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Research paper

Novel approach to recognition of predicted HIV-1 Gag B*3501-restricted CD8 T-cell epitopes by HLA-B*3501$^+$ patients: Confirmation by quantitative ELISpot analyses and characterisation using multimers

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ABSTRACT

Exploring the intricacies of CD8$^+$ T-cell epitope recognition using emerging technologies to combine assessment of affinity, phenotype and resulting polyfunctional efficacy advances our understanding of HIV-1 immunopathogenesis and disease progression.

Complexities within T-cell antigen recognition, such as epitope:MHC binding, stability and affinity, appear to influence the distinction between protective and ineffective anti-HIV-1 immune responses, which are thought to govern rate of disease progression.

This study utilises the novel ProImmune REVEAL and ProVE® technology of rapid peptide synthesis, binding and affinity assays, and pentamer synthesis in conjunction with flow cytometry and simultaneous assessment of multiple CD8$^+$ T-cell effector functions in response to HLA-B*3501-restricted HIV-1 Gag peptides, to discover new T-cell epitopes.

The predicted HLA-B*3501-restricted peptides, HPVHAGPIA and YPLTSLRSL, and relevant pentamers were used in parallel to validate T-cell epitopes on clinical HIV-1$^+$ samples, confirming correlation between the expected superior immunogenicity of newly discovered epitopes and the *ex vivo* T-cell response. Such a platform should be employed in prophylactic and therapeutic vaccine settings.

1. Introduction

A more comprehensive understanding of both HIV-1 immunopathogenesis and specific T-cell efficacy is fundamental to the generation of future prevention and treatment of HIV-1 infection (Appay et al., 2008). Following HIV-1 infection the rate at which an individual progresses to AIDS varies. The genetic profile of an individual is reported to have a large influence on the rate of HIV-1 disease progression (reviewed in Deeks and Walker, 2007). Studies of the polymorphic human leukocyte antigen (HLA) loci have revealed correlations between certain HLA-types and the rate of HIV-1 disease progression. For example, HLA-B35 (Carrington et al., 1999) and -B8 (Kaslow et al., 1990) have been associated with rapid disease progression in HIV-1$^+$ individuals.

To clear a viral infection a robust, virus-specific, CD8$^+$ cytotoxic T-lymphocyte (CTL) response is required (McMichael, 2006). In HIV-1$^+$ individuals viral replication, disease progression and opportunistic infection inversely correlate with fully functional virus-specific T helper (T_H) and CTL responses (Rosenberg et al., 1997; Migueles et al., 2002). It has been demonstrated that in most cases of chronic HIV-1

Abbreviations: AIDS, acquired immune deficiency syndrome; APC, Allophycocyanin; CD, cluster of differentiation; CMV, cytomegalovirus; CTL, cytotoxic T lymphocyte; DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbant assay; ELISpot, enzyme-linked immunospot; FITC, fluorescein isothiocyanate; Gag, group associated antigen; HAART, highly active antiretroviral therapy; HCP5, HLA complex p5; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; IC_{50}, inhibitory concentration for 50% inhibition; IFN, interferon; IL, interleukin; mAb, monoclonal antibody; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cell; PE, phycoerythrin; PerCP, Peridinin chlorophyll protein; Pol, polymerase; RNA, ribonucleic acid; SFC, spot forming cells; t1/2, half life; TCM, T central memory; TEM, T effector memory; TEMRA, terminally differentiated RA$^+$; T_H, helper T lymphocyte; TNF, tumour necrosis factor.

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infection there is a lack of IL-2 production by HIV-1-specific $CD4^+$ T_H cells, and consequently an absence of T-cell proliferation, when stimulated with HIV-1 antigens (Wilson et al., 2000; Imami et al., 2001; Imami et al., 2002). However, the secretion of other antiviral cytokines, such as TNF-α and IFN-γ, remains unimpaired (Wilson et al., 2000; Imami et al., 2001). An effective, fully functional virus-specific CTL-mediated immune response fails to be mounted in chronic HIV-1 infection, likely due to the lack of $CD4^+$ T_H cell orchestration and support, and a resulting inability of HIV-1-specific $CD8^+$ T cells to mature and differentiate into fully functional, proliferative, perforin-producing 'killer' cells (Champagne et al., 2001; Migueles et al., 2002).

HIV-1 group-specific antigen (Gag) has been indicated as an important immune target in previous studies, where the breadth of the $CD8^+$ T-cell response to previously defined epitopes within the Gag protein (number of epitopes targeted) was found to inversely correlate with viral burden in chronically infected HIV-1^+ individuals (Kiepiela et al., 2007). By utilising new technology, available through ProImmune, to "REVEAL and ProVE®" potential $CD8^+$ T-cell epitopes, this study aims to further dissect the HIV-1 Gag-specific $CD8^+$ T-cell immune response mounted by HLA-$B35^+$ individuals, to elucidate possible mechanisms to which an increased rate of disease progression can be attributed. HLA-B*3501 is one of the HLA alleles currently able to be screened using REVEAL and ProVE® technology, and so possible HLA-B*3501 restricted $CD8^+$ T-cell epitopes within the HIV-1 Gag protein were investigated.

In conjunction with the new technology described here we sought to confirm predicted $CD8^+$ T-cell epitopes, and assay the function of epitope-specific $CD8^+$ T cells, by more conventional ELISpot assays, looking into IFN-γ, Perforin, IL-2 and IL-4 production. Previously published HLA-B*3501-restricted CD8 T-cell epitopes, discovered by functional assays using matrices of overlapping peptides (Goulder et al., 1997; McMichael and Walker, 1994), provide a comparison for the epitopes predicted by the ProImmune assays.

HIV-1-specific $CD8^+$ T cells have been shown to exhibit skewed maturation, being arrested at the pre-terminally differentiated stage of differentiation (Champagne et al., 2001). The differentiation and maturation profile of $CD8^+$ T cells, specific for epitopes predicted by ProImmune REVEAL and ProVE® assays, will be investigated by flow cytometry to evaluate the phenotype, indicating the stage of differentiation, as well as the functional ability of the epitope-specific $CD8^+$ T cells.

2. Methods

2.1. Study populations

Informed consent and Ethics Committee approval was obtained for the studies described. Blood samples were taken from four HLA-B*3501^+, asymptomatic, chronically infected HIV-1^+ individuals from the Chelsea and Westminster Hospital cohort (Stebbing et al., 2006). All individuals are infected with clade B HIV-1, showing >94% DNA sequence similarity between autologous proviral *gag* and the consensus HXB2 sequence. Median CD4 T-cell count was 315 cells/μl blood (range: 255–1,489 cells/μl blood) CD8 count was 1,389 cells/μl blood (range: 680–2,014 cells/μl blood) and median HIV-1 load was 127 copies/ml plasma (range: <50–37,019 copies/ml plasma). Details of HLA-type and patient characteristics at time of sample collection are shown in Table 1.

2.2. Collection and separation of whole blood

Blood was collected into Heparinised and EDTA Vacutainers™ (Becton Dickinson, Oxford, UK), for functional and flow cytometric analysis respectively. Fresh PBMC were isolated by density gradient centrifugation as previously described (Imami et al., 2002).

2.3. Plasma HIV-1 RNA load

The Versant HIV-1 RNA 3.0 branched DNA assay (Siemens Healthcare, Camberley, UK) was used to quantitate the copy number of HIV-1 RNA in the plasma, with a lower detection limit of 50 copies/ml plasma.

2.4. Lymphocyte subsets

Murine, anti-human monoclonal antibodies (mAb) to: CD3, CD4, CD8, CD45, CD56 and CD19 (Tetra One, Beckman Coulter, High Wycombe, UK) were used to mark lymphocyte subsets within whole blood and then evaluated using a Cytomics FC 500 flow cytometer (Beckman Coulter) and Tetra CXP (version 2.2) software.

2.5. Genomic DNA extraction and high definition HLA typing

Genomic DNA was extracted from 5×10^6 PBMC using the Nucleon Genomic DNA Extraction Kit (Tepnel Life Sciences, Manchester, UK) according to the manufacturer's instructions. DNA was resuspended to a final concentration of 50 ng/μl in

Table 1
HLA-types and patient characteristics at the time of sample collection

Patient ID	CD4 T-cell count, cells μl $blood^{-1}$	CD8 T-cell count, cells μl $blood^{-1}$	Plasma viral load, HIV RNA copies/ml plasma	HAART regimen	Length of time on HAART, months	HAART effectiveness, months until viral suppression	Length of confirmed HIV-1 infection, years	Class I HLA-type
01	284	1207	205	AZT, NVP, 3TC	2	3	0.5	A2, A11, B27, B35
02	345	1570	<50	AZT, 3TC, EFV	2	2	7	A9, A11, B35, B61
03	1489	680	<50	NVP, ABC, 3TC	275	2	14	A3, A29, B35, B44
04	255	2014	37,019	none	0[a]	2	15	A1, A2, B35, B57
Median	315	1389	127	N/A	2	2	10.5	N/A

[a] Patient 04 discontinued HAART after 246 months of effective treatment. The patient has not been receiving HAART for the past 54 months.