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Antibody response to tetravalent influenza subunit vaccine in patients infected with human immunodeficiency virus type 1

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Abstract

The capacity of patients infected with human immunodeficiency virus (HIV) to develop an adequate antibody response to influenza vaccination in relation to the CD4 cell count has been studied in a prospective study. A total of 73 subjects (54 HIV-infected patients and 19 healthy control persons) were vaccinated with influenza subunit vaccine containing 15 μg hemagglutinin of each of the following strains: A/Beijing/353/89(H3N2), A/Singapore/6/86(H1N1), B/Panama/45/90, and B/Beijing/1/87. Hemagglutinin inhibition (HI) antibody titers were determined prior to vaccination, 3 weeks afterwards, and at the end of the influenza season.The percentage of subjects with HI antibody titers above the assumed protective level was significantly lower in the HIV-infected patients for all 4 vaccine strains compared with those in the control group (7–26% and 42–74%, respectively). There was an association between CD4 cell count and antibody response to the B/Panama strain only.The serologic response to tetravalent subunit influenza vaccine is severely impaired in the majority of HIV-infected patients compared with control subjects. The results of this study challenges the recommendation to vaccinate HIV-infected patients.

Keywords: Vaccination; Influenza vaccine; HIV seropositivity; AIDS; CD4 cell count

1. Introduction

Influenza causes increased morbidity and mortality in the elderly and patients with certain chronic diseases [1–4]. The influenza incidence is low among adults and HIV-related immunodeficiency seems to increase the influenza risks only minimally [5,6]. However since the pandemic occurrence of the human immunodeficiency virus type 1 (HIV) in the 1980s, it has been discussed whether subjects carrying HIV, have an increased risk of serious complications after an acute influenza infection [7,8]. Therefore the Public Health Service Advisory Committee on Immunization Practices has been advising influenza vaccination in HIV-infected patients as a 'prudent precaution' [2,9].

The ability of HIV-infected patients to produce sufficient protective antibody to influenza vaccine has been disputed. Only one study found that asymptomatic HIV-infected patients, patients with generalized lymphadenopathy and patients with AIDS-related complex had a normal antibody response to influenza vaccination [10]. In contrast, several other studies showed antibody levels to be lower in HIV-infected patients after a single dose of influenza vaccine, even in asymptomatic subjects with minimally decreased CD4 cell counts, than in healthy control subjects [11–14]; the degree of impairment correlated with the severity of immunologic dysfunction [15]. Booster vaccination after four weeks did not improve the prevalence of assumed protective antibody levels [14]. The effect of HIV on the persistence of antibodies induced by influenza vaccine has not been studied yet.

Studies which present data on short- and long-term

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side effects of inactivated influenza vaccine in HIV carriers show that the vaccine is safe: direct side effects (local and systemic reactions) are negligible [10,11,14].

Thus, in view of these unanswered questions regarding influenza vaccination, the current study was conducted to assess the efficacy of a single dose of tetravalent influenza subunit vaccine to induce an antibody response in HIV-infected patients compared with healthy adults. The antibody response in relation to the concentration of CD4 lymphocytes in peripheral blood was examined. We also determined the antibody titers after the influenza season, to provide serologic evidence of infection with natural influenza and to establish the persistence of vaccine-induced antibodies with time. Side effects were not monitored in this study.

2. Materials and methods

2.1. Study population

Volunteers were recruited in November 1991 and were included when they were not taking immunosuppressive agents (other than antiretroviral drugs), were not known to be hypersensitive to eggs, had no history of anaphylactic reaction to influenza vaccine, and were not pregnant or lactating. The history of previous influenza vaccinations was recorded for all participants.

The HIV seropositive group consisted of patients who were positive for anti-HIV type 1-antibody (enzyme-linked immunoabsorbent assay and Western blot) and who were being treated in the HIV outpatient clinic of the University Hospital Utrecht, The Netherlands. The stage of the HIV disease was determined according to the criteria of the Centers for Disease Control and Prevention [16]. The control subjects were recruited from the house staff of the University Hospital Utrecht without additional selection.

The study design was approved by the Investigational Review Board of the University Hospital Utrecht. All patients gave their informed consent.

2.2. Vaccine and study design

Tetravalent influenza subunit vaccine (Solvay-Duphar B.V., The Netherlands) for the 1991–1992 season contained 15 microgram hemagglutinin of each of the following strains: A/Beijing/353/89(H3N2), A/Singapore/6/86(H1N1), and B/Panama/45/90, B/Beijing/1/87, in 0.5 ml aqueous solution. The vaccine composition was according to the advice of the Dutch Health Council and recommendation of the World Health Organization. Each subject received an intramuscular injection of one dose in the right or left deltoid muscle. Venous blood specimens were drawn immediately before vaccination (prevaccination), 3 weeks afterwards (postvaccination), and after the winter season 1991–1992 (postseason). CD4 cell counts were performed within 3 weeks of vaccination.

2.3. Laboratory tests

For antibody determination, sera were coded, separated, and kept frozen at −20°C within 24 hours after blood collection. The sera were tested in a blinded fashion, simultaneously, and in duplicate by using a hemagglutination inhibition (HI) assay, with two-fold dilution steps [17]. The strains used in the titrations were the 4 vaccine strains, and 2 endemic strains, A/Ned/968/92(H1N1) and A/Ned/969/92(H3N2), isolated during the 1991–92 outbreak of influenza in the Netherlands. Influenza B strains were treated with ether according to Berlin et al. [18].

2.4. Calculations and statistical analysis

HI titers are expressed as the reciprocals of the dilution showing 50% inhibition of hemagglutination with three hemagglutination units of the antigen. Titers between 0 and 9 were arbitrarily regarded as 5. With the method used, protection against infection, for the immunocompromised patients and the control subjects, is assumed to be associated with a HI titer ≥ 100 for influenza A strains [19] and ≥ 200 for influenza B strains [20]. Percentiles were calculated and differences between groups were tested with the Wilcoxon rank sum test. Differences in response between the HIV patients and controls were tested with Chi-square and Fisher exact. The association between CD4 cell count and antibody response was tested with Chi-square for trend. For this purpose patients were categorized in three groups according to their CD4 cell count: group 1 with a CD4 cell count $\leq 200/mm^3$, group 2 with a CD4 cell count between 200–500/mm^3, and group 3 with a CD4 cell count $\geq 500/mm^3$.

A P-value of 0.05 or less was considered to indicate statistical significance. Confidence intervals had 95% limits. All calculations were done on a personal computer using the SAS computer program [21].

3. Results

3.1. Study population

A total of 82 subjects were enrolled in the study in November 1991. Sixty-two persons were HIV-seropositive patients and 20 persons were healthy controls. All subjects were immunized with tetravalent influenza subunit vaccine.

Both pre- and postvaccination blood specimens were available from 73 participants. The second blood speci-

men was missing of seven HIV-seropositive patients and of one control subject, because of noncompliance. The prevaccination sample of one HIV-seropositive patient was lost. The HIV-seropositive group ($n = 54$) and the control group ($n = 19$) were balanced with respect to age, time period between pre- and postvaccination serum samples, and previous vaccinations (Table 1). The HIV seropositive group contained 51 males and 3 females, whereas the control group contained 14 males and 5 females (Table 1). In contrast to the 19 control subjects, who were heterosexual, 48 of the 54 HIV infected patients were homosexual (Table 1).

The distribution of HIV-seropositive patients according to the CDC classification [15] resulted in 19 patients in class II, 7 patients in class III, 20 patients in classes IVC1 and IVC2, and 8 patients in classes IVA, B, D and E. CDC classes II and III were associated with higher CD4 cell counts and a lower treatment percentage with antiretroviral agents (Table 1). CDC classification and CD4 cell count were not affected by age or previous vaccinations. Antiretroviral therapy was significantly correlated with low CD4 cell counts.

3.2. Vaccine-induced HI antibodies

Serum hemagglutin inhibition (HI) antibody titers before vaccination differed between the two study groups. However, in both HIV-seropositive patients and control subjects prevaccination titers were below the assumed protective level for all four vaccine strains (Table 2). These low prevaccination titers were to be expected, because none of the healthy adults and only four of the HIV-infected patients were vaccinated previously. Postvaccination titers were significantly lower for all 4 vaccine strains in the HIV-seropositive group compared with the control group (A/Singapore (H1N1), $P = 0.001$; A/Beijing (H3N2), $P = 0.004$; B/Panama, $P = 0.0001$, and B/Beijing, $P = 0.0001$; Table 2).

The percentage of subjects with HI titers above the assumed protected level was low in the HIV patients compared with the controls, for all four vaccine strains (A/Beijing (H3N2), A/Singapore (H1N1), B/Panama, and B/Beijing: 17%, 13%, 26% and 7%, and 53%, 63%, 74% and 42%, respectively, Table 3).

The antibody response was significantly lower in the HIV-infected patient than in the controls. There was no significant association between CD4 cell count and the antibody response, except for the response to the B/Panama strain. In HIV patients with a CD4 cell count ≥ 500 per mm^3, 4 out of 10 patients responded to vaccination of the B/Panama strain; in patients with CD4 cell count between 200 and 500 per mm^3, 6 out of 15 responded, but in patients with a CD4 cell count ≤ 200 per mm^3, only 4 out of 29 responded (Table 3).

The use of antiretroviral therapy was highly related to CD4 cell count and was not an independent factor affecting the antibody response. Previous vaccination did not affect the postvaccination antibody response as was expected because of the small number of previously vaccinated subjects.

Table 1
Base-line characteristics of the study groups

Variable	Patients	Controls
No. of patients	54	19
Age (years)		
Mean	39	35
Range	24–64	24–42
Male sex	51/54	14/19
Risk factors for HIV infection		
Homosexual or bisexual	46	0
i.v. drugs or receipt of bloodproducts	8	0
No. of patients with previous vaccinations against influenza	4	0
Time (days) between pre- and post-vaccination samples		
Mean	23	21
Range	15–37	20–30
CD4 cell count (per mm^3)		
Median	179	697
Range	2–1550	317–1079
Disease stage (No. of patients using antiretroviral drugs)		
II	19 (3)	
III	7 (1)	
IV C1–C2	20 (17)	
IV A, B, D, E	8 (6)	

3.3. Incidence of infections during influenza season

Postseason blood specimens were available for 59 of the 73 participants (collected 111–169 days after the postvaccination specimens). Fourteen patients in the HIV-seropositive group dropped out. Seven HIV-seropositive patients died of AIDS and 6 HIV-infected patients were noncompliant. Blood samples from 1 patient were lost.

More than four-fold titer rises between postvaccination and postseason sera, a conventionally accepted criterium for an infection in the period between the two blood samples [19], occurred in 2 of 58 HIV-infected patients (3%) and 1 of 18 control subjects (6%) for the A-H3N2 strain, which indeed circulated in The Netherlands during the winter season. There was no evidence for a difference in the incidence of infection between HIV infected patients and control subjects.

3.4. Antibody persistence

To assess the decrease of HI antibody titer with time,

Table 2
Serum hemagglutination inhibition antibody titers to tetravalent influenza subunit vaccine before, 3 weeks after vaccination and at the end of the influenza season

	A/Singapore			A/Beijing			B/Panama			B/Beijing		
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
HIV patients	$N = 54$	$N = 54$	$N = 40$	$N = 54$	$N = 54$	$N = 40$	$N = 54$	$N = 54$	$N = 40$	$N = 54$	$N = 54$	$N = 40$
Median	5	9	5	5	24	5	12	51	26	5	12	6
P25–75*	5–5	5–43	5–15	5–5	5–54	5–26	5–34	15–242	12–65	5–5	5–96	5–12
mean	11	42	20	11	93	43	52	213	88	9	58	20
Controls	$N = 19$	$N = 19$	$N = 19$	$N = 19$	$N = 19$	$N = 19$	$N = 19$	$N = 19$	$N = 19$	$N = 19$	$N = 19$	$N = 19$
Median	5	192	108	5	106	48	68	626	484	8	96	82
P25–75	5–12	96–384	48–272	5–5	22–272	15–136	12–96	192–1935	121–968	5–19	48–1219	24–610
Mean	12	398	641	7	181	145	62	1583	1069	24	634	543
P-value**		$p = 0.001$	$p = 0.0001$		$p = 0.004$	$p = 0.0002$		$p = 0.0001$	$p = 0.0001$		$p = 0.0001$	$p = 0.0001$

S1: serum HI antibody titers prevaccination; S2: serum HI antibody titers 3 weeks after vaccination; S3: serum HI antibody titers at the end of the influenza season.
*P25 = 25th Percentile; P75 = 75th Percentile.
**P-value represents the difference between the titer measured in HIV-infected patients and the one measured in controls.

subjects were identified, of whom HI antibody titers were available 3 weeks after vaccination and at the end of the season (40 HIV-infected patients and 19 control subjects). Subsequently the difference between the postseason (S3) HI antibody titers and the postvaccination (S2) titers of the HIV-infected patients were compared to the difference between those titers (S2 and S3) of the control subjects for each vaccine strain. The differences in HI antibody titers between S2 and S3 were equal in controls and HIV-infected patients for any of the vaccine strains except for the Influenza A/Singapore strain ($P = 0.05$).

4. Discussion

This study shows that HIV-infected patients have a severely impaired antibody response to tetravalent influenza subunit vaccine compared to control persons. The median postvaccination titers of hemagglutination inhibition (HI) antibodies to all 4 vaccine strains, (A/Beijing/353/89 (H3N2), A/Singapore/6/86(H1N1), B/Panama/45/90, and B/Beijing/1/87 were significantly lower in HIV-infected patients than in control subjects (Table 2). Moreover the percentage of subjects with postvaccination HI antibody titers above the assumed protective level was also significantly lower in the HIV-infected group than in the control group (7–26% and 42–74%, respectively; Table 3). Remarkably all HIV-infected patients had low HI antibody titers postvaccination, independent of the subgroup, according to their CD4 cell count, they were divided in (Table 3). This finding seems to be in contrast to several earlier reports, which found that HIV patients with higher CD4 cell counts (and/or patients who are asymptomatic have a better response [10,14]. We statistically analyzed the influence of several demographic and clinical variables on these postvaccination titers. First, the HIV-infected patients differed from the controls with respect to the proportion of homosexuals and heterosexuals (Table 1). Several studies showed a difference in postvaccination HI antibody titers to some strains of the influenza vaccine between homosexual and heterosexual HIV-seronegative males [13–14]. However, the clinically more relevant percentage of subjects with assumed protective postvaccination HI antibody titers seemed not significantly different in those studies. Therefore, the difference in the proportion of homosexual subjects probably did not have a significant influence on the outcome of the current study. Second,

Table 3
The number (%) of HIV-infected patients with hemaglutination inhibition titers above the assumed protective level after influenza vaccination, categorized in three groups according to their CD4 cell count.

	CD4 ≤ 200	CD4 = 201–499	CD4 ≥ 500	Chi-square for trend	Total HIV patients		
						Control	Chi-square
A/Singapore	2/29	4/15	1/10	NS	7/54 (13)	12/19 (63)	$p < 0.0001$
A/Beijing	3/29	6/15	0/10	NS	9/54 (17)	10/19 (53)	$p = 0.002$
B/Panama	4/29	6/15	4/10	$P = 0.05$	14/54 (26)	14/19 (74)	$p < 0.0001$
B/Beijing	1/29	2/15	1/10	NS	4/54 (7)	8/19 (42)	$p < 0.0001$

the prevaccination HI antibody titers significantly affected the level of postvaccination titers, as was expected. However, although prevaccination titers differed between the two study groups, all prevaccination titers were below the assumed protective level (Table 2). After elimination of the influence of prevaccination titers on the postvaccination titers the differences between the two study groups with respect to all 4 vaccine strains remained significant. The impairment of the antibody response after vaccination in HIV-infected patients was more severe in patients in CDC class IV, than in patients in CDC classes II and III. This confirms the results of previous studies [11,13–14]. Finally, there were no differences in the postvaccination HI antibody titers between HIV-infected patients who used antiretroviral drug therapy and HIV-infected patients who did not receive these drugs. Thus, the use of antiretroviral therapy did not affect the antibody response to influenza vaccination in this study group.

HI antibody titers were also measured at the end of the influenza season. Postseason vaccination titers were lower in both study groups. There was no significant difference between the two study groups with respect to the decrease of HI antibody titers between 3 weeks after vaccination and at the end of the season, except possibly for the A/Singapore strain.

Although this study was not designed to assess the incidence of natural influenza infection during the winter of 1991–92, the available data showed that the HIV-infected patients did not have a higher incidence of natural infections than the controls subjects (3% versus 6%, respectively). However, given the low rate of breakthrough infections and the small number of participants, the study power may have been to low to show a difference in protection if it was present.

In our study, the impairment of antibody response in all HIV-infected patients to influenza vaccine, independent of their CD4 cell count was much more severe than in other studies published so far [10–14]. This heterogenicity among studies has already been described for influenza research in other patient groups [22,23]. Earlier, the influenza vaccine was reported to have no deleterious effect on the level of serum p24 antigen or the CD4 cell count [10,11,14]. However a recent study showed that HIV patients had a statistically significant higher number of plasma HIV copies/ml, one month after influenza vaccination than HIV patients who were vaccinated with placebo-vaccine. Furthermore 3 months after vaccination, CD4% dropped in the vaccine recipients and had risen slightly in the control group [24]. The question therefore arises if routine vaccination of any HIV-infected patients is warranted at all, given its low efficacy and the probability of increased plasma HIV burden after vaccination. However additional chemoprophylaxis with amantadine or rimantadine should be considered during an influenza A outbreak.

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