DIFFERENTIAL IMMUNODOMINANCE HIERARCHY OF CD8+ T CELL RESPONSES IN HLA-B*27:05 AND B*27:02-MEDIATED CONTROL OF HIV-1 INFECTION

Emily Adland1, Matilda Hill1, Nora Lavandier1, Anna Csala1, Anne Edwards2, Fabian Chen3, Marek Radkowski4, Justyna D. Kowalska4, Dimitrios Paraskevis5, Angelos Hatzakis5, Humberto Valenzuela-Ponce6, Katja Pfafferott7, Ian Williams8, Pierre Pellegrino8, Persephone Borrow7, Masahiko Mori1, Jürgen Rockstroh9, Julia G. Prado10, Beatriz Mothe10,11, Judith Dalmau10, Javier Martinez-Picado8,10,11,12, Gareth Tudor-Williams13, John Frater7,14, Anette Stryhn15, Soren Buus15, Gustavo Reyes Teran6, Simon Mallal16, Mina John17, Susan Buchbinder18, Gregory Kirk19, Jeffrey Martin20, Nelson Michael21, Jacques Fellay22, Steve Deeks19, Bruce Walker23, Santiago Avila-Rios6, David Cole24,25, Christian Brander10, Mary Carrington23,26, Philip Goulder11

1 Department of Paediatrics, University of Oxford, UK
2 Department of GU Medicine, The Churchill Hospital, Oxford University NHS Foundation Trust, Oxford, UK
3 Department of Sexual Health, Royal Berkshire Hospital, Reading, UK
4 Department of Immunopathology of Infectious and Parasitic Diseases, Hospital for Infectious Diseases, Medical University of Warsaw, Warsaw, Poland
5 Medical School, National and Kapodistrian University of Athens, Mikras Asias 75, 11527, Athens, Greece
6 Centre for Research in Infectious Diseases, National Institute of Respiratory Diseases, Mexico City, Mexico
7 Nuffield Department of Medicine, University of Oxford, Oxford, UK
8 Centre for Sexual Health and HIV Research, Mortimer Market Centre, London, UK
9 Department of Medicine I, University Hospital Bonn, Bonn, Germany
AIDS Research Institute IrsiCaixa, Institut d’Investigació en Ciències de la Salut Germans Trias i Pujol (IGTP), Universitat Autònoma de Barcelona, Badalona, Spain

University of Vic-Central University of Catalonia (UVic-UCC)

Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain;

Department of Paediatrics, Imperial College, London, UK

Oxford Martin School, University of Oxford, Oxford, United Kingdom

Dept of Immunology and Microbiology, University of Copenhagen, Denmark

University of Vic-Central University of Catalonia (UVic-UCC)

Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain;

Department of Paediatrics, Imperial College, London, UK

Oxford Martin School, University of Oxford, Oxford, United Kingdom

Dept of Immunology and Microbiology, University of Copenhagen, Denmark

Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee, USA

Institute of Immunology and Infectious Diseases, Murdoch University, Perth, Australia

San Francisco Department of Public Health, HIV Research Section, San Francisco, CA

Department of Epidemiology, Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD

Department of Epidemiology and Biostatistics, University of California, San Francisco, CA

U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD

School of Life Sciences, EPFL, Lausanne, Switzerland

Ragon Institute of MGH, MIT and Harvard, Boston, Massachusetts, USA

Cardiff University School of Medicine, Henry Wellcome Building, Heath Park, Cardiff

Immunocore Limited, 101 Milton Park, Abingdon, Oxon, OX14 4RX, UK.

Cancer and Inflammation Program, Leidos Biomedical Research, Frederick National Laboratory for Cancer Research, Maryland, USA

* Corresponding author- Philip Goulder; philip.goulder@paediatrics.ox.ac.uk

RUNNING TITLE: HLA-B*27:02-MEDIATED CONTROL OF HIV-1

ABSTRACT

The well-characterised association between HLA-B*27:05 and protection against HIV disease progression has been linked to immunodominant HLA-B*27:05-restricted CD8+ T-cell responses towards the conserved Gag 263-272 ('KK10') and Pol 901-909 'KY9' epitopes. We here studied the impact of the 3 amino acid differences between HLA-B*27:05 and the closely-related HLA-B*27:02 on the HIV-specific CD8+ T-cell response hierarchy and on immune control of HIV. Genetic epidemiological data indicate that both HLA-B*27:02 and HLA-B*27:05 associate with slower disease progression and lower viral loads. The effect of HLA-B*27:02 appears consistently stronger than that of HLA-B*27:05. In contrast to HLA-B*27:05, the immunodominant HIV-specific HLA-B*27:02-restricted CD8+ T-cell response is to a Nef epitope (residues 142-150, ‘VW9’), with Pol-KY9 subdominant and Gag-KK10 further subdominant. This selection was driven by structural differences in the F-pocket, mediated by a polymorphism between these two HLA alleles at position 81. Analysis of autologous virus sequences showed that in HLA-B*27:02-positive subjects all three of these CD8+ T-cell responses impose selection pressure on the virus, whereas in HLA-B*27:05-positive subjects there is no Nef-VW9-mediated selection pressure. These studies demonstrate that HLA-B*27:02 mediates protection against HIV disease progression that is at least as strong or stronger than that mediated by HLA-B*27:05. In combination with the protective Gag-KK10 and Pol-KY9 CD8+ T-cell responses that dominate HIV-specific CD8+ T-cell activity in HLA-B*27:05-positive subjects, a Nef-VW9-specific response is additionally present and immunodominant in HLA-B*27:02-positive subjects, mediated through a polymorphism at residue 81 in the F-pocket, that contributes to selection pressure against HIV.
CD8+ T-cells play a central role in successful control of HIV infection, and have the potential also to mediate the eradication of viral reservoirs of infection. The principal means by which ‘protective’ HLA class I molecules, such as HLA-B*27:05 and HLA-B*57:01, slow HIV disease progression, is believed to be via the particular HIV-specific CD8+ T cell responses restricted by those alleles. We focus here on HLA-B*27:05, one of the best-characterised ‘protective’ HLA molecules, and the closely-related HLA-B*27:02, which differs by only 3 amino acids, and which has not been well-studied in relation to control of HIV infection. We show that HLA-B*27:02 is also protective against HIV disease progression, but the CD8+ T-cell immunodominance hierarchy of HLA-B*27:02 differs strikingly from that of HLA-B*27:05. These findings indicate that the immunodominant HLA-B*27:02-restricted Nef response adds to protection mediated by the Gag and Pol specificities that dominate anti-HIV CD8+ T-cell activity in HLA-B*27:05-positive subjects.

INTRODUCTION

HLA-B*27:05 is strongly associated with slow progression in HIV infection (1-3). It has been proposed that it is the particular HIV-1 specific CD8+ T cell responses restricted by HLA-B*27:05 that provide a likely mechanism for protection. HLA-B*27:05 mediates an immunodominant response towards an epitope in p24 Gag, ‘KK10’: KRWIILGLNK (Gag 263-272). HLA-B*27 has a unique structure among HLA-B class I molecules in having an absolute requirement for Arginine at position 2 (P2) in the binding peptide (4). Loss of immune control and progression to AIDS in HIV-infected HLA-B*27:05-positive individuals appeared to be precipitated by selection of an escape mutation at Gag residue 264, most commonly R264K or R264G (5-7). This R264X escape mutant is selected prior to, and not as a result of, the sharp rise in viral load (6). The Arg-264 substitution results in reduced binding of the epitope to HLA-B*27:05 and reduced recognition of virus-infected cells.
More recently, an HLA-B*27:05 restricted Pol epitope KY9 (KRKGGIGGY) (Pol residues 900-909) has been described (8). The magnitude of responses to this epitope is only marginally lower than that to KK10 (8). At position 908 (P8) within the KY9 epitope, a viral escape mutant with an amino acid change from Glycine to Glutamic Acid emerges soon after the R264X mutation in the Gag KK10 epitope. This would imply that both the KK10 and KY9 are imposing selection pressure on the virus at the same time and highlights KY9 as a contributing factor to HLA-B*27:05 mediated immune control of HIV.

The most prevalent subtype of HLA-B*27 worldwide is HLA-B*27:05, although many other natural variants of the molecule have been described to date, from HLA-B*27:01 to B*27:99 (http://www.ebi.ac.uk/ipd/imgt/hla/align.html). Most of these differences have a direct impact on the peptide binding groove and therefore on the nature of the peptides binding that particular HLA-B*27 subtype. The particular peptides presented by these subtypes could potentially explain differences in disease susceptibility. HLA-B*27 has been especially well studied because of its strong association with ankylosing spondylitis (AS), and it is noteworthy that some HLA-B*27 subtypes are associated with AS and others are not. For example, HLA-B*27:01, B*27:02, B*27:04, B*27:05, B*27:07 and B*27:08 have been linked with AS, whereas B*27:06 and B*27:09 are not associated with AS (13).

HLA-B*27:02 reportedly comprises 1-10% of B*27-positive subjects in Northern Europe, 20% in Spain and Portugal, 35-50% in Greece and ~55% in Arab and Jewish populations (13-15). However, in Germany this figure is 14% in a population where B*27 prevalence is ~10% (16) and in Poland this is 26-29% in a population where B*27 prevalence is ~14% (17).

As stated above, there are three amino acid differences between HLA-B*27:05 and HLA-B*27:02. These 3 amino acids line the F-pocket of the peptide binding groove, therefore influencing the type of residue at the carboxy-terminal anchor position (PC). HLA-B*27:02 has Asparagine at HLA residue 77 whereas HLA-B*27:05 uses the negatively charged
Aspartate. The F-pocket of HLA-B*27:02 is therefore lined with residues conferring a lower overall negative charge than HLA-B*27:05, and for this reason the peptide binding motif does not include basic residues (Lys or Arg) at PC in HLA-B*27:02-binding peptides but these are a feature of many of the peptides binding to HLA-B*27:05. Additionally, HLA-B*27:05 has Isoleucine at residue 81, compared to Alanine in HLA-B*27:02. Thus, the F-pocket in HLA-B*27:02 is potentially slightly larger and may be able to accommodate amino acids with bulkier side chains. Together, these differences provided a unique opportunity to investigate the differences in the HIV epitopes presented by HLA-B*27:05 and HLA-B*27:02 and their respective roles in immune control of HIV.

MATERIALS AND METHODS

Study Cohorts

We studied treatment-naïve subjects with chronic HIV-1 infection from 1) Warsaw, Poland; 2) Athens, Greece; 3) Mexico City, Mexico; 4) Bonn, Germany; 5) Boston, USA; 6) Barcelona, Spain; 7) Thames Valley, UK. Several cohorts were used for the study because of the paucity of HIV-infected subjects expressing HLA-B*27:02. Subjects were included in the study if they were HIV-infected, HLA-B*27:02-positive and samples were available for either elispot assays or viral sequencing. The absolute CD4 counts of these study subjects was 460 cells/ul (IQR 287-647) and median viral load was 11,399 copies/ml plasma (IQR 437-29,592). Study subjects from all cohorts gave written informed consent for their participation. The study was approved by the institutional review boards of the University of Oxford, University of Warsaw, Medical School, National and Kapodistrian University of Athens, University of Bonn, National Institute of Respiratory Diseases in Mexico City, Ragon Institute, and University Hospital ‘Germans Trias i Pujol’ in Badalona (Barcelona, Spain).
For survival analyses we included 783 individuals from five studies: AIDS Linked to the Intravenous experience (ALIVE, n=12) (18), Multicenter AIDS Cohort Study (MACS, n=417) (19), Multicentre Haemophilia Cohort Study (MHCS, n=243) (20), San Francisco City Clinic Cohort (SFCCC, n=74) (21) and the DC Gay Cohort Study (DCGCS, n=37) (22) with prospective follow-up and known dates of seroconversion. For HIV mean viral load analyses we included 3280 individuals enrolled in one of five prospective studies: Multicenter AIDS Cohort Study (MACS, n=1583), Military HIV Research Program (MHRP, n=191), the Ragon Institute of MGH, MIT and Harvard HIV Controller study (n=975), Study of the Consequences of the Protease Inhibitor Era (SCOPE, n=386) and the Swiss HIV Cohort study (n=145).

There was an overlap of 414 subjects between the two analyses. Measures of disease outcome in the survival analyses of HIV-infected subjects were: an absolute CD4 count of <200 cells/mm$^3$; meeting the 1987 CDC definition of AIDS (AIDS defining illness); meeting the 1993 CDC definition of AIDS (AIDS defining illness or decline to absolute CD4 count of <200 cells/mm$^3$; and death.

The frequency of amino acid polymorphisms among HLA-B*27-negative, B-clade infected individuals within the Gag, Pol and Nef HLA-B*27 epitopes shown in Table 2 was determined from analysis of 555 AIDS Clinical Trials Group and 245 Western Australia HIV Cohort Study subjects (23).

**HLA Typing**

HLA genotyping was performed either by PCR-SSOP (sequence-specific oligonucleotide probing), PCR-SBT (sequence based typing) using the Sanger sequencing technology recommended by the 13th International Histocompatibility Workshop (http://www.ihwg.org), or next generation sequencing using the Roche 454 platform (24).
Amplification and sequencing of HIV genes by polymerase chain reaction (PCR)

Gag, Pol and Nef sequences were generated from either viral RNA or genomic DNA. DNA was extracted from whole blood, viral RNA was extracted from plasma using RNA extraction Minikit (Qiagen UK) in accordance with the manufacturer’s instructions. Reverse-transcription of RNA to cDNA was undertaken using Superscript III One-Step Reverse Transcriptase kit (Invitrogen) as a one-step reaction combined with outer PCR according to the manufacturers instructions and amplified by nested PCR to obtain population sequences. Sequencing was undertaken using the Big Dye Ready Reaction Terminator Mix (V3) (Applied Biosystems UK) analysed using Sequencher v4.8 (Gene Codes Corporation) and manually aligned using Se_Al software.

IFN-gamma ELISpot Assays

We tested ex vivo PBMCs against a panel of 410 overlapping peptides (OLPs) spanning the entire HIV-1 proteome to screen for IFN-gamma ELISpot responses (25). We additionally tested putative optimal epitopes by ELISpot using ex vivo PBMCs from HLA-B*27:02 and B*27:05+ subjects.

Cell staining and flow cytometry

Cell staining was undertaken from cryopreserved PBMCs using anti-CD3-Pacific orange (Invitrogen), anti-CD8-Alexa Fluor 700 (BD Biosciences), and HLA-B*27 tetramers conjugated to PE. Dead cells were gated out using “live/dead” viability kit (Invitrogen).

Peptide – MHC-I binding assay
Peptide affinity to HLA Class I molecules were determined using a luminescent oxygen channeling immunoassay (LOCI) (26). Briefly, peptides were dissolved in PBS/0.1% Lutrol F68 by sonication for 10 min. Peptides were titrated in 384 well microplates using a Microlab® STAR liquid handling robot (Hamilton Robotics). Recombinant, denatured HLA-C heavy chain was diluted into PBS/0.1% Lutrol® F68/100 mM Tris/maleate (pH 6.6) containing prefolded, recombinant β₂m on ice. The HC/β₂m mix was added 1:1 to the peptide titrations and incubated for 48 hours at 18°C to allow peptide-MHC-I complex folding. After complex folding, samples were transferred to 384 well Optiplates™ and streptavidin coated donor beads (Perkin Elmer 6760002) and W6/32 conjugated acceptor beads (Perkin Elmer 6762001, in-house conjugated with W6/32) diluted in PBS/0.1% Lutrol F68 were added to a final concentration of 5 µg/ml of each. The Optiplates™ were incubated overnight and luminescence was measured in an EnVision™ 2103 Multilabel reader.

Structural analysis

The HLA-B*27:05-KK10 structure (from PDB: 4G9D) (27) was used to model analyze C-terminal peptide residue interaction with the F-pocket in which 10-Lys was mutated to 10-Trp. This structure was also used to model the F-pocket of HLA-B*27:02 (D77N, T81I and L81A) with both 10-Lys and 10-Trp. Sequences were adjusted with COOT (28) and graphical representations were prepared with PYMOL. (The PyMOL Molecular Graphics System, Version 1.8 Schrodinger, LLC).

RESULTS:

HLA-B*27:02 is associated with protection against HIV disease progression

In cohorts that have been studied to determine the impact of host genetic factors on HIV disease outcome, the prevalence of HLA-B*27:02 is relatively low, approximately 10-fold lower than that of HLA-B*27:05. Survival analyses were performed on seroconverter cohorts.
and, for all four outcomes studied, B*27:02 associated more strongly with slower progression than did B*27:05, though the sample sizes are very limited in the B*27:02 group (Table 1, Fig. 1). Both B*27:02 and B*27:05 associated significantly with lower mean viral loads as compared to those without these alleles, and there was no significant difference between the two alleles on viral control. Although, unexpectedly, in the current analyses HLA-B*27:05 was not significantly associated with slow disease progression, HLA-B*27:05 has been consistently and in many studies associated with control of HIV viraemia, as well as with slow disease progression (1-3, 5). Our subtype-specific analyses here, both of viral setpoint and disease progression, suggest slightly better protection in both cases conferred by B*27:02 than B*27:05. However, in a previous study of viral setpoint only, involving 2,767 subjects, also of European descent, suggested that HLA-B*27:05 (odds of being an immune controller versus progressor = 3.34) was slightly more protective than HLA-B*27:02 (odds ratio 2.53) (2). Thus these data together would indicate that HLA-B*27:02 is associated with protection against HIV disease progression and provides a similar or possibly even a somewhat greater degree of protection to that provided by HLA-B*27:05.

Nef-VW9 is the immunodominant HLA-B*27:02-restricted CD8+ T-cell epitope

To characterise the HIV-specific CD8+ T-cell responses associated with HLA-B*27:02 and immune control of HIV infection, we tested in IFNγ Elispot assays recognition of a panel of 410 overlapping peptides spanning the B clade proteome (25). The immunodominant response among 7 HLA-B*27:02-positive subjects whose HIV-specific CD8+ T-cell responses were analysed, was to an epitope in Nef, defined using peptide-MHC tetramers as the 9mer VRYPLTFGW (Nef residues 133-141), confirmed via tetramer staining (Fig 2).

Summarising the HLA-B*27-restricted CD8+ T-cell responses observed in these 7 HLA-B*27:02-positive subjects and in 19 HLA-B*27:05-positive subjects (Fig 3A) confirms the reversal of the immunodominance pattern observed in HLA-B*27:05-positive subjects, in
which the Gag-KK10 is dominant and the Pol-KY9 response co-dominant or subdominant, and typically there is no HLA-B*27:05-restricted Nef-specific response. In the HLA-B*27:02-positive subjects, the Nef-VW9 response was dominant, Pol-KY9 subdominant, and Gag-KK10 below Pol-KY9 in the hierarchy (Fig 3AB).

HLA-B*27:05 and HLA-B*27:02 have distinct peptide binding preferences

To help explain the differences observed in the CD8+ T-cell immunodominance patterns observed between HLA-B*27:02-positive and HLA-B*27:05-positive subjects, the peptide-MHC binding avidity was determined for each of the HLA-B*27-restricted epitopes. Consistent with the differences in peptide binding motifs between HLA-B*27:05 (inability to bind Trp, ability to bind basic residues Lys/Arg/His in the F pocket) and HLA-B*27:02 (ability to bind Trp, inability to bind basic residues Lys/Arg/His in the F pocket) we observe that the Gag-KK10 epitope that is immunodominant in HLA-B*27:05 positive subjects binds poorly to HLA-B*27:02; conversely, the Nef-VW9 epitope that is immunodominant in HLA-B*27:02-positive subjects does not bind well to HLA-B*27:05 (Fig 3C). Overall it is striking that none of the B*27-restricted peptides tested that had a basic residue Lys/Arg at the carboxy-terminal position (PC) bound successfully to HLA-B*27:02: namely, Gag-IK9, Gag-KK10, Gag-QK10, Pol-FR9, Env-CR9. Also, as has been well-described (29, 30), strong peptide-MHC binding avidity does not necessarily translate into high immunogenicity. For example, the Env-HRLRDLLLl (HI9) binds well to both HLA-B*27:02 and HLA-B*27:05 but responses were detected only in subjects expressing HLA-B*27:02. Thus, adequate binding is a requirement for immunogenicity, but is not sufficient to predict it.

Structural modelling of the impact of HLA-B*27:05/HLA-B*27:02 polymorphisms on F-pocket amino acid compatibility
The crystal structure of HLA-B*27:05 in complex with the KK10 peptide has been previously determined, but there is currently no crystal structure of HLA-B*27:02. Thus, the polymorphisms in the F-pocket that distinguish HLA-B*27:05 from HLA-B*27:02 (27, 31) (D77N, T80I and L81A, respectively) were modelled to explore the distinct C-terminal peptide (PC) residue preferences between the two HLA alleles (Fig 4). KK10 epitope PC residue 10-Lys forms a ‘peg-in-hole’ type interaction in the HLA-B*27:05-KK10 structure, forming a stabilizing van der Waal interaction with HLA residue 81-Leu. This interaction is likely disrupted in HLA-B*27:02 because of the smaller side chain at residue 81 (81-Ala in HLA-B*27:02 compared to 81-Leu in HLA-B*27:05) (Fig 4A). This polymorphism also results in the widening of the F-pocket in HLA-B*27:02, that would tend to increase mobility of the Lys-10 side chain that could destabilize the peptide.

Additionally, HLA-B*27:05 encodes Asp at residue 77 that provides a negative charge that is favorable for the binding of positively charged basic amino acids. In contrast, HLA-B*27:02 encodes Asn at residue 77 that is neutral in charge and less favorable for binding to basic amino acids. Although the structural modelling did not reveal an obvious difference in the ability of Asp-77, or Asn-77 to form a hydrogen bond the main chain N group of Lys-10 in the KK10 peptide, these difference in charge could also play a role in the differential selection of amino acids based on their C-terminal residue between HLA-B*27:05 and HLA-B*27:02.

Together, these observations are consistent with the low KK10 peptide-MHC binding avidity to HLA-B*27:02 (Fig 3C) and our observation that KK10 is not the dominant HIV epitope in HLA-B*27:02 positive individuals (Fig 3B).

We next modelled the interaction between HLA-B*27:05 and HLA-B*27:02 with Trp as the C-terminal peptide residue. The tighter F-pocket in HLA-B*27:05 is unlikely to accommodate KK10 peptide PC 10-Trp because of a potential steric clash with HLA residue 81-Leu.
contrast, the wider F-pocket in HLA-B*27:02 is ideally suited to bind Trp, which could form van der Waals contacts with 81-Ala (Fig 4B). These observations are consistent with the switch in immunodominant responses from KK10 in HLA-B*27:05 individuals, to VW9 in HLA-B*27:02 individuals, and demonstrate the extreme peptide selectivity of HLA alleles that differ at only a few key residues in the peptide binding groove.

**Immune escape selection pressure matches CD8+ T-cell immunodominance hierarchy**

A hallmark of disease progression in subjects expressing HLA-B*27:05 is the impact of escape within Gag-KK10 as a precipitant. To date, progression to AIDS (CD4<200) has not been observed in an HLA-B*27:05-positive subject without escape occurring in this epitope. However, the kinetics of viral escape are strongly influenced by the immunodominance hierarchy of the CD8+ T-cell responses (32). Analysis of autologous viral sequences in HLA typed subjects demonstrated that, in HLA-B*27:05-positive subjects, the strongest selection pressure is imposed by the dominant Gag-KK10 response, followed closely by the co-dominant or sub-dominant Pol-KY9 response, and as expected no selection pressure was evident within the Nef-VW9 epitope (Table 2: odds ratios for escape in HLA-B*27:05+ves versus HLA-B*27:negatives at R264X (Gag) and K903X (Pol) were 15 and 10, respectively). In contrast, strong immune selection pressure was observed on the Nef-VW9 and Pol-KY9 epitopes in HLA-B*27:02-positive subjects and, although escape mutation at R264X was observed in a minority of HLA-B*27:02-positive subjects (3 of 22 subjects studied), the selection of escape variants in Gag-KK10 was clearly less frequent than in Nef-VW9 or Pol-KY9 (odds ratios for escape in HLA-B*27:02+ves versus HLA-B*27:negatives at R264X (Gag), R902X (Pol) and L137X (Nef) were 4, 15 and 15, respectively). Consistent with previous studies comparing the footprints of closely-related HLA types on the same epitope (33, 34) we observed in Pol-KY9 a different HLA-B*27:02 footprint (dominant footprint R902X) compared to HLA-B*27:05 (dominant footprint in this epitope K903X).
DISCUSSION

These studies focus on HLA-B*27, and the mechanisms by which this HLA molecule is associated with protection in HIV infection. We show that HLA-B*27:02 expression is associated with slower progression to HIV disease and also with lower viral loads, compared to non-expression of HLA-B*27. Compared to HLA-B*27:05, HLA-B*27:02 appears at least as protective, and may be slightly more protective. The immunodominant HLA-B*27:02-restricted HIV-specific CD8+ T-cell response is directed towards an epitope in Nef (VRYPLTFGW, Nef 133-141) that is not an HLA-B*27:05-restricted epitope. The two principal HLA-B*27:05-restricted epitopes, Gag-KK10 and Pol-KY9, are both also HLA-B*27:02-restricted epitopes, but Gag-KK10 is the subdominant HLA-B*27:02-restricted response. These distinct HIV-specific CD8+ T-cell hierarchies are confirmed in the studies showing the selection of escape mutants within these epitopes. The strongest selection pressure in the HLA-B*27:02-positive subjects is for escape within Nef-VW9 and Pol-KY9, with weak pressure evident for escape within Gag-KK10. These data suggest that, while the immunodominant Gag-KK10 response may play an important part in HLA-B*27:05-mediated immune control of HIV, access to the Nef-VW9 epitope and alteration of the CD8+ T-cell immunodominance hierarchy in subjects expressing HLA-B*27:02 does not reduce HLA-B*27-associated protection against HIV disease progression.

As described above, the prevailing hypothesis is that HLA-B*27 is protective against HIV disease progression because the immunodominant response, Gag-KK10, is highly efficacious, killing virus-infected target cells very soon after viral entry (8) and escape mutants are typically selected late in the course of infection (5) because of the crippling impact of the R264K or R264G mutation in the absence of a simultaneous compensatory mutation at S173T or E260D, respectively (35, 36). This hypothesis has been extended to
explain HLA-B*57-mediated control also, a broad Gag-specific response (37) here being even more protective than that generated in HLA-B*27-positive subjects, and multiple mutations required in several Gag epitopes each likely to occur at a detriment to viral fitness (38, 39).

Although there are exceptions – HLA-B*14:02 and HLA-B*51:01 being two of these (40, 41), - most of the HLA-B alleles that have shown a well-documented association with favourable control of viraemia, including HLA-B*27:05, HLA-B*57, HLA-B*58:01, HLA-B*13:02, HLA-B*52:01 and HLA-B*81:01 (42-45) have an immunodominant anti-HIV CD8+ T cell response within p24 Gag. In general, HLA molecules associated with more rapid progression to AIDS such as HLA-B*18:01, HLA-B*35:01 and HLA-B*58:02 show dominant responses directed at non-Gag epitopes such as Nef or Env (30, 37, 46-48). Here we show that the HLA-B*27:02 is at least as protective as HLA-B*27:05, and the immunodominant HIV-specific epitope is located in Nef. Although Nef targeting may not generally be associated with improved control of HIV (37, 49, 50), a study of SIV infection in Mamu-B*08 rhesus macaques, an animal model for HLA-B*27-mediated elite control (51) showed that the frequency of the CD8+ T cell response against a Nef epitope correlated significantly with reduced acute phase viraemia (52). This is one of the first models to demonstrate that a vaccine-induced Nef specific CD8+ T cell response can control replication of an AIDS virus in an animal model of MHC class-I-associated control. Indeed, elite control of SIV in macaques expressing the protective MHC alleles Mamu-B*08 or Mamu-B*17 have little or nothing in the way of Gag responses. Protective responses appear to lie exclusively in Nef or Vif. Indeed it is striking that the immunodominant Mamu-B*17 epitope in Nef, IRYPKTFGW (56), corresponds exactly with the immunodominant HLA-B*27:02-restricted described epitope here, VRYPLTFGW. Mamu-B*17 in fact bears strong similarity with HLA-B*27:02 in binding peptides that carry Arg at P2 and Trp at PC (57). It seems remarkable and not coincidental that two MHC class I molecules that have evolved independently, but by convergent evolution (62) possess the
ability to bind similar peptides, can mediate, independently, control of SIV and HIV infection, respectively.

Thus, although immunodominant p24 Gag-specific immune responses are generally associated with effective immune control of HIV, and Nef-specific CD8+ T-cell responses are not, this does not exclude the fact that certain non-Gag-specific CD8+ T-cell responses may also contribute to successful suppression of viral replication. Furthermore, as evidenced by the finding of escape mutations within Gag-KK10 albeit in a minority if HLA-B*27:02-positive subjects, the Gag-KK10-specific response may continue to contribute to control of HIV in HLA-B*27:02-positive subjects, even if it is not the dominant response. Numerous previous examples have been presented of subdominant responses being more efficacious in control of virus infections including HIV (53).

The reasons for the differences observed between HLA-B*27:05 and HLA-B*27:02 in the CD8+ T-cell immunodominance hierarchy were explored using structural modelling. These analyses demonstrated a clear structural difference within the F-pocket of the two alleles, mainly attributed to the L81A polymorphism. The narrower F-pocket in HLA-B*27:05 (governed by 81-Leu), although ideally suited for binding to peptides with Lys at the C-terminus, would likely be unable to accommodate the larger Trp side chain in the Nef-VW9 peptide. On the other hand, the wider F-pocket in HLA-B*27:02 (governed by 81-Ala) would likely form a less stable interaction with 10-Lys, but is ideally suited for interactions with peptides with Trp at the C-terminus. The resulting low binding avidity of HLA-B*27:02 for Gag-KK10 and high binding avidity for Nef-VW9, and the converse for HLA-B*27:05, largely explains the altered immunodominance patterns and consequent escape hierarchies (32, 54) observed.
Comparisons between the CD8+ T-cell responses restricted by HLA-B*27:02 and HLA-B*27:05 have not been made previously in HIV infection, but a similar study has been undertaken in HCV infection (58). As in HIV, HLA-B*27 is associated with improved HCV disease outcome (59, 60), and, also as in HIV, HLA-B*27:02 appears to present more epitopes than HLA-B*27:05. Of three HLA-B*27-restricted HCV NS5B-specific epitopes, two are presented by both HLA-B*27:02 and HLA-B*27:05 (NS5B amino acid sequences ARMILMTHF and GRAAICGKY) and a third by HLA-B*27:02 only (amino acid sequence ARHTPVNSW). It is striking that this HLA-B*27:02-restricted epitope ARHTPVNSW carries Trp at PC, just as the Nef-VW9 HLA-B*27:02-restricted epitope described here, which from the structural considerations above would not be expected to be accommodated within the smaller F pocket of HLA-B*27:05. Also, it is notable that these HLA-B*27:02-specific epitopes, ARHTPVNSW and Nef-VW9, in HCV and HIV, respectively, are both clearly the immunodominant responses among HLA-B*27:02-positive individuals. This is consistent with findings from comparisons of HLA-B*44:02 and HLA-B*44:03 (61), HLA molecules differing by only a single amino acid residue, demonstrating that the greater capacity within the HLA-B*44:03 peptide-binding groove allows a larger repertoire of peptides to bind. Also, structural studies of peptide binding to HLA-B*57:03 (31) which, like HLA-B*27:02, has Ala at HLA residue 81, show that large residues such as Trp binding into the appropriately-sized F pocket make greatly increased numbers of interatomic van der Waal’s contacts that contribute to stability of the peptide-MHC complex and therefore to immunodominance of the response.

The limitations of the current study include the fact that HIV-infected HLA-B*27:02-positive subjects were very hard to find and therefore only a relatively small number were studied. In addition, other than defining the specificity of the HIV-specific CD8+ T-cell responses and the seeking selection pressure on the virus through the three main specificities of interest, sample availability limited further analyses to investigate the ability of the HLA-B*27:02-Nef
response to inhibit viral replication. In addition, the study has focused solely on the HLA-B*27-restricted CD8+ T-cell response, although it is known that HLA-mediated effects on HIV disease outcome may arise via other mechanisms (9, 10, 55). In particular, the finding that Bw4-80I-expressing alleles in combination with high expressing KIR3DL1 alleles is associated with more effective control of HIV (9) provides an additional potential explanation for the improved action of HLA-B*27:02 (a Bw4-80I allele) in comparison with HLA-B*27:05 (a HLA-Bw4-80T allele) in control of HIV.

In conclusion, despite the sub-dominance of the Gag-KK10 in HLA-B*27:02-positive subjects, HLA-associated protection against HIV disease progression is at least as strong as that mediated by HLA-B*27:05. The immunodominant Nef-VW9-specific response may contribute to this additional immune control, in combination with contributions made via the Gag-KK10 and Pol-KY9-specificities that are shared with HLA-B*27:05. In addition, there may be additional mechanisms, such as the HLA-B*27:02 interaction with KIR3DL1, operating to supplement further the antiviral immune effects of HLA-B*27:02 against HIV.

ACKNOWLEDGEMENTS

This work was funded by grants from the National Institutes of Health (RO1AI46995 to PJRG), the Wellcome Trust (WT104748MA to PJRG). This project has been funded in whole or in part with federal funds from the Frederick National Laboratory for Cancer Research, under Contract No. HHSN261200800001E (to MC). The MACS is funded primarily by the National Institute of Allergy and Infectious Diseases (NIAID), U01-AI35042 (Johns Hopkins University Bloomberg School of Public Health, Joseph Margolick, PI), U01-AI35039 (Northwestern University, Steven Wolinsky, PI), U01-AI35040 (University of California, Los Angeles, Steven Wolinsky, PI).
Angeles, Roger Detels and Oto Martinez MPI), U01-AI35041 (University of Pittsburgh, Charles Rinaldo, PI), and UM1-AI35043 (Johns Hopkins University Bloomberg School of Public Health, Lisa Jacobson, PI). The SCOPE cohort was supported by the UCSF/Gladstone Institute of Virology and Immunology CFAR (P30 AI027763) and the CFAR Network of Integrated Systems (R24 AI067039). Additional support was provided by the Delaney AIDS Research Enterprise (DARE; AI096109, A127966) and the amfAR Institute for HIV Cure Research (amfAR 109301). PB is a Jenner Investigator. IW and PP are funded by MRC Programme grant MR/K012037. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors have declared that no competing interests exist.

The authors wish to thank Dr James Goedert for facilitating collection of specimens for the Multicentre Haemophilia Cohort Study (MHCS) included in these analyses.

REFERENCES


Brown, Burack, J. Bush LM, Cafaro, V. Campbell O, Campbell J, Carlson
RH, Carmichael J K, Casey KK, Cavacuti C, Celestin G, Chambers ST, Chez
N, Chirch LM, Cimoch PJ, Cohen D, Cohn LE, Conway B, Cooper DA, Cornelson
B, Cox DT, Cristofano MV, Cuchural G Jr, Czartoski JL, Dahman JM, Daly JS, Davis
BT, Davis K, Davod SM, DeJesus E, Dietz CA, Dunham E, Dunn ME, Ellerin
R, Freedberg KA, French NK, Fuchs JD, Fuller JD, Gaberman J, Gallant JE, Gandhi
I, Goepfert PA, Gottlieb MS, Goulston C, Groger RK, Gurlay TD, Haber S, Hardwicke
R, Hardy WD, Harrigan PR, Hawkins TN, Heath S, Hecht FM, Henry WK, Hladek
CA, Kirchner JT, Kogelman L, Kojic EM, Korthuis PT, Kurisu W, Kwon DS, LaMar
MR, Loy D, Mohammed DY, Man A, Mansour MK, Marconi VC, Markowitz
M, Marques R, Martin JN, Martin HL Jr, Mayer KH, McElrath MJ, McGeorge
TA, McGovern BH, McGowan K, McIntyre D, Mcleod GX, Menezes P, Mesa
G, Metroka CE, Meyer-Olson D, Miller AO, Montgomery K, Mounzer KC, Nagami
EH, Nagin I, Nahass RG, Nelson MO, Nielsen C, Norene DL, O’Connor DH, Ojikutu
BO, Okulicz J, Oladehin OO, Oldfield EC 3rd, Olender SA, Ostrowski M, Owen WF
Jr, Pae E, Parsonsen J, Pavlamos AT, Perlmutter AM, Pierce MN, Pincus JM, Pisani
L, Price L, Proia L, Prokesch RC, Pujet HC, Ramgopal M, Rathod A, Rausch
M, Ravishankar J, Rhome FS, Richards CS, Richman DD, Rodes B, Rodriguez
M, Rose RC 3rd, Rosenberg ES, Rosenthal D, Ross PE, Rubin DS, Rumbaugh
E, Saenz L, Salvaggio MR, Sanchez WC, Sanjana VM, Santiago S, Schmidt
W, Schultemaker H, Sestak PM, Shalit P, Shay W, Shirvani VN, Silebi VI, Sizemore
JM Jr, Skolnik PR, Sokol-Anderson M, Sosman JM, Stabile M, Stapleton JT, Starrett
S, Stein F, Stellbrink HJ, Sterman FL, Stone VE, Stone BR, Tambussi G, Taplitz
RA, Tedaldi EM, Teioli A, Theisen W, Torres R, Tosiello L, Tremblay C, Tribble
A, Wlodaver CG, van’t Wout A, Wright DP, Yang OY, Yurdin DL, Zabukovic
BW, Zachary KC, Zeeman B, Zhao M. 2010. The major genetic determinants of HIV-1

L. Mann. 1996. Influence of combinations of human major histocompatibility complex
genes on the course of HIV-1 infection. Nat Med 2:405-11.

dimensional structure of HLA-B27 at 2.1 Å resolution suggests a general mechanism

5. Goulder, P. J., R. E. Phillips, R. A. Colbert, S. McAdam, G. Ogg, M. A. Nowak, P.
Giangrande, G. Luzzi, B. Morgan, A. Edwards, A. J. McMichael, and S. Rowland-
Jones. 1997. Late escape from an immunodominant cytotoxic T-lymphocyte response
associated with progression to AIDS. Nat Med 3:212-7.


restricted, conserved, and immunodominant cytotoxic T lymphocyte epitopes in slow progressors in HIV type 1 infection. AIDS Res Hum Retroviruses 12:1691-8.


Fig 1. HLA-B*27:02 is associated with protection against HIV disease progression.

HLA differences by log rank test are shown as follows: [A] Fraction of B27:02+ (n=12) and B27- (n=721) subjects remaining AIDS free (Absolute CD4 <200/ul) and [B] Fraction of B*27:02+ (n=13) and B*27:05+ (n=64) subjects remaining AIDS free by the 1987 CDC definition for AIDS.

Fig 2 Characterisation of HLA B*27:02 restricted CTL responses. [A] IFNγ ELISpot assay recognition of a panel of 410 overlapping peptides spanning the B clade proteome. Amino acid sequence of overlapping peptide and confirmed optimal epitope and HLA restriction are shown: Gag-KK10 is KRWIILGLNLK, Pol-KY9 is KRKGGIGGY, Nef-VW9 is VRYPLTFGW. [B] Confirmative FACS staining of a B*27:02+ subject R120 with an HLA mis-matched tetramer on the left and the B*27:02 restricted VRYPLTFGW (VW9) on the right.

Fig 3. Differential recognition of HLA-B*27-restricted epitopes. [A] Epitope responses from one representative B*27:05 subject (top panel) and one B*27:02 subject (bottom panel). CD8 is shown on the X axis and Tetramer-PE on the y axis. [B] Left panel: Percentage of B*2702-positive subjects (n=7) and B2705-positive subjects (n=19) making responses to the indicated epitopes. **=p<0.001, Fishers Exact test. Right panel: Differential recognition of B27 epitopes is related to peptide-MHC binding avidity. Peptide-MHC binding avidity is measured as Kd (nM) and shown as log10 (1/Ka). Shaded are the three epitopes Gag-KK10 (KRWIILGLNLK), Pol-KY9 (KRKGGIGGY), Nef-VW9 (VRYPLTFGW).
Fig 4. Structural modelling of the impact of B*27:05/B*27:02 polymorphisms on F-pocket amino acid compatibility. The crystal structure of B*27:05 in complex with the KK10 peptide (27, 31) was used to model how the polymorphisms in B*2702 might impact on C-terminal anchoring of peptide epitopes. Wincoot™ was used to generate a model with the following mutations in the B*27:05 F-pocket; D77N, T81I and L81A and the peptide was modelled with a Trp or a Lys at position 10. A. KK10 peptide is shown in red cartoon, with 10-Lys in red sticks. Left panels show the B*2705 F-pocket in grey surface with 77-Asp, 80-Thr and 81-Leu in grey sticks. Dotted line represents a van der Waal contact between 10-Lys and 81-Leu.
Right panels show the modelled B*2702 F-pocket in cyan surface with 77-Asn, 80-Ile and 81-Ala in cyan sticks. Red cross represents the loss of interactions between 10-Lys and 81-Ala in B*27:02. B. KK10 peptide modelled with Trp at position 10 is shown in blue cartoon, with Trp10 in blue sticks. Left panels show the B*2705 F-pocket in grey surface with 77-Asp, 80-Thr and 81-Leu in grey sticks. Red circle shows the steric clash that would occur between 10-Trp and 81-Leu. Right panels show the modelled B*2702 F-pocket in cyan surface with 77-Asn, 80-Ile and 81-Ala in cyan sticks. Black dotted lines represent a van der Waal contacts, red dotted lines represent hydrogen bonds.

Table 1 Protective effect of B*27:02 Top panel: Data from the Multicenter AIDS Cohort Study (MACS) showing comparisons between B*27-negatives (referred to as "others"), and B*27:02-positives and B*27:05 positives and time to CD4<200 mm³, AIDS defined by the CDC 1987 criteria; AIDS defined by the CDC 1993 criteria; death. Cox proportional hazards model shown. Bottom panel: ANOVA analysis of median viral loads from the MACS cohort comparing B*27-negatives and B*27:02-positives and B*27:05 positives.

Table 2. Analysis of selection pressure imposed by HLA-B*27:05+ and B*27:02+ subjects on immunodominant viral epitopes. Selection pressure on Gag KK10 (263-272), Pol KY9 (901-
and Nef VW9 (133-141) epitopes was examined in B*27:05+ (n=21), B*27:02+ (n=22), and B*27- (n=458) subjects. Odds ratio shown and p values obtained from Fishers exact test.
**Fig 1**

**A**

- Fraction AIDS free (CD<sub>4</sub>-<sub>200</sub>)

- HLA-B<sup>27</sup>-negative
- HLA-B<sup>27</sup>02

- p=0.04

**B**

- Fraction AIDS free (AIDS<sub>1987</sub>)

- HLA-B<sup>27</sup>05
- HLA-B<sup>27</sup>02

- p=0.04
### Fig 3

#### A

<table>
<thead>
<tr>
<th>Subject</th>
<th>Gag K110</th>
<th>Pol K99</th>
<th>Nef V90</th>
</tr>
</thead>
<tbody>
<tr>
<td>B*27:05+</td>
<td>1.04%</td>
<td>1.7%</td>
<td>0%</td>
</tr>
<tr>
<td>B*27:02+</td>
<td>0%</td>
<td>0.63%</td>
<td>1.14%</td>
</tr>
</tbody>
</table>

#### B

<table>
<thead>
<tr>
<th>Peptide</th>
<th>B*2705</th>
<th>B*2702</th>
</tr>
</thead>
<tbody>
<tr>
<td>YRPITFSGW</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>RQGLERAL</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>CRASHPR</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>HRLNDLLE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRKQINM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FRIQGQHSSR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VHRHPRWIL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RRRRERQQIQ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KRKQIGGY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCVPKTIH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GQNFNQRK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KRWIIGLINK</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>IRLPGKGG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Y-axis: % Responders
- X-axis: log 1/Kd (Kd nM)
Fig 4

A

B*27:05

B*27:02

80-Thr

80-Ile

80-Leu

77-Asp

77-Asn

Lys

X

81-Ala

81-Leu

81-Lys

81-Leu

81-Lys

81-Leu

81-Ala

81-Lys

81-Ala

Trp

Trp

Trp

Trp

B

B*27:05

B*27:02

80-Thr

80-Ile

80-Leu

77-Asp

77-Asn

Lys10

X

Lys10

X

Lys10

X

Lys10

X

Lys10

X
## Table 1

<table>
<thead>
<tr>
<th>Condition</th>
<th>Group 1</th>
<th>Group 2</th>
<th>p</th>
<th>HR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 &lt;200</td>
<td>B27:02 vs others</td>
<td>12 vs 721</td>
<td>0.04</td>
<td>0.35</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>B27:05 vs others</td>
<td>59 vs 674</td>
<td>0.31</td>
<td>0.82</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>B27:02 vs B27:05</td>
<td>12 vs 59</td>
<td>0.15</td>
<td>0.46</td>
<td>0.16</td>
</tr>
<tr>
<td>AIDS B7</td>
<td>B27:02 vs others</td>
<td>13 vs 770</td>
<td>0.02</td>
<td>0.09</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>B27:05 vs others</td>
<td>64 vs 719</td>
<td>0.13</td>
<td>0.71</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>B27:02 vs B27:05</td>
<td>13 vs 64</td>
<td>0.04</td>
<td>0.12</td>
<td>0.02</td>
</tr>
<tr>
<td>AIDS B9</td>
<td>B27:02 vs others</td>
<td>12 vs 721</td>
<td>0.03</td>
<td>0.33</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>B27:05 vs others</td>
<td>59 vs 674</td>
<td>0.22</td>
<td>0.78</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>B27:02 vs B27:05</td>
<td>12 vs 59</td>
<td>0.13</td>
<td>0.44</td>
<td>0.15</td>
</tr>
<tr>
<td>Death</td>
<td>B27:02 vs others</td>
<td>13 vs 770</td>
<td>0.04</td>
<td>0.13</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>B27:05 vs others</td>
<td>64 vs 719</td>
<td>0.24</td>
<td>0.76</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>B27:02 vs B27:05</td>
<td>13 vs 64</td>
<td>0.07</td>
<td>0.15</td>
<td>0.02</td>
</tr>
</tbody>
</table>

### Additional Table

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>mean log VL</th>
<th>SE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>B*27:02</td>
<td>33</td>
<td>3.46</td>
<td>0.2</td>
<td>4.00E-03</td>
</tr>
<tr>
<td>others</td>
<td>3247</td>
<td>4.04</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>B*27:05</td>
<td>299</td>
<td>3.6</td>
<td>0.07</td>
<td>7.00E-12</td>
</tr>
<tr>
<td>others</td>
<td>2981</td>
<td>4.08</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>B*27:02</td>
<td>30</td>
<td>3.49</td>
<td>0.22</td>
<td>0.6</td>
</tr>
<tr>
<td>B*27:05</td>
<td>296</td>
<td>3.6</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Variant</td>
<td>HLA-B*27:05+</td>
<td>HLA-B*27-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>---------------</td>
<td>------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>902R</td>
<td>1</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>903K</td>
<td>6</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>908G</td>
<td>8</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>902R</td>
<td>6</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>903K</td>
<td>4</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>908G</td>
<td>2</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>137L</td>
<td>1</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>137F</td>
<td>0</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>137L</td>
<td>4</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>137F</td>
<td>4</td>
<td>9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Variant</th>
<th>HLA-B*27:05+</th>
<th>HLA-B*27-</th>
</tr>
</thead>
<tbody>
<tr>
<td>264R</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>264L</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>268R</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>268L</td>
<td>7</td>
<td>15</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variant</th>
<th>HLA-B*27:05+</th>
<th>HLA-B*27-</th>
</tr>
</thead>
<tbody>
<tr>
<td>902R</td>
<td>21</td>
<td>413</td>
</tr>
<tr>
<td>903K</td>
<td>16</td>
<td>418</td>
</tr>
<tr>
<td>908G</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>902R</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>903K</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>908G</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>137L</td>
<td>24</td>
<td>434</td>
</tr>
<tr>
<td>137F</td>
<td>24</td>
<td>434</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variant</th>
<th>HLA-B*27:05+</th>
<th>HLA-B*27-</th>
</tr>
</thead>
<tbody>
<tr>
<td>264R</td>
<td>18</td>
<td>433</td>
</tr>
<tr>
<td>268R</td>
<td>18</td>
<td>433</td>
</tr>
<tr>
<td>268L</td>
<td>7</td>
<td>380</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variant</th>
<th>HLA-B*27:05+</th>
<th>HLA-B*27-</th>
</tr>
</thead>
<tbody>
<tr>
<td>902R</td>
<td>21</td>
<td>413</td>
</tr>
<tr>
<td>903K</td>
<td>16</td>
<td>418</td>
</tr>
<tr>
<td>908G</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>902R</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>903K</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>908G</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>137L</td>
<td>24</td>
<td>434</td>
</tr>
<tr>
<td>137F</td>
<td>24</td>
<td>434</td>
</tr>
</tbody>
</table>