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Dynamics of HIV-1 replication following influenza vaccination of HIV$^+$ individuals

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SUMMARY

Levels of HIV-1 have been reported to increase in peripheral blood after influenza vaccination of HIV$^+$ individuals. In this study we have evaluated the dynamics of these changes. Ten HIV-1$^+$ individuals classified in revised CDC clinical categories B and C as well as five seronegative healthy controls were vaccinated with the recommended influenza strains. HIV viral RNA and proviral DNA were sequentially quantified in serum and blood lymphocytes, respectively. Nine of the 10 HIV$^+$ individuals had an increase in the frequency of infected CD4 cells 2 weeks after influenza vaccination. Individuals with low viral load had a rapid increase in viraemia and a small increase in frequency of infected cells in peripheral blood. In contrast, individuals with high viral load had a small drop in viraemia followed by a significant rise in the rate of infected cells. The observed changes may resemble those taking place during intercurrent infections in HIV$^+$ individuals. The effects of the relative increases in infectious virus after the transient viraemic phase should be further investigated to evaluate potential risks of vaccination.

Keywords HIV haemagglutination inhibition influenza vaccination viral load

INTRODUCTION

Infection with HIV leads from an often asymptomatic acute infection to the development of AIDS. A characteristic of HIV infection is the progressive loss of CD4$^+$ T cells (CD4 cells) in peripheral blood as well as changes in the functional subsets of these cells, thereby depleting an individual of immunocompetent T cells [1].

Acute influenza is in most individuals, regardless of HIV status, characterized by respiratory as well as generalized symptoms, and may be lethal if complicated by bacterial infections in patients of defined risk groups [2]. The case fatality rate (CFR) of acute influenza and its complications among HIV$^+$ subjects is not known, but previous studies have suggested that for asymptomatic HIV$^+$ individuals it is comparable to that of HIV$^-$ controls [3–6].

In the USA as well as in Norway, where this study took place, prophylactic influenza vaccination is recommended for HIV$^+$ individuals, based on the assumption that vaccination 'will result in protective antibody levels in many recipients' [7]. It has been documented that individuals with CD4 cell levels of <100/μl are less likely to respond to the vaccine [7,8], but in general there is no correlation between CD4 cell levels and the ability to respond to vaccination.

Before the present study there were conflicting data concerning potential harmful effects following influenza immunization of HIV$^+$ individuals. Some reports indicated an increase in blood levels of HIV in the vaccine recipients, whereas others where unable to confirm this [9,10]. Vaccination with T cell-dependent immunogens leads to T cell activation and proliferation, which in turn could enhance HIV replication and thereby result in measurable increases in blood levels of HIV.

In the present study, we describe the dynamics of changes in HIV burden measured as virion RNA in serum (termed 'viral load') and relative number of CD4 cells carrying infectious provirus (termed 'infected cells') in relation to the biological phenotype of the virus as well as the immune response to parenteral influenza vaccination. Our results are in line with those recently published by O'Brien *et al.* [11]. A similar transient increase in viral load was observed, but in contrast to the latter data, we found a substantial increase in the rate of HIV-infected cells in peripheral blood of most vaccine recipients. Influenza vaccination elicited a protective antibody response to both subtypes of influenza type A as well as to type B in most individuals, irrespective of HIV status.

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Table 1. Clinical and laboratory status of the HIV^+ participants

Subject no.	Clinical status*	CD4 cells/μl	Infected cells†	Viral load‡	HIV phenotype§
1	B1	569	108	200	s/l
2	B2	354	25	5000	s/l
3	B3	151	5	77 500	s/l
4	B2	349	8	400	s/l
5	B2	355	4	400	s/l
6	B1	619	6	800	s/l
7	C3	167	1570	140 000	r/h
8	B1	864	44	43 900	r/h
9	C2	451	164	22 200	r/h
10	B2	363	618	6000	r/h

* Defined by CDC classification [12].

† Number of cells containing replication-competent virus per million CD4 cells in peripheral blood before vaccination.

‡ Copies of HIV RNA per ml of plasma or serum before vaccination.

§ r/h, Rapid/high, syncytia-inducing phenotype; s/l, slow/low, non-syncytia-incuding phenotype.

MATERIALS AND METHODS

Study participants and vaccine components

Ten HIV^+ individuals and five HIV^- controls were given one intramuscular injection of 0·5 ml Fluzone 1994/95 split virus vaccine (formalin-inactivated and Triton X-100-treated influenza-virus) containing 15 μg haemagglutinin (HA) of each of the recommended A/Shangdong/9/93 (H3N2)-like, A/Singapore/6/86 (H1N1)-like and B/Panama/45/90-like, strains. Written consent was obtained from all participants and ethical approval was given by the Regional Medical Ethical Committee. Eight of the 10 participants were classified as asymptomatic (B) and two as symptomatic (C), according to the revised CDC classification [12] when included in the study. Clinical stage and laboratory data of the HIV^+ participants are summarized in Table 1.

Cell separation

Whole blood was collected by vacutainers containing ACD. Peripheral blood mononuclear cells (PBMC) were separated by gradient centrifugation (Lymphoprep; Nycomed, Oslo, Norway). CD4 cells were positively selected by immunomagnetic beads coated with antibodies to the CD4 molecule (Dynabeads; Dynal, Oslo, Norway) as described [13]. The CD4 cells were > 98% pure as determined by flow cytometry. Cells were resuspended at a concentration of 10^6 cells/ml in RPMI medium (GIBCO LT, Gaithersburg, MD) containing 10% heat-inactivated fetal calf serum (FCS; GIBCO), 5 U/ml recombinant IL-2 (Amersham DK ApS, Gjettum, Norway) and antibiotics (IL-2 medium).

HIV isolation and quantification

Virus was isolated from PBMC as described [14]. It was characterized as being of the slow/low, non syncytia-inducing (NSI) or rapid/high, syncytia-inducing (SI) phenotype by cocultivating virus-positive lymphocytes with cells of the MT-2, U-937 and Jurkat-tat lines [15].

Serum and plasma were obtained at days 0, 5 and 14 post-vaccination. Viral load was determined by one or the other of two commercially available quantitative methods: the Nucleic Acid Sequence Based Assay (NASBA; Organon Teknika AB, Gothenburg, Sweden) and a reverse transcriptase-polymerase chain reaction (RT-PCR) assay (Amplicore HIV Monitor; Roche Diagnostics, Stockholm, Sweden) following instructions provided by the manufacturers.

Infected $CD4^+$ T cells (CD4 cells) in peripheral blood were quantified by limiting dilution performed at days 0 and 14 post-vaccination, based on principles described earlier [16]. Briefly, purified CD4 cells were cocultured in decreasing numbers (five-fold dilutions) with uninfected allogeneic CD4 cells from an HIV^- donor. The cells were stimulated with MoAbs to the T cell receptor (TCR; MoAb T10B9, kindly provided by Professor J. S. Thompson (University of Kentucky Medical Centre, Lexington, KY)) coated onto immunomagnetic beads (Dynabeads M450; Dynal). IL-2 medium was changed twice weekly and analysed for the presence of p24 antigen at day 14 by an in house p24 antigen ELISA assay [17]. The number of infected CD4 cells was calculated according to the Poisson distribution formula based on the inverse fraction of the number of cells required to give 63% $p24^+$ culture supernatants [16].

In vitro *stimulation and determination of CD69 expression*

Purified CD4 cells were stimulated by MoAb T10B9 coated onto the bottom of 48-well culture plates. Coating was performed by addition of 300 μl MoAb-containing ascites diluted 1:2500 in sterile PBS and incubating the plate for 2 h at 37°C followed by washing off excess antibody. CD4 cells (0·5 ml) were added to the coated wells, incubated at 37°C and analysed for expression of the CD69 receptor as a marker for induction of T cell activation. CD69 expression was determined by flow cytometry on a Coulter EPICS XL, using a FITC-conjugated anti-CD69 MoAb (Clone Leu-23; Becton Dickinson, La Jolla, CA).

Haemagglutination inhibition assay

Haemagglutination inhibition (HI) titres to the various vaccine strains were determined in serum samples taken at day 0 (pre-vaccination titres), day 5 and finally at day 14. Briefly, the sera were treated for removal of non-specific inhibitors with receptor-destroying enzyme (RDE) and with packed turkey erythrocytes for removal of agglutinins. The sera were then tested for antibodies against the various vaccine components by serial two-fold dilutions against 8 HA units of virus and 0·7% v/v turkey erythrocytes as described elsewhere [18]. Antibody titres $\geqslant 40$ were used as an indication of protection in the vaccine recipients [19].

Statistical analysis

For comparison of changes in viral load, non-parametric (Kruskal–Wallis) one-way analysis of variance method was used. Differences were regarded as statistically significant when $P \leqslant 0{\cdot}05$.

RESULTS

Dynamics of HIV replication

A significant increase ($P < 0{\cdot}05$) in viral load was observed 5 days after vaccination (Fig. 1) in subjects with low viral load ($< 2\times10^5$ RNA copies/ml) before vaccination compared with the group with high viral load ($> 2\times10^5$ copies/ml). However, median values returned to baseline 2 weeks later.

More importantly, in nine of the 10 vaccinees an increase in the relative number of infected CD4 cells was observed which persisted after the reversal of the initial increase in viral load

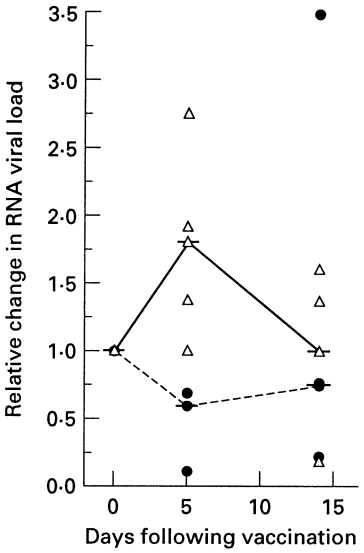

Fig. 1. Post-immunization data showing changes in viral load following influenza vaccination in individuals with viral load higher than 2×10^5 (●) and lower than 2×10^5 (△) HIV RNA copies/ml. The dotted and full lines connect the median values of the two groups, respectively.

(Fig. 2). Seven individuals (including all those with rapid/high type virus) had > 50% increase, two individuals had < 50% increase (41% and 32%, respectively), and one individual had no increase in the relative number of infected CD4 cells (Fig. 2). The rise in frequency of HIV-infected peripheral CD4 cells was particularly pronounced in individuals with high viral load. On average, more than 10-fold increases in number of infected cells were observed in these patients in contrast to the two-fold increase in individuals with low virus load (Fig. 3). Three individuals receiving antiviral (Zidovudine) treatment had 0·38-, 1·55- and 25-fold increases in rate of infected cells, respectively. All vaccinees had been followed with regular provirus determinations before influenza vaccination and asymptomatic individuals had remained stable for up to 2 years (data not shown). Increase in viral burden correlated with neither *in vitro* T cell reactivity nor induction of influenza antibodies.

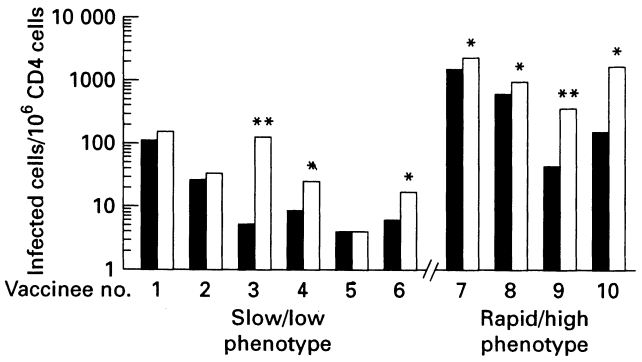

Fig. 2. Changes in number of infected CD4 cells in peripheral blood in individuals harbouring HIV of the slow/low and rapid/high phenotypes, respectively, before (■) and after (□) vaccination. *More than 50% increase in the number of infected cells; **10-fold or higher increase in the number of infected cells.

Cell stimulation and proliferation

The percentage of CD4 cells expressing CD69 after *in vitro* stimulation was used as an indicator for T cell activation and was compared with the changes in viral load and frequency of infected cells as well as with the average increase in titre to all vaccine components. CD69 expression on CD4 cells from patients harbouring HIV of the rapid/high phenotype was found numerically but not statistically elevated (median 68·45, range 22·4–75·1) compared with cells from individuals with slow/low type virus (median 40·05, range 9·7–66·6). There was no correlation between induction of CD69 expression and changes in viral load, number of infected cells or vaccine response to influenza (data not shown).

Vaccine response

HI titres following vaccination are summarized in Table 2. Two weeks after vaccination, nine of the 10 HIV+ individuals had protective titres to the various vaccine strains (i.e. HI titres $\geqslant$ 40). Protection against one vaccine strain did not result in protection against the others, but all individuals were protected against at least one strain. However, most individuals had protective levels already before vaccination, and only two, seven and four of the 10 study participants had a four-fold or higher increase in titres to influenza A/(H1N1), A/(H3N2) and B/Panama, respectively (Table 2). The immune responses to the various components were comparable to those seen in the HIV− control group except for the response to influenza A/(H1N1), which was lower in the HIV+ group.

DISCUSSION

In the present study we have documented an increase in the relative number of HIV-infected CD4 cells in peripheral blood in nine out of 10 HIV+ individuals following influenza vaccination. In addition, four of six individuals with low viral loads showed increases in viral load 5 days after vaccination. The increase in the number of infected cells persisted after the reversal of plasma viraemia.

Several publications have described a transient increase in HIV replication following influenza vaccination of HIV+ individuals

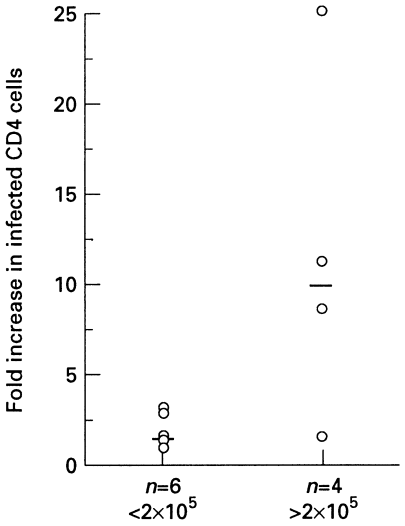

Fig. 3. The bars represent median fold increase in infected CD4 cells observed in peripheral blood 2 weeks after vaccination of individuals in the groups with viral load lower than and higher than 2×10^5 HIV RNA copies/ml serum.

Table 2. Immune response following influenza vaccination of HIV^+ and HIV^- individuals

	A/H1N1			A/H3N2			B/Panama		
	$\geqslant 40$*	Four-fold†	GMT‡	$\geqslant 40$	Four-fold	GMT	$\geqslant 40$	Four-fold	GMT
HIV-positive									
Day 0	7/10		45	0/10		12	9/10		46
Day 5	7/10		60	1/10		13	10/10		61
Day 14	9/10	2/10	160	9/10	7/10	45	10/10	4/10	160
HIV-negative									
Day 0	3/5		53	2/5		23	2/5		46
Day 5	3/5		70	2/5		20	4/5		80
Day 14	5/5	3/5	211	3/5	2/5	61	5/5	3/5	160

* Number of sera with HI titres higher than 40.
† Number of sera from the vaccinees in each group having a four-fold increase in antibody titre against the various influenza strains.
‡ Geometric mean titre of the groups.

[9,11], but so far vaccination has not been regarded as unsafe for HIV^+ individuals. The viraemia following immunization occurs rapidly and declines equally fast in accordance with recent knowledge about the rapid immune response to influenza vaccination that takes place in lymphatic tissues [20]. Protein-based, T cell-dependent vaccines are processed by antigen-presenting cells and presented to CD4 cells located in lymphoid tissues. This leads to CD4 cell activation that may result in virus production from infected CD4 cells having antigen specificity for the vaccine used. During viral infections, antigens are presented to CD4 as well as to CD8 cells. In HIV^+ individuals this could lead to increased HIV replication, and our findings may thus resemble changes occurring during intercurrent infections of HIV^+ individuals [21].

Current routine methods do not allow us to determine whether the observed increase in rate of infected cells in peripheral blood represents a real increase in the total number of virus-containing T cells, or whether it is due to redistribution of infected cells from lymphatic tissues. We found that high viral load correlated with smaller changes in HIV RNA and a large increase in the number of infected cells in peripheral blood, whereas low viral load correlated with a significant increase in HIV RNA from baseline values, but a smaller increase in the number of infected cells. This difference could speak in favour of a cellular redistribution. Low viral load is generally associated with early stage disease, a relatively intact morphology of the lymphoid organs and low frequencies of infected cells. Based on our current findings we can neither rule out *de novo* infection, nor say that the observed increase in infected cells is harmless, and these topics should therefore be further evaluated. Since both transient increases in plasma viral load and high levels of PBMC carrying infectious HIV are reported to correlate with accelerated disease progression [22,23], these conditions should be avoided in HIV^+ individuals.

There is in general no correlation between CD4 levels in peripheral blood and immune responses in asymptomatic HIV^+ individuals. We therefore evaluated the induction of the early activation marker CD69 on CD4 cells as an indicator for T cell reactivity. CD69 is normally not present on cells in peripheral blood, but is detectable on the cell surface a few hours after mitogen-induced stimulation and acts as a costimulatory molecule [24]. Patients with rapid/high type virus had a numerically but not significantly higher CD69 expression than patients with slow/low phenotype, and the interpretation of this is thus uncertain. Since CD69 expression correlated neither with overall vaccine responses nor with changes in viral burden, the induction of this marker could not be used as an indicator of *in vivo* T cell reactivity in our protocol.

Nine of the 10 HIV^+ individuals had protective influenza antibody levels after vaccination (HI titre $\geqslant 40$). However, a significant response evaluated by a four-fold increase in HI titre to the different vaccine components was seen only in a minority of patients. Previous studies have indicated a lower response to strains of influenza A, but HIV^+ individuals, both children [25] and adults [8], do in general respond adequately with induction of protective levels of antibodies to all vaccine components.

Today, the CFR for HIV^+ individuals in the acute phase of influenza disease is not known, but previous studies, including larger retrospective data, indicate that the CFR among HIV^+ individuals is comparable to that of HIV^- healthy controls [3–6]. There is therefore little evidence to support the use of prophylactic influenza vaccination to protect from disease and death in HIV^+ individuals. A dilemma is that the individuals who are likely to respond to the vaccine are less likely to develop severe influenza during outbreaks, as these individuals are immunologically quite competent, whereas the individuals who would benefit from an efficient vaccine respond poorly due to their immunodeficiency. Previous studies have concluded that for this reason vaccination of HIV^+ individuals with less than 100 CD4 cells/μl should not be recommended [8]. Vaccination of individuals infected with HIV should be a subject of close evaluation, because the target cell of the virus is an effector cell in terms of induction of immunity to a number of other infectious agents. The benefit of vaccination must therefore be clearly documented and outweigh the potential risk of accelerating the immunodeficiency.

In conclusion, the large increase in infected cells that we observe in patients with high viral load should be a cause of concern for the general recommendation to individuals in this patient group. The key question whether the observed increase in HIV load necessarily leads to a worsening of the HIV disease remains unanswered. Previous reports based on short-term follow up have not found any evidence of clinical deterioration, evaluated by changes in CD4 cell counts, induced by influenza immunization [8], and our material is too small in size to draw conclusions from. Until further answers on harmful effects of vaccination are given,

we suggest consideration of other methods of influenza prevention [3,26,27] to avoid potential progression of HIV disease.

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Note added in proof

During revision of the present manuscript, a study by Staprans and coworkers was published, describing changes in plasma levels of HIV following influenza vaccination of HIV$^+$ individuals (Staprans SI, Hamilton BL, Follansbee SE, Elbeik T, Barbosa P, Grant RM, Feinberg MB. Activation of virus replication after vaccination of HIV-1-infected individuals. J Exp Med 1995; **182**:1727–37). They document that increases in plasma viraemia correlate with CD4 cell counts and T cell proliferative responses to vaccine antigens, which is in agreement with our data.

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