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# Immunodominant HIV-1-specific HLA-B- and HLA-C-restricted CD8+ T cells do not differ in polyfunctionality

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ABSTRACT

HIV-1 specific HLA-B-restricted CD8+ T cell responses differ from HLA-C-restricted responses in antiviral effectiveness. To investigate possible reasons for these differences, we characterized the frequency and polyfunctionality of immmunodominant HLA-B*57/B5801- and HLA-Cw*07-restricted CD8+ T cells occurring concurrently in nine study subjects assessing IFN-γ, TNF-α, IL-2, MIP-1β, and CD107a by flow cytometry and analyzed sequence variation in targeted epitopes. HLA-B*57/5801 and HLA-Cw*07 restricted CD8+ T cells did not differ significantly in polyfunctionality ($p = 0.84$). Possession of three or more functions correlated positively with CD4+ T cell counts ($r = 0.85$; $p = 0.006$) and monofunctional CD8+ T cells inversely correlated with CD4 cell counts ($r = -0.79$; $p = 0.05$). There were no differences in polyfunctionality of CD8+ T cells specific to wildtype versus mutated epitopes. These results suggest that loss of polyfunctionality and increase in monofunctional HIV-1-specific CD8+ T cells are associated with disease progression independent of restricting HLA allele. Furthermore, sequence variation does not appear to significantly impact CD8+ T cell polyfunctionality in chronic HIV-1 infection.



## Introduction

HIV-specific CD8+ T cells play a vital role in the control of HIV replication and disease progression (Altfeld et al., 2006; Borrow et al., 1994; Koup et al., 1994; Schmitz et al., 1999). However, differences exist in their antiviral effectiveness based on their HLA restriction, epitope specificity, functional epitope avidity and targeted viral protein (Bennett et al., 2007; Bihl et al., 2006; Kiepiela et al., 2007). In particular, major histocompatibility complex (MHC) class I molecules have been shown to differ in their ability to mediate the control of HIV and SIV replication in humans and non-human primates respectively (Goulder and Watkins, 2008; Kiepiela et al., 2004). For example, virus-specific Gag CD8+ T cell responses restricted by HLA-B*57, HLA-B*5801 and HLA-B*27 are associated with low viral loads or slow disease progression in HIV infection (Kiepiela et al., 2007; Klein et al., 1998; Migueles and Connors, 2001; Novitsky et al., 2003) while Mamu-A*01 and Mamu-B*17 restricted responses are associated with control in SIV infection of rhesus macaques (Chung et al., 2007; Loffredo et al., 2007; Maness et al., 2008; Migueles et al., 2003).

In contrast to the beneficial outcomes associated with the protective MHC allele-restricted Gag CD8+ T cell responses, Gag HLA-C-restricted CD8+ T cell responses were found to be associated with high viral loads (Kiepiela et al., 2007). Paradoxically, although HLA-C-restricted CD8+ T lymphocytes appear to contribute little or even negatively to viral control *in vivo*, HLA-C is not down-regulated by HIV-1 Nef from the surface of infected cells to the same extent that HLA-A and HLA-B molecules are (Cohen et al., 1999; Collins et al., 1998). Overall, the mechanisms underlying control of HIV by protective alleles such as HLA-B*57/5801, or the lack of control by HLA-C alleles remain unclear and this limited understanding has important implications for rational vaccine design.

HIV-specific CD8+ T cells may also display different differentiation status and activation profiles (Appay and Sauce, 2008; Papagno et al., 2004). It has been suggest that these phenotypic differences are associated with divergent functional antiviral capacities of virus-specific T cells (Almeida et al., 2009). Some studies have suggested that polyfunctional CD8+ T cells, able to secrete up to five different effector functions (IFN-γ, IL-2, TNF-α, MIP-1β and CD107a), have better antiviral activity (Betts et al., 2006; Daucher et al., 2008; Precopio et al., 2007). CD8+ T cell responses restricted by HLA-B*27

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and HLA-B*57 alleles were reported to be polyfunctional when compared to CD8+ T cell responses restricted by HLA-A alleles within the same patients (Harari et al., 2007). However, recent data have suggested that the functional profile of CD8+ T cells is largely a consequence of the duration and level of antigen load, with prolonged continuous exposure to high levels of antigen resulting in exhausted CD8+ T cells characterized by a monofunctional effector profile (Rehr et al., 2008; Streeck et al., 2008a).

Here, we studied the polyfunctionality profiles of immunodominant HLA-B and HLA-C-restricted CD8+ T cells in a cohort of HIV-1 clade C chronically infected individuals displaying both responses. This provided the unique opportunity to examine HLA-B and C restricted responses in the context of matched viral loads and CD4 cell counts. We focused on the immunodominant HLA-B*57/*5801 epitopes in Gag p24 and the immunodominant HLA-Cw*07 restricted epitope KY11 in Nef, in persons possessing both responses. We hypothesized that HLA-B CD8+ T cells will display a more polyfunctional phenotype compared to HLA-C-restricted CD8+ T cells. We also aimed to determine whether sequence variation within epitopes presented by these two alleles has a bearing on the magnitude and polyfunctionality of epitope-specific CD8+ T cell responses. The relationship between the frequency of polyfunctional HIV-1-specific CD8+ T cells and CD4+ T cell counts and viral loads was also investigated.

## Results

### *Characteristics of study subjects*

The study subjects were seven females and two males, with a median age of 40 (range 27–58) years, all coexpressing HLA-B*57/5801 and Cw*07. The subjects were selected on the basis of possession of concurrent dominant HLA-B*57/5801 and HLA-Cw*07 restricted HIV-1 specific CD8+ T cell responses ≥500 SFC/$10^6$ on IFN-γ ELISPOT. The median plasma viral load was 11,500 (range 2530–750,000) RNA copies/ml and the median CD4 count was 271 (range 202–411) cells/μl. The subject gender, age, CD4 count, viral load, HLA type and epitopes examined for each subject are shown in Table 1. These subjects were selected from the Sinikithemba cohort, which compromised of 451 HIV-1 infected individuals whose time of infection was unknown. In the cohort, 37 of 451 (8.2%) participants coexpressed HLA-B*57/5801 and HLA-Cw*07, 51 (11.3%) expressed HLA-B*57/5801 without HLA-Cw*07 and 81 (18%) expressed HLA-Cw*07 without HLA-B*57/5801. The median age CD4 cell count and viral load for the 37 subjects who coexpressed HLA-B*57/5801 and HLA-Cw*07 was 37 years, 490 cells/μl and 6700 copies/ml respectively. Of these 37 individuals only the nine further studied here had concurrent immunodominant HLA-B*57/5801- and HLA-Cw*07-restricted IFN-γ ELISPOT responses defined as ≥500 SFC/$10^6$ PBMCs.

### *Magnitude and breadth of HLA-B*57/5801 and HLA-C restricted responses by the IFN-γ ELISPOT assay*

All subjects included in this study made HIV-specific CD8+ T cell responses to known HLA-B*57/5801 and HLA-Cw*07 epitopes as determined by IFN-γ ELISPOT (Fig 1A). Only a few individuals within the Sinikithemba study cohort had responses ≥500/$10^6$ SFCs for both HLA-B*57/5801 and HLA-Cw*07; these high magnitude responses were examined further in subsequent assays. The immunodominant HLA-B*57/5801-restricted responses were to the following four epitopes: TSTLQEQIAW (TW10), ISPRTLNAW (ISW9), QATQDVKNW (QW10) and KAFSPEVIPMF (KF11). In contrast, only one HLA-C response was targeted by the study subjects: KRQEILDLWVY (KY11) restricted by HLA-Cw*07 (Fig 1A; Suppl. Table 1). The overall magnitude of the responses targeted by HLA-Cw*07 restricted epitopes was significantly higher than the magnitude of HLA-B*57/5801 restricted responses ($p = 0.0012$, Mann–Whitney test; Fig 1B).

The nine study patients were representative of an additional 28 individuals (total $n = 37$) (data not shown) with HLA-B*57/5801-restricted responses in terms of breadth of positive IFN-γ epitope-specific ELISPOT responses detected. ISPRTLNAW (ISW9) and KAFSPEVIPMF (KF11) were dominantly presented by HLA-B*5703; whereas the presentation of QW9 and TW10 was variable between B*5702 and B*5801 and were least presented by HLA-B*5703 (Fig. 1C). Notably, none of the B*5702 subjects presented ISW9 and KF11.

### *Functionality profiles of HLA-B*57/5801 and HLA-C restricted HIV-1 specific CD8+ T cell epitopes*

Previous studies of long term non-progressors have shown that HIV-1-specific CD8+ T cells restricted by protective alleles such as HLA-B*57 and HLA-B*27, may be more polyfunctional than CD8+ T cells restricted by other HLAs (Champagne et al., 2001; Harari et al., 2007; Zimmerli et al., 2005). To determine the functionality of HIV-specific CD8+ T cells restricted by alleles with different disease outcomes (HLA-B*57/5801 and HLA-Cw*07); we assessed the polyfunctionality of these responses in those who possessed them concurrently.

HIV-specific CD8+ T cells polyfunctionality was evaluated using multicolor flow cytometry by simultaneous measurement of five functions: IFN-γ, TNF-α, IL-2, MIP-1β, and CD107a as previously described in other studies (Betts et al., 2006; De Rosa et al., 2004; Streeck et al., 2008a). On single function gating (Supp. Fig. 1), IFN-γ expression was lower on HLA-B*57/5801 than on HLA-Cw*07 restricted HIV-1 specific CD8+ T cell epitopes ($p = 0.06$; Mann–Whitney test) (Fig. 2A), consistent with ELISPOT data. A similar trend was observed for the other individual functions although the magnitude of other responses was lower than for IFN-γ.

**Table 1**
Characteristics of study subjects.

| Patient ID | Sex | Age (years) | CD4 count (cell/ml) | Viral load (copies/ml) | HLA type | HLA-B*57/5801 epitopes | HLA-C epitopes |
|---|---|---|---|---|---|---|---|
| SK 009 | Male | 32 | 291 | 47,000 | A*2301/74 B*1503/5702 Cw*0202/0701 | TSTLQEQIAW (p24) | KRQEILDLWVY(Nef) |
| SK 215 | Female | 35 | 202 | 34,800 | A*6802/74 B*0702/5703 Cw*07/07 | ISPRTLNAW (p24) | KRQEILDLWVY(Nef) |
| SK 236 | Female | 37 | 411 | 9900 | A*02/3002 B*0801/5801 Cw*07/07 | ISPRTLNAW (p24) | KRQEILDLWVY(Nef) |
| SK 251 | Female | 58 | 271 | 2530 | A*02/3001 B*4201/5801 Cw*07/1701 | QATQDVKNW (p24) | KRQEILDLWVY(Nef) |
| SK 318 | Female | 27 | 370 | 3600 | A*33/74B*0702/5703 Cw*07/07 | KAFSPEVIPMF (p24) | KRQEILDLWVY(Nef) |
| SK 358 | Female | 42 | 264 | 750,000 | A*0202/2301 B*08/5701 Cw*07/07 | ISPRTLNAW (p24) | KRQEILDLWVY(Nef) |
| SK 364 | Female | 38 | 305 | 11,500 | A*02/3001 B*4201/5801 Cw*07/1701 | QATQDVKNW (p24) | KRQEILDLWVY(Nef) |
| SK 379 | Male | 44 | 267 | 4310 | A*0205/0208 B*0702/5801 Cw*07/07 | TSTLQEQIAW (p24) | KRQEILDLWVY(Nef) |
| SK 428 | Female | 45 | 214 | 272,000 | A*0205/0208 B*1401/5801 Cw*07/08 | TSTLQEQIAW (p24) | KRQEILDLWVY(Nef) |
| Median | | 38 | 271 | 11,500 | | | |