone, we have only tested a very limited repertoire of TW10/B57 and KF11/B57 HIV-specific T cell clones from 4 HIV-seropositive individuals. Testing this phenomenon in a larger cohort including for other HIV epitopes or for other virus specificities will be important. Also tracking expansion and/or activation of TW10/B57 restricted T cells in PBMC samples from patients diagnosed with the abacavir HSR reaction would provide supportive information, and identifying the presence of crossreactive T cells in the skin of patients undergoing HSR would provide important in-vivo confirmation.

Therefore we propose a model whereby pathogen peptide/self-HLA restricted memory T cells may contribute to immunopathological disease in the setting of altered HLA (transplant rejection) or altered self-peptide repertoire (drug HSR), via heterologous immunity (Supplementary figure 1). Results presented here may have major clinical implications for investigation and management of drug HSR reactions.

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Figure and table legends

Figure 1. Generation of HIV-specific T cell clones. (A) TW10/HLA-B57 tetramer staining of peripheral blood mononuclear cells from patient 2. The identified HIV Gag TW10 specific T cell population then underwent single cell sorting into 96 well plates **(B)** HIV-specific T cell clone was generated using single cell sorting based on Gag TW10/HLA-B*57:01 tetramer staining. Clone 457 shown here. Clone 464 is identical to clone 457 and gave identical results. **(C)** TCR sequence of abacavir/HLA-B57 reactive clones. Clonality of HIV Gag TW10/B57-restricted CD8 memory T cell clones 457 and 464 was confirmed by single cell sorting and TCR sequencing. Both clones expressed identical TCR CDR3 and cross-reacted against autologous HLA-B57 only in the presence of abacavir.

Figure 2. A HIV Gag TW10/HLA-B57 restricted T cell clone recognizes autologous HLA-B57 only in the presence of abacavir.

(A and B) Abacavir exposure can induce *de-novo* recognition of autologous HLA-B57 by HIV-specific T cells. Experiments were performed neat (no drug) and in the presence of abacavir. Identical Gag TW10/HLA-B57 specific T cell clone 457 and 464, from an HLA-B*57:01 expressing HIV seropositive individual (patient 2), did recognize autologous HLA-B*57:01 only in the presence of abacavir, using IFNγ production and CD137 upregulation as readout. Abacavir did not induce recognition of any other autologous HLA molecules (supplementary table 2). Other clones from the same patient with the same specificity, but with different TCR TRBV usage did not recognize autologous HLA-B57 in the presence of abacavir (Supplementary table 3). Therefore exposure to a drug can induce de novo recognition of

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autologous (self) HLA molecules by virus-specific CD8 T cells. IFNγ experiments performed in duplicate. *** $p<0.001$.

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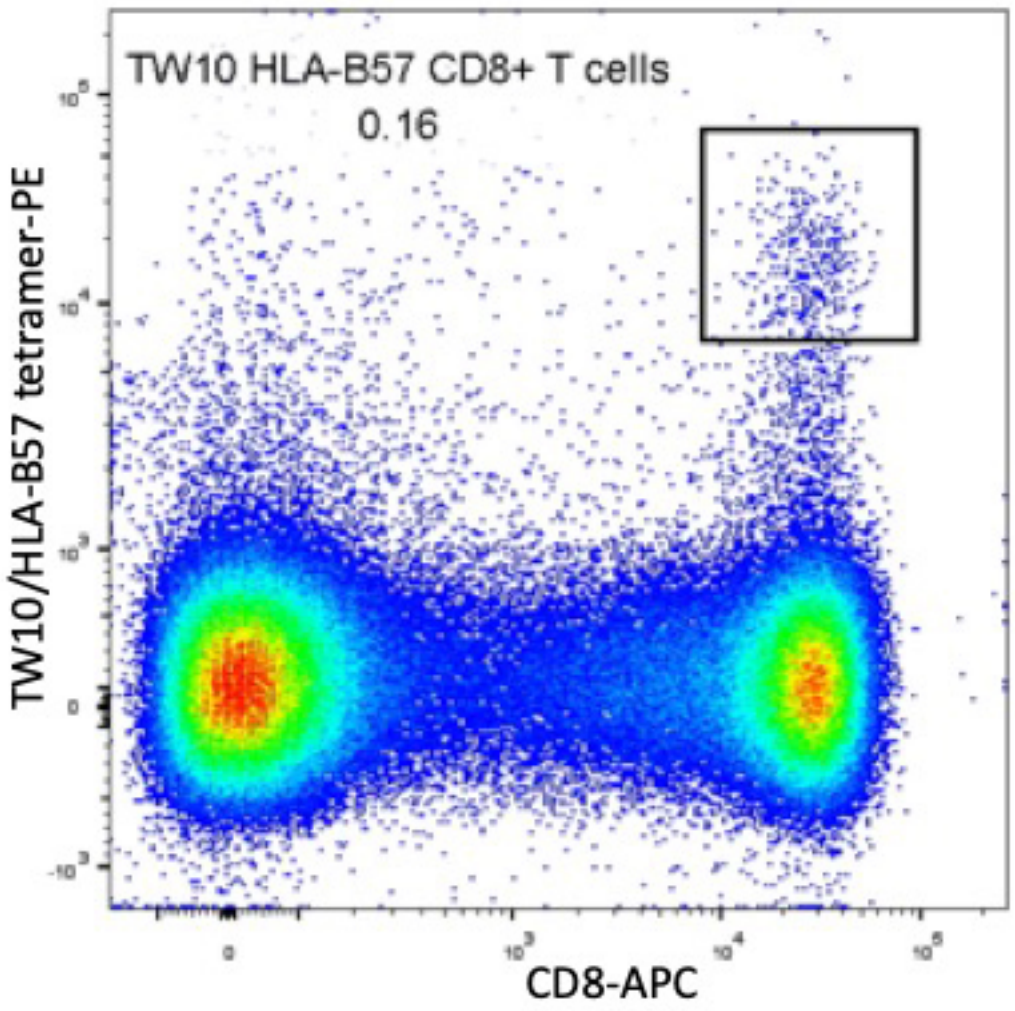

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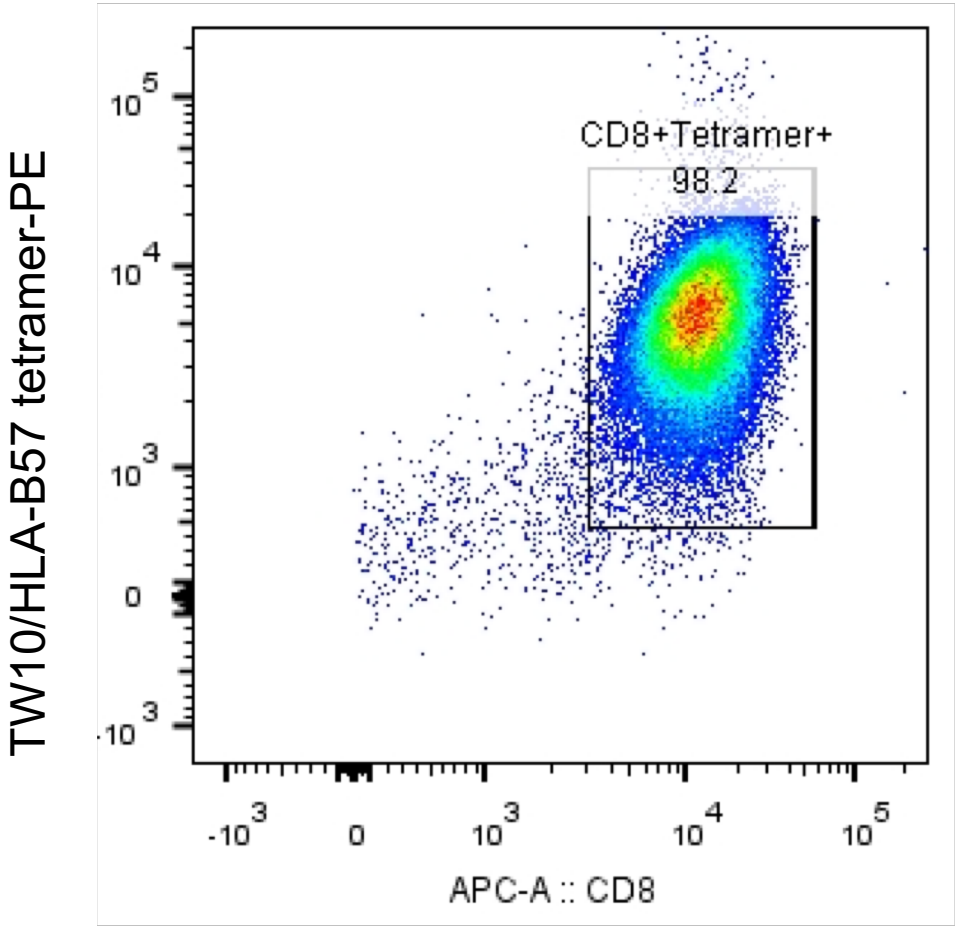
TW10/HLA-B57 tetramer-PE
CD8+Tetramer+
98.2
APC-A :: CD8

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Clone	Specificity	V	D	J	CDR3 AA	CDR3 DNA
457	HIV Gag TW10/HLA-B57	TRAV39*01		TRAJ40*01	CAVDINTSGTYKYIF	
		TRBV10-3	TRBD1*01	TRBJ2-5*01	CAISRQGARQETQYF	TGTGCCATCAGTAGACAGGGGGCGAGGCAAGAGACCCAGTACTTC
464	HIV Gag TW10/HLA-B57	TRAV39*01		TRAJ40*01	CAVDINTSGTYKYIF	
		TRBV10-3	TRBD1*01	TRBJ2-5*01	CAISRQGARQETQYF	TGTGCCATCAGTAGACAGGGGGCGAGGCAAGAGACCCAGTACTTC

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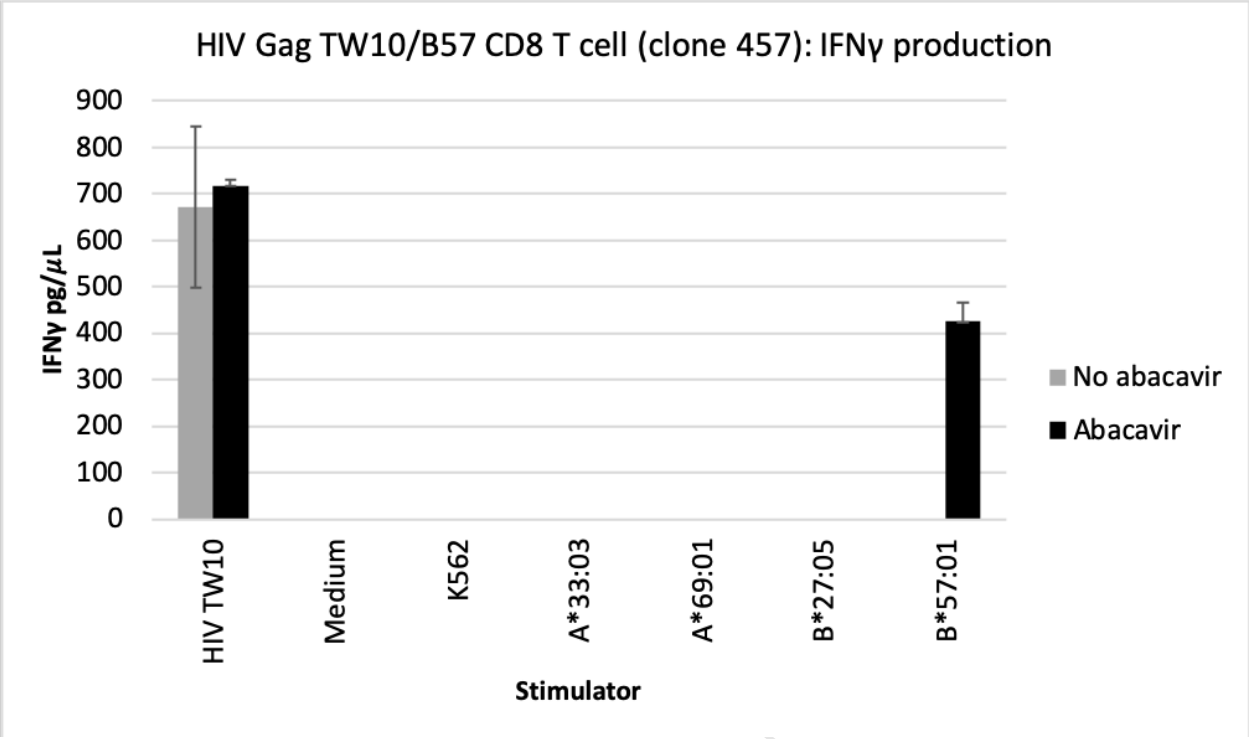

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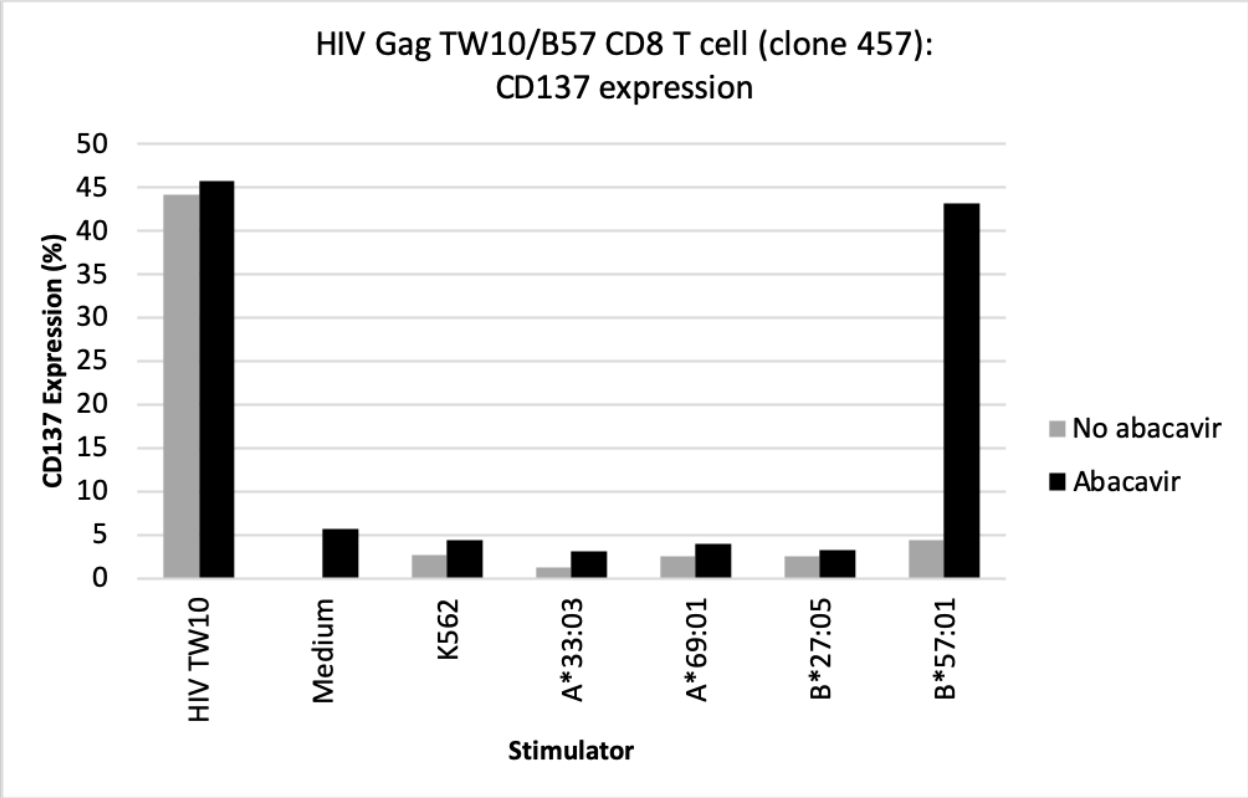

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Patient ID	HLA-A	HLA-A	HLA-B	HLA-B	HLA-C	HLA-C	HLA-DRB1	HLA-DRB1	HLA-DQB1	HLA-DQB1	HLA-DPB1	HLA-DPB1	Unique TW10/B57-restricted clones identified	Unique KF11/B57-restricted clones identified
1	02:01	26:01	45:01	57:01	06:02	06:02	07:01	15:01	03:03	06:02	04:01	23:01	1	3
2	01:01	03:01	08:01	57:01	06:02	07:01	03:01	04:04	02:01	03:02	01:01	04:01	5	0
3	03:01	32:01	14:01	57:01	06:02	08:02	07:01	13:01	02:02	06:02	04:BGDSB	05:BKHVM	8	3
4	02:01	03:01	35:01	57:01	04:01	06:02	04:04	07:01	03:03	03:ADAJH	03:01	04:01	2	4

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A locus	B locus
A*01:01	B*07:02
A*02:01	B*08:01
A*03:01	B*13:01
A*11:01	B*14:02
A*24:02	B*15:01
A*26:01	B*27:05
A*31:01	B*35:01
A*32:01	B*38:01
A*33:03	B*39:01
A*69:01	B*40:01
	B*40:02
	B*44:02
	B*44:03
	B*45:01
	B*46:01
	B*49:01
	B*55:01
	B*57:01

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Patient	Clone	V	D	J	CDR3 AA	CDR3 DNA	TW10/B57 restricted	Abacavir/B57 reactive
2	257	TRAV9-2		TRAJ8	CALNTGFQKLVF		Y	N
		TRBV14	TRBD1	TRBJ2-1	CASSPLNSGTGNEQFF	TGTGCCAGCAGCCCACTGAATAGTGGGACGGGCAATGAGCAGTTCTTC	Y	N
2	259	TRAV4		TRAJ42	CLVGNYGGSQGNLIF		Y	N
		TRBV20-1		TRBJ2-7	CSARQRDLSYEQYF	TGCAGTGCCCGTCAGCGGGATCTCTCCTACGAGCAGTACTTC	Y	N
2	262	TRAV4		TRAJ36	CLRATGANNLFF		Y	N
		TRBV20-1	TRBD1	TRBJ2-4	CSARAQGAAKNIQYF	TGCAGTGCTAGAGCACAGGGGGCGGCCAAAAACATTCAGTACTTC	Y	N
2	355	TRAV3		TRAJ4	CAVRDTGGYNKLIF		Y	N
		TRBV7-6	TRBD1	TRBJ2-3	CASSPTTGADTQYF	TGTGCCAGCAGTCCCACCACAGGGGCAGATACGCAGTATTTT	Y	N
2	457	TRAV39		TRAJ40	CAVDINTSGTYKYIF		Y	Y
		TRBV10-3		TRBJ2-5	CAISRQGARQETQYF	TGTGCCATCAGTAGACAGGGGGCGAGGCAAGAGACCCAGTACTTC	Y	Y
2	458	TRAV26-1		TRAJ39	CIVRFRSNAGNMLTF		Y	N
		TRBV14	TRBD1	TRBJ2-1	CASSPLNSGTGNEQFF	TGTGCCAGCAGCCCACTGAATAGTGGGACGGGCAATGAGCAGTTCTTC	Y	N
2	464	TRAV39		TRAJ40	CAVDINTSGTYKYIF		Y	Y
		TRBV10-3		TRBJ2-5	CAISRQGARQETQYF	TGTGCCATCAGTAGACAGGGGGCGAGGCAAGAGACCCAGTACTTC	Y	Y

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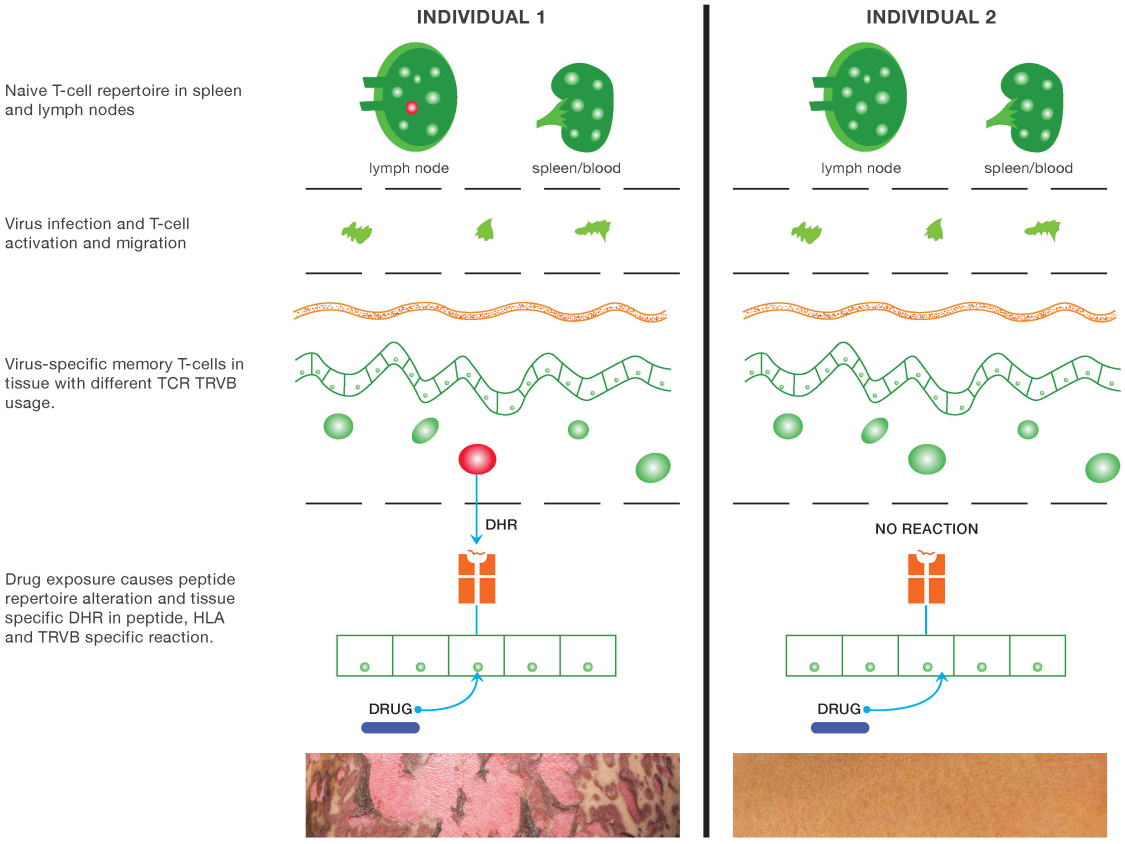

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Supplementary Table 1: HLA typing results and number of unique TW10/B57 or KF11/B57 T cell clones single cell sorted from the 4 HIV seropositive, abacavir naïve, individuals as part of this study.

Supplementary Table 2: HLA-A and HLA-B Single Antigen Cell lines (SALs) used in this study. SALs are a K562 cell line that have been transfected with a single HLA-A or HLA-B molecule. The HLA-B*57:01 SAL was tested in the presence or absence of abacavir.

Supplementary Table 3: TW10/HLA-B57 restricted clonotypes sorted from patient 2. T cell clones were single cell sorted based on viral peptide/HLA-tetramer complex staining. Multiple TW10/HLA-B57 restricted clonotypes were generated from this same patient. The TCR usage of the various clones is listed here. Clones 457 and 464 are identical and are the subject of the current report. Results presented here confirm that drug induced recognition of autologous HLA molecules by virus-specific T cells is specific to the cognate antigen, HLA restriction and TCR TRBV usage of the responding T cell clonotype.

Supplementary Figure 1: Model for drug induced recognition of autologous HLA by virus-specific memory T cells. Virus (pathogen) infection activates naïve T cell responses, leading to generation of tissue resident memory T cell populations. The tissue resident T cells of the same specificity will have unique TCR TRBV usage between different individuals. Virus-specific T cells may then crossreact against a drug altered peptide repertoire presented on autologous HLA

in a cognate antigen, HLA restriction and TCR TRBV usage specific mechanism, also termed heterologous immunity.

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