Possession of HLA Class II *DRB1*1303* Associates with Reduced Viral Loads in Chronic HIV-1 Clade C and B Infection

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Background. The HLA class II molecules play a central role in the generation of human immunodeficiency virus (HIV)–specific CD4^{+} T-helper cells, which are critical for the induction of cytotoxic CD8^{+} T cell responses. However, little is known about the impact of HLA class II alleles on HIV disease progression.

Methods. In this study we investigated the effect of HLA class II alleles on HIV disease outcome and HIV-specific T cell responses in a cohort of 426 antiretroviral therapy–naive, HIV-1 clade C–infected, predominantly female black South Africans.

Results. The HLA class II allele *DRB1*1303* was independently associated with lower plasma viral loads in this population ($P = .02$), an association that was confirmed in a second cohort of 1436 untreated, HIV-1 clade B–infected, male European Americans, suggesting that *DRB1*1303*-mediated protection is independent of ethnicity, sex, and viral clade. Interestingly, *DRB1*1303* carriage was not associated with an increased frequency of interferon (IFN) γ–positive HIV-specific CD4^{+} T cell responses.

Conclusions. These data demonstrate the independent effect of an HLA class II allele, *DRB1*1303,* on HIV disease progression, in the absence of increased IFN-γ–positive HIV-specific CD4^{+} T cell frequencies, suggesting that the protective activity of *DRB1*1303* may be mediated via an alternative mechanism.

CD4^{+} T cells play a pivotal role in adaptive immunity, because they are required for the development and maintenance of memory and cytotoxic CD8^{+} T cells

[1–4]. On the other hand, human immunodeficiency virus (HIV)–specific CD4^{+} T cells serve as the primary target cell population for viral replication [5]. Thus, induction of HIV-specific CD4^{+} T cells may have pleiotropic effects, on the one hand protecting against HIV through its role in adaptive immune responses but, at the same time, generating a pool of target cells for HIV propagation.

HLA class II molecules are involved in presenting antigen to CD4^{+} T cells, and their role is therefore considered central for the development and maintenance of HIV-specific CD4^{+} T-helper cells. Of the 10 genes that encode for HLA class II molecules (reported as of January 2010), the *DRB1* gene at the *DRB* locus is the most diverse, encoding for 762 distinct alleles (http://hla.alleles.org/nomenclature/stats.html).

Despite the potential role of HLA class II alleles in HIV infection, only a limited number of alleles have been associated with HIV disease outcome [6–12]. In

particular, several *DRB1*13* alleles and the *DRB1*13-DQB1*06* haplotype have been reported to confer a certain degree of protection, but all of these reports focused on highly selected patient subsets, including long-term nonprogressors, subjects treated during acute infection, and children with vertically transmitted HIV. The role of HLA class II alleles in chronic progressive HIV infection remains unknown.

To investigate the effect of HLA class II alleles on disease progression in chronic HIV infection, we studied a well-characterized cohort of 426 antiretroviral therapy (ART)–naive, HIV-1 clade C–infected, mainly female black South Africans, as well as 1436 HIV-1 clade B virus–infected persons from the Multicenter AIDS Cohort Study (MACS). Here we report that the *DRB1*1303* allele is associated with reduced viral loads (VLs) in both B and C clade populations, although *DRB1*1303* expression was not associated with an increased frequency of interferon (IFN) γ–positive HIV-specific CD4$^+$ T cell responses. This suggests that the protective activity of specific HLA class II alleles may be mediated via an alternative mechanism.

MATERIALS AND METHODS

Ethics Statement

For the Sinikithemba cohort, written consent was obtained from all study participants. The institutional review boards of Massachusetts General Hospital and the National Institutes of Health and the ethics committee of the University of KwaZulu-Natal granted ethical approval. For the MACS cohort, protocols were approved by the institutional review boards at Johns Hopkins Bloomberg School of Public Health; Howard Brown Health Center, Feinberg School of Medicine, Northwestern University; Cook County Bureau of Health Services; University of California, Los Angeles; and University of Pittsburgh; informed written consent was obtained from all participants.

Study Population

For the Sinikithemba cohort, 426 HIV-1–infected subjects (80.8% female [$n = 344$], 19.2% male [$n = 82$]) from the local Zulu/Xhosa population were recruited at the Sinikithemba Care Center at McCord Hospital in Durban, South Africa, from 2003 to 2006. All subjects were ART naive at the time of enrollment, with a mean VL of 4.6 $\log_{10}$ HIV RNA copies/mL plasma (range, 1.7–6.8) and a mean CD4$^+$ T cell count of 350 cells/μL whole blood (range, 6.5–1161); all longitudinal CD4$^+$ T cell count and VL values were collected before initiation of ART. Mean CD4$^+$ T cell count and VL values were calculated using all data available for each study subject. We included an average number of 7 VL and 7 CD4$^+$ T cell values per patient (range, 1–18) collected over a period of >2 years (range, .23–5.03 years). *DRB1* typing data was available from 426 subjects, and *DQB1* typing data from 398. HLA class I typing, mean CD4$^+$ T cell counts, and plasma VL data were available for all 426 study subjects.

We also analyzed 1436 HIV-1–infected male white subjects from the MACS, a prospective, observational study of natural histories of HIV-1 infection in the United States [13]. HLA class I and *DRB1* typing, as well as mean CD4$^+$ T cell counts (mean, 553 cells/μL; range, 26–1913) were available from all 1436 subjects. Because some individuals in this cohort were recruited before plasma VL quantification became a routine procedure, this information was available for only 1359 study subjects (mean $\log_{10}$ VL, 4.1 HIV RNA copies/mL plasma; range, 1.6–6.4). Mean CD4$^+$ T cell counts and VLs were calculated using all data available for each study subject before ART was initiated. For the MACS cohort, an average of 20 VL values and 20 CD4$^+$ T cell counts per patient were available (range, 3–57), spanning >10 years of follow-up (range, .5–24.5 years).

HLA Class I and II Genotyping

For both cohorts, high-resolution (4-digit allele level) HLA genotyping was carried out using the standard sequence-based typing protocols developed by the 13th International Histocompatibility Workshop (HLA typing and informatics. In: Hansen JA, ed. Immunobiology of the human MHC. Seattle, Washington: IHWG Press, 2006:179–477). Briefly, HLA class I genes were amplified by polymerase chain reaction (PCR), with locus-specific primers spanning exons 2 and 3. PCR products were sequenced on a 3730xl DNA Analyzer (Applied Biosystems). Class II sequence-based typing was based on exon 2. For *DRB1* sequence-based typing, generic *DR* types were first elucidated by sequence-specific PCR followed by group-specific PCR amplification and sequencing of exon 2. Sequencing results were interpreted with the help of the ASSIGN 3.5 software developed by Conexio Genomics. Ambiguities were resolved by using allele-specific PCR primers to isolate each of the 2 alleles in heterozygous individuals.

Intracellular Cytokine Staining

The intracellular cytokine assay was conducted using peripheral blood mononuclear cells isolated from whole blood within 4 h of phlebotomy, as described elsewhere [14]. From the Sinikithemba cohort, 403 patients were screened for CD4$^+$ and CD8$^+$ T lymphocyte responses against a peptide pool spanning the HIV-1 protein Gag; 318 were also screened for responses against the proteins Pol, Env, Nef, Acc, and Reg (based on HIV-1 clade C consensus sequence 2001). Control conditions were established by using 2 tubes of unstimulated peripheral blood mononuclear cells per patient (with the average background subtracted from the response for the experimental tubes). A response was considered positive for HIV-1–specific T cells if it was still greater than the background after subtraction of the average background. Gag specific CD4$^+$ T cell responses as well as HIV-specific CD8$^+$ T cell responses from a subset of the Sinikithemba cohort have been reported elsewhere [15, 16].

Statistical Analysis

SAS software (version 9.1; SAS Institute) was used for statistical analyses, and graphs were plotted using GraphPad Prism software (version 4). The VLs were transformed into log scale, and the CD4$^+$ T cell counts are shown as square roots to account for normal distribution. Procedure PROC GLM was used for analysis of variance, and Bonferroni correction was performed for multiple comparisons. To eliminate confounding by the effect of *HLA B*57* in the Sinikithemba cohort, this factor was used as a covariate in the analysis; both *HLA B*27* and *HLA B*57* were used as covariates in the analysis of the MACS cohort. Further analysis with adjustment for sex or age did not change the overall results (data not shown). T cell responses were compared using the Mann-Whitney test. Survival analysis was performed using the Cox proportional hazard model.

RESULTS

Frequencies of HLA Class II Molecules in HIV-1 Clade C–Infected Zulu/Xhosa from South Africa

We first determined allele frequencies at the *DRB1* and *DQB1* loci ($n = 426$ for *DRB1*, $n = 385$ for *DQB1*) in a cohort of chronically HIV-1–infected, untreated Zulu/Xhosa subjects (Sinikithemba cohort) in KwaZulu Natal Province, South Africa, where the overall seroprevalence rates are among the highest on the African continent (UNAIDS 2008; http://data. unaids.org/pub/Report/2008/20080904_southafrica_anc_2008_ en.pdf). *DRB1*0302* was the most common *DRB1* allele observed ($n = 140$; 16.43%) followed by *DRB1*1101* ($n = 115$; 13.50%) and *DRB1*1301* ($n = 101$; 11.85%), whereas *DRB1*1303* was expressed in only 1.9% ($n = 16$) of the population. In African Americans, *DRB1*1303* has been reported to be expressed at slightly higher rates (3.5%–3.7% of individuals (http://www.allelefrequencies.net), compared with ∼1% of European Americans). Allele frequencies for HIV-uninfected black South Africans are not available. In the Sinikithemba cohort, *DQB1*06* was the most common *DQB1* allelic group expressed ($n = 249$; 32.4%), followed by *DQB1*03* ($n = 159$; 20.56%), *DQB1*04* ($n = 143$; 18.57%), and *DQB1*02* ($n = 134$; 17.40%).

Protective Effect of *DRB1*1303* on HIV Disease Outcome in HIV-1 Clade C–Infected Zulu/Xhosa from South Africa and Clade B–Infected White Subjects from the United States

Having defined the frequencies of these class II alleles in this population, we next investigated potential associations between HLA class II allele expression, plasma RNA VLs, and CD4$^+$ T cell counts by comparing the mean VL (or CD4$^+$ T cell count) of those individuals expressing a particular HLA class II allele with values for the rest of the population (Table 1). In these infected Zulu/Xhosa subjects, *DRB1*1303* was the only allele associated significantly with a lower mean VL and an increased mean CD4$^+$ T cell count (mean VL, 3.93 vs 4.62 log$_{10}$ HIV RNA

copies/mL; square root CD4$^+$ T cell count, 20.47 vs 17.73 cells/ μL: $P = .002$ and $P = .04$, respectively) (Figure 1A). In this African population, *DRB1*1303* is in linkage disequilibrium with *HLA B*5703* (D' = .48), an *HLA* class I allele known to be associated with lower HIV VL [17]. However, after correction for *HLA B*57*, the protective effect of *DRB1*1303* on mean VL remained significant, although the association was weaker (log$_{10}$ VL, 3.93 vs 4.48 RNA copies/mL; $P = .02$). In contrast, the effect on mean CD4$^+$ T cell counts was lost (square root CD4$^+$ T cell for *DRB1*1303* carriers vs noncarriers, 20.47 vs 18.57; $P = .20$). The lack of seroconversion data in the Sinikithemba cohort, however, prevents a clear interpretation of the latter result. These data suggest that *DRB1*1303* has an independent protective effect on the level of HIV plasma viremia.

Given the extensive polymorphism at the *DRB1* locus, the protective effect of *DRB1*1303* did not remain significant for CD4$^+$ T cell counts and was only borderline significant for VL after correction for multiple tests ($P = .048$). Thus, to validate the protection conferred by *DRB1*1303* observed in the Sinikithemba cohort, we tested for a potential effect of *DRB1*1303* specifically in a second, non-African cohort. We compared mean VL and CD4$^+$ T cell counts between individuals in the MACS cohort who expressed this allele (28 with VL and 31 with CD4$^+$ T cell data) and those who did not (1231 with VL and 1405 with CD4$^+$ T cell data). As in the analysis performed in the Sinikithemba cohort, we corrected statistically for the effects of *HLA B*57* (B*5701). We also included *HLA B*27* as a covariable in the model, because this allele, predominantly expressed in white ethnicities, is associated with a better disease course in HIV infection, as is *HLA B*57*. Once again, the *DRB1*1303* allele was associated with lower mean VLs (mean plasma VL, 3.33 vs 3.78 log$_{10}$ HIV RNA copies/mL; $P = .02$) in this predominantly clade B–infected European American cohort. In contrast to the results in the African cohort, mean CD4$^+$ T cell counts in the clade B cohort were significantly higher in the 31 *DRB1*1303* carriers than in the rest of the cohort ($n = 1405$) (mean square root CD4$^+$ T cell count, 26.11 vs 23.57 cells/μL; $P = .03$) (Figure 1B). Among 395 seroconverters from the MACS cohort, for whom seroconversion dates were known, 7 expressed *DRB1*1303*. Although statistical power was very limited, a consistent trend toward slower disease progression was observed in the *DRB1*1303* carriers (for the end points of CD4$^+$ T cell counts <200 cells/μL, AIDS diagnoses in 1987 or 1993, and death, hazard ratios ranged from .54 to .67), although this was not significant.

Because *HLA B*57* and *DRB1*1303* are in linkage disequilibrium in African populations, we tested whether *DRB1*1303* confers protection in the absence of *B*57*. Half of the Sinikithemba samples (8/16) but only 1 of 30 MACS samples carried both *DRB1*1303* and HLA-*B*57*. The 8 subjects who were carriers of *DRB1*1303* but not *B*57* had a mean plasma VL of 4.15 log$_{10}$ HIV RNA copies/mL, compared with 4.65 log$_{10}$ HIV RNA copies/mL for the *DRB1*1303* noncarriers ($P = .13$). Although

Table 1. Association between *DRB1/DQB1* Allele Expression and Mean CD4^{+} T Cell Counts and Viral Loads in the Sinikithemba Cohort[a]

DRB1/DQB1 Type	CD4^{+} T Cell Count, Mean Square Root, Cells/μL (No. of Subjects)			Viral Load, Mean, log$_{10}$ HIV RNA copies/mL (No. of Subjects)		
	Allele Expressed	Allele Not Expressed	P	Allele Expressed	Allele Not Expressed	P
DRB1*0102	19.77 (22)	17.72 (404)	.08	4.40 (21)	4.61 (400)	.31
DRB1*0301	17.23 (65)	17.91 (361)	.33	4.67 (64)	4.59 (357)	.49
DRB1*0302	18.03 (128)	17.74 (298)	.61	4.54 (126)	4.62 (295)	.40
DRB1*0401	16.49 (25)	17.91 (401)	.20	4.94 (25)	4.58 (396)	.05
DRB1*0404	21.36 (11)	17.74 (415)	.03[b]	4.53 (11)	4.60 (410)	.81
DRB1*0701	17.45 (67)	17.90 (359)	.52	4.76 (67)	4.57 (354)	.11
DRB1*0804	18.80 (33)	17.75 (393)	.28	4.46 (31)	4.61 (390)	.36
DRB1*1001	17.95 (19)	17.82 (407)	.92	4.34 (19)	4.61 (402)	.20
DRB1*1101	17.39 (109)	17.98 (317)	.32	4.62 (108)	4.59 (313)	.78
DRB1*1102	18.06 (32)	17.81 (394)	.80	4.50 (31)	4.61 (390)	.53
DRB1*1201	16.92 (30)	17.90 (396)	.34	4.64 (30)	4.59 (391)	.80
DRB1*1301	17.59 (94)	17.90 (332)	.63	4.62 (94)	4.59 (327)	.76
DRB1*1302	18.66 (38)	17.75 (388)	.31	4.66 (37)	4.59 (384)	.68
DRB1*1303	20.47 (16)	17.73 (410)	.04[b]	3.93 (16)	4.62 (405)	.002[b]
DRB1*1501	20.06 (9)	17.78 (417)	.21	4.78 (9)	4.59 (412)	.55
DRB1*1503	17.37 (76)	17.93 (350)	.41	4.52 (75)	4.61 (346)	.42
DQB1*02	17.45 (125)	17.90 (273)	.43	4.66 (124)	4.61 (270)	.61
DQB1*03	17.64 (149)	17.83 (249)	.74	4.64 (147)	4.61 (247)	.76
DQB1*04	18.25 (135)	17.51 (263)	.19	4.54 (133)	4.67 (261)	.17
DQB1*05	18.45 (79)	17.59 (319)	.20	4.53 (78)	4.64 (316)	.32
DQB1*06	17.72 (205)	17.80 (193)	.89	4.66 (204)	4.58 (190)	.35

NOTE. [a] *DRB1* and *DQB1* alleles with frequencies >1% of total populations are shown. Mean CD4^{+} T cell counts and human immunodeficiency virus type 1 (HIV-1) viral loads are shown for each allele.

[b] Significant differences (p<0.05).

the difference in VL was not significant, the trend was consistent with protection. As expected, *DRB1*1303* in the absence of *B*57* was significantly associated with lower VLs ($P = .02$) and higher CD4^{+} T cell counts ($P = .03$) after excluding the single carrier of both *DRB1*1303* and *B*57* from the analysis.

Given the well-described protection conferred by *HLA B*57* [17], we attempted to determine in the Sinikithemba cohort whether *DRB1*1303* enhances *HLA B*57* protection. We compared the mean VL of individuals expressing *HLA B*57* plus *DRB1*1303* with that of individuals expressing *HLA B*57* but not *DRB1*1303*. Interestingly, subjects with both *HLA B*57* and *DRB1*1303* expression had a lower mean VL than those who expressed *HLA B*57* but not *DRB1*1303*, though this difference did not reach significance (mean log$_{10}$ plasma VL, 3.7 vs 4.36 HIV RNA copies/mL, respectively; $P = .11$). Thus, *DRB1*1303* may enhance the protective effect of *HLA B*57*, potentially through a mechanism mediated by CD4^{+} T-helper cells, but further studies are necessary to confirm or negate this hypothesis.

Expression of *DRB1*1303* Associates with Decreased HIV-Specific CD4^{+} T Cell Responses
It has recently been shown that virus-specific CD4^{+} T cells targeting the Gag protein are associated with reduced VLs in

chronic HIV infection [15], but it remains unclear whether these conserved CD4^{+} T cell responses are a cause or consequence of reduced antigen exposure in vivo. To identify a potential mechanism by which *DRB1*1303* mediates reduced viral levels, T cell responses among individuals expressing *DRB1*1303* were compared with those in noncarriers. The total magnitude and protein specificity of HIV-specific CD4^{+} and CD8^{+} T cells in 403 subjects of the Sinikithemba cohort were determined by measuring intracellular IFN-γ production after stimulation with HIV peptide pools spanning all HIV proteins. The median frequency of Gag-specific CD4^{+} T cells was significantly reduced in subjects expressing *DRB1*1303* (.06% vs .15% of total CD4^{+} T cells in *DRB1*1303* carriers vs noncarriers, respectively; $P = .0067$) (Figure 2A). Although we did observe a trend toward lower total HIV-specific CD4^{+} T cell counts in the *DRB1*1303* carriers (.23% vs .42% of total CD4^{+} T cells; $P = .09$) (Figure 2B), this trend was driven by the Gag responses, because total HIV responses excluding Gag (ie, Pol, Env, Nef, Acc, and Reg only) showed no significant difference between the *DRB1*1303* carriers and noncarriers (data not shown). In contrast, no significant differences were observed between *DRB1*1303* carriers and noncarriers in Gag-specific (.86% vs .84%; $P = .99$) or total
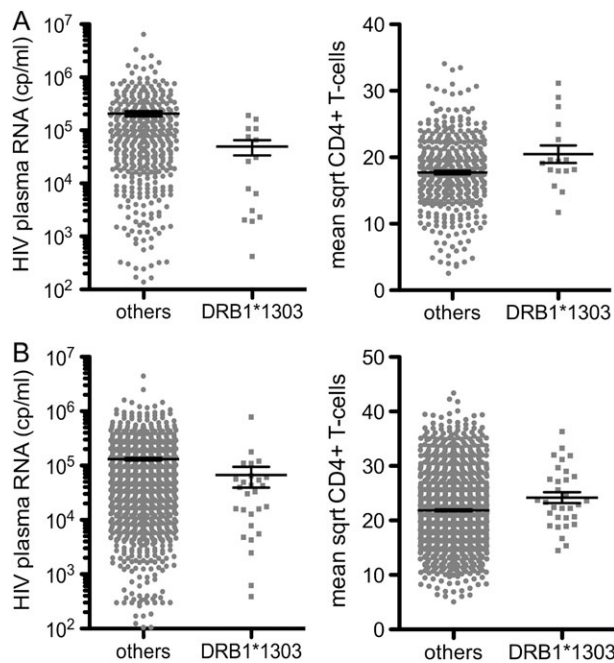
Figure 1. Mean human immunodeficiency virus (HIV) plasma RNA levels and mean square root (sqrt) CD4$^+$ T cell counts of *DRB1*1303* noncarriers versus carriers in the Sinikithemba cohort (*A*) (after correction for *B*57*, $P = .02$ for viral load and $P = .2$ for CD4$^+$ T cell count) and the Multicenter AIDS Cohort Study cohort (*B*) (after correction for *B*27* and *B*57*, $P = .02$ for viral load and $P = .03$ for CD4$^+$ T cell count). Bars represent mean values ($\pm$ standard errors of the mean).

(3.11% vs 3.96%; $P = .23$) CD8$^+$ T cell responses (Figure 3A and B). Adjustment of T cell responses for mean VLs had no influence on the results (data not shown), suggesting that lower Gag-specific CD4$^+$ T cell counts in *DRB1*1303* carriers are not a consequence of antigen exposure. Rather, reduction of the Gag-specific CD4$^+$ T cell population, which accounts for ∼40% of the total HIV-specific CD4$^+$ T cell pool, might contribute to reducing VLs by limiting the number of target cells available for HIV to infect.

DISCUSSION

There is strong evidence for the association between HLA class I alleles and HIV disease outcome [16–19], but the role of HLA class II alleles in chronic HIV-1 infection has not yet been examined in such detail. Because HLA class II molecules determine the antigen specificity of CD4$^+$ T cells, they might be indirectly involved in the control of HIV infection; HIV-specific CD4$^+$ T-helper cells are required for the development of memory and cytotoxic CD8$^+$ T cells [1–4].

To understand better the interaction between HLA class II alleles, HIV disease outcome, and HIV-specific CD4$^+$ T cell responses, we examined 426 subjects in the Sinikithemba cohort, consisting of ART-naive, HIV-1 clade C–infected, predominantly female black South Africans. We hypothesized that
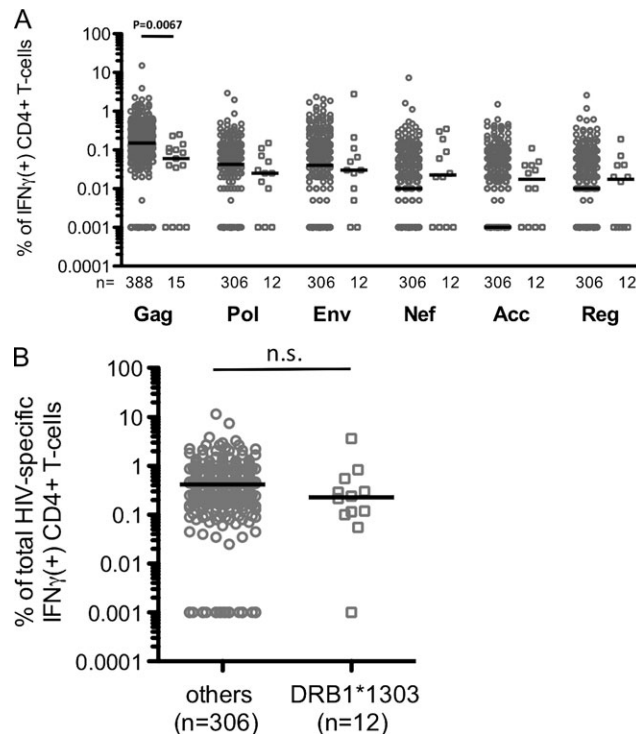

Figure 2. Human immunodeficiency virus (HIV) protein-specific magnitude (*A*) and total HIV magnitude (*B*) of interferon (IFN) γ–positive CD4$^+$ T cell responses in subjects with or without *DRB1*1303* expression. Bars represent median values. Undetectable results are represented at an arbitrary assay cutoff of .001. Only statistically significant differences are displayed; n.s., not significant.

certain HLA class II alleles are associated with reduced VLs in chronic HIV-1 clade C infection, as has been shown for several HLA B-alleles in this same cohort [16]. Individuals expressing the *DRB1*1303* allele showed significantly lower mean plasma VLs compared with the rest of the cohort, even after correction for the protective HLA class I allele *B*57*, which is in linkage disequilibrium with *DRB1*1303* in populations of African descent. Furthermore, an analysis of the *DRB1*1303* allele in 1436 chronically HIV B clade–infected, untreated white male subjects from the MACS cohort in the United States also revealed that carriers of the *DRB1*1303* allele had significantly lower VLs and increased CD4$^+$ T cell counts, thus supporting our initial observation of a moderate protective effect of this allele. Our data suggest that the effect of *DRB1*1303* on mean plasma VLs is mediated independently of ethnic background or infecting HIV-1 clade. Additionally the effect of *DRB1*1303* appears to be independent of sex as well, because >80% of subjects in the African cohort were female, but the MACS cohort was entirely male.

The observation that *DRB1*13* confers protection has been shown in other infections, in particular hepatitis B virus infection, where this allele is associated with a higher frequency of clearance and/or better clinical disease course [20–24]. Furthermore, studies in human papillomavirus (HPV) infection
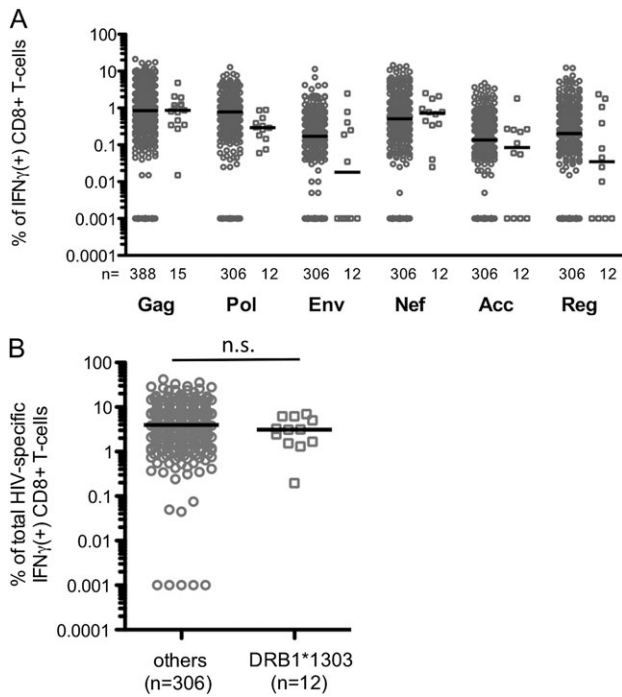
Figure 3. Human immunodeficiency virus (HIV) protein-specific magnitude (*A*) and total HIV magnitude (*B*) of interferon (IFN) γ–positive CD8$^+$ T cell responses in subjects with or without *DRB1*1303* expression. Bars represent median values. Undetectable results are represented at an arbitrary assay cutoff of .001. Only statistically significant differences are displayed; n.s., not significant.

have associated *DRB1*13* with protection against the HPV-16 serotype, and *DRB1*13* was negatively correlated with the occurrence of cervical carcinoma [25, 26]. In contrast, *DRB1*13* enrichment has also been reported in subjects with autoimmune diseases, including anti–citrullinated protein antibody–negative rheumatoid arthritis [27], severe forms of Henoch-Schönlein purpura [28], and type 1 autoimmune hepatitis [29]. *DRB1*1303* might therefore share some similarities with the HLA class I allele *B*2705,* which is associated with protection in HIV and HCV infection [16, 17, 30] but is common among patients with autoimmune disorders, such as ankylosing spondyloarthritis [31]. It has been proposed that the effect of *DRB*13* on disease pathogenesis may be related to particularly effective antigen presentation capacity, which confers protection against viral infections, but may trigger autoimmunity through molecular mimicry with self-antigens [32]. It remains uncertain, however, whether the observed associations can be attributed to a stronger and more effective T cell response or whether other mechanisms mediate the *DRB1*13* effect.

Because increased frequencies of HIV-specific CD4$^+$ T cells have recently been associated with viral control in chronic HIV-1 infection [15, 33], we reasoned that stronger and conserved CD4$^+$ T-helper responses might explain the lower VL levels in individuals expressing the *DRB1*1303* allele. We therefore

determined the total magnitude and protein specificity of HIV-specific CD4$^+$ and CD8$^+$ T cells in 403 subjects in the Sinikithemba cohort by measuring intracellular IFN-γ production after stimulation with HIV peptide pools spanning all HIV proteins. Interestingly, carriers of *DRB1*1303* tended to have lower frequencies of total HIV-specific IFN-γ–positive CD4$^+$ T cells than carriers of other allelic types, primarily owing to significantly reduced Gag-specific responses in these individuals. However, we might have underestimated the true frequency of HIV-specific CD4$^+$ T cells by limiting our detection assay to IFN-γ–secreting cells and not taking into account other cytokine activity, such as interleukin 2 production, a characteristic of central memory CD4$^+$ T cells [34]. Another possible explanation for reduced HIV-specific CD4$^+$ T cells along with lower VLs in *DRB1*1303* carriers could be the decreased induction of these cells, resulting in fewer targets for HIV infection, as reported by Douek et al [5]. HIV-specific CD4$^+$ cells are preferentially infected by HIV and contain more HIV viral DNA than other memory CD4$^+$ T cells. The lack of an expanding pool of Gag-specific CD4$^+$ cells could contribute to "passive" control of the infection. More in-depth studies investigating maturation phenotype, polyfunctionality, epitope specificity, and proviral loads of CD4$^+$ T cells in individuals expressing *DRB1*1303* are needed to fully understand the association between the genetics and T cell immunology.

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