HLA-A*7401–Mediated Control of HIV Viremia Is Independent of Its Linkage Disequilibrium with HLA-B*5703


J Immunol 2011; 186:5675-5686; Prepublished online 15 April 2011; doi: 10.4049/jimmunol.1003711
http://www.jimmunol.org/content/186/10/5675

Supplementary Material
http://www.jimmunol.org/content/suppl/2011/04/15/jimmunol.1003711.DC1

References
This article cites 56 articles, 27 of which you can access for free at:
http://www.jimmunol.org/content/186/10/5675.full#ref-list-1

Subscription
Information about subscribing to J Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
HLA-A*7401–Mediated Control of HIV Viremia Is Independent of Its Linkage Disequilibrium with HLA-B*5703

Philippa C. Matthews,* Emily Adland,* Jennifer Listgarten,† Alasdair Leslie,‡ Nompumelelo Mkwanazi,§ Jonathan M. Carlson,† Mikkel Harndahl,* Anette Stryhn,* Rebecca P. Payne,* Anthony Ogwu,§§ Kuan-Hsiang Gary Huang,* John Frater,¶ Paolo Paioni,* Henrik Kloeverpris,* Pieter Jooste,** Dominique Goedhals,†† Cloete van Vuuren,‡‡ Dewald Steyn,‡‡ Lynn Riddell,§§ Fabian Chen,§§ Graz Luzzi,¶¶ Thembia Balachandran,## Thumbi Ndung’u,§ Søren Buus,* Mary Carrington,***††† Roger Shapiro,‖†‡‡ David Heckerman,¶ and Philip J. R. Goulder*§§

The potential contribution of HLA-A alleles to viremic control in chronic HIV type 1 (HIV-1) infection has been relatively understudied compared with HLA-B. In these studies, we show that HLA-A*7401 is associated with favorable viremic control in extended southern African cohorts of >2100 C-clade–infected subjects. We present evidence that HLA-A*7401 operates an effect that is independent of HLA-B*5703, with which it is in linkage disequilibrium in some populations, to mediate lowered viremia. We describe a novel statistical approach to detecting additive effects between class I alleles in control of HIV-1 disease, highlighting improved viremic control in subjects with HLA-A*7401 combined with HLA-B*57. In common with HLA-B alleles that are associated with effective control of viremia, HLA-A*7401 presents highly targeted epitopes in several proteins, including Gag, Pol, Rev, and Nef, of which the Gag epitopes appear immunodominant. We identify eight novel putative HLA-A*7401–restricted epitopes, of which three have been defined to the optimal epitope. In common with HLA-B alleles linked with slow progression, viremic control through an HLA-A*7401–restricted response appears to be associated with the selection of escape mutants within Gag epitopes that reduce viral replicative capacity. These studies highlight the potentially important contribution of an HLA-A allele to immune control of HIV infection, which may have been concealed by a stronger effect mediated by an HLA-B allele with which it is in linkage disequilibrium. In addition, these studies identify a factor contributing to different HIV disease outcomes in individuals expressing HLA-B*5703.

The Journal of Immunology, 2011, 186: 5675–5686.

Human leukocyte Ag class I genotype is a major determinant of HIV-1 viremic control and progression to AIDS (1–5). Much of this effect is mediated by the presentation of HIV-specific epitopes by different HLA class I molecules for recognition of virus-infected cells by CD8+ T cells (6–12). HLA-B is the class I locus with the strongest influence on median viral load in chronic infection (13), and HLA-B*5703 is the allele associated with most effective suppression of viremia in C-clade–infected subjects (6, 14–17). However, an effect on viremic control has also previously been identified in association with some HLA-A alleles (18–21), and with the HLA-Cw class I locus (3, 22, 23).

The starting point of these studies was the observation that HLA-A*7401 is associated with the lowest median HIV-1 viral load of

*Department of Paediatrics, University of Oxford, Oxford OX1 3SY, United Kingdom; ‡Microsoft Research, eScience Group, Los Angeles, CA 90024; §Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Headington, Oxford OX3 9DS, United Kingdom; ¶HIV Pathogenesis Programme, Doris Duke Medical Research Institute, University of KwaZulu-Natal, Durban, 4013 South Africa; ‡‡Laboratory of Experimental Immunology, Faculty of Health Sciences, University of Copenhagen, DK-2200 Copenhagen, Denmark; §§Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana; ¶¶Suffield Department of Medicine, University of Oxford, Oxford OX1 3SY, United Kingdom; ††Paediatric Department, University of Free State, Kimberley Hospital, Kimberley 8300, Northern Cape, South Africa; ‡‡‡Department of Medical Microbiology and Virology, University of the Free State/National Health Laboratory Services, Bloemfontein 9300, South Africa; †§Department of Internal Medicine, University of the Free State, Bloemfontein 9300, South Africa; †¶Department of Genitourinary Medicine, Northamptonshire Healthcare NHS Foundation Trust, Northampton General Hospital, Cliftonville, Northampton NN1 SBD, United Kingdom; †‖Department of Sexual Health, Royal Berkshire Hospital, Reading RG1 5AN, United Kingdom; ‡§Sexual Health at Wycombe Clinic, Wycombe Hospital, High Wycombe, Bucks HP11 2TT, United Kingdom; ‡¶Department of Sexual Health, Luton and Dunstable Hospital, Luton LU4 0DZ, United Kingdom; §§§Cancer and Inflammation Program, Laboratory of Experimental Immunology, SARC-Frederick, National Cancer Institute at Frederick, Frederick, MD 21702; §§‡Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology and Harvard, Boston, MA 02129; and §§¶Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA 02115

Received for publication November 8, 2010. Accepted for publication March 4, 2011.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1003711

This work was supported by the United Kingdom Medical Research Council (to P.C.M.), the Oxford Radcliffe Hospitals Medical Research Fund (to P.C.M.), National Institutes of Health Grant 2R01AI046995, National Institute of Allergy and Infectious Diseases Contract HSNS27220090045C, the South African AIDS Vaccine Initiative, the Wellcome Trust (to A.L. and P.J.R.G.), the National Cancer Institute, National Institutes of Health Contract HHSN261200800001E, and the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research. P.J.R.G. is an Elizabeth Glaser Pediatric AIDS Foundation Scientist.

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Address correspondence and reprint requests to Prof. Philip J.R. Goulder, University of Oxford, Peter Medawar Building for Pathogen Research, South Parks Road, Oxford OX1 3SY, United Kingdom. E-mail address: philip.goulder@paediatrics.ox.ac.uk

The online version of this article contains supplemental material.

Abbreviations used in this article: HIV-1, HIV type 1; LD, linkage disequilibrium; LOCI, luminescent oxygen channeling immunoassay; OLP, overlapping peptide; P2, position two of epitope; PC, C-terminal position of epitope.
all the 23 HLA-A alleles expressed at ≥0.5% phenotypic frequency in a large cohort of HIV-1–infected subjects from Durban, South Africa (3). Furthermore, in this C-clade–infected cohort, we noted that HLA-A*7401 is associated with the selection of escape mutations in Gag, Pol, and Nef (3, 5). These two strands of evidence both suggest that HLA-A*7401–restricted CD8+ T cell responses may contribute to disease control in chronic HIV-1 infection and are in keeping with recently published data documenting an association between HLA-A*7401 and disease control in Tanzania and Kenya (19, 24).

The role of HLA-A*7401 in viremic control in Durban is potentially confined by its occurrence in linkage disequilibrium with HLA-B*5703 (23, 25). This raises the possibility that the apparent benefits to viremic control seen among subjects with HLA-A*7401 are, in fact, mediated by a linked HLA-B*5703–restricted response. Alternatively, it is possible that the impact of HLA-A*7401 is independent of HLA-B*5703 but obscured by the stronger effects of the HLA-B allele. Indeed, although HLA-B*5703 is the allele most strongly associated with successful control of viremia in sub-Saharan African populations, considerable variation in viral set point is seen among subjects possessing this allele (23). We therefore hypothesized that some of the stratification in disease outcome among subjects with HLA-B*5703 might be accounted for by the presence or absence of HLA-A*7401 in the haplotype.

HLA-A*7401 alleles belong to the HLA-A19 serotype, which comprises HLA-A*29, -A*30, -A*31, -A*32, and -A*74, and has been included within the HLA-A*03 supertype (26, 27). Although HLA-A*7401 is prevalent in sub-Saharan African populations, occurring at a phenotypic frequency of ~10%, [http://www.allelefrequencies.net (28)], the peptide binding motif for HLA-A*7401 has yet to be defined.

The aims of this study were, therefore, as follows: first, to investigate the contribution of HLA-A*7401 to control of HIV-1 infection, both alone and in combination with HLA-B*5703 in southern African populations; and second, to investigate the means by which HLA-A*7401 might contribute to viremic control via the presentation of HLA-A*7401–restricted CD8+ T cell epitopes.

**Materials and Methods**

**Study cohorts**

We studied a total of 2126 treatment-naive, adult subjects with chronic HIV-1 C-clade infection from southern Africa. We recruited subjects via five cohorts as follows: 1) Durban, South Africa (n = 1218), as previously described (3, 13, 16, 29); 2) Bloemfontein, South Africa [n = 261]; in this cohort, HLA types were ascertained only for subjects with CD4+ T cell counts <100 or >500 cells/mm3, as previously described (30); 3) Kimberley, South Africa (n = 261 postnatal mothers); 4) Gaborone, Botswana (n = 514 antenatal women) (10); and 5) 100/100 (100%) of these subjects typed to 4 digits, and 100/100 (100%) of these subjects typed to 4 digits had HLA-A*7401 confirmed by high-resolution typing. Of 81 HLA-A*74+ subjects from the Thames Valley, Botswana, Kimberley, and Bloemfontein cohorts typed to 4-digits, again 100% had HLA-A*74 confirmed by high-resolution typing. To test the generality of these findings, we also examined a random sample of 90 individuals of African descent with HLA-A*74 recently typed by the National Institutes of Health, and found that ~97% had HLA-A*74 (M. Carrington, unpublished observations). In this study, we have therefore classified all HLA-A*74+ subjects together as HLA-A*74+.

**Amplification and sequencing of proviral DNA**

Gag sequences were generated from genomic DNA extracted from PBMCs, amplified by nested PCR to obtain population sequences, as previously described (16, 31). Sequencing for the Durban and Bloemfontein cohorts was undertaken using the Big Dye Ready Reaction Terminator Mix (V3) (Applied Biosystems, U.K.) and manually aligned using Se_Al software, as previously described (31, 32). Sequencing of subjects from the Thames Valley, Botswana, and Kimberley cohorts was performed by Macrogen (South Korea). Sequences were analyzed using Sequencher v4.8 (Gene Codes Corporation). Online sequences are available at GenBank, FJ198407-FJ199088 (Durban) and FJ497801-FJ497950 (Botswana).

**HLA-A*7401 motif prediction and confirmation; HLA–peptide binding studies**

Amino acid sequences for HLA-A*74+ and related HLA-A alleles were downloaded from the International Immunogenetics Project (http://www.ebi.ac.uk/imgt/hla/). HLA-A*7401 optimal epitopes were initially predicted using motif inference based on the comparison of HLA-A*7401 with HLA-A alleles that share sequence homology for the B and F pockets of the peptide binding groove (33). These two pockets determine the preferred residues bound at the “anchor” position of the epitope (corresponding to position two [P2] and the carboxy-terminal position [PC]) (34). The validity of the predicted HLA-A*7401 peptide binding motif was tested by two methods. Optimal epitopes were first determined using IFN-γ ELISPOT assays to test recognition of peptides that corresponded to the predicted optimal epitope, and of peptides differing by one amino acid in length from each predicted optimal. We used Los Alamos HIV databases (epitopes “A-list”; http://www.hiv.lanl.gov) to search for previously published HLA-A*7401–restricted epitopes.

Binding of these predicted optimal peptides to HLA-A*7401 was tested in HLA–peptide–binding studies, undertaken using a luminescent oxygen channeling immunoenzymaas (LOCI), as previously described (35).

**IFN-γ ELISPOT assays**

We tested ex vivo PBMCs from 1010 study subjects from the Durban cohort against a panel of 410 overlapping peptides (OLPs) spanning the entire C-clade HIV-1 proteome to screen for IFN-γ ELISOT responses, as previously described (29). A total of 119 of these subjects had HLA-A*7401 (91 of these 119 [76%] were confirmed to be HLA-A*7401 at 4-digit resolution; the remainder were typed to only 2 digits). Fisher’s exact test was performed to identify responses to OLPs that were significantly associated with expression of HLA-A*7401. Analysis was also repeated in the absence of the 28 subjects for whom HLA-A*74 was typed to only 2-digit resolution to remove any potential bias from subjects not confirmed to have HLA-A*7401 as the high-resolution type. We tested putative optimal epitopes by ELISPOT using ex vivo PBMCs from HLA-A*74+ subjects recruited through the Thames Valley and Durban cohorts.

**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), Pacific orange (Invitrogen), and anti–CD3–Alexa Fluor 700 (BD Biosciences), and HLA-A*7401-RR9 tetramer conjugated to PE. Dead cells were gated out using “live/dead” viability kit (Invitrogen).

**Statistical analysis**

We used Fisher’s exact test to screen for LD between HLA class I alleles, using the online tool available at http://www.hiv.lanl.gov/content/immunology/hla/hla_linkage.html, corrected for multiple comparisons using a Bonferroni approach. To identify MHC class I haplotypes, we used PyPop software [Python for Population Genomics, http://www.pypop.org (36)] (Supplemental Table I). Other statistical analysis was undertaken using Prism GraphPad software v. 5.0a.

To assess whether any possible pairs of HLA class I alleles might perform better than a single allele in mediating disease control in chronic infection,
we devised a novel statistical test for additive effects using pooled data from a total of 2126 C-clade–infected adult subjects from southern Africa. Each HLA combination was tested to see whether an additive model for two HLA alleles together, irrespective of linkage disequilibrium, performed better in predicting disease control than a model that considered only the most predictive of each single HLA allele. For the purposes of this test, each allele was collapsed to a two-digit resolution only, apart from exceptions in which it is known that the four-digit types cross supertype boundaries or have a strong bearing on outcome (HLA-A*68XX, HLA-B*15XX, and HLA-B*58XX) (13, 27). For disease control based on the log of the absolute viral load and the log of the CD4+ T cell count, we used the log likelihood of linear-regression-based models to look for additive effects. In particular, we defined the null model for a given HLA pair as the one of the two models, ϕ0 or ϕ1 [defined next], that had the maximum likelihood under a maximum likelihood parameter setting. Intuitively, it is a model where at most only one HLA at a time can have an effect within an individual patient; the HLA chosen is the one with the larger overall effect in such a model. Thus, the null model was either ϕ0 (phen|X, Y) = normal(α0 + ωX + ωY + ωXY, σ α), where X′ = 0 for people with X = 1, and otherwise X′ = X, or ϕ0′(phen|X, Y) = normal(α0 + ωX + ωY′ + ωXY′, σ α), where Y′ = 0 for people with X = 1, and otherwise Y′ = Y. The alternative model used is given by ϕ1(phen|X, Y) = normal(α0 + ωX + ωY + ωXY, σ α).

The variable "phen" is, for example, the viral load; X and Y are binary variables representing two HLA alleles; ωX and ωY are parameters in the model for the effect of each allele; σ α is the variance parameter of the normal distribution; and ω0 is an offset parameter. Parameters were fit independently for each model. The test statistic for an HLA pair was the difference in log likelihood between these null and alternative models after fitting each by maximum likelihood. The values were obtained by 50,000 permutations of one HLA allele in the test. In addition, any pair of alleles for which one of the alleles did not have a univariate correlation in the direction of control (as defined by the phenotype of interest) was assigned a test statistic of zero. Note that two alleles in perfect linkage disequilibrium (LD) or anti-LD could not come up as having an additive effect with this test statistic. For binary outcomes (disease control based on criteria of viral load ≤ 2000 RNA copies/ml plasma and CD4+ T cell count > 250 cells/μl), we used an analogous test based on logistic regression.

To account for possible differences between cohorts that might cause artifact either by enhancing or obscuring additive effects between HLA alleles (i.e., confounding), we also corrected for geographical origin of the subject by adding a set of binary cohort covariates to each model (one for recruitment in each of Durban, Kimberley, Bloemfontein, or Gaborone; we removed Thames Valley subjects who originate from a wide variety of locations). An LRT test showed these cohort covariates to be highly significant (e.g., p = 2 × 10−59) in predicting the log CD4+ T cell count, so these covariates were used in all models. Note that all HLA pairs coming where HLA-B*5703 in South African subjects from Durban, Kimberley, and Bloemfontein (n = 1510, p = 1.1 × 10−26, Fisher’s exact test) (Fig. 1A). In the epidemiologically unlinked study population of adults in Botswana (n = 514), we did not identify statistically significant LD between these two alleles (Fig. 1A, 1B). The apparent lack of LD between HLA-A*7401 and HLA-B*5703 in Botswana does not appear to be attributable to a smaller sample size than our South African cohorts, because in the Botswana cohort, only 1/46 (2.2%) HLA-A*7401* subjects also carried HLA-B*5703, whereas in the South African cohorts, 25/157 (15.9%) HLA-A*7401* subjects also carried HLA-B*5703. The HLA-A*7401-B*5703 haplotype has previously been identified in African Americans [http://www.allelefrequencies.net (28); both alleles resolved to two digits only in this source (3)], but to our knowledge has not been reported in other African populations to date.

To confirm the haplotypes in which HLA-A*7401 is transmitted, we analyzed HLA data for Durban, Bloemfontein, and Gaborone using PyPop software [http://www.pypop.org (36)] (Supplemental Table I). In keeping with our analysis of LD (Fig. 1), this demonstrates that HLA-A*7401 is transmitted on a haplotype with HLA-B*1503, HLA-B*3501, and HLA-B*5703 in South Africa. The association of HLA-A*7401 with HLA-B*1503 and HLA-B*3501 is also seen in Botswana, but in this population, there is no HLA-A*7401-B*5703 haplotype. In addition, these data show a weaker haplotype of HLA-A*7401-B*4201 in all three cohorts, but the LD between HLA-A*7401 and this allele was not statistically significant.

HLA-A*7401 is statistically associated with lowered viral load in chronic C-clade HIV-1 infection

To assess the relation between HLA-A*7401 and viral load in chronic infection, we compared subjects with and without HLA-A*7401 in four different cohorts: Durban, Gaborone, Bloemfontein, and the Thames Valley cohorts (excluding Kimberley because of small numbers). In each case, HLA-A*7401 was associated with a reduction in viral load in chronic infection (p = 0.0007 (Fig. 2A), p = 0.02, p = 0.2, and p = 0.05, respectively, Mann–Whitney U test). The trend toward lowered viremia in association with HLA-A*7401 in Bloemfontein may not have reached statistical significance at least in part because of the small number of HLA-A*7401 subjects in Bloemfontein (n = 8). In all southern African subjects, HLA-A*7401 was strongly associated with lowered viremia (median viral set point, 14600 versus 32450 RNA copies/ml in HLA-A*7401+ versus HLA-A*7401−; n = 1965 subjects with viral load data available; p < 0.0001, Mann–Whitney U test) (Fig. 2B).

In subjects from Gaborone (where HLA-A*7401 is not in linkage disequilibrium with HLA-B*5703), CD4+ T cell counts were statistically higher in subjects with HLA-A*7401 (p = 0.0005, Mann–Whitney U test) (Fig. 2C). This illustrates that HLA-A*7401 may mediate a protective effect that is independent of linkage disequilibrium with HLA-B*5703. Associations between absolute CD4+ T cell count and expression of HLA-A*7401 did not reach statistical significance in the Durban, Bloemfontein, or Thames Valley cohorts (data not shown). However, in pooled data from all subjects, there was a significant association between the presence of HLA-A*7401 and higher CD4+ T cell count (median CD4+ cell count, 409 versus 350 in HLA-A*7401+ versus HLA-A*7401−; n = 1962 subjects with CD4+ cell count available; p = 0.001, Mann–Whitney U test) (Fig. 2D).

HLA-A*7401 and HLA-B*5703 have an additive effect in lowering viral load in chronic C-clade HIV-1 infection

The relation between the presence of HLA-A*7401 and improved disease control of HIV-1 may be confounded by haplotype, whereby the observed effect of HLA-A*7401 on viral load arises only as a consequence of linkage with favorable alleles, in particular, with HLA-B*5703 in South African subjects (Fig. 1A). To mitigate this haplotype effect, we next investigated the impact of HLA-A*7401 on viral load after removing subjects with HLA-B*5703 from the analysis. Among subjects with HLA-A*7401, there remained an association with lowered viremia in the absence of HLA-B*5703 (p = 0.002 in the pooled cohort, p = 0.09 in the Durban cohort, Mann–Whitney U test) (Fig. 3A, 3B). Strikingly,
however, the combination of HLA-A*7401 and HLA-B*5703 resulted in significantly lower viral loads than in the presence of either allele alone, suggesting an additive effect of these alleles in mediating control of disease (Fig. 3A, 3B), as seen previously (23). Additional evidence for the independent role of HLA-A*7401 in lowering viremia, irrespective of the presence of HLA-B*5703, comes from the Botswana cohort in which there is no LD between these alleles (Fig. 1), but HLA-A*7401 is still associated with favorable outcomes (see earlier and Fig. 2C).

As HLA-A*7401 is also transmitted in strong LD with HLA-B*1503 and HLA-B*3501 in both Botswana and South Africa (Fig. 1A), we also sought to exclude either of these alleles as the driving force behind the apparent benefit of HLA-A*7401. There was no relation between HLA-A*7401 or HLA-B*3501 and lowered viremia in this cohort (data not shown); indeed, in Durban, HLA-B*1503 was actually statistically associated with higher viral loads (p = 0.03, Mann–Whitney U test; data not shown). The overall relation between HLA-A*7401 and lowered viral load is, therefore, not likely to be attributable to linkage with either of these alleles.

To further investigate the possible additive effect of HLA-A*7401 on disease control in subjects with HLA-B*5703, we ranked HLA-B*5703+ subjects from the whole pooled southern African cohort (n = 97) and from Durban (n = 55) according to viral load. We found significant enrichment of HLA-A*7401 in subjects with viral loads less than the median in each case (p = 0.015 in the whole cohort, p = 0.003 in Durban, Fisher’s exact test). Division of these HLA-B*5703+ subjects into four pools by viral load quartiles confirmed enrichment of HLA-A*7401 in subjects with the lowest viral loads (Fig. 3C, 3D). This phenomenon is not associated with the presence of other favorable HLA-B alleles, such as HLA-B*5702, -B*5801, or -B*8101, in subjects with the lowest viral loads (data not shown), suggesting that HLA-A*7401 may indeed be responsible for mediating the effect.

To investigate whether the beneficial effect of HLA-A*7401 occurs significantly in combination with HLA alleles other than HLA-B*5703, we undertook a computational analysis of all 2126 southern African subjects. Using this newly developed test, we sought evidence for additive effects of any combination of two HLA alleles (collapsed to two-digit resolution), irrespective of LD in improving disease control compared with the effect of any one allele alone. After correction for cohort location, we identified statistically significant additive effects between HLA-A*74 and two favorable HLA-B alleles, HLA-B*57 and HLA-B*81, in mediating control of viral load (Table II). No significant effect of HLA-A*74 was determined in conjunction with any other allele in

---

**Table I. Summary of 2126 C-clade HIV-1–infected study subjects from five southern African study cohorts**

<table>
<thead>
<tr>
<th>Cohort Location</th>
<th>Number (%) with HLA-A*7401&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Viral Load (RNA Copies/ml Plasma)</th>
<th>CD4 T Cell Count (Cells/mm&lt;sup&gt;3&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Median</td>
<td>IQR</td>
</tr>
<tr>
<td>Durban, South Africa</td>
<td>1218</td>
<td>135 (11.1)</td>
<td></td>
</tr>
<tr>
<td>Bloemfontein, South Africa&lt;sup&gt;b&lt;/sup&gt;</td>
<td>261</td>
<td>21 (8.0)</td>
<td></td>
</tr>
<tr>
<td>Kimberley, South Africa</td>
<td>31</td>
<td>1 (3.2)</td>
<td></td>
</tr>
<tr>
<td>Gaborone, Botswana</td>
<td>514</td>
<td>46 (8.9)</td>
<td></td>
</tr>
<tr>
<td>Thanes Valley, southern Africa</td>
<td>102</td>
<td>13 (12.7)</td>
<td></td>
</tr>
<tr>
<td>All Southern Africa</td>
<td>2126</td>
<td>216 (10.2)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>The 4-digit HLA type was determined in 181/216 subjects (84%); in all 181 of these (100%), the 4-digit type was HLA-A*7401. In the remaining 35 cases typed to 2 digits, the 4-digit type was undetermined.

<sup>b</sup>Bloemfontein data are for 261 HLA-typed subjects with CD4+ T cell count < 100 or > 500 cells/mm<sup>3</sup>. In an extended Bloemfontein cohort (including subjects without HLA types), median CD4+ cell count was 229 cells/mm<sup>3</sup>, with interquartile range (IQR) of 122–366 cells/mm<sup>3</sup> (n = 885), and median viral load was 76,000, with IQR of 20,000–21,000 cells/mm<sup>3</sup> (n = 500).

---

**FIGURE 1.** LD between HLA-A*7401 and other class I alleles in southern African populations. A, All HLA-B and HLA-Cw alleles that are significantly linked to HLA-A*7401 in a pooled cohort from South Africa (Durban, Bloemfontein, and Kimberley cohorts, n = 1510) and in Botswana (n = 514) are shown. The p values were calculated by Fisher’s exact test and corrected for multiple comparisons using the Bonferroni approach, using the online tool at: http://www.hiv.lanl.gov/content/immunology/hla/hla_linkage.html. No significant linkage associations were identified for HLA-A*7401 among Zimbabweans recruited via the Thames Valley cohort (n = 73, data not shown). All subjects with HLA-B*35 are pooled together as HLA-B*35(01). B, Percentage of HLA-A*7401+ subjects with HLA-B*5703 in each of five southern African cohorts. Statistically significant LD between these alleles is present only in the Durban cohort.
Having predicted a peptide binding motif for HLA-A*7401, we identified regions of the HIV-1 proteome-containing potential HLA-A*7401–restricted epitopes using two approaches. First, we screened IFN-γ ELISPOT responses from 1010 Durban subjects. We identified two statistically significant IFN-γ responses made by 119 subjects with HLA-A*7401: these were to OLP-19 in p24 Gag (p = 1.7 × 10⁻⁵ and OLP-100 in Rev (p = 1.8 × 10⁻³; Fisher’s exact test) (Fig. 4, Table IV). Even after exclusion of the minority of subjects with HLA-A*74 restricted to only two digits (n = 28), both of these associations remained statistically significant (p = 2.5 × 10⁻⁴ and p = 1.8 × 10⁻³, respectively). To define the optimal epitope within each of these OLPs, we identified a putative HLA-A*7401–restricted epitope based on the earlier predicted binding motif for HLA-A*7401 (LI/M/Q at P2 and R/K at PC) (Table III). Within OLP-19 (IVNLQQGMVHQAISPR), the peptides GR11 (GQMVHQAISPR, p24-Gag 8–18) and QR10 (QMVHQAISPR, p24-Gag 9–18) fitted this motif. Within the Rev 17mer OLP-100 (RWRARQQRHISILER), two putative overlapping HLA-A*7401–binding peptides were identified: Rev-RR9 (RQRHISILER, Rev 50–58) and Rev-RR11 (RQRHISILER, Rev 48–58).

The second approach we used to identify HIV-specific HLA-A*7401–restricted epitopes involved reference to previously determined HLA-A*7401–associated HIV amino acid polymorphisms within Gag, Pol, and Nef population sequences from the Durban cohort (3). Six sites of HIV polymorphism associated with HLA-A*7401 were identified (3, 40) (Table V). Based on the predicted HLA-A*7401 binding motif, we used these sites of point mutation to identify further putative HLA-A*7401–restricted epitopes, p17-KR9 (p17 12–20, KLDKWEKIR), Nef-RR11 (RQRQIHISILER), two putative overlapping HLA-A*7401–binding peptides were identified: Rev-RR9 (RQRHISILER, Rev 50–58) and Rev-RR11 (RQRQIHISILER, Rev 48–58).

The amino acid sequence determining the B pocket of the peptide binding groove of HLA-A*7401 allele is identical to that of HLA-A*3201 and closely related to that of HLA-A*0101 (37) (Table III, top half). HLA-A*0101 preferentially binds epitopes bearing a small aliphatic residue, such as Thr/Ser/Ile/Leu, at position 2 (33, 37, 38), but differs from the HLA-A*7401 allele by predicting preferential binding of the basic residues Arg or Lys (27, 33, 39).

**Identification of putative HLA-A*7401-restricted epitopes in HIV-1**

To confirm the optimal epitope, we used IFN-γ ELISPOT assays to quantify CD8⁺ T cell responses to the optimal peptide and variant truncations of the peptide in individual subjects selected on the basis that they made a response to the relevant OLP. Using this method, we acquired data to support the optimal epitope in three of our predicted HLA-A*7401–restricted epitopes: p24-Gag-GR11 (p24 Gag 8–18, GQMVHQAISPR), RT-GR9 (RT 269–277, QYPGIKVR), and Rev-RR9 (Rev 50–58, RQRQIHISILER) (Fig. 5A–C, respectively). In the case of Rev-RR9, we also synthesized the peptide–MHC class I tetramer to confirm the HLA restriction of the response (Fig. 5D).

We also used LOCI (35) to confirm binding of HLA-A*7401 to optimal peptides Gag-GR11, RT-GR9, and Rev-RR9 (Fig. 6). These binding studies also add to the evidence for two other putative HLA-A*7401–restricted epitopes, p17-KR9 and Nef-RR9.
There was no substantial difference in the strength of binding of overlapping variants of our three confirmed epitopes (p24-Gag epitopes p24-GR11/QR10, RT-SR10/QR9, and Rev-RR11/RR9) (Fig. 6), suggesting that the optimal peptide for presentation is not necessarily determined by differences in HLA class I binding. Together, these ELISPOT, tetramer, and binding data strongly support three new HLA-A*7401–restricted epitopes (Gag-GR11, RT-QR9, and Rev-RR9) and point to two further likely epitopes (Gag-KR9 and Nef-SR9). These data also substantiate our proposed motif for HLA-A*7401 as L/I/M/Q at P2. At the PC of the epitope, R appears to be the preference (our two putative epitopes bearing K at this position, p17-SK10 and RT-SK9, were both low binders to HLA-A*7401). We also tested binding of the only HLA-A*7401 HIV-1 epitope that has previously been reported by Los Alamos HIV databases (http://www.hiv.lanl.gov), Prot-IV9 (Pol 58–67, ITLWQRPLV) (41); this does not match our motif prediction at the PC, and was also a low binder.

In the presence of HLA-A*7401, the HLA-B*5703 Gag footprint is diminished and HLA-B*5703–restricted IFN-γ ELISPOT responses are preserved

To investigate the way in which HLA-A*7401 may ameliorate disease progression in subjects with HLA-B*5703, we investigated the impact of HLA-A*7401 on the selection of polymorphisms and...
on IFN-γ ELISPOT responses associated with HLA-B*5703. HLA-B*5703 is strongly associated with the selection of five mutations in p24 Gag, at positions A146, I147, A163, S165, and T242, within and flanking the epitopes ISW9 (p24 Gag 147–155, ISPRTLNAW), KF11 (p24 Gag 162–172, KAFSPEVIPMF), and TW10 (p24 Gag 240–249, TSTLQEIQAW) (3, 6, 16, 17).

In 64 subjects with HLA-B*5703, there was a trend toward preservation of the wild-type amino acid in the presence of HLA-A*7401 at 4 of the 5 sites of polymorphism (I147, A163, S165, and T242), although this only reached statistical significance at S165 (p = 0.004, Fisher’s exact test; Fig. 7A). The exception was A146, flanking the ISW9 epitope. However, selection at this position may occur as a consequence of many different alleles because of the extensive overlap of CD8+ T cell epitopes at this position (including HLA-B*3910, HLA-B*1510, HLA-B*5801, and HLA-B*4801) (10). Subjects with HLA-A*7401 were also less likely than HLA-A*7401+ individuals to have mutations at all five sites (p = 0.03, Fisher’s exact test) (Fig. 7A).

To determine whether the presence of HLA-A*7401 has an impact on HLA-B*5703 responses to Gag epitopes, we studied ELISPOT data from 46 HLA-B*5703+ southern African subjects to have mutations at all five positions making response/total number of subjects. Although responses to individual OLPs containing HLA-B*5703 epitopes were not statistically associated with the presence or absence of HLA-A*7401, we found overall preservation of an IFN-γ response to these OLPs in the presence of HLA-A*7401 (p = 0.006 for response to any OLP containing an HLA-B*5703 epitope, Fisher’s exact test) (Fig. 7B). The trend toward maintenance of these IFN-γ ELISPOT responses is in keeping with the sequence data showing preservation of wild-type epitopes in the presence of HLA-A*7401.

FIGURE 4. Proportion of subjects with and without HLA-A*7401 making IFN-γ ELISPOT responses to HIV-1 C-clade OLPs. IFN-γ ELISPOT responses to OLP-19 and OLP-100 are significantly enriched among subjects with HLA-A*7401 (Durban cohort; n = 1010; p values by Fisher’s exact test). Subjects with HLA-B*1510 are removed from the analysis of responses to OLP-100 to avoid potential confounding because of the presence of an overlapping HLA-B*1510 epitope IL-9 (HHSISERIL, Rev 52–60) (29, 56), accounting for higher than expected rate of responses in the HLA-A*7401- population (method as previously described [13]). n = number making response/total number of subjects.

Discussion

These studies add to recently published literature (19, 23) substantiating the beneficial influence of HLA-A*7401 on disease control in adult subjects with HIV-1 infection, both alone and in tandem with HLA-B*5703. HLA-A*7401 is common in black African populations (occurring at a 10.2% phenotypic frequency in our extended cohort of >2100 southern African subjects), but has been relatively understudied compared with many HLA-B alleles.

The transmission of HLA-A*7401 in LD with HLA-B*5703 in the Durban cohort highlights the difficulties of appreciating the influence of a single allele. Although our calculations to verify LD do not, of course, demonstrate, in an individual subject, on which haplotype the HLA-A*7401 and HLA-B*5703 alleles are located, they show that statistically these alleles are likely to arise on the same haplotype when coexpressed in a particular individual, and this is verified by haplotype analysis using PyPop (36) (Supplemental Table I). Given the well-established benefits of HLA-B*5703, it would be easy to dismiss the apparent association between HLA-A*7401 and low viremia as simply caused by linkage with HLA-B*5703. However, in addition to our data demonstrating a significant HLA-A*7401–restricted response through CD8+ T cell epitopes, we also present two further strands of evidence for an independent role for HLA-A*7401. First, the favorable effect of this allele on viremic control observed in a Botswana cohort (in which these two alleles are not transmitted in LD) suggests a true independent benefit of HLA-A*7401. Second, we have identified a consistent additive effect of HLA-A*74 and HLA-B*57 through a novel computational approach to the analysis of all possible pairs of two alleles at the A, B, and C loci in >2100 subjects.

Our results demonstrate that HLA-A*7401 is associated with lower viral loads and higher CD4+ T cell counts in several
southern African cohorts. Although these associations do not reach statistical significance in every cohort, the trends are broadly consistent in different settings. There are several possible explanations for the lack of strong correlation with CD4+ T cell counts in cohorts other than Gaborone. First, viral load and CD4+ T cell count are correlated, but not always strongly (typical correlation coefficients reported are of $r^2 = 0.28$) (42), so an association between HLA-A*7401 and lowered viremia does not necessarily translate into a statistically significant association with higher CD4+ T cell count, especially where cohort size is small. Indeed, this same observation has been made in a previous study of HLA associations with viral set point or absolute CD4+ T cell count (23), in which some alleles were significantly associated with low viral set point, for example, but not with high absolute CD4+ T cell count. An alternative possible explanation of this observation might be that certain HLA alleles have a stronger impact on CD4 count than on viral load, or vice versa, for reasons unknown.

Using motif prediction undertaken according to previously validated methodology (33), in combination with ELISPOT and sequence data, we identified eight potential sites of HLA-A*7401–restricted CD8+ T cell epitopes in HIV-1 Gag, Pol, Rev, and Nef (Tables IV, V). Putative epitopes at five of these sites are intermediate or good binders to HLA-A*7401. In particular, the presence of epitopes in Gag may contribute to viremic control in two specific ways. First, the presence of Gag epitopes suggests the potential for an HLA-A*7401–restricted response to emerge early in the course of acute infection because of the abundance of Gag protein at the initiation of infection (43). Second, of the six sequence polymorphisms we identified in association with HLA-A*7401, three have previously been predicted to revert to wildtype after transmission to an HLA-mismatched host (Table V), suggesting a fitness cost imposed by the mutation (3). In these instances, we predict that selection of the mutation itself may contribute to suppression of viremia in subjects with HLA-A*7401 through an effect on viral infectivity and/or replicative capacity. This effect has previously been shown in mutations in HLA-B*57 epitopes that contribute to a reduced fitness virus (6, 17) and are therefore associated with clinical control of disease (44–46). In particular, the presence of two reverting sites in Gag highlights specifically that HLA-A*7401 may contribute to viremic control through the selection of costly mutations in the highly structurally constrained Gag protein (17, 47–49).

To date, only one putative HIV-1 epitope restricted by HLA-A*7401 is listed by Los Alamos HIV databases “A-list” (http://www.hiv.lanl.gov), Prot-IV9 (Pol 58–67 ITLWQRPLV) (41). However, this epitope sequence was determined through pre-
diction, and the HLA restriction has not been demonstrated. The binding studies shown in this article suggest this peptide is a very low binder of HLA-A*7401 (Fig. 6), and together with two of the putative epitopes presented in this article (Prot-NR10 and Prot-SK9), it is therefore less likely to be a commonly targeted HLA-A*7401–restricted epitope. A further two putative overlapping HLA-A*7401–restricted epitopes have previously been proposed on the basis of an HLA-A*7401–associated polymorphism at position Gag-441 (24). One of these did fit the HLA-A*7401 binding motif proposed in this article, with Lys at the PC; however, the other lacked a positively charged residue at PC. No HLA-A*7401 binding data were provided for these proposed epitopes, and these peptide sequences did not meet the more rigorous criteria for epitopes set out in the Los Alamos Immunology Database “A-list.” These criteria include titration curves showing recognition of the optimal epitope at serial peptide dilutions compared with four additional peptides, each differing from the optimal by one amino acid, either longer or shorter by one amino acid at the N or C termini, respectively (see Fig. 5A, 5B), or unequivocal staining with a peptide–MHC tetramer (see Fig. 5D). Peptides have been described that do not precisely fit the established peptide binding motif for the respective HLA molecule (50), but these are the exception rather than the rule. In contrast, more typically, a peptide that does not fit the peptide binding motif (such as Prot-IV9; Fig. 6) proves not to bind once the peptide–MHC binding assays are undertaken.

Notably, the approach we adopted using HLA-associated polymorphisms to identify location of an epitope successfully enabled us to identify optimal HLA-A*7401–restricted epitopes, but this approach is challenging because, by definition, few HLA-A*7401+ individuals make responses to epitopes where escape mutations are so readily selected. Despite screening cryopreserved PBMCs from 15 HLA-A*7401+ subjects from the Thames Valley cohort against OLPs and optimal peptides for putative epitopes restricted by this allele, we identified only two responses: one each in subjects H005 and R070 (Fig. 5). On a follow-up visit (after an interval of 23 mo), H005 had lost the IFN-γ response to RT-QR9, and we were therefore unable to proceed with further restriction of this epitope. These findings suggest that HLA-A*7401 CD8+ T cell responses may be short-lived (arising early in the course of infection and being lost as a consequence of immune escape), or may be subdominant compared with greater magnitude responses restricted by HLA-B alleles, and therefore difficult to detect using this IFN-γ ELISPOT screening method. However, despite the challenges of confirming HLA-A*7401–restricted responses, in this article, we present in vitro data (binding studies and tetramer data) that support three new epitopes: Gag-GR11 (GQMVHQAISPR), RT-QR9 (QIYPGIKVR), and Rev-RR9 (RQIHSISER).

In these in vitro experiments, HLA-A*7401 constructs were used, confirming the binding of this allele (resolved to high resolution) to predicted peptides. Although ELISPOT responses to a particular peptide cannot necessarily be attributed to one specific allele (in this case, HLA-A*7401), the combined approach of analysis of ELISPOT and sequence data, motif inference, in vitro binding studies, and tetramer binding is a robust method of highlighting putative novel epitopes.

**FIGURE 6.** Binding of HLA-A*7401 to putative optimal epitopes, including overlapping variants of epitopes in p17, RT, and Rev and sequence variants of the epitope in Nef. Binding affinity KD (nM) of HLA-A*7401 to optimal peptides assessed by LOCI (35). Strength of binding is classified on a log scale in accordance with previous methods (35).

**FIGURE 7.** Relation between HLA-A*7401 status and frequency of HLA-B*5703–restricted CD8+ T cell mutations/IFN-γ ELISPOT responses. A. Frequency of HLA-B*5703–selected Gag polymorphisms [defined by previous studies (3, 6, 16, 17)], in 64 subjects with HLA-B*5703 according to the presence/absence of HLA-A*7401. p24-Gag sequences from Bloemfontein (n = 8), Durban (n = 38), Gaborone (n = 11), and Thames Valley cohorts (n = 7), with sequence data available for TW10 (n = 63), KF11 (n = 64), ISW9 (n = 63), and all three epitopes (n = 62). B. Frequency of IFN-γ ELISPOT response to three HLA-B*5703 epitopes in p24-Gag in 46 subjects with HLA-B*5703 according to the presence or absence of HLA-A*7401. The p values were by Fisher’s exact test. Data from Durban (n = 31) and Thames Valley cohorts (n = 15).
The mechanism by which the additive effect of HLA-A*7401 and HLA-B*5703 arises is uncertain. The preservation of wild-type HLA-B*5703 epitopes and the related maintenance of an HLA-B*5703-restricted CD8+ T cell response in the presence of HLA-A*7401 may be the cause or effect of lowered viremia. Several potential mechanisms may explain the favorable effect of this haplotype. First, there may simply be an additive effect of multiple CD8+ T cell responses consequent on the dual presentation of epitopes restricted by both alleles on the surface of infected cells, particularly given the Gag-directed responses restricted by both alleles. The preservation of wild-type sequence in HLA-B*5703-restricted Gag epitopes in the presence of HLA-A*7401 suggests the possibility that subdominant responses mediated by HLA-B*5703 on control of viremia (3, 6, 13, 16, 23), we demonstrate that, for HLA-A*7401, this is indeed the case. As cohort sizes increase, it will become possible to disentangle the haplotypic effects arising from the presence of several distinct HLA and non-HLA alleles on the same chromosome.

In addition to HLA-A*7401, there may be other examples of HLA-A alleles that are associated with good viremic control but in which the benefit is obscured by the influence of HLA-B alleles with which they are linked. One such example is HLA-A*6601. This occurs in LD with HLA-B*5802 in Durban (p value for linkage = 3.5 × 10^-7, Fisher’s exact test), an allele associated with poor viremic control and rapid progression to AIDS (13, 55). When ranked according to median viremia in chronic infection, HLA-A*6601 appears to be among the worst performing of HLA-A alleles; however, when subjects with HLA-B*5802 are removed from the analysis, a significant association between HLA-A*6601 and good control of viremia emerges (23).

In summary, this work represents investigation of HLA-A*7401 and the HLA-A*7401-B*5703 haplotype. Although this allele has recently been recognized to be associated with favorable viremic control (19, 23, 24), the mechanism for this phenomenon has not previously been studied. Despite the strong favorable effect mediated by HLA-B*5703 on control of viremia (3, 6, 13, 16, 23), we show in this article that the HLA-A*7401-B*5703 haplotype is more favorable than HLA-B*5703 alone. Our data do not demonstrate a single explanation for this effect, but we present compelling evidence for an independent CD8+ T cell response restricted by HLA-A*7401. These findings highlight the poten-

Table IV. Putative HLA-A*7401–restricted epitopes predicted using motif inference (32) combined with analysis of IFN-γ ELISpot assays

<table>
<thead>
<tr>
<th>Protein</th>
<th>OLP Number</th>
<th>C-clade OLP Sequence</th>
<th>p Value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P&lt;sub&gt;2&lt;/sub&gt; Value</th>
<th>C Terminus</th>
<th>Epitope Designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>p24-Gag</td>
<td>19</td>
<td>IVQNLGQMQMVHQAISPR</td>
<td>1.7 × 10^-4</td>
<td>Q MVHQAISP R</td>
<td>(Gag-QR10)</td>
<td></td>
</tr>
<tr>
<td>Rev</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
<td>RWRARQRQIHSISERIL</td>
<td>1.8 × 10^-5</td>
<td>R QIHSISE R</td>
<td>(Rev-RR9)</td>
<td></td>
</tr>
</tbody>
</table>

Two C-clade 17-mer OLPs significantly associated with IFN-γ ELISpot responses in subjects expressing HLA-A*7401 are shown (Durban and Thames Valley cohorts, as shown in Fig. 4; p values by Fisher’s exact test). Arrows indicate the anchor positions at which the optimal binds the class I allele (position 2 and C terminus); these residues are shown in bold and underlined.

<sup>a</sup>The <i>p</i> values both remain statistically significant if analysis repeated after removal of minority of subjects with HLA-A*74 typed to only two-digit resolution: OLP-19, <i>p</i> = 2.5 × 10^-5; OLP-100, <i>p</i> = 1.8 × 10^-5.

<sup>b</sup>Responses to OLP-100 are corrected by removal of subjects with HLA-B*1510 from analysis, because of presence of HLA-B*1510 epitope IL9 (HISISERIL, Rev 52–60; http://www.lanl.gov).

Table V. Putative HLA-A*7401–restricted epitopes predicted using motif inference (32) combined with analysis of HLA-A*7401 associations with sequence polymorphisms

<table>
<thead>
<tr>
<th>Protein</th>
<th>HXB2 Position of Polymorphism</th>
<th>A*7401 Polymorphism</th>
<th>Reversion</th>
<th>p Value</th>
<th>q Value</th>
<th>P&lt;sub&gt;2&lt;/sub&gt; Value</th>
<th>C Terminus</th>
<th>Epitope Designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>p17 Gag</td>
<td>12</td>
<td>GARASLRLGERXLDKWEK1LR</td>
<td>R</td>
<td>2.71E-14</td>
<td>0.00</td>
<td>S LRGKELDR</td>
<td>(Gag-SK10)</td>
<td></td>
</tr>
<tr>
<td>p17 Gag</td>
<td>20</td>
<td>GRLDLKWEK1RRLPGKKKYM</td>
<td>R</td>
<td>6.47E-10</td>
<td>0.00</td>
<td>K DKW KEI</td>
<td>(Gag-KR9)</td>
<td></td>
</tr>
<tr>
<td>Protease</td>
<td>9</td>
<td>F E R N L A F Q G E A R F P E S E</td>
<td>4.83E-05</td>
<td>0.02</td>
<td>N L A F P G K</td>
<td>(Prot-NR10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT</td>
<td>277</td>
<td>A S Q I Y P G K V Q L C K L L R G K A K</td>
<td>3.13E-04</td>
<td>0.13</td>
<td>Q Y P G K V</td>
<td>(RT-QR9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT</td>
<td>476</td>
<td>I V S L T E T T N Q K T E L Q A I Q L A L</td>
<td>6.03E-04</td>
<td>0.15</td>
<td>S L T E T T N Q</td>
<td>(RT-SK9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nef</td>
<td>192</td>
<td>K W R F D S L A R H L A R L H P E Y</td>
<td>R</td>
<td>4.95E-05</td>
<td>0.00</td>
<td>S L L A R H L A</td>
<td>(Nef-SR9)</td>
<td></td>
</tr>
</tbody>
</table>

Six sites of HLA-A*7401–associated polymorphism identified from lineage-corrected analysis of sequences from a total of 710 Durban subjects (q < 0.2), as previously published (3). The position of the polymorphism is marked ▼, with consensus sequence 10 as upstream and downstream of the site of polymorphism. Arrows indicate anchor positions (shown in bold and underlined) as in Table IV. R indicates polymorphisms that are predicted to revert to wild-type after transmission to an HLA-mismatched recipient [methods as previously described (17, 57)].

<sup>a</sup>Squences based on Durban consensus sequence from Gag (n = 446) and Nef (n = 436) subjects.
tial for HIV-1 disease, and demonstrate the importance of neutralizing antibodies in controlling HIV-1 disease control, and demonstrate the importance of a broad CD8⁺ cell response, especially targeting Gag peptides, and suggest the utility of combining multiple CD8⁺ T-cell responses in a potential vaccine.

**Disclosures**

The authors have no financial conflicts of interest.

**References**

4. O’Brien, S. J., S. Gao, and M. Carrington. 2001. HIV-1 disease control, and demonstrate the importance of neutralizing antibodies in controlling HIV-1 disease control, and demonstrate the importance of a broad CD8⁺ cell response, especially targeting Gag peptides, and suggest the utility of combining multiple CD8⁺ T-cell responses in a potential vaccine.

**References**

4. O’Brien, S. J., S. Gao, and M. Carrington. 2001. HIV-1 disease control, and demonstrate the importance of neutralizing antibodies in controlling HIV-1 disease control, and demonstrate the importance of a broad CD8⁺ cell response, especially targeting Gag peptides, and suggest the utility of combining multiple CD8⁺ T-cell responses in a potential vaccine.

**References**


Supplementary Table I: MHC Class I haplotypes involving HLA-A*7401 in three Southern African cohorts, identified using PyPop software (31).

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Number of subjects</th>
<th>MHC Class I Haplotype</th>
<th>Haplotype frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>(A:B:Cw)</em></td>
<td></td>
</tr>
<tr>
<td>Durban, South Africa</td>
<td>1211</td>
<td>7401:1503:0202</td>
<td>1.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7401:35(01):0401</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7401:57(03):07(01)</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7401:1503:0210</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7401:4201:1701</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7401:4901:0701</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7401:8101:0401</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7401:5801:0302</td>
<td>0.10</td>
</tr>
<tr>
<td>Gaborone, Botswana</td>
<td>490</td>
<td>7401:1503:02(10)</td>
<td>1.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7401:3501:04(01)</td>
<td>1.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7401:4201:1701</td>
<td>0.45</td>
</tr>
<tr>
<td>Bloemfontein, South Africa</td>
<td>259</td>
<td>7401:1503:0210</td>
<td>1.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7401:4201:1701</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7401:3501:0401</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7401:5703:0701</td>
<td>0.39</td>
</tr>
</tbody>
</table>

*a* Four digit types in parentheses indicate pooled subjects with the two digit and four digit allele

*b* Haplotypes involving HLA-A*7401 that occur in >1 subject are listed