EFFECT OF AN ORAL THERAPEUTIC HIV-1 VACCINE ON AIDS PATIENTS WITH CD4 COUNT ABOVE 250 CELLS/MM^3

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Summary. – A simple delivery route, e.g. oral, would greatly facilitate the acceptance of an AIDS vaccine by a large target population. We developed a vaccine that took advantage of inherent properties of mucosal immunity in response to oral antigen challenge, namely the V-1 Immunitor vaccine (V1), which is a polyvalent oral Human immunodeficiency virus 1 (HIV-1) vaccine. The vaccine, currently manufactured in Thailand, contains pooled, inactivated viral antigens. In order to compare this vaccine with HIV-1 therapeutic vaccines reported earlier we analyzed retrospectively 13 HIV-1-positive patients that had the baseline CD4 T-cell counts greater than 250 cells/mm^3 (range 270–605 cells/mm^3). The patients self-administered one 850 mg vaccine tablet at breakfast and dinnertime for an average of 32 weeks (median 26 weeks). The treatment was well tolerated without any toxic effect. Twelve of thirteen patients (92%) and 9 of 13 patients (69%) experienced an elevation in CD4 and CD8 cells. The mean increase in absolute CD4 and CD8 cell counts across this group was 98 (22%; P = 0.02) and 324 (26%; P = 0.05) cells/mm^3, respectively. Viral plasma load was measured by PCR in six patients. The observed viremia reduction was within 1 log unit. Subjective parameters, i.e., appetite, energy, and sense of well-being were reported by patients as being markedly improved, reflected in a mean body weight gain of 2.75 kg (P = 0.0008). Oral administration of HIV-1 immunogens provides compelling clinical response, especially when patients are treated earlier.

Key words: antiviral therapy; cellular immunity; clinical trials; gut, immune-based therapy; therapeutic vaccine

Introduction

Anti-HIV-1 cocktail drugs are toxic and effective for a limited period of time, and, most importantly, they are too expensive for the majority of HIV-1-infected individuals. One of the alternatives in solving this problem is the development of an inexpensive therapeutic vaccine. A great variety of preventive vaccines against HIV-1 are now being developed and many of them have been first tested in clinical trials as a therapeutic modality administered to already infected individuals. There has been a considerable experience with this type of approach, with over 30 clinical trials reported over the last fifteen years. Most, if not all, have demonstrated no clinical benefit and had no effect on CD4 or CD8 T cell counts or viral load (Kinloch-de Loes and Autran, 2002; Lisziewicz *et al*., 2003).

The availability of simple delivery route, i.e., oral, would greatly improve the acceptance of a vaccine by a large population of patients, especially in developing countries. The development of an oral AIDS vaccine is a task fraught with many challenges including the antigen degradation in the stomach. The insight gained from our earlier groundwork dealing with transmucosal transmission of retroviruses has enabled us to develop an oral HIV-1 vaccine (Bourinbaiar

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Abbreviations: AIDS = acquired immunodeficiency syndrome; ALVAC = recombinant vaccinia virus-based vaccine; CTL = cytotoxic lymphocyte; HIV-1 = Human immunodeficiency virus1; MIP-1 alpha = macrophage inflammatory protein-1 alpha; NYVAC = recombinant vaccinia virus-based vaccine; rgp = recombinant glycoprotein; V1 = V-1 Immunitor; VLP = virus-like particle

and Minowada, 1989; Bourinbaiar, 1994; Bourinbaiar and Lee-Huang, 1995). The preliminary results of an open-label trial with the oral HIV-1 therapeutic vaccine in patients with advanced disease have been published earlier (Jirathitikal and Bourinbaiar, 2002). However, almost every published therapeutic AIDS vaccine has been tested in relatively healthy patients in early stages of the disease.

In order to compare the results of our study with those of the published studies on HIV-1 therapeutic vaccines, which have been mostly tested on asymptomatic patients with high CD4 cell counts, we included in this report only the patients with initial CD4 cell counts over 250 cells/mm^3.

Materials and Methods

Study population. Forty Thai patients with documented history of HIV-1 infection were available for the analysis at the time this retrospective study has been carried out. In this study all patients who had over 250 CD4 cells/mm^3 at baseline and had at least one pair of "before" and "after" data were considered for the analysis. One test (the "before" test) was performed prior to or shortly after the enrollment and the "after" test was carried out post immunization. The patient group consisted of 10 females (77%) and 3 males (23%). The age ranged from 22 to 50 years with mean age of 32.3 years (the median of 30 years). After obtaining written informed consent the subjects were given a monthly dose of the vaccine. Patients self-administered two 850 mg vaccine tablets b.i.d. at breakfast and dinnertime. None of the patients received any conventional antiretroviral therapy during the treatment period. Only one patient (No. 6) was treated with various reverse transcriptase antivirals; the treatment was stopped 2 months before the enrollment.

The vaccine has been manufactured in Thailand according to a process developed by the first author of this article. It was a polyvalent preparation containing heat-inactivated HIV-1 antigens derived from pooled blood of HIV-1-positive donors. The exact protocol of manufacturing and composition of V1 cannot be described as it represents a still pending patent. The vaccine has been produced as 850 mg coated tablets sealed in a blister; after 3 years of storage at ambient temperature the vaccine was not inactivated. It has been first registered in the Chachoengsao Province on October 15, 1999 as a food under permit No. 1/2542 and then licensed as a food supplement by the Thai FDA on July 13, 2001 (the license No. 152/44).

In September 2000 the vaccine received the R&D Permit No. 1A1874/43 from the Thai FDA for producing drug samples for clinical testing. Thus V1 has to date also a status of an experimental drug which will eventually lead to its licensing as a vaccine.

Flow cytometry. CD3/CD4 and CD3/CD8 T lymphocytes were determined in samples of whole blood by two-color immunofluorescence in a commercial lab using known amount of fluorescent beads as reference.

Viral load assay. The number of HIV-1 RNA copies per ml of the blood was assayed by RT-PCR in a commercial lab using an RNA PCR Kit according to the manufacturer's instructions (Roche Diagnostic Systems, Inc., USA).

Statistical analysis. The significance of differences in CD4, CD8 and body weight gain data was tested by the Student's *t* test (StatMost Version 2.5, DataMost Corporation, USA). The viral load data were evaluated by the Pearson correlation test, useful for identifying a potential correlation (r) of two sets of data. Differences with $P \leq 0.05$ were considered significant.

Results

V1 was well tolerated by the study participants; no safety concerns or adverse effects arose during the therapy. In contrary to patients in terminal stage of the disease no deaths occurred in this group of patients (Metadilogkul *et al*., 2002). On follow-up visits none of the patients presented with a new complaint or new opportunistic infection, which is quite unusual among HIV-1-positive patients not treated with any medication.

A common manifestation of progressive HIV-1 infection is weight loss. This parameter provides an objective assessment of the quality of life. The mean body weight of immunized patients increased from baseline by 2.75 kg (baseline mean ± S.E. was 52 ± 1.9 kg and the post-immunization value was 54.75 ± 2.2 kg; P = 0.0008). The weight range at the entry was 45–67 kg and following the therapy it rose to 46–71. These results are consistent with our recent data from 650 patients treated with V1 (Jirathitikal *et al*., 2004).

When lymphocyte counts were followed in 13 patients treated with the vaccine for an average of 32 weeks (median 26 weeks) there was an elevation of CD4 (22%) from 445 ± 28 to 542 ± 43 (P = 0.02) and CD8 (26%) from 1241 ± 125 to 1565 ± 211 (P = 0.05).

When only those patients who did not show the decline in T cell counts were taken in consideration, the mean CD4 cell count increased by 128 cells (30%) from 433 ± 27 to 561 ± 43 (P = 0.0001) and the mean CD8 cell count increased by 521 cells (40%) from 1312 ± 165 to 1833 ± 256 (P = 0.02). A decrease in CD4 cell count was observed in only one patient (No. 3) and the CD8 cell count decreased by 119 cells in 4 patients (11%) from 1080 ± 165 to 961 ± 89; this decrease, however, was insignificant (P = 0.23). Out of 13 patients only 1 experienced a decline in both CD4 and CD8 cell counts, but at the same time the level of viremia in this patient decreased from 9,574 to 5,754 viral copies per ml (Table 1).

The viral load measurement by RT-PCR was performed in only 6 patients (Table 1). In three subjects viral load decreased while in one viral load increased. In the remaining two subjects, who showed no detectable virus, viral load was measured after initiation of the treatment but not at the baseline. However, due to sample size the observed differences were insignificant.

Table 1. Summary of viral load measurements

Patient No.	Viral load before the treatment	Viral load after the treatment
1	2,940	1,876
2	8,179	3,465
3	9,574	5,745
4	ND	917, 725[a]
5	ND	123, <50[a]
6[b]	27,931	40,265

ND = not done. Viral load = No. HIV-1 RNA copies per ml of blood.
[a]Results of two measurements.
[b]This patient was on intermittent antiretroviral monotherapy (AZT) for 8 years.

Despite the limited number of patients the correlation analysis showed that the observed trend towards decrease in a viral load was significant and was unlikely to be random ($r = 0.98$; Fisher's $z = 2.35$; $P = 0.02$). No significant correlation between the viral load and the CD4/CD8 cell count was established (data not shown).

Discussion

How does V1 compare with other HIV-1 vaccines? A considerable number of reports on AIDS vaccines used as a therapeutic modality have been published. Zagury *et al*. (1987) have been the first to attempt this approach. The most extensively studied product is Remune or HIV-1 Immunogen vaccine. Unlike V1, which is available as an oral pill, Remune is an injectable preparation of whole inactivated HIV-1 depleted of envelope gp120 protein (Immune Response Corporation, USA). In a recent report, 223 Thai patients with initial CD4 cell count over 300 have been treated for over 2 years with Remune (Sukeepaisarn-charoen *et al*., 2001). An increase in CD4 cell count by 36 cells and in CD8 cell count by 415 cells was recorded. The weight gain was 1.08 kg while the viral load remained essentially the same. In contrast, the interim results of a randomized, double-blind study of 2527 HIV-1-infected adults in the USA have shown that the changes in viral load, CD4 cell count or body weight attributable to Remune were insignificant (Kahn *et al*., 2000). However, in a subset of these patients the vaccine appeared to produce a significant beneficial effect (Turner *et al*., 2001).

The results of a series of multinational trials with a recombinant gp160, which have been first launched by Redfield *et al*. (1991) showed unequivocally that rgp160 had little efficacy as a therapeutic vaccine even though a transient improvement was reported in some studies (Redfield *et al*., 1991; Birx *et al*., 2000; Valentine *et al*., 1996; Sandstrom and Wahren, 1999; Goebel *et al*., 1999; Pontesilli *et al*., 1998). Other envelope-based therapeutic vaccines did not seem to exert a better effect (Lambert *et al*., 1998). In a pediatric study, the rgp160 (IIIB. MicroGeneSys) was compared with rgp120 (SF-2, Chiron) and rgp120 (MN, Genentech). In this and other unrelated studies neither the CD4 cell count nor viremia differed significantly between vaccinated and control groups (Wright *et al*., 1999; Smith *et al*., 2001; Benson *et al*., 1999). It has been thus concluded that recombinant HIV-1 envelope vaccines may not be suitable as therapeutic modalities (Essajee *et al*., 2002).

Synthetic HIV-1 peptides have been tested in a number of trials. The response to the F46 vaccine consisted of only weak antibody production (Schwander *et al*., 1994). The C4-V3 vaccine consisting of four peptides has been studied in 8 HIV-1-positive patients; however, no changes in both the CD4 cell count and plasma HIV-1 RNA level were observed (Bartlett *et al*., 1998). Envelope-based synthetic peptides have been studied in a trial but both the viral load and CD4 cell count did not change markedly (Pinto *et al*., 1999). Another vaccine, a lipid-conjugated gag peptide has been applied to 6 HIV-1-seropositive subjects without augmenting significantly HIV-1-specific CTLs or decreasing viremia (Seth *et al*., 2000). A pilot trial based on use of dendritic cells pulsed with rgp160 or viral core peptides did not show any evidence of clinical benefit or a reduced viremia (Kundu *et al*., 1998). Vacc-4x containing four peptides of p24 has been tested in 11 HIV-1-infected individuals with or without drug therapy, however, both the plasma HIV-1 RNA level and CD4 cell count did not change appreciably (Asjo *et al*., 2002).

British and Australian studies evaluating p24-VLP vaccine have failed to show any significant effect on CD4 cell count or other parameters (Smith *et al*., 2001; Benson *et al*., 1999). The study with another VLP vaccine has shown both humoral and cellular immune response *in vitro* but no effect on the T cell count or viral load (Veenstra *et al*., 1996).

DNA-based vaccine containing env and rev genes has been tested in 15 patients with the CD4 cell count over 500/ mm^3. Whereas a specific lymphocyte proliferative response and an increased MIP-1 alpha level were detectable, no significant changes in immune parameters were observed (Boyer *et al*., 1999). A recombinant vaccinia virus-based vaccines, ALVAC and NYVAC initially appeared to delay the viral rebound due to the antiviral drug interruption (Tubiana *et al*., 1997). However, in a more recent study such an effect has not been reproduced (Markowitz *et al*., 2002). Retroviral vector-transduced autologous fibroblasts expressing HIV-1 Env/Rev proteins induced significant in vitro CTL activity in two of four patients (Ziegner *et al*., 1995). Finally, an anti-CD4-idiotype vaccine tested in 158 patients has shown a response in both the HIV-1 neutralizing antibody titer and gp120 antigen binding titer but no clinical effect (Schedel *et al*., 1999).

Table 2. Summary of results with various AIDS vaccines

No.	Vaccine	Reference	N	Duration	CD4 at baseline	CD4	CD8	Viral load
1	gp 160	Redfield *et al*., 1991	30	6 m	≥600	+0.6%	Unknown	Unknown
2	gp 160	Valentine *et al*., 1996	45	60 w	≥400	–1%	Unknown	No change
3	gp 160	Sandstrom *et al*., 1999	416	3 y	>200	↓	Unknown	No change
4	gp 160	Pontesilli *et al*., 1998	34	2 y	>400	↓	Unknown	Increased
5	gp 160	Birx *et al*., 1998	608	5 y	>400	↓	Unknown	Unknown
6	gp 160	Goebel *et al*., 2000	208	21 m	>500	↓	Unknown	No change
7	gp 160MN	Kundu-Raychaudhuri *et al*., 2001	15	>6 m	>500	No change	Unknown	No change
8	gp1 160MN/HL AGag/Pol peptide	Kundu *et al*., 1998	6	>6 m	>400	No change	Unknown	No change
9	gp 120MN	Eron *et al*., 1996	573	15 m	>600	↓	Unknown	Unknown
10	gp 120MN	Wright *et al*., 1999	26	5 m	>400	↓	Unknown	No change
11	rgp 120(SF-2) rgp 160 (IIIB) rgp 120 (MN)	Lambert *et al*., 1998	12	6 m	≥500	No change	No change	No change
12	Remune	Sukeepaisarncharoen *et al*., 2001	223	2 y	>300	↑36 cells	↑ 415 cells	No change
13	Remune	Kahn *et al*., 2000	1262	3 y	>300	↑10 cells	Unknown	No change
14	VLP-24	Benson *et al*., 1999	31	6 m	>400	No change	No change	Unknown
15	p17/p24	Veenstra *et al*., 1996	74	48 w	>350	No change	Unknown	No change
16	Lipopeptide	Seth *et al*., 2000	9	6 m	>500	Unknown	Unknown	No change
17	F46 peptide	Schwander *et al*., 1994	29	270 days	>100	No change	Unknown	Unknown
18	DNA vaccine	Boyer *et al*., 1999	15	20 w	ı500	Unknown	Unknown	No change
19	p24 peptides	Asjo *et al*., 2002	11	6 m	>300	No change	No change	No change
20	Anti-idiotype	Schedel *et al*., 1999	80	12 m	>350	↓	Unknown	No change
21	V1	Present study	13	6 m	>250	↑ 98 cells	↑ 324 cells	Decreased

The reviews mentioned above list almost every AIDS vaccine currently available but the results of their application show unequivocally that whereas the *in vitro* immune parameters changed as a result of vaccination the CD4 or CD8 cell counts either did not change or declined over time. Similarly, the viral load in the treated patients was either stable or rising from baseline levels.

Little is known as to how V1 operates since the mechanisms underlying mucosal immunity in response to oral antigenic challenge are still unclear. The original premise in developing this vaccine took into consideration the natural history of sexual, transplacental, and breast-milk-borne HIV-1 transmission across the mucosal lining of reproductive and digestive organs (Bourinbaiar and Minowada, 1989; Bourinbaiar, 1994; Bourinbaiar and Lee-Huang, 1995). Intraepithelial mucosal lymphocytes specialized in handling dietary antigens and microbial/viral pathogens crossing the mucosal barrier represent up to 90% of immunocompetent cells in the human body (Bourinbaiar, 1994). Delivery of inactivated HIV-1 antigens at the mucosal site of viral entry was postulated to provide an adequate priming of HIV-1-specific cells. The expected mucosal immune response would theoretically favor non-responsiveness and oral tolerance. Our results, nevertheless, suggest that mucosal delivery of viral antigens provides a better clinical outcome than prior HIV-1 vaccines, supplied exclusively in injectable form and thus targeting systemic immunity. No studies on neutralizing antibodies, CTL activity, secreted cytokines and other *in vitro* studies were carried out in this work. They would certainly reveal interesting correlates of the oral tolerance.

In conclusion, V1 is safe and very well tolerated. Subjective parameters of the quality of life have improved markedly, but except weight gain measurement no systematic evaluation was carried out. The statistical analysis of surrogate markers has indicated that, even though the sample size was small, the gains in CD4 and CD8 cell counts and the decrease in viral load were not random (Phanuphak *et al*., 2002). Thus, oral immunization seems to hold promise as a potentially beneficial therapeutic and prophylactic intervention (Jirathitikal *et al*., 2003b). While the dosage of V1 was daily and hence more frequent than that of injectable vaccines this practice is not unusual and has been commonly employed in the past with oral vaccines for treatment of bacterial infections. The observed increase in CD4 and CD8 cell counts was even more pronounced than in our earlier study comprising patients who were mostly in later disease stage (Jirathitikal and Bourinbaiar, 2002). However, considering the discrepancy between the Remune trial results in Thailand (Churdboonchart *et al*., 1998; Churdboonchart *et al*., 2000) and USA (Kahn *et al*., 2000; Turner *et al*., 2001), contribution of the placebo effect cannot be ruled out (Sabin, 2002). Nevertheless, this study supports our general impression that V1 provides better clinical benefit when patients are treated earlier rather than later in the disease (Metadilogkul *et al*., 2002).

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