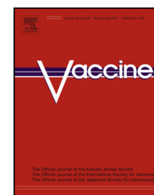
Vaccine 31 (2013) 3739–3746

Contents lists available at SciVerse ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine



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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ARTICLE INFO

Article history:
Received 26 December 2012
Received in revised form 11 April 2013
Accepted 8 May 2013
Available online 21 May 2013

Keywords:
HIV-1
HIV-1 vaccine
Viral controllers
CD4$^+$ T cell response

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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with human immunodeficiency virus (HIV) worldwide, the global

dence [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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http://dx.doi.org/10.1016/j.vaccine.2013.05.021

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 ≥7 years, and had repeated and most recent CD4$^+$ T cell counts ≥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 ≥500/μL, in the absence of ART or AIDS-related symptoms. Typical progressors had repeated and most recent CD4$^+$ T cell counts ≤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

Table 1
Patient characteristics.

ID	Sex	Age	Origin	Time[a] (years)	Transmission[b]	CCR5 genotype	HIV-1 subtype[c]	CD4 count[d] (cells/μL)	Viral load[d] (copies/mL)
Long-term non-progressors									
LTNP01	M	44	Hispanic	19.3	MSM	wt/wt	B	570	1778
LTNP02	F	45	Caucasian	18.8	HE	wt/wt	F1	434	2344
LTNP03	M	34	Caucasian	22.9	BT	wt/Δ32	B	477	7586
LTNP04	F	46	Caucasian	8.9	HE	wt/wt	02_AG	696	468
LTNP05	F	56	Caucasian	12.1	HE	wt/wt	G	486	2042
LTNP06	F	69	Caucasian	9.8	HE	wt/Δ32	02_AG	1080	<50
LTNP07	F	33	African	7.9	HE	wt/wt	02_AG	534	210
LTNP08	F	42	Caucasian	17.0	IVD	wt/wt	?	885	<50
Median [IQR]		45 [13]		14.6 [9.7]				552 [309]	1123 [2087]
Recently infected individuals									
RI01	M	35	Hispanic	1.2	MSM	wt/wt	B	496	64,565
RI02	M	24	Caucasian	0.7	MSM	wt/wt	B	593	10,2239
RI03	M	62	Caucasian	1.6	MSM	wt/wt	B	918	23,442
RI04	M	32	Caucasian	1.4	MSM	wt/wt	B	651	3090
RI05	M	36	Caucasian	1.3	MSM	wt/wt	B	563	643
RI06	M	36	Caucasian	1.9	MSM	wt/wt	B	1360	1122
RI07	F	31	African	1.9	?	wt/wt	A1	451	35,481
RI08	M	27	Caucasian	2.0	MSM	wt/wt	B	568	57,544
RI09	M	48	Caucasian	0.9	MSM	wt/wt	B	756	43,652
RI10	M	53	Caucasian	1.8	MSM	wt/Δ32	B	559	20,417
Median [IQR]		35 [17]		1.5 [0.7]				581 [197]	29,462 [54,454]
Typical early progressors									
TEP01	M	32	Caucasian	2.5	MSM	wt/wt	B	341	114,815
TEP02	M	30	Caucasian	2.1	HE	wt/wt	B	334	44,668
TEP03	M	42	Caucasian	0.5	MSM	wt/wt	B	334	151,356
TEP04	M	48	Caucasian	2.5	MSM	wt/wt	B	199	18,197
TEP05	M	36	Caucasian	2.0	MSM	wt/Δ32	B	242	53,703
TEP06	F	28	African	1.0	HE	wt/wt	C	239	380
TEP07	M	31	Asian	1.0	MSM	wt/wt	B	260	66,069
TEP08	M	41	Caucasian	0.5	MSM	wt/wt	B	230	25,400
TEP09	M	40	Caucasian	2.1	HE	wt/wt	01_AE	248	5888
TEP10	M	28	Caucasian	1.7	MSM	wt/Δ32	B	162	24,547
Median [IQR]		34 [11]		1.8 [1.1]				245 [104]	35,034 [47,872]
Viral controllers									
VC01	M	27	Asian	3.0	MSM	wt/wt	01_AE	864	642
VC02	F	40	African	11.8	HE	wt/wt	C	442	<50
VC03	F	50	African	20.7	HE	wt/wt	A1	321	<50
VC04	F	33	African	3.7	HE	wt/wt	02_AG	518	56
VC05	M	43	African	3.8	HE	wt/wt	01_AE	613	541
VC06	M	30	Caucasian	3.9	MSM	wt/wt	B	503	163
VC07	F	55	Caucasian	1.3	HE	wt/Δ32	?	1210	<50
VC08	M	32	African	4.3	HE	wt/wt	02_AG	904	<50
VC09	F	44	Caucasian	5.7	HE	wt/wt	?	1010	<50
VC10	M	43	Caucasian	8.1	MSM	wt/wt	C	413	<50
Median [IQR]		41 [12]		4.1 [4.4]				566 [462]	<50 [113]

[a] Time since diagnosis of HIV-1 infection at time of blood sampling.
[b] HIV-1 transmission route. MSM, men-who-have-sex-with-men; HE, heterosexual; BT, blood transfusion; IVD, intravenous drug use; and ?, unknown.
[c] HIV-1 subtype. ?, unknown.
[d] Most recent $CD4^+$ T cell count and HIV-1 viral load at time of blood sampling (range 0–8.3 months, median time between CD4 count or viral load and inclusion 0.2 or 0.4 months, respectively).

comparisons between groups were carried out on conditions identified by the Kruskal–Wallis test. All statistics were performed using PASW Statistics 18 (SPSS Inc., Chicago, IL).

3. Results

3.1. Clinical characteristics

A total of 38 HIV-infected subjects were studied, including 8 LTNP, and 10 subjects in each of the three other groups (RI, TEP, and VC). Their demographic, clinical and virological characteristics are listed in Table 1. Patient LTNP08 was co-infected with hepatitis B and hepatitis C virus (HCV); patients RI08 and VC02 were both co-infected with HCV. Although patients were randomly selected, there was a predominance of clade B infections in the RI and TEP groups, whereas the LTNP and VC patients were infected with a more heterogeneous mix of HIV-1 subtypes. Since the duration of infection in the RI and TEP was shorter than that in LTNP and VC, this difference probably results from specific characteristics of the local epidemic with HIV-1 subtype B infected Caucasian men-who-have-sex-with-men (MSM) accounting for the majority of recent local HIV transmissions while the majority of non-B infections are found in other ethnic groups [32]. Not surprisingly, there was some overlap between groups, with four LTNP also fulfilling the criteria for inclusion in the VC group. Statistical significance of the data described below was not affected if those four patients were added to the VC group. The VC group contained six so-called elite controllers with undetectable viral load.

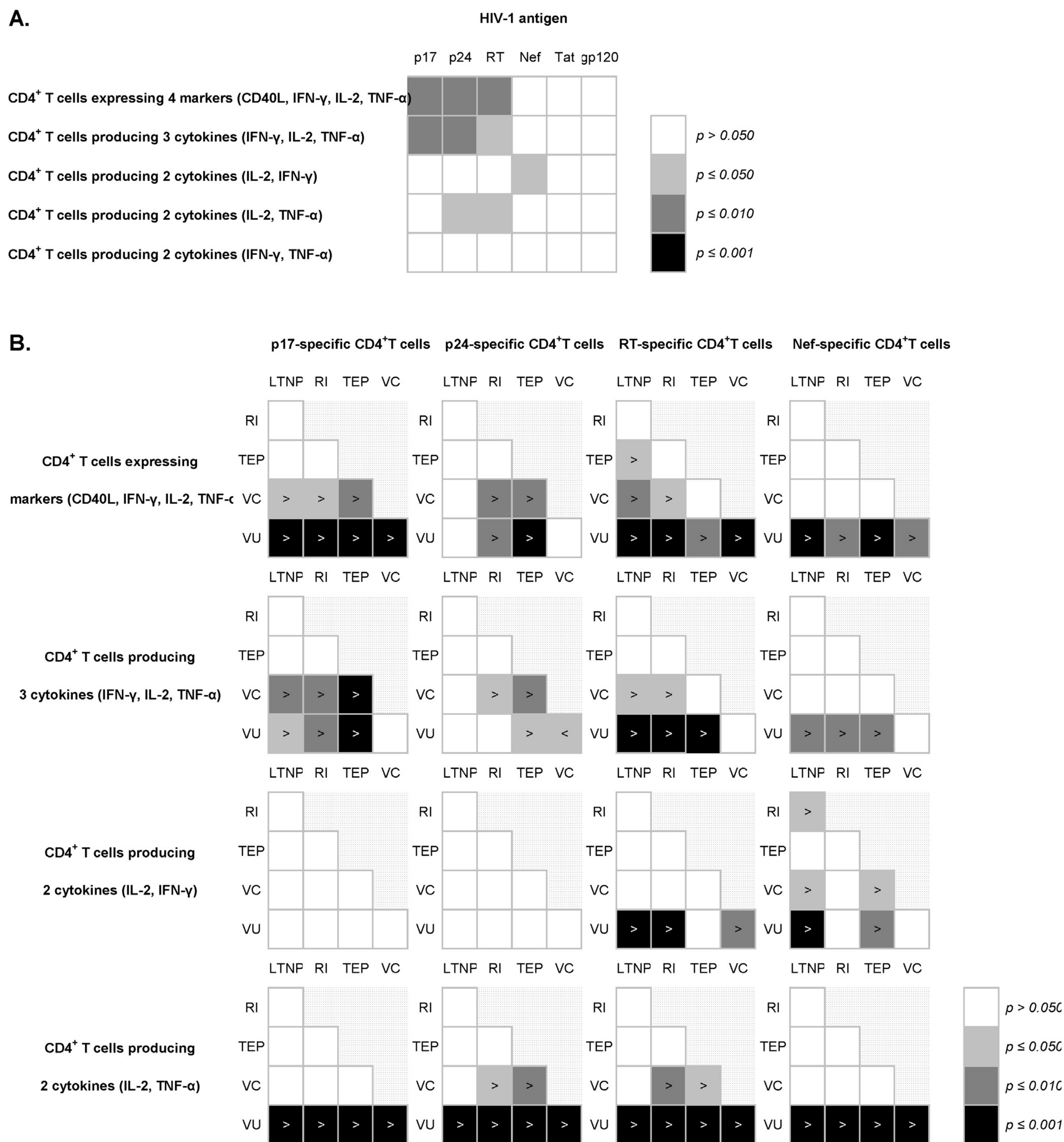

Fig. 1. Overview of polyfunctional HIV-specific CD4+ T cell responses. (A) Significant differences between the four HIV-1-infected patient groups (LTNP, RI, TEP, VC) were first identified using a Kruskal–Wallis test as a screening method. The rows indicate various combinations of markers expressed by CD4+ T cells. The columns indicate the different HIV-1 antigens used for stimulation. The significance levels are indicated by the gray intensities. (B) Two-by-two Mann–Whitney comparisons were performed on those conditions identified by the Kruskal–Wallis test, including the vaccinated uninfected cohort (VU). The symbols ">" and "<" indicate greater and smaller than, respectively, when reading the charts from the left to the right.

3.2. CD4+ T cell responses

Inverse correlations were observed between HIV plasma RNA levels and different expressions of CD4+ T cell polyfunctionality. A lower viral load correlated with co-expression of four markers (CD40L, IL-2, IFN-γ and TNF-α) after stimulation with p24 ($p < 0.001$), with triple cytokine production (IL-2, IFN-γ and TNF-α) after stimulation with p17, p24 (both $p < 0.001$) and RT ($p = 0.005$), and with co-production of IL-2 and TNF-α after stimulation with p24 ($p = 0.003$) and RT ($p = 0.005$). No correlations were found with CD4 responses to gp120, Nef and Tat.

The CD4+ T cell responses against p17, p24, RT and Nef, induced by the F4co/AS01 vaccine candidate in healthy volunteers, were generally higher than those observed in infected patients. We considered the responses that were different among HIV-1-infected patient groups as the most relevant ones, identified by a

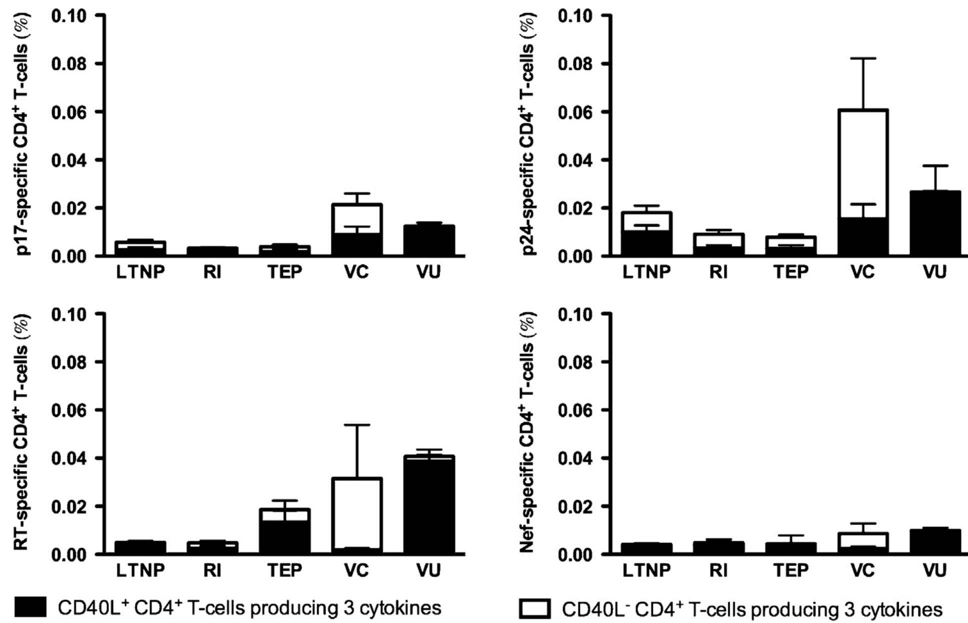

Fig. 2. HIV-specific CD4+ T cells producing 3 cytokines with or without CD40L expression after stimulation with p17, p24, RT or Nef. Results (group means, SEM) are represented as the percentage of CD4+ T cells co-producing 3 cytokines (IL-2, IFN-γ and TNF-α), with or without simultaneous CD40L expression. All responses shown are background subtracted.

Kruskal–Wallis test (Fig. 1A) and subsequently further differentiated between the four patient groups and the vaccinees (Fig. 1B). Simultaneous expression of 4 markers was higher in VU than in most HIV-1-infected patients (Figs. 1 and 2). Among HIV-1-infected patients, VC had the highest frequencies of polyfunctional CD4+ T cells co-expressing 4 markers or co-producing 3 cytokines when stimulated with p17, p24 and RT antigen (Figs. 1 and 3). There was no statistically significant difference between elite and viremic controllers. After stimulation with Nef, VC had more CD4+ T cells expressing both IL-2 and TNF-α than other patient groups. For all antigens, the differences between VC and other HIV-1-infected patients fade out when combinations of only two markers or only one marker are considered. After stimulation with Tat and gp120, no significant differences between groups were observed (Fig. 1A). Fig. 4 summarizes all antigen-stimulated conditions of CD4+ T cells expressing 1, 2 or 3 cytokines among IFN-γ, IL-2 and TNF-α.

3.3. CD8+ T cell responses

The $CD8^{bright}$ T cell responses observed in the HIV-infected patient groups are available online as supplementary data. The F4/AS01 vaccine candidate induced no detectable CD8+ T cell responses.

4. Discussion

This is the first report of a head-to-head comparison of CD4+ T cell responses induced by HIV infection in individuals with different patterns of disease progression and CD4+ T cell responses induced by an HIV-1 vaccine candidate in healthy volunteers. The data show that vaccination of healthy HIV-uninfected volunteers with an adjuvanted polyprotein vaccine induced polyfunctional CD4+ T cell responses to p17, p24, RT and Nef of the same magnitude and quality as those observed in HIV-infected patients who spontaneously control the virus.

HIV-infected VC had significantly more CD4+ T cells co-expressing IL-2, IFN-γ and TNF-α after stimulation with p17, p24 and RT antigen than other HIV patient categories. Differences between study groups decreased with lowering polyfunctionality. With exception of the combination of IL-2 and TNF-α, the production of only one or two cytokines by antigen-specific CD4+ T cells was comparable for all HIV patient groups, irrespective of the viral load. Our results are in line with previous studies, wherein Gag was

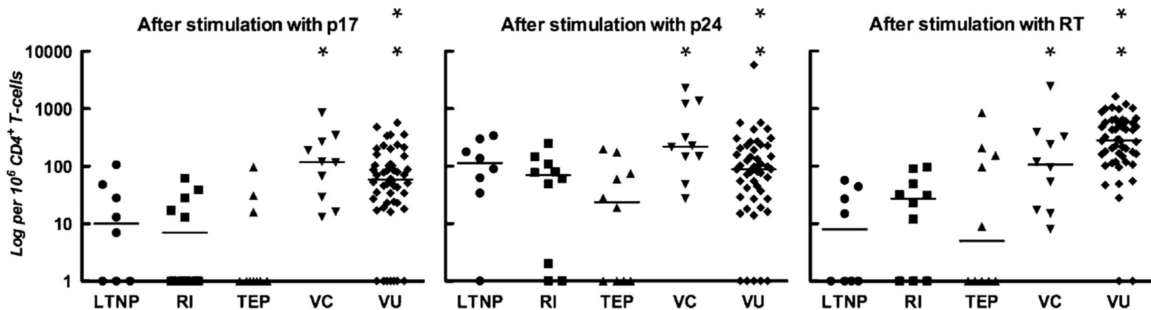

Fig. 3. CD4+ T cells co-expressing IL-2, IFN-γ and TNF-α. Horizontal lines represent group medians. All responses shown are background subtracted. *VC significantly higher than TEP ($p = 0.001$), RI ($p = 0.003$) and LTNP ($p = 0.007$) after p17 stimulation, than TEP ($p = 0.005$), RI ($p = 0.019$) and VU ($p = 0.026$) after p24 stimulation, and than LTNP ($p = 0.011$) and RI (0.034) after RT stimulation. **VU significantly higher than TEP ($p = 0.001$), RI ($p = 0.002$) and LTNP ($p = 0.020$) after p17 stimulation, than TEP ($p = 0.033$) after p24 stimulation, and than RI, LTNP and TEP (all $p \leq 0.001$) after RT stimulation.

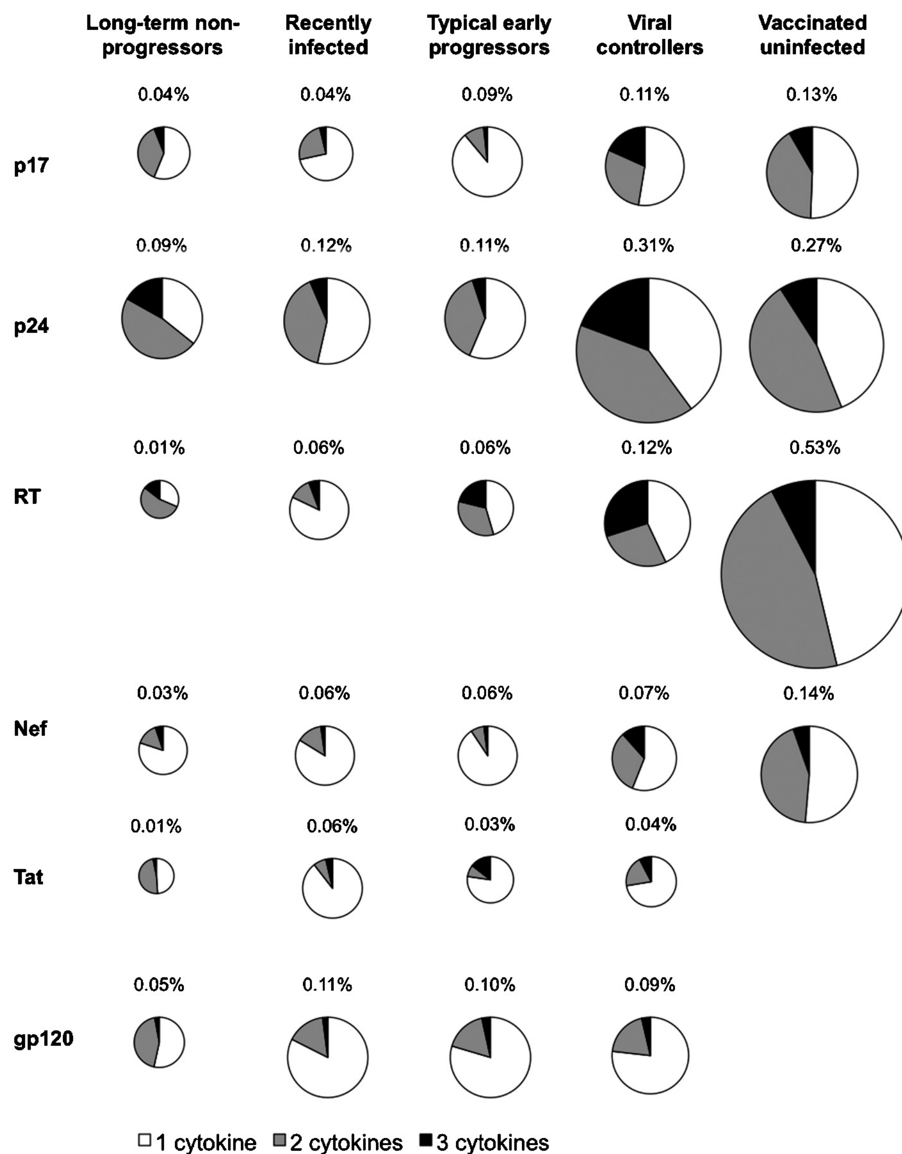

Fig. 4. Overview of cytokine expression by HIV-1 antigen-specific CD4⁺ T cells per study group. Results (group means) were expressed as the percentage of the total cytokine-producing CD4⁺ T cells expressing 1, 2 or 3 cytokines (IL-2, IFN-γ or TNF-α). Frequencies (%) of total CD4⁺ T cells producing at least 1 cytokine are indicated above each pie chart. The sizes of the pies reflect these percentages. The F4/AS01 vaccine candidate contained no Tat or gp120 antigens. Responses shown are background subtracted.

most often used as a stimulating antigen. Both non-progression and durable control of HIV replication have been associated with high levels of Gag-specific IL-2⁺ IFN-γ⁺ CD4⁺ T cells in studies wherein TNF-α expression was not measured [23,26]. Strong p24-specific CD4⁺ T cell responses have also been linked to efficient viral control in primary HIV-1 infection [25] and a stable p24-specific proliferative response to control of viremia and retention of non-progressor status [29]. Kannanganat et al. demonstrated that CD4⁺ T cells producing three cytokines are functionally superior to those producing single cytokines and that control of HIV-1 is associated with the maintenance of highly functional Gag-specific CD4⁺ T cells co-producing IL-2, IFN-γ and TNF-α [28,33]. A recent analysis of CD4⁺ T cell responses to the entire HIV proteome in 93 subjects at different stages of HIV infection, indicated Gag as the target of IFN-γ⁺ CD4⁺ T cells most robustly associated with lower levels of viremia [34]. Responses to three distinct Gag epitopes were linked to spontaneous HIV control.

We also found that TEP had lower frequencies of HIV-specific CD8⁺ T cells producing IL-2 alone or in combination with TNF-α, when compared to VC and also RI. This is consistent with the findings of Betts et al., who demonstrated that CD8⁺ T cells of non-progressors retain the capacity to produce TNF-α and IL-2 in conjunction with other functions, such as degranulation and chemokine production [35]. Another study, using IFN-γ enzyme-linked immunospot assays, indicated that Gag-specific responses were associated with lowering viremia, while Env-specific and Accessory/Regulatory protein-specific responses were associated with higher viremia [36]. In our patients, we also observed that TEP had more gp120- and Nef-specific CD8^{bright} T cells expressing IFN-γ (in combination with CD40-L) than VC.

The CD4⁺ T cell responses of the LTNP in our study resembled better those of TEP and RI than of VC. This may be due to the fact that most LTNP in our cohort had detectable plasma HIV RNA levels and had reached a certain phase of disease progression at the time of inclusion. The two LTNP with undetectable viral load, subjects LTNP06 and 08, showed the highest functional CD4 responses of the LTNP group, the magnitude of which was comparable to the group medians of the VC. Our explanation of this unexpected finding is supported by the results of a longitudinal study which showed that detectable viremia at study entry was predictive of loss of LTNP

status and/or disease progression and differentiated slow progressors from elite LTNP who retained potent virological control [29]. Together these data suggest that the viral load is a better predictor of functional HIV-specific immunity than the CD4 count.

The magnitude and quality of the CD4+ T cell responses in VC were comparable to those observed in F4/AS01 vaccine recipients. This was most pronounced after stimulation with RT, when co-expression of IL-2, IFN-γ and TNF-α was similar to VC but more frequent than in TEP, RI and LTNP. No comparisons were possible at the CD8 level because the F4/AS01 vaccine induced no detectable CD8 responses. Our results demonstrate that in healthy subjects an adjuvanted polyprotein HIV vaccine can induce CD4+ T cell responses that share several characteristics with the HIV-specific immune response detected in the peripheral blood of HIV-infected patients able to spontaneously control HIV viremia. Both prospective studies and vaccination trials in HIV-infected patients are needed to provide further evidence on the hypothesis that these immune responses are cause rather than consequence of viral suppression. The results of these vaccine studies will determine which subgroups within all prevalent HIV-infections may benefit from such immunotherapy. A CD4+ T cell-inducing vaccine targeting the appropriate set of HIV antigens might be able to induce a non-progression status in vaccinated, chronically infected patients.

The CD40L protein delivers activating signals to B-cells and antigen presenting cells when expressed by activated T cells. Administration of the adjuvanted HIV vaccine to healthy volunteers induced CD40L expression on practically all polyfunctional HIV-specific CD4+ T cells co-producing 3 cytokines. Interestingly, in HIV-infected controllers a similar pool of triple cytokine producing CD4+ T cells was found but the majority of these cells did not express CD40L (Fig. 2). This finding is consistent with the HIV-induced impairment of CD40L expression on CD4+ T cells described by other groups, which is thought to contribute to antigen-presenting cell dysfunction in HIV infection [37,38].

A possible weakness of this study is the heterogeneity in viral subtypes seen in the LTNP and VC groups, whereas in the other groups patients were mainly infected with viruses of clade B. An influence of varying viral fitness can therefore not be excluded. However, although all peptides used in the ICS assays were derived from clade B viruses, we observed the most vigorous responses in the VC group in which only one clade B infection was present. This suggests a high degree of cross-reactivity at the T cell level between the different clades, similar to what has been observed before in the F4/AS01 vaccinees with clades A and C peptides [30]. Since we have not examined the patients' HLA types we cannot estimate the possible effects of protective class I alleles, such as B*5701 and B*27 [39]. Genotyping for the CCR5 Δ32-mutation that has been associated with slower disease progression revealed no homozygous and a balanced representation of heterozygous patients in all study groups.

This comparison of HIV-specific CD4+ T cell responses induced by natural infection on the one hand and by a candidate HIV vaccine on the other hand, provides for the first time direct evidence that administration of an adjuvanted polyprotein vaccine can induce an immune response with qualities similar to that observed in HIV-infected patients who spontaneously control the virus. If a causal relation between this observed immune response and viral control can be proven further, administration of an adjuvanted multi-antigenic vaccine might produce an immune status that will direct the disease course toward the VC status in case of subsequent HIV infection. Controlling the viral load is not only beneficial for the individual patient but has also impact on the spread of the virus in the community. Our observations need to be corroborated but warrant further exploration of the F4/AS01 vaccine candidate in HIV-1-infected patients as an immunotherapeutic approach.

Acknowledgements

We are indebted to all study participants, and the following staff members of the Aids Reference Center at Ghent University Hospital for selection and recruitment of HIV patients: Prof. Dr. S. Blot, Dr. F. Van Wanzeele, Dr. B. Van Der Gucht, Dr. J. Pelgrom, Mrs. T. James. The PRO HIV-005 vaccine study was carried out at Center for Vaccinology, Ghent University Hospital, with the help of study physicians, trial nurses and lab technicians. Viral load, CCR5 and viral genotyping results were obtained from the Aids Reference Laboratory, Ghent University. Statistical support was kindly provided by Prof. Dr. G. Van Maele, Division of Biostatistics, Ghent University. We gratefully acknowledge GSK Biologicals for providing access to the raw data from the Phase I/II trial with their F4/AS01 vaccine candidate (PRO HIV-005, NCT00434512) and for kindly providing the HIV-1 antigens used in the flow cytometry assays in the HIV-1-infected patient groups. We would like to acknowledge, while accepting full responsibility for the content of this manuscript, open discussions with Drs. G. Voss, M. Koutsoukos, P. Bourguignon, M. Janssens and L. McNally (GSK Biologicals). *Potential conflicts of interest*: G.L.R. was principal investigator for clinical studies of a variety of candidate vaccines for Baxter, GSK Biologicals, Novartis, and SanofiPasteur and has also performed consulting services for GSK Biologicals and Novartis. Ghent University and University Hospital received sponsoring for the conduct of these studies. E.V.B., I.D., F.C., L.V., C.V. and D.V. have no conflict of interest in relation to this article. *Funding*: This work was supported by internal funding of the Center for Vaccinology. The PRO HIV-005 study in healthy volunteers was sponsored by GlaxoSmithKline (GSK) Biologicals, Rixensart, Belgium. L.V. is supported by the Flemish Fund for Scientific Research.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2013.05.021.

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