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Immunodominance of HLA-B27-restricted HIV KK10-specific CD8+ T-cells is not related to naïve precursor frequency

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ABSTRACT

The factors that determine the immunodominance, efficacy and almost ubiquitous presence of CD8+ T-cell responses to the HLA-B27-restricted HIV-1 p24 Gag-derived KK10 epitope remain to be fully elucidated. Here, we show that neither the precursor frequency nor the priming capacity of KK10-reactive CD8+ T-cells within the naïve pool differ substantially in comparison to other specificities. These data implicate alternative mechanisms in the relative protection conferred by CD8+ T-cell responses to this epitope.

It is well established that the expression of certain HLA class I molecules, such HLA-B27 and HLA-B57, is associated with prolonged AIDS-free survival in HIV-1 infection [1]. Furthermore, a number of studies indicate that CD8+ T-cell responses restricted by such HLA molecules present superior functional properties (*e.g.* proliferative capacity, HIV suppressive capacity and polyfunctionality), which may render them more effective [2–4]. Nonetheless, the mechanistic basis for the acquisition of protective attributes within these CD8+ T-cell populations remains unclear.

A high frequency of antigen-specific precursors in the naïve pool may confer both quantitative and qualitative advantages during the generation of effective CD8+ T-cell responses. In addition to the obvious numerical and kinetic benefits associated with a high precursor frequency, greater repertoire diversity within such naïve antigen-specific populations could provide a rich foundation for the optimal selection and priming of high quality clonotypes. This is important given the fundamental role of individual clonotypes as determinants of efficacy within CD8+ T-cell responses to specific viral antigens [5–7]. Thus, in theory at least, the dominance and functional properties of HIV-specific CD8+ T-cell populations could be influenced by the initial frequency of antigen-reactive precursors [8]. In line with this hypothesis, recent studies in murine models indicate that naïve precursor frequencies can vary widely between T-cell populations with distinct antigen specificities; these differences, in turn, impact immunodominance patterns, differentiation kinetics and functional efficacy [9–13]. Although evidence in humans is scarce, it is reasonable to predict that naïve precursor frequency may similarly shape T-cell memory in response to antigen challenge. Indeed, the widespread incidence and dominance of CD8+ T-cell responses to the Melan-A/MART-1 epitope EV10 in HLA-A2+ melanoma patients is thought to be associated with a particularly high frequency of naïve antigen-reactive precursors [14], which can be observed in the majority of HLA-A2+ individuals [15–17]. Moreover, a recent study indicates that the immunodominance pattern of HLA-A2-restricted HCV-specific CD8+ T-cell responses can be determined by the frequency of naïve-precursors reactive for HCV epitopes [18–20].

To tackle this hypothesis in the context of HIV-1 infection, we evaluated the frequency and priming capacity of naïve CD8+ T

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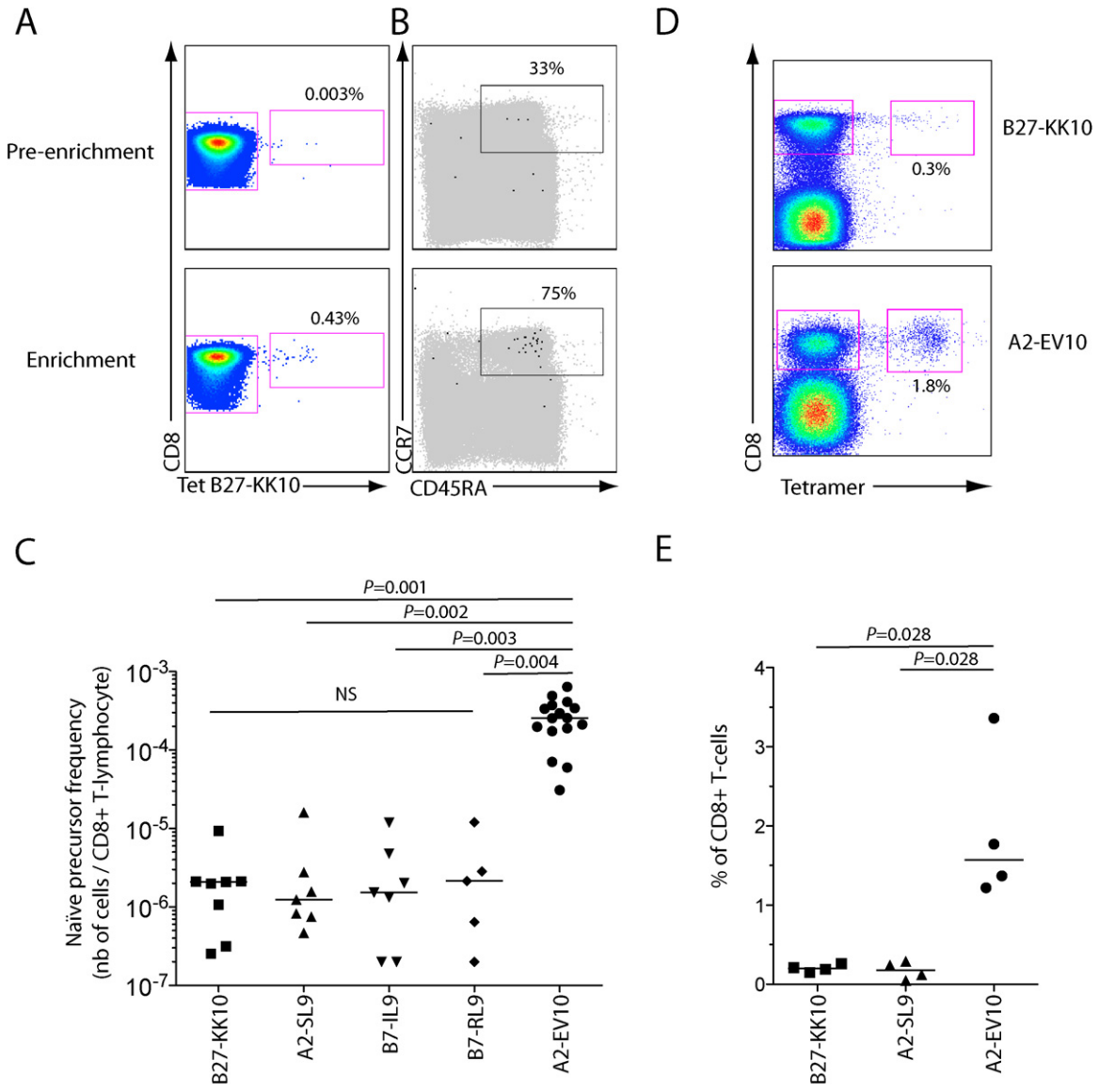

Fig. 1. Antigen-reactive naïve CD8$^+$ T-cell precursor frequencies in healthy donors. (A) Representative staining of KK10/HLA-B*2705 tetramer$^+$ CD8$^+$ T-cells in one uninfected donor pre- and post-enrichment from 10^8 PBMCs. Percentages of tetramer$^+$ cells within the total CD8$^+$ T-cell population are indicated. (B) Representative CD45RA and CCR7 staining of total (gray) or KK10/HLA-B*2705 tetramer$^+$ (black) CD8$^+$ T-cells in one uninfected donor pre- and post-enrichment. (C) Frequencies of antigen-reactive CD8$^+$ T-cell precursors in healthy donors. The indicated genotype-matched tetramers were produced using the HLA-A*0201 (SL9 and EV10), HLA-B*0702 (IL9 and RL9) and HLA-B*2705 (KK10) heavy chains. Statistical analyses were conducted using the Mann–Whitney *U*-test. (D) Representative stainings of EV10/HLA-A*0201 and KK10/HLA-B*2705 tetramer$^+$ CD8$^+$ T-cells after priming with peptide-pulsed autologous dendritic cells and *in vitro* expansion for 20 days. Percentages of tetramer$^+$ cells within the total CD8$^+$ T-cell population are indicated. (E) Percentages of expanded tetramer$^+$ CD8$^+$ T-cells in *HLA-A*0201*$^+$ and *HLA-B*2705*$^+$ healthy donors.

-cells specific for HIV-derived epitopes. In particular, we compared these parameters for the HLA-B27-restricted p24 Gag-derived epitope KK10 (KRWIILGLNK$_{263–272}$), which elicits protective CD8$^+$ T-cell responses, *versus* epitopes restricted by HLA-A2 (p17 Gag SL9$_{77–85}$) and HLA-B7 (gp160 Env IL9$_{843–851}$ and Nef RL9$_{77–85}$) that are not associated with efficacious immunity. The HIV-specific CD8$^+$ T-cell response in HLA-B27$^+$ individuals almost invariably targets the KK10 epitope [21–23]. These cells display potent effector functions [24,25], and represent the prototypic effective CD8$^+$ T-cell response against HIV-1. To eliminate potentially confounding effects related to the influence of HIV infection on priming and precursor consumption *in vivo*, all analyses were performed using samples obtained from healthy HIV-seronegative donors, screened for *HLA-A*0201*, *HLA-B*0701* and *HLA-B*2705*.

Although the number of virus-reactive precursors in the total pool of human T-cells is generally low, their frequency can be measured directly *ex vivo* in peripheral blood [18–20]. Accordingly, to quantify HIV-derived epitope-specific naïve CD8$^+$ T-cell precursors in healthy donors, we first enriched these cells from samples of 10^8 peripheral blood mononuclear cells (PBMCs) using genotype-matched peptide-HLA class I tetramers and magnetic beads (Fig. 1A). Parallel analyses of Melan-A/MART-1 EV10-reactive CD8$^+$ T-cell precursors were conducted in *HLA-A*0201*$^+$ donors. Eight donors were analyzed for the HLA-B*2705 restricted KK10 epitope, necessitating to screen up to 102 healthy donors for *HLA-B*2705* (which is an infrequent allele). For comparison, seven donors were analyzed for the HLA-A*0201 restricted SL9 and HLA-B*0701 IL9 epitopes, five for the HLA-B*0701 RL9 epitope, and sixteen for the HLA-A*0201 restricted EV10 epitope. The naïve phenotype of antigen-reactive precursors was verified by flow cytometry assessment of CD45RA, CCR7 and CD27 expression on tetramer$^+$ cells (Fig. 1B). Precursor frequencies were calculated