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OPEN

Subdominant Gag-specific anti-HIV efficacy in an HLA-B*57-positive elite controller

Despite the discovery of HIV over three decades ago, the 2008 'Berlin patient' is the only case of sustained HIV remission. Other cases of apparent 'cure' eventually relapsed [1] and although early antiretroviral therapy (ART) has recently gained traction as a factor contributing to remission [2,3], most cases are likely to relapse [4]. In contrast, relapse in 'elite controllers' of HIV infection is less common. These are ART-naïve individuals who spontaneously suppress viremia to undetectable levels. Approximately 40% of elite controllers express HLA-B*57 [5], an example being the original 1999 'Berlin patient', in whom virologic control has been maintained for >15 years to date [6].

Mechanisms proposed to explain HLA-B*57-mediated immune control include immunodominant $CD8^+$ T-cell-targeting of multiple conserved Gag epitopes from which mutational escape is detrimental to viral fitness, characteristics of the T-cell receptor on HLA-B*57-restricted $CD8^+$ T cells, HLA-B*57-peptide binding affinity, and HLA-B*57 cross-talk with innate immune cells [7]. However, some HLA-B*57-positive elite controllers have no detectable Gag-specific responses without ex-vivo expansion [8,9]. Here, we studied one such elite controller, to determine whether the immunodominant $CD8^+$ T-cell response in such cases mediates the most potent antiviral efficacy, as Gag-specific $CD8^+$ T-cell responses typically have greater capacity to inhibit viral replication than non-Gag specificities [10,11].

An African-Caribbean female was recruited in the UK at 52 years of age in 2013. She had been diagnosed with HIV in 1991, an estimated 2 years after heterosexual transmission in Jamaica (and hence is referred to here as the '1991 Jamaica patient'). Our study was approved by the Oxford Research Ethics Committee and the patient provided written informed consent.

For more than 24 years, she has remained ART-naïve and aviremic with a healthy $CD4^+$ T-cell count (median 1237 cells/μl) (Fig. 1a). Despite being HLA-B*57 : 03-positive, she demonstrated only two HIV-specific $CD8^+$ T-cell responses detectable by ELISPOT assay, neither greater than 60 spot forming units (SFC)/million peripheral blood mononuclear cell (PBMC) and none detectable by tetramer staining (Fig. 1b and c). This is in contrast to the '1999 Berlin patient', who had a dominant HLA-B*57-restricted Nef-HW9 response of 3000 SFC/million PBMC [6] (Fig. 1b). One of the two significant ELISPOT responses in the 1991 Jamaica patient was also against this same Nef-HW9 epitope (Fig. 1b). However, via peptide stimulation of memory T-cell responses [8], we identified five HLA-B*57-restricted responses (Fig. 1c), three of which we tested for their ability to inhibit HIV replication. Bulk $CD8^+$ T cells demonstrated weak ex-vivo ability to suppress viral replication (Fig. 1d and e), fitting the profile of a subset of HLA-B*57-positive elite controllers [12]. Of the three expanded HLA-B*57-restricted $CD8^+$ T-cell specificities tested, Gag-TW10-specific $CD8^+$ T cells were significantly the most potent in suppressing HIV replication, followed by Nef-KF9 and then Nef-HW9 (Fig. 1d and e).

The study of this HLA-B*57-positive individual confirms that, in spite of HIV-specific responses being low frequency or undetectable by tetramer staining or ELISPOT assay, strong responses could be 'recalled' from memory, as previously reported [8]. Among these, Gag-TW10-specific $CD8^+$ T cells were more potent at inhibiting viral replication than Nef-KF9-specific cells, despite the latter being a stronger response in ELISPOT assays. These data support previous findings in subjects chronically infected with HIV [13] indicating that subdominant responses may be more efficacious in terms of control of viremia. The findings here are extended also to the case of an HLA-B*57-positive elite controller. Although, like the 1999 Berlin patient, this is a single case report, the data are consistent with the hypothesis that HLA-B*57-mediated Gag-specific targeting by $CD8^+$ T cells confers benefit to the host in HIV infection [7,14] and that vaccine induction of broad Gag-specific $CD8^+$ T-cell responses would tend to increase immune control in HIV infection [15].

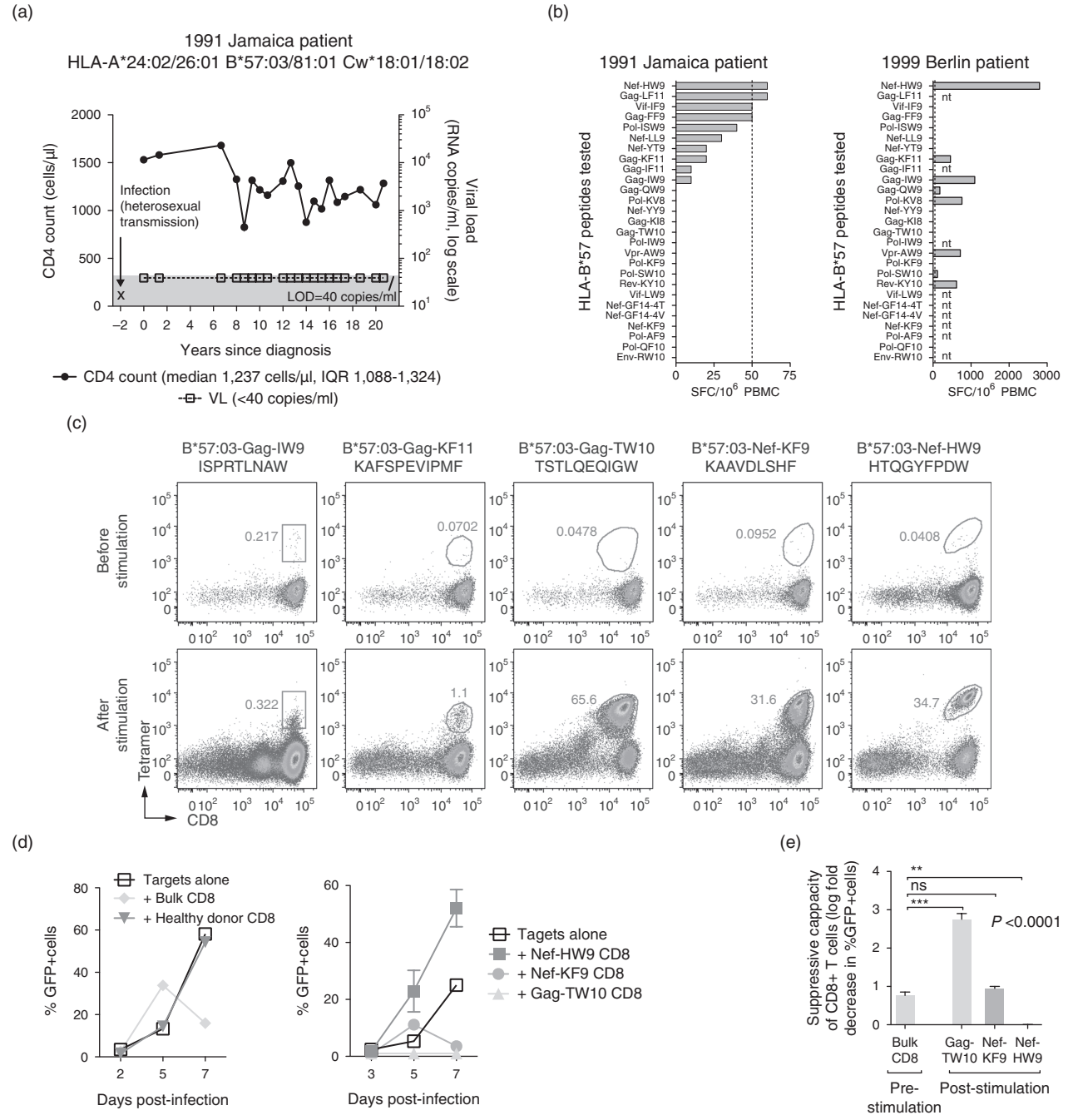

Fig. 1. Clinical profile and anti-HIV suppressive activity of the 1991 Jamaica patient. (a) $CD4^+$ T-cell count and HIV RNA viral load measurements. '0' is time of diagnosis. Limit of detection (LOD) for viral load is 40 copies/ml (gray area) and measurements are shown at 40 copies/ml for convenience. Although sequencing was unsuccessful due to lack of circulating virus, the 1991 Jamaica patient was likely infected with subtype-B HIV predominant in Jamaica. (b) ELISPOT $CD8^+$ T-cell responses in unstimulated peripheral blood mononuclear cells (PBMCs) in the 1991 Jamaica patient (22 years postdiagnosis) to subtype-B consensus HLA-B*57-restricted defined optimal epitopes. Responses were considered positive if they were at least three times the mean number of spot forming colonies (SFC) in the four negative control wells and had to be greater than 50 SFC/10^6 PBMC (dotted line). $CD8^+$ T-cell responses for the 1999 Berlin patient are shown to highlight the different patterns of responses (not for direct comparisons as the assays were done in different laboratories at different times). Nt = not tested. (c) PBMC (23 years postdiagnosis) were stimulated with a panel of 30 HLA-B*57/81 : 01-restricted optimal peptides. Five previously undetectable HLA-B*57-restricted responses were discovered poststimulation, but no HLA-B*81 : 01-restricted responses. Three of these five responses were successfully expanded and tested in (d). Gated on live $CD3^+CD4^-$ cells around $CD8^+$tetramer+ population; numbers indicate % tetramer+$CD8^+$ cells (of $CD3^+CD4^-$). (d) Viral replication in HLA-B*57 : 03-expressing H9 cells infected with NL4-3-GFP and cultured alone ('targets alone'), with unstimulated bulk $CD8^+$ T cells (left) or stimulated epitope-specific

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Conflicts of interest

There are no conflicts of interest.

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Fig. 1 *(continued)*
$CD8^+$ T cells (right) of the 1991 Jamaica patient (23 years postdiagnosis). Each symbol represents the mean of three replicates, error bars represent the SEM. (e) Suppressive capacity (log_{10} fold decrease in % of infected GFP^+ cells) of bulk unstimulated $CD8^+$ T cells or stimulated epitope-specific $CD8^+$ T cells of the 1991 Jamaica patient. Results were compared with 'bulk CD8' (ANOVA with Dunnett's multiple comparison post-test). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns = not significant ($P > 0.05$).