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Reduced viral burden amongst high responder patients following HIV-1 p24 peptide-based therapeutic immunization

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Abstract

We have previously shown that HIV p24-like peptides (Vacc-4x) via activation of skin dendritic cells induced immune responses in 90% of HIV patients on highly active antiretroviral treatment (HAART). These patients ($n = 38$) were here subjected to a final 14-week interruption of HAART. Patients with the highest delayed type hypersensitivity (DTH) responses to Vacc-4x-peptides before treatment interruption tended to achieve lower actual HIV RNA levels at the end of the study compared to Vacc-4x DTH low-responders ($p = 0.08$) and significantly so in terms of viral loads relative to their individual pre-HAART HIV RNA set-points ($p = 0.04$). CD4+ lymphocyte counts were maintained only among DTH high responders but decreased in the other patients during recurrent viremia ($p \leq 0.02$). No other pre-study differences in HIV history or p24-responses were found.

Keywords: HIV; Immunotherapy; Delayed type hypersensitivity

1. Introduction

Immunity against human immunodeficiency virus type-1 (HIV) is insufficient during chronic HIV infection [1,2]. Therapeutic immunization aims to attenuate disease progression by modulating HIV-specific immune responses, but correlates of effective immunity remain to be defined [1,3,4]. So far, most immune response-inducing candidates have neither been linked with clinical benefit nor with sustained control of viral replication [2,4,5]. However, preliminary data by Lu et al. showed that autologous monocyte-derived dendritic cells, pulsed ex vivo with inactivated autologous virus, induced prolonged reduction of HIV RNA in a substantial fraction of patients highlighting the potential of immunotherapy strategies targeting dendritic cells [6].

The approach used in this study involved activation of Langerhans' dendritic cells in situ by intracutanous injection of low-dose granulocyte macrophage-colony stimulating factor (GM-CSF) and subsequent direct priming with a mixture of four HIV p24-like peptides (Vacc-4x). In the initial immunization part of this phase IIa dose-finding clinical trial where patients on highly active antiretroviral treatment (HAART) received Vacc-4x, we found that 90% of the patients developed partly dose-dependent cellular immune responses in terms of Vacc-4x-specific CD4+ and CD8+ T cell proliferation in vitro and delayed type hypersensitivity test (DTH) reactions in vivo [7]. We now present data from the 26-week observation phase of the same trial with no further immunizations but instead two consecutive interruptions of

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HAART lasting 4 and 14 weeks, respectively, with 8 weeks on HAART in between.

2. Materials and methods

2.1. Patients and study protocol

Forty HIV-infected patients stable on HAART, with a median value of 550 CD4+ T cells/μl and HIV RNA < 50 copies/ml at inclusion, were initially randomized into two dosage-arms receiving 0.4 and 1.2 mg Vacc-4x peptides, respectively. Thirty-eight patients completed the study (LD $n = 18$, HD $n = 20$). In the first phase of this study, described in Ref. [7], the Vacc-4x p24-like peptides [8] were injected intradermally over a period of 26 weeks under continuous HAART, using low-dose granulocyte macrophage-colony stimulating factor (GM-CSF) as a local adjuvant. The second phase of the study, described here, consisted of a 26-week observation period that included two consecutive interruptions of HAART lasting 4 and 14 weeks, respectively, as outlined in Fig. 1 (bottom, right panel). The study was conducted with informed patient consent and approved by the Regional Ethics Committee and Norwegian Medicines Agency.

2.2. Immune response assays in vitro and in vivo

DTH responses were used to detect specific cellular responses in vivo at weeks 38 and 52 and were measured as palpable skin infiltrate areas 48 h after intradermal injection of Vacc-4x-peptides without GM-CSF as described previously [7]. Vacc-4x- and recombinant HIV p24 protein-specific proliferative responses in vitro were measured in peripheral blood mononuclear cells (PBMC) that were drawn immediately before injection of DTH antigens. PBMC were then pulsed with carboxyfluorescein diacetate succimidylester (CFSE) and subsequently challenged with antigen for 7 days. Proliferating cells were defined by a reduction of CFSE-fluorescense, and proliferative responses were expressed as the fraction of proliferating T cell blasts (weighed percent divided, WPD) [7,9]. Plasma HIV RNA levels and CD4+ and CD8+ lymphocyte counts were obtained by standard assays [7].

2.3. Statistical analysis

Non-parametric statistics were used throughout the study, and data are presented as median (interquartile range). Mann-Whitney *U*-test, Spearman Rank test, and Wilcoxon matched pairs test were used for comparison of patient groups, calculating correlations, and for paired comparisons, respectively.

3. Results

3.1. Overall results and adverse events

No serious adverse events were reported, but two patients experienced transient DTH-associated systemic allergic re-

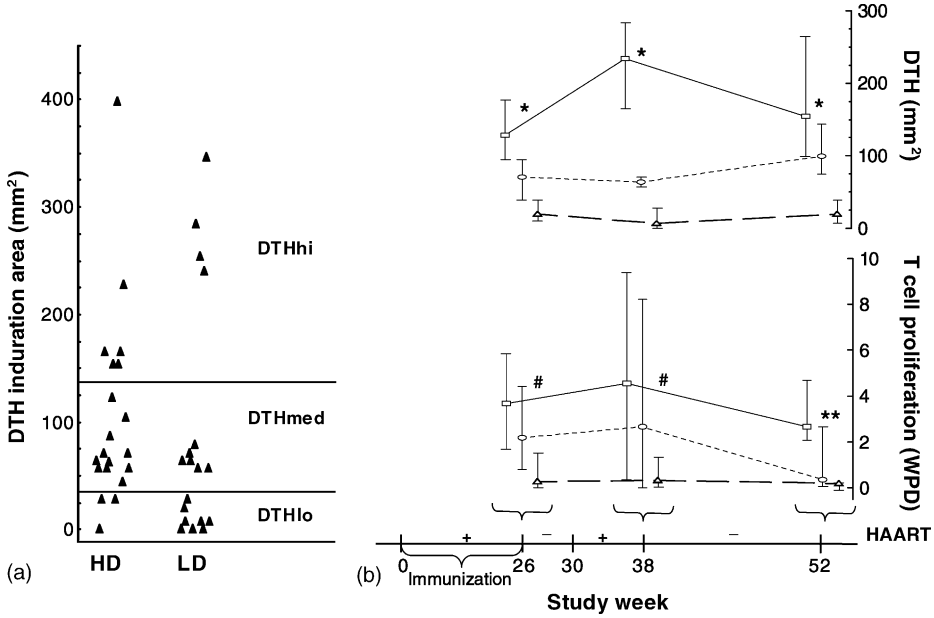

Fig. 1. Vacc-4x peptide-specific T cell responses. (a) DTH induration areas for all patients at week 38 in the Vacc-4x high (HD) and low (LD) dosage arms. The two horizontal lines represent the 25- and 75-percentiles that define the three DTH responder groups, as indicated. (b) Changes in Vacc-4x-specific T cell responses over time, independent of Vacc-4x immunization dose, and their relation to HAART, as indicated on the abscissa. DTH induration areas (upper panel) and Vacc-4x-specific CD3+ T cell proliferation (lower panel) at weeks 26, 38 and 52 are shown for the DTHhi (□, solid lines), DTHmed (○, dotted lines) and DTHlo (Δ, dashed lines) patient groups (medians, interquartile ranges indicated). Proliferation expressed as weighed percent divided (WPD), denoting the fraction of proliferating T cell blasts. Differences between the DTHhi and DTHlo patients are indicated with $^{*}p < 0.001$, $^{\#}p < 0.1$ or $^{**}p = 0.006$, respectively.