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T-cell vaccination against anti-CD4 autoimmunity in HIV-1 subtypes B and C-infected patients—An extended open trial

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Abstract

This study is an extended clinical trial of the one initiated and reported in the Journal of Clinical Virology 2004;31S:S48–54. Thirteen HIV-1 patients (eight subtype B and five subtype C) that manifested T-cell autoimmunity to recombinant human CD4 (rCD4) were treated with T-cell vaccine composed of glutaraldehyde-treated autologous anti-CD4 reactive T-cells and compared to historical seven non-vaccinated HIV-1-infected subjects. This study proved to be feasible and safe. Follow-up study revealed that 7/8 subtype B and 2/4 subtype C patients (one has just received the first TCV injection) responded with a persistent increase in their blood CD4 T-cell levels and four subtype B patients manifested decreased anti-CD4 autoimmunity. Despite highly active antiretroviral therapy (HAART), the persistence of CD4 T-cell lymphopenia may be associated with anti-CD4 autoimmunity. T-cell vaccination (TCV) may decrease such autoimmunity and elevate CD4 T-cell numbers.

Keywords: T-cell vaccination; HIV-1 subtypes B and C; Autoimmunity

1. Introduction

HIV-1 infection is characterized by a continuous attrition of CD4 T-cells leading progressively to immune deficiency [1]. It is unclear whether HIV infection of CD4 T-cells may, account for the massive CD4 T-cell loss in HIV-infected persons [2]. It was suggested that other indirect effects of HIV on the host immune system may participate in the CD4 decline. The persistence of CD4 T-cell leukopenia, despite a reduction in the viral load by highly active antiretroviral therapy (HAART) [3], indeed supports this proposal. HIV infection may provoke an autoimmune against to the CD4 molecule [4] causing the destruction of uninfected CD4 T-cells.

T-cell vaccination is a procedure in which killed autoimmune T-cells are used as vaccines to induce immune regulation against autoimmunity. T-cell vaccination has been studied in experimental animals [5], and 1ately has been used clinically to down-regulate human autoimmune diseases such as multiple sclerosis [6,7]. Atlan et al. have suggested that autologous anti-CD4 T-cells might be used as vaccines against anti-CD4 autoimmunity in HIV-infected patients [8].

This study investigates T-cell autoimmunity to CD4 in HFV-1 subtypes B and C patients and initiates an open trial of T-cell vaccination where we vaccinated 13 patients with their own anti-CD4 T-cells, and were followed up for 1–2 years. Seven control-infected subjects were also followed up.

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2. Materials and methods

2.1. Protocol for the preparation of the T-cells used for T-cell vaccination (TCV)

Recombinant human CD4 (rCD4)-reactive T-cells were generated from 30 to 40 ml of blood obtained from each patient for vaccine preparation [7]. Briefly, PBMC were isolated from the blood of each of the patients, cultured with 3 μg/ml phytohemaglutinine (PHA) (Murex Diagnostic Ltd., England), and expanded with 5–20 U/ml recombinant interleukin-2 (rIL-2) (Boehringer Mannheim GmbH Mannheim, Germany) for 11 days. The cultures were re-stimulated on day 14, with irradiated rCD4-pulsed antigen presenting cells (APCs) as described [7], and were further grown for another 7 days. On day 21, the cells were inactivated for 5 min at room temperature with 1.0% glutaraldehyde (Sigma Chemical Co., St. Louis, MO, USA) and prepared for injection. Aliquots of 10×10^6 CD4 reactive T-cells were frozen in liquid nitrogen, to be used later for second and third booster vaccinations at 2–6-month intervals.

2.2. TCV and patient follow-up

Medical personnel administered vaccinations in an AIDS clinic to thirteen HIV-infected patients: P1–P13 (12 males and one female; median age 41) (Table 1). The effects of the TCV were evaluated clinically, immunologically, and virologically before, during and following the first injection for 1–2 years. Seven HIV subtype B-infected patients receiving HAART (three males and four females; median age 53) (Table 3B) were not vaccinated and were followed as controls for changes in CD4 counts in the absence of TCV (three subtype C-vaccinated control patients were added, data not shown). The clinical follow-up of the patients consisted of a complete physical examination and blood tests including a CBC and biochemistry (liver function and renal function). Following each injection, the patients were monitored at the clinic for 2 h for any side effects, and remained under close surveillance by the treating physician for an additional 7 days to record any adverse events. Adverse effects were monitored according to the WHO protocol. The immunological follow-up consisted of: (a) total lymphocytes and T-cell subset counts (CD4 and CD8) at the indicated time points and (b) specific proliferative responses of PBMC to rCD4, gp120 before and after vaccination. The virological follow-up consisted of an assessment of HIV plasma viral load, 2 weeks after the first injection, then 4 weeks later, and thereafter, 1 and 3 months.

2.3. Flowcytometry

Percentages of CD4, CD8 and activation markers of T-cells were assessed by flow cytometry analysis using FACSCalibur and monoclonal antibodies specific for CD3, CD4 and CD8 from Becton-Dickinson Immuno-cytometry Systems, San Jose, CA, USA.

3. Results

3.1. Anti-CD4 autoimmunity

We screened 23 HIV-1 subtype B-infected patients and 20 healthy Caucasian blood donors as well as 20 HIV subtype C patients and 18 African healthy controls. These subjects were tested for T-cell proliferation responses to recombinant human CD4 (rCD4), recombinant gpl20 (rgpl20), tetanus toxoid and *Candida*. Proliferation responses were measured in stimulation index (S.I.) as described previously [9]. Responses of both HIV subtype B and C patients to rCD4 were significantly higher 31/43 (72%) compared to healthy controls 8/38 (29%).

Table 1
Clinical features of HIV-1 subtypes B and C-infected patients enrolled for TCV

Subject #	Sex/age	Subtype	Disease duration (years)	Duration of HAART (years)	Viral load (copies/ml)	CD4		Proliferation (S.I.)	
						(cells/μl)	(%)	rgpl20	rCD4
P1	M/63	B	11	2.3	<400	348	21	1.6	4.2
P2	M/35	B	9	2	14,000	394	28	1	4.5
P3	M/36	B	10	2.3	1500	327	26	nd	7
P4	M/55	B	5	1.8	<400	289	29	1	2
P5	M/41	B	2	3.2	2000	172	17	2.9	2
P6	M/50	B	12	0.5	37,000	247	14	1	5.7
P7	M/40	B	13	2	<400	388	23	1	7.8
P8	M/59	B	2	1.5	<400	531	25	3.6	6.9
P9	M/51	C	3	3	<400	473	29	nd	2.2
P10	M/38	C	2	2	<400	386	19	nd	2
P11	M/49	C	3	2	<400	194	23	nd	2.8
P12	M/38	C	5	3	2200	229	17	1.4	4.9
P13	F/29	C	2	2	<400	255	24	1.7	2
Median	41	–	5	2	2200	327	23	1.2	4.4
Mean	–	–	–	–	–	–	–	1.7 ± 0.9	4.15 ± 2.1

nd: not determined.

Thus, the HIV patients showed a significantly enhanced T-cell response to CD4 (mean S.I. 3.8 ± 2.1) compared to controls (mean S.I. 1.4 ± 0.36); $P = 0.0001$ (data not shown) except for patients receiving TCV (Table 1). Thirteen patients from the 43 screened were selected for receiving TCV (eight subtype B and five subtype C) (Table 1). Patients enrolled responded to rCD4 (mean S.I. 4.15 ± 2.1 and only two of eight tested responded to rgpl20).

3.2. T-cell vaccination

A phase I open trial of T-cell vaccination against CD4 autoimmunity was carried out in 13 of the HIV patients (P1–P13), all receiving HAART (Table 1). The cells used for vaccines were derived from peripheral blood mononuclear cells, stimulated and expanded for 21 days (see Section 2). The CD4 reactive T-cells were enriched in CD8 T-cells, (Table 2) and manifested high IFN-γ and low IL-10 expression, in addition to cytotoxic activity from 10–67% (T1-type). Prior to the vaccination, the cells were fixed with 1.0% glutaraldehyde, so as to kill them and inactivate any live HIV viruses. No virus was detectable after 21 days of in vitro culture in the T-cells of patients with viral loads in the blood; patients P2, P3, P5 and P12 had plasma viral loads of 14,000, 1500, 2000 and 2200 copies/μl, respectively, and 5, 11, 19 and 19% of CD4 T-cells, respectively, remained in the T-cell vaccine preparation. P6, despite having only 7% remaining CD4 T-cells in the T-cell vaccine preparation, had a plasma viral load of 37,000 copies/μl blood (Tables 1 and 2) (Amplicor, Hoffman-LaRoche, Basel, Switzerland). Nevertheless, the T-cell cultures used for vaccination were free of virus even before fixation.

The autologous T-cell vaccine preparations were injected subcutaneously with about 10^7 T-cells in 1 ml of saline administered three or four times at intervals of 2–6 months. No clinical or laboratory side-effects were observed during the 1 year (P5 and P7), 2 years (P1, P2, P3, P4, P6), or several months (P8 received only two injections of TCV and all type C patients are still undergoing TCV) follow-up period. Nine of the 13 vaccinated subjects responded with increased levels (above 50%) of circulating CD4 T-cells compared to the mean levels of CD4 T-cells observed during the 2-year period preceding the T-cell vaccination (Table 3A). Among the subtype C patients, two patients (P9 and P11) manifested an increase in CD4 T-cell levels (above 50%). *No decrease in CD4 cell levels was reported in all TCV patients*. Furthermore, as can be seen in Table 3B, a spontaneous rise of CD4 levels was not observed in the control group of HIV-infected patients that also received HAART and had a similar degree of HIV suppression. We also included three untreated HIV-1 subtype C patients in our follow-up studies and none manifested above 20% in CD4 T-cell levels with no significant changes in viral load (data not shown).

In addition, among the subtype B patients, no increase in the plasma viral loads were detected after 1 year in patients P5 and P7, after 2 years in patients P1, P3, P4 and P6, and after half a year (two injections) in patient P8 following TCV. In fact, patients P5 and P6 manifested a significant decrease from 2000 copies/μl to below detection, and from 37,000 to 5600 copies/μl, respectively (Table 3A). Patient P2 is the only exception; in spite of unstable viral load was unusually unstable from the beginning with ups and downs, which continued after TCV. In addition, his CD4 cell count improved significantly during the 2 years following TCV. Among the subtype C patients, P9, P10, P11 and P13 remained below detection levels during a period of up to 1 year. Patient P12 manifested increase in the viral load (34,200) (was not tolerating HAART); however, after his HAART therapy was recently altered, his viral load became below detection.

4. Discussion

This study describes an initial trial of T-cell vaccination. Thirteen of the 43 HIV-infected patients that were selected

Table 2
T-cells vaccine characteristics

Patient	Fresh PBMC		T-cell vaccine			
	CD4 (%)	CD8 (%)	CD4 (%)	CD8 (%)	IFN-γ (pg/ml)	IL-10 (pg/ml)
P1	28	27	15	53	3090	34
P2	19	52	5	92	2560	82
P3	26	56	11	73	1390	78
P4	30	43	27	61	1760	170
P5	16	39	19	70	5530	38
P6	14	40	7	88	1240	110
P7	23	55	9	86	nd	nd
P8	28	37	nd	nd	nd	nd
P9	nd	nd	13	65	nd	nd
P10	nd	nd	14	43	nd	nd
P11	36	27	9	89	nd	nd
P12	nd	nd	19	62	nd	nd
P13	27	20	15	60	nd	nd

nd: not determined. CTL activity was from 10–67%.

Table 3
Peripheral blood CD4 T-cell numbers and viral load

Patient number	CD4 cells/μl blood			HIV Plasma viral load (copies/ml)	
	[a]Pre-vaccination	[b]Post-vaccination	Change (%)	[a]Pre-vaccination	[b]Post-vaccination
A. TCV-treated HIV-1 patients					
P1	353 ± 100	600	70	<400	<400
P2	413 ± 112	701	69	14,000	229,000
P3	290 ± 52	523	80	1500	1490
P4	275 ± 41	512	86	<400	<400
P5	187 ± 54	277	48	2000	<400
P6	252 ± 106	385	52	37,000	5690
P7	237 ± 96	450	89	<400	<400
P8	402 ± 180	926	130	<400	<400
P9	422 ± 81	639	51	<400	<400
P10	413 ± 92	440	6.5	<400	<400
P11	204 ± 38	409	100	<400	<400
P12[c]	165 ± 50	199	20	9940	<400
P13	290 ± 27			<400	<400
B. Untreated HIV-1 subjects					
CP1	142 ± 29	167	18	5130	958
CP2	202 ± 43	256	27	6930	6890
CP3	136 ± 33	220	61	<400	<400
CP4	190 ± 52	224	18	3790	<400
CP5	197 ± 62	166	−15	<400	<400
CP6	153 ± 41	235	53	3890	1220
CP7	311 ± 124	282	−9	8300	5830

[a] Mean of 2 years before vaccination.
[b] Measured after 1 year in patients P5 and P7, after 2 years in patients P1–P4, P6, and less than 1 year in the rest of patients.
[c] Prior to screening, patient P12's viral load was 2200 copies/ml and prior to vaccination, his viral load was 9940 copies/ml.

and screened for TCV manifested T-cell autoimmunity to the CD4 molecule. Their autoreactivity to CD4 appears to be independent of the plasma HIV viral load and the HIV subtype of the virus infected (subtype B or C) because no correlation was found between the viremia or HIV type (Table 1) and the in vitro T-cell proliferative response to CD4 (correlation coefficient = 0.2298).

Although the study lacked a placebo control group, it generated some positive results. T-cell vaccination in HIV-infected patients is feasible; it was indeed possible to raise a sufficient number of autologous T-cells to develop a vaccine, even in persons with detectable viremia. The resulting vaccines were composed of T-cells that produced more IFN-γ than IL-10, suggesting T1 type of cells (Table 2). Some of the subjects appeared to respond to the vaccination by a reduction in anti-CD4 autoimmunity (data not shown) and by a rise in absolute numbers of peripheral blood CD4 T-cells. All but three of the patients (P5, P10 and P12) showed an above 50% change (median 70%; Table 3A); this was not seen in a control group of non-vaccinated patients; only two patients (CP3 and CP6) showed a change to 61% and 53%, respectively (median 27%; $P = 0.007$; Table 3B). In no instance did we see any aggravation of the patient's condition, in particular any decrease in CD4 cell numbers. In two patients (P2, P12), the viral load was highly unstable before and after TCV, but a significant increase in CD4 cell counts from 400 to 700 was observed in patient P2 2 years after TCV (Table 3A). Although patient P12's CD4 cell counts increased from 165 to 199 only after the first injection, we cannot draw any conclusions since this patient is still undergoing TCV and, as mentioned, his viral load is presently below detection.

Finally, as mentioned above some patients entered the study with relatively high viral loads, and the T-cell preparations used as vaccines still contained appreciable numbers of CD4 cells. Nevertheless, no virus could be detected in the supernatants of the cell cultures. This suggests that some of the T-cells in the culture may have had anti-HIV activity in vitro. However, it is unlikely that the vaccination produced any undesirable immune response against HIV-specific CTLs, since there was no significant clinical deterioration following vaccination. With the exception of patient P2 discussed above, none of the patients showed an increase in viral load after TCV (patient P12 received only one injection out of three). Actually, two vaccinated subjects showed a significant decrease in their viral load 2 years after TCV indicating, at least, that TCV did not decrease the efficacy of the ongoing antiviral therapy. How HIV infection may activate autoimmunity to CD4 is unknown, but conceivably, it could be related to an immunogenic alteration of the CD4 molecule following the binding of gpl20 to CD4 [8], and/or to the abnormal state of activation of the immune system in HIV-infected persons [2].

Interestingly, in concurrence with published paper [10], patients infected by either subtype B or C showed no difference in anti CD4 autoimmunity and responded equally to the TCV treatment.

To summarize, our study shows that TCV against anti-CD4 cells in HIV-infected patients is feasible and safe with no adverse effects. Both HIV subtypes of patients B and C manifested the same anti-CD4 autoimmunity and responded the same to TCV, albeit subtype C patients are still undergoing TCV treatment. However, because of the small number of test subjects, our study does not allow us to draw firm conclusions regarding its therapeutic efficacy. Obviously, a confirmatory Phase II trial would be warranted.

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