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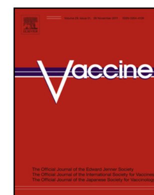

Polyfunctional CD4+ T cell responses in HIV-1-infected viral controllers compared with those in healthy recipients of an adjuvanted polyprotein HIV-1 vaccine☆

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ABSTRACT

A recombinant fusion protein (F4) consisting of HIV-1 p17, p24, reverse transcriptase (RT) and Nef, adjuvanted with AS01, induced strong and broad CD4+ T cell responses in healthy volunteers. Here we compare these vaccine-induced CD4+ T cell responses with the ones induced by natural infection in patients with varying disease courses.

Thirty-eight HIV-infected, antiretroviral treatment-naïve subjects were classified into four categories: 8 long-term non-progressors (infection ≥7 years; CD4+ T cells ≥500/μL), 10 recently infected individuals (infection ≤2 years; CD4+ T cells ≥500/μL), 10 typical early progressors (CD4+ T cells ≤350/μL), and 10 viral controllers (plasma HIV-1 RNA <1000 copies/mL). Peripheral blood mononuclear cells were stimulated *in vitro* with p17, p24, RT and Nef peptide pools and analyzed by flow cytometry for expression of IL-2, IFN-γ, TNF-α and CD40L. CD4+ T cell responses were compared to those measured with the same method in 50 HIV-uninfected subjects immunized with the F4/AS01 candidate vaccine (NCT00434512).

After *in vitro* stimulation with p17, p24 and RT antigen viral controllers had significantly more CD4+ T cells co-expressing IL-2, IFN-γ and TNF-α than other HIV patient categories. The magnitude and quality of these responses in viral controllers were comparable to those observed in F4/AS01 vaccine recipients. In contrast with viral controllers, triple cytokine producing CD4+ T cells in vaccinees also expressed CD40L.

Subjects who spontaneously control an HIV infection display polyfunctional CD4+ T cell responses to p17, p24, RT and Nef, with similar magnitude and qualities as those induced in healthy volunteers by an adjuvanted HIV candidate vaccine (F4/AS01).

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1. Introduction

With an estimated 33.3 million people currently infected with human immunodeficiency virus (HIV) worldwide, the global HIV/AIDS pandemic has completed its third decade [1]. A fully efficacious vaccine providing durable protection against HIV type 1 (HIV-1) would definitely have the biggest impact on HIV incidence [2]. However, a disease-modifying HIV vaccine, generating an immune response that helps to control virus load, prevents progression of disease in HIV-infected patients, and reduces viral transmission, remains a valuable alternative [3].

The lack of natural protective immunity against HIV is the main obstacle in defining immune correlates of protection [4,5] and suggests that an effective vaccine will need to generate an immune response that is superior to the natural immune response [6]. The quality of the CD4+ (or CD8+) T cell cytokine response, estimated by enumerating polyfunctional T cells co-producing gamma interferon (IFN-γ), interleukin-2 (IL-2) and tumor necrosis factor alpha (TNF-α), is considered a possible correlate of vaccine-induced protection [7].

As the main target of HIV [8], CD4+ T cells are pivotal in HIV pathogenesis and wide controversy exists concerning their role in control versus promotion of HIV replication [9]. Nevertheless, the role of CD4+ T cells in induction and maintenance of efficient

Abbreviations: AIDS, acquired immune deficiency syndrome; ARC, AIDS Reference Center; ART, antiretroviral therapy; AS, adjuvant system; CD40L, CD40-ligand; F4, recombinant fusion protein comprised of HIV-1 p17, p24, RT and Nef; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IFN-γ, gamma interferon; IL-2, interleukin 2; LTNP, long-term non-progressors; MSM, men-who-have-sex-with-men; PBMC, peripheral blood mononuclear cells; RT, reverse transcriptase; TNF-α, tumor necrosis factor alpha; TEP, typical early progressors; RI, recently infected individuals; VC, viral controllers; VU, vaccinated uninfected individuals.

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memory $CD8^+$ T cell and B cell responses is well established and considered of pivotal importance [10–12]. Moreover, evidence supporting direct antiviral effects of HIV-specific $CD4^+$ T cells is growing [13–15]. It is therefore generally believed that an effective HIV vaccine will need to elicit a robust $CD4^+$ T cell response [9,16]. This is illustrated by the modest success of the Thai vaccine trial, in which decreased HIV acquisition was observed with a $CD4^+$ T cell-inducing vaccine [17].

Studying subpopulations of untreated HIV-infected patients with slow disease progression, as defined by immunologic or virologic parameters, allows us to obtain more insights into the protective mechanisms [18,19]. Long-term non-progressors (LTNP) remain asymptomatic for many years and maintain high CD4 cell counts without antiretroviral therapy (ART), whereas viral controllers (VC) spontaneously suppress viral replication [19]. VC and LTNP share some characteristics but have distinct clinical phenotypes, while in the literature varying definitions are encountered [3,19–21]. Some VC experience clinical progression despite viral control, and some LTNP maintain high levels of viremia [3].

HIV-specific $CD4^+$ T cell-mediated immune responses have been shown to correlate with LTNP and/or VC phenotypes [22–29]. Both non-progression and control of HIV replication are believed to be associated with high frequencies of HIV-1 Gag-specific $CD4^+$ T cells secreting both IFN-γ and IL-2 [23,26]. Subsequent studies have linked suppression of viremia to maintenance of highly functional $CD4^+$ T cells co-producing IFN-γ, IL-2 and TNF-α in response to Gag and preserved proliferative responses to p24 [28,29]. In addition, $CD4^+$ T cells from LTNP exhibited strong proliferative responses and IFN-γ secretion after stimulation with Nef-peptides [27].

Assuming that these $CD4^+$ T cell responses directly contribute to the more benign course of the infection in VC, the challenge is to design a vaccine that induces these beneficial cellular immune responses [16]. The present study has been performed to compare $CD4^+$ T cell responses to a wide range of HIV-1 antigens, induced by natural HIV-1 infection in patients with different disease courses, with the responses induced in healthy volunteers by vaccination with the same set of antigens. The latter responses were induced *in vivo* and analyzed *in vitro* during a recent clinical trial in which HIV-uninfected volunteers were immunized with a recombinant fusion protein (F4) comprised of HIV-1 p17 and p24 Gag, reverse transcriptase (RT) and Nef, adjuvanted with AS01 [30]. The immune markers measured *in vitro* were the simultaneous production of IFN-γ, IL-2 and/or TNF-α, and the expression of CD40-ligand (CD40L) as a T cell activation marker.

2. Materials and methods

2.1. Study design and participants

This was a single center cross-sectional observational study. The HIV-infected patients were recruited at the AIDS Reference Center (ARC), Ghent University Hospital (Ghent, Belgium). The data from the vaccinated uninfected (VU) volunteers were obtained from a clinical trial conducted at the Center for Vaccinology, Ghent University and Hospital, registered with the ClinicalTrials.gov registry (NCT00434512) [30]. The study was approved by the local independent ethics committee (Ghent University Hospital) and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was obtained from all subjects prior to study entry.

Thirty-eight HIV-1-infected male and female adults between 18 and 69 years of age were recruited and divided into four study groups. Standard eligibility criteria were used for enrolment into the study. Exclusion criteria included receipt of live attenuated vaccines within 30 days, other vaccine or antigen injections within 14 days, or blood products or immunoglobulins 120 days prior to enrolment, as well as chronic administration of immunosuppressants or immune-modifying drugs within 6 months prior to enrolment. The group-specific inclusion criteria were pre-defined as follows: LTNP were diagnosed with HIV-1 infection since $\geq$7 years, and had repeated and most recent $CD4^+$ T cell counts $\geq$500/μL in the absence of ART or AIDS-related symptoms. Recently infected (RI) individuals had a documented HIV-1 infection since <2 years, and repeated and most recent $CD4^+$ T cell counts $\geq$500/μL, in the absence of ART or AIDS-related symptoms. Typical progressors had repeated and most recent $CD4^+$ T cell counts $\leq$350/μL in the absence of ART and were named typical early progressors (TEP) since the median time since diagnosis was only 1.8 years (Table 1). A VC status was defined as repeated and most recent HIV plasma RNA levels (viral load) <1000 copies/mL in the absence of ART or AIDS-related symptoms. Subjects were selected from the ARC database of patients in regular follow up and included consecutively in order of appearance at the HIV clinic ($n = 8$ for the LTNP group, $n = 10$ for each of the RI, TEP and VC groups). All patients were ART-naïve, except for subject F04 who was treated during a pregnancy 3 years before inclusion. Clinical data were recorded retrospectively.

The vaccinated uninfected (VU) subjects were healthy male and female adults aged 18–41 years at low risk of HIV infection who had received two doses of the F4/AS01 study vaccine (GlaxoSmithKline (GSK) Biologicals, Rixensart, Belgium) with 1 month interval [30]. This vaccine consisted of 10 μg per dose of F4 recombinant protein adjuvanted with AS01. F4 is a recombinant fusion protein comprised of four HIV-1 subtype B antigens, namely p24, RT, Nef and p17. AS01 is a liposome-based adjuvant system containing 50 μg MPL and 50 μg QS21. The immunogenicity data of month 2 (day 60) were used for this comparative study [30].

2.2. T cell responses

T cell responses were evaluated by intracellular cytokine staining (ICS) following *in vitro* stimulation with pools of 15-mer peptides overlapping by 11 amino acids (Eurogentec, Liège, Belgium) covering the sequences of HIV-1 clade B p17, p24, RT and Nef to assess the expression of CD40L and/or the production of IL-2, IFN-γ and/or TNF-α. The ICS was performed on thawed peripheral blood mononuclear cells (PBMC) that had been isolated from venous blood by standard Ficoll-Isopaque density gradient centrifugation and cryopreserved in liquid nitrogen. This ICS procedure has been described elsewhere [30]. PBMC from the HIV-infected patient groups were also tested after stimulation with gp120 and Tat peptide pools (Eurogentec), as described in a previous vaccine study report [31]. Analyses were performed with an LSR II flow cytometer (BD Biosciences, Erembodegem, Belgium) and FACSDiva software (BD Biosciences). The mean number of $CD4^+$ T cells measured for each condition was 52,638 (minimum 10,942, maximum 121,295). The ICS results were expressed as the percentage of the total $CD4^+$ and $CD8^{bright}$ T cells expressing the immune markers IL-2, IFN-γ, TNF-α and/or CD40L in response to stimulation with p17, p24, RT, Nef, Tat or gp120 antigens minus the frequency of $CD4^+$ and $CD8^{bright}$ T cells expressing these cytokines upon *in vitro* culture in medium only.

2.3. Statistical analysis

The frequency of $CD4^+$ and $CD8^+$ T cells expressing each marker and combinations of markers following *in vitro* stimulation by each individual antigen was determined. Spearman correlations with viral load were calculated for all HIV-1-infected patients, irrespective of the patient group. To minimize bias induced by multiple comparisons, significant differences between HIV-1-infected patient groups were first identified using a Kruskal–Wallis test as a screening method. Subsequently, two-by-two Mann–Whitney

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