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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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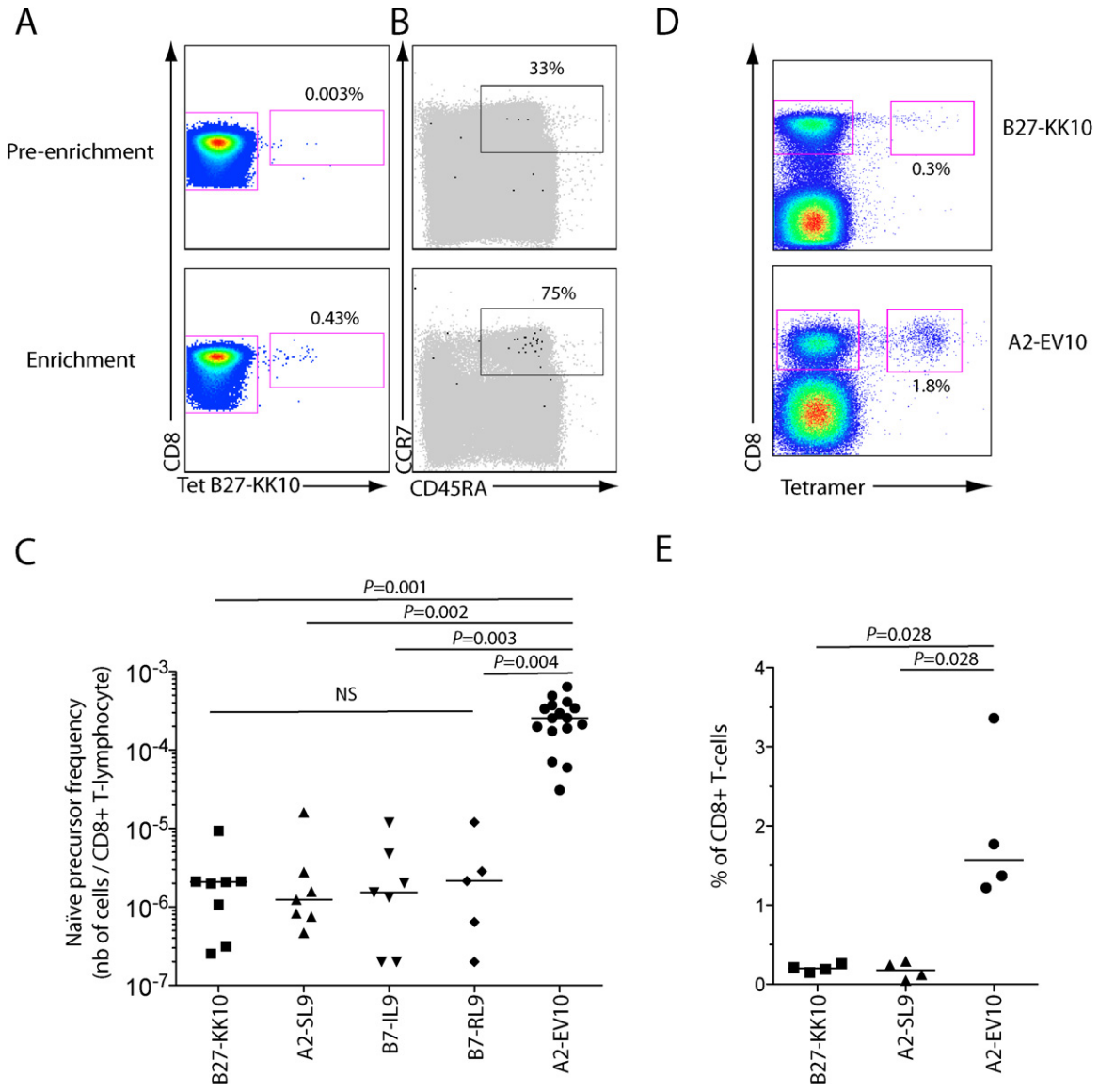

Fig. 1. Antigen-reactive naïve CD8$^+$ T-cell precursor frequencies in healthy donors. (A) Representative staining of KK10/HLA-B*2705 tetramer$^+$ CD8$^+$ T-cells in one uninfected donor pre- and post-enrichment from 10^8 PBMCs. Percentages of tetramer$^+$ cells within the total CD8$^+$ T-cell population 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

according to recently published procedures [18]. The frequency of EV10-reactive CD8+ T-cell precursors was consistently high (>100 cells per million CD8+ T-lymphocytes), in line with previous reports [18,19]. In contrast, however, the frequency of KK10-reactive precursors was low (approximately 1 cell per million CD8+ T-lymphocytes) and not substantially different from those measured for other HIV epitope specificities (Fig. 1C).

In further experiments, we analyzed the expansion of antigen-reactive naïve CD8+ T-cells during *in vitro* priming with peptide-pulsed autologous dendritic cells, which were generated from PBMCs by differentiation with GM-CSF and IL-4, then matured with a cytokine cocktail composed of TNF-α, IL-1β, PGE2 and IL-7 [26]. The percentages of CD8+ T-cells specific for KK10, SL9 or EV10 that expanded *in vitro* from healthy donor PBMC samples were compared by tetramer staining after 20 days (Fig. 1D). The individuals tested for *in vitro* stimulation were selected on the basis that they displayed both *HLA-A*0201* and *HLA-B*2705* alleles, in order to compare HLA-A*0201 and HLA-B*2705 restricted T-cell expansions in individual donors ($n = 4$) and thus avoid a potential bias associated with inter-donor variability. In line with the *ex vivo* measurements of precursor frequency, antigen-reactive CD8+ T-cell percentages for KK10 and SL9 were equivalent after *in vitro* expansion, and significantly lower than those for EV10 in the same cultures (Fig. 1E).

Collectively, these results show that the frequency of KK10-reactive CD8+ T-cell precursors in the naïve pool is not significantly elevated relative to other HIV specificities. It will be of interest to see if the same finding applies also to other immunodominant CD8+ T-cell responses important for the control of HIV or SIV replication (*e.g.* epitopes restricted by HLA-B5701 or HLA-B5801 in humans, or Mamu-B*08, Mamu-B*17 and Mamu-A*01 in macaques). Nonetheless, our data indicate that the frequency of the naïve precursors *per se* cannot explain either the acquisition of superior functional attributes by KK10-specific CD8+ T-cell populations or the almost universal immunodominance of this response in HLA-B27+ individuals infected with HIV-1. Alternative explanations for the observed immunodominance of this response may be related to the TCR affinity or avidity of the naïve cells that make up the KK10 reactive precursor population. For a still undetermined reason, this might be particularly high. The relative rarity of these cells in healthy donors precluded detailed analyses of their clonotypic composition and functional attributes (*e.g.* TCR avidity), thus leaving open the possibility that intrinsic features of the KK10-reactive precursor population may confer particular advantages *in vivo*. Interestingly, the number of epitope precursors generated during antigen processing, or epitope abundance, which is particularly high in the case of KK10, has been proposed to impact on CD8+ T-cell response hierarchies and play a role in the immunodominance of the KK10 specific response [27]. Upon priming, this parameter, together with the seemingly rapid kinetics of KK10 epitope presentation [28], may influence the KK10 specific T-cell population avidity and clonality, thought to be key factors of the functional efficacy of this population [24,29]. Eventually, these features may also play a role in the acquired capability of KK10-specific CD8+ T-cells to escape suppression by regulatory T-cells, as recently reported [30], thus supporting their expansion capacity and immunodominance. Further studies will be needed to reach an exact understanding of the sophisticated mechanisms underlying the selection and maintenance of the CD8+ T-cells that are required for an effective immune response against HIV.

Conflict of interest statement

The authors declare that they have no competing financial interests.

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