MAJOR ARTICLE

Randomized, Double-Blind Comparative Trial of Subunit and Virosomal Influenza Vaccines for Immunocompromised Patients

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Background. To our knowledge, no study to date has compared the effects of a subunit influenza vaccine with those of a virosomal influenza vaccine on immunocompromised patients.

Methods. A prospective, double-blind, randomized study was conducted to compare the immunogenicity and reactogenicity of subunit and virosomal influenza vaccines for adult patients who had an immunosuppressive disease or who were immunocompromised as a result of treatment.

Results. There were 304 patients enrolled in our study: 131 with human immunodeficiency virus (HIV) infection, 47 with a chronic rheumatologic disease, 74 who underwent a renal transplant, 47 who received long-term hemodialysis, and 5 who had some other nephrologic disease. There were 151 patients who received the subunit vaccine and 153 patients who received the virosomal vaccine. A slightly higher percentage of patients from the subunit vaccine group were protected against all 3 influenza vaccine strains after being vaccinated, compared with patients from the virosomal vaccine group (41% vs. 30% of patients; $P = .03$). Among HIV-infected patients, the level of HIV RNA, but not the CD4 cell count, was an independent predictor of vaccine response. Among renal transplant patients, treatment with mycophenolate significantly reduced the immune response to vaccination. The 2 vaccines were comparable with regard to the frequency and severity of local and systemic reactions within 7 days after vaccination. Disease-specific scores for the activity of rheumatologic diseases did not indicate flare-ups 4–6 weeks after vaccination.

Conclusions. For immunosuppressed patients, the subunit vaccine was slightly more immunogenic than the virosomal vaccine. The 2 vaccines were comparable with regard to reactogenicity. Vaccine response decreased with increasing degree of immune suppression. Among HIV-infected patients, the viral load, rather than the CD4 cell count, predicted the protective immune response to the vaccine.

Clinical trials registration. NCT00783380.

Influenza virus is responsible for annual epidemics associated with substantial morbidity and excess mortality, particular among elderly persons, persons with end-organ disease, and immunocompromised persons. Prevention of influenza is usually achieved by the use of vaccination programs. The efficacy of influenza vaccination depends on the antigenic shift and drift of the virus and on the ability of a person's immune system to adequately respond to the vaccine antigens. Unfortunately, immunocompromised persons are not only at a higher risk of morbidity and mortality due to influenza infection but also show lower immune response rates to vaccination [1–4]. The aim of the development of new vaccine formulations has been to increase immunogenicity in patients while maintaining an acceptable number of adverse effects in patients. Since their introduction in 1943, several generations of influenza vaccines have become available. Whole-cell influenza vaccines have been replaced by split and subunit vaccines, which result in a lower number of adverse effects in patients because the viral components of these vaccines, other than the vaccine antigen hemagglutinin (HA), are excluded or because the number of viral components is reduced. In virosomal vaccines, the antigens

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bind to phosphatidylcholine membranes with the aim of imitating the natural routes of infection and thereby increasing immunogenicity in patients.

Trials that have compared virosomal influenza vaccines with conventional vaccine formulations are scarce and have yielded conflicting results. Two studies found higher seroconversion rates and higher protective anti-HA antibody titers after virosomal vaccination than after whole-cell or subunit vaccination among residents of nursing homes and elderly people [5, 6]. These results could not be reproduced in another study of a comparable patient population that compared a virosomal vaccine with subunit and split vaccines [7]. One of these 2 studies investigated the adverse effects of these vaccines and could not find a clear advantage of virosomal vaccines over their comparators [5, 7].

No study has compared the immunogenicity and reactogenicity of virosomal vaccines with those of other vaccine formulations in human immunodeficiency virus (HIV)–infected patients. One trial evaluated a virosomal vaccine for both adults and former intravenous drug users and found significantly lower response rates among HIV-infected persons, irrespective of CD4 cell count, than among HIV-uninfected participants [8]. Similarly, in another trial [9], a virosomal vaccine had a lower rate of immunogenicity among HIV-infected children (even after suppression of HIV viremia) than among healthy control subjects.

We conducted a randomized, double-blind, comparative trial of subunit and virosomal vaccines for 304 patients with immunosuppression due to a variety of underlying diseases and treatments. We assessed the immunogenicity and safety of these vaccines, including the eventual reactivation of underlying conditions such as HIV infection and chronic rheumatologic disease. To our knowledge, such a study has not been conducted before.

METHODS

Our study was performed during the period from 17 October through 16 December 2005 at the University Hospital Bern in Bern, Switzerland. The study protocol was approved by the local ethical review board and registered with ClinicalTrials.gov (identifier NCT00783380).

Subjects. We enrolled immunosuppressed adults (≥16 years of age) who were observed in the outpatient clinics of the departments of rheumatology, nephrology, and infectious diseases. Immunosuppression was attributable to either long-term hemodialyis, long-term peritoneal dialysis, renal transplantation, or treatment for HIV infection or rheumatologic disease. These patient groups fulfilled the national recommendations for yearly influenza vaccination (from the Federal Office of Public Health in Switzerland; available at: http://www.bag.admin.ch). Excluded from the study were inpatients, patients with an acute febrile illness at the time of vaccination, and patients who, in the past, had an allergic reaction to an influenza vaccine or to proteins derived from chicken. All patients gave written informed consent before enrollment.

Vaccines. The subunit vaccine (Influvac; Solvay Pharma AG) and the virosomal vaccine (Influvac plus; Solvay Pharma AG) were commercially available for use during the 2005–2006 influenza season. Both vaccines contained 15 μg of A/California/20/99 (H3N2)–like HA, 15 μg of A/New Caledonia/20/99 (H1N1)–like HA, and 15 μg of B/Shanghai/361/2002-like HA. Patients were block randomized from each outpatient clinic. At baseline, a serum sample was obtained for determination of anti-HA antibody titers before vaccination. Patients with rheumatologic disease had disease-specific scores that were based on the Disease Activity Score for rheumatoid arthritis [10], the Bath Ankylosing Spondylitis Disease Activity Index for ankylosing spondylitis [11], the European Consensus Lupus Activity Measurement for lupus erythematodes [12], and the Disease Extend Index for Wegener granulomatosis [13].

The vaccine was administered by deep intramuscular injection into the deltoid muscle. For patients with a contraindication for intramuscular injection, the vaccine was administered subcutaneously. The depth of the injection and the immediate reaction of the patient were recorded. Patients received a questionnaire for documentation of local and systemic adverse effects during the first 7 days after vaccination. After 4–6 weeks, a second serum sample was obtained, and patients with rheumatologic disease were examined for disease activity.

Serologic testing. Baseline and follow-up serum samples were stored at −20°C until further analyses. Anti-HA antibody titers were determined by the World Health Organization Collaborating Centre for Reference and Research on Influenza at the Université Claude Bernard (Lyon, France). The antigens used were the H3N2 A Panama strain for A California, the H1N1 A New Caledonia for the A Caledonia, and the B Hong Kong 330/2001 for the B Shanghai strain. The anti-HA antibody titers were determined using the method described by Palmer et al. [14]. Immunogenicity was defined as the attainment of a protective anti-HA antibody titer of ≥40 or a seroconversion rate of 1:4 for all vaccine strains at follow-up and in comparison with baseline values.

Statistical analysis. All analyses were performed in Stata, version 9 (StataCorp), using a cutoff of $P < .05$ for 2-tailed tests. Differences between mean values were tested by use of the Student's t test or the Mann-Whitney U test, and proportions were compared by use of the χ^2 test or Fisher's exact test, as appropriate. Univariate und multivariate logistic regression analyses were performed to analyze the factors that influenced the vaccine's performance.

Table 1. Characteristics of 304 immunosuppressed patients randomized to receive a subunit or virosomal influenza vaccine.

Patient characteristic	Subunit vaccine group (n = 151)	Virosomal vaccine group (n = 153)	P
Age, years	50.6 ± 14.3	50.4 ± 14.3	.7
Male sex	103/151 (68.2)	106/153 (69.3)	.9
Creatinine clearance, mL/min[a]	97.7 ± 37.1	91.1 ± 36.4	.1
Current smoker	42/151 (27.8)	40/153 (26.1)	.6
Charlson comorbidity index,[b] mean score (range)	1 (0–5)	1 (0–5)	.7
Subcutaneous administration of vaccine	44/140 (31.4)	38/138 (27.5)	.4
Duration of time from vaccination to follow-up, days	53.5 ± 48.3	50.0 ± 45.0	.5
Never vaccinated against influenza	20/151 (13.2)	26/153 (17.0)	.4
Patients with rheumatologic disease			
All	28/151 (18.5)	19/153 (12.4)	.8
Chronic idiopathic arthritis	18/28 (64.3)	10/19 (52.6)	
Spondylarthritis	7/28 (25.0)	6/19 (31.6)	
Autoimmune connectivitis	1/28 (3.6)	2/19 (10.5)	
Sarcoidosis	1/28 (3.6)	1/19 (5.3)	
Vasculitis	1/28 (3.6)	0/19 (0.0)	
Patients with rheumatologic disease who received treatment			
With ⩽1 immunosuppressive drug	9/28 (32.1)	4/19 (21.1)	.7
With 2 immunosuppressive drugs	11/28 (39.3)	10/19 (52.6)	
With ⩾3 immunosuppressive drugs	8/28 (28.6)	5/19 (26.3)	
Patients with rheumatologic disease who were never vaccinated	15/28 (53.6)	8/19 (42.1)	.5
Patients with nephrologic disease			
All	60/151 (39.7)	66/153 (43.1)	.8
Long-term dialysis	21/60 (35.0)	26/66 (39.4)	
Renal transplant recipient	37/60 (61.6)	37/66 (56.1)	
Other	2/60 (3.3)	3/66 (4.5)	
Patients with nephrologic disease who received treatment			
Without immunosuppressive drugs	23/60 (38.3)	24/66 (36.4)	.9
With 1 immunosuppressive drug	1/60 (1.7)	2/66 (3.0)	
With 2 immunosuppressive drugs	13/60 (21.7)	15/66 (22.7)	
With ⩾3 immunosuppressive drugs	23/60 (38.3)	25/66 (37.9)	
Patients with nephrologic disease who underwent transplantation			
Time since transplant, years	8.4 ± 6.5	6.8 ± 5.9	.2
Time since start of dialysis, years	5.2 ± 4.7	5.0 ± 6.2	.8
Patients with nephrologic disease who were never vaccinated	1/60 (1.7)	9/66 (13.6)	.01
HIV-infected patients			
All	63/151 (41.7)	68/153 (44.4)	
Risk groups[c]			
Men who have sex with men	27/63 (42.9)	28/68 (41.2)	.3
Heterosexuals	30/63 (47.6)	26/68 (38.2)	
Intravenous drug users	5/63 (7.9)	9/68 (13.2)	
Stage of HIV infection[d]			
A	32/63 (50.8)	31/68 (45.6)	.5
B	21/63 (33.3)	25/68 (36.8)	
C	10/63 (15.9)	12/68 (17.6)	
HIV-infected patients with HIV RNA <50 copies/mL	43/63 (68.3)	43/68 (63.2)	.3
CD4 cell count of HIV-infected patients, cells/μL	463 ± 238.9	461 ± 236.3	.9
CD4 cell percentage of HIV-infected patients	23 ± 9.3	25 ± 8.2	.2
HIV-infected patients who received HAART[e]	48/63 (76.2)	53/68 (77.9)	.8
HIV-infected patients who were never vaccinated	4/63 (6.3)	9/68 (13.2)	.1

NOTE. Data are proportion (%) of patients or mean values ± standard deviation, unless otherwise indicated. HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus.

[a] Values for patients who underwent dialysis are excluded.

[b] Charlson et al. [15].

[c] Risk factors were unknown for 1 patient in the subunit vaccine group and for 5 patients in the virosomal vaccine group.

[d] Acccording to definition of the Centers for Disease Control and Prevention [16].

[e] HAART with at least 3 active drugs.

Table 2. Immunogenicity of a subunit influenza vaccine and a virosomal influenza vaccine in immunosuppressed patients.

Vaccine characteristic	Subunit vaccine group (n = 151)	Virosomal vaccine group (n = 153)	P
Geometric mean anti-HA antibody titers (95% CI)			
A1 California strain			
Baseline	25.4 (21.0–30.7)	26.2 (21.8–31.5)	.5
Follow-up	100.0 (80.4–125.7)	107.5 (86.8–133.1)	.8
A1 Caledonia strain			
Baseline	29.8 (23.0–36.1)	30.9 (25.1–38.1)	.9
Follow-up	69.5 (55.4–87.2)	64.0 (52.3–78.3)	.9
B Shanghai strain			
Baseline	25.6 (20.6–31.8)	21.9 (18.1–26.3)	.3
Follow-up	52.7 (42.2–65.7)	36.8 (30.6–44.4)	.1
Seroconversion[a] rate			
A1 California strain	94/151 (62.3)	90/153 (58.8)	.4
A1 Caledonia strain	63/151 (41.7)	61/153 (40.0)	.1
B Shanghai strain	41/151 (27.2)	31/153 (20.3)	.4
Protective anti-HI antibody titers of ⩾40			
A1 California strain			
Baseline	43/151 (28.5)	39/153 (25.5)	.5
Follow-up	109/151 (72.2)	116/153 (75.8)	.4
A1 Caledonia strain			
Baseline	41/151 (27.2)	41/153 (26.8)	.9
Follow-up	99/151 (65.6)	97/153 (63.4)	.6
B Shanghai strain			
Baseline	31/151 (20.5)	21/153 (13.7)	.6
Follow-up	74/151 (49.0)	59/153 (38.6)	.06
Seroprotection rate for patients with nonprotective baseline titers			
A1 California strain	66/108 (61.1)	77/114 (67.5)	.3
A1 Caledonia strain	58/110 (52.7)	56/112 (50.0)	.6
B Shanghai strain	43/120 (35.8)	40/132 (30.3)	.3
Protective anti-HA antibody titers after vaccination			
In all immunosuppressed patients			
⩽2 strains	89/151 (59.0)	107/153 (70.0)	.038
3 strains	63/151 (41.7)	46/153 (30.1)	
In renal transplant recipients			
⩽2 strains	27/37 (73.0)	28/37 (75.7)	.7
3 strains	10/37 (27.0)	9/37 (24.3)	
In patients who received long-term dialysis			
⩽2 strains	10/21 (47.6)	18/26 (69.2)	.1
3 strains	11/21 (52.4)	8/26 (30.8)	
In patients with rheumatologic disease			
⩽2 strains	18/28 (64.3)	16/19 (84.2)	.1
3 strains	10/28 (35.7)	3/19 (15.8)	
In patients with HIV infection			
⩽2 strains	32/63 (50.8)	43/68 (63.2)	.1
3 strains	31/63 (49.2)	25/68 (36.8)	
Seroconversion[a] rate			
In all immunosuppressed patients			
⩽2 strains	118/151 (78.1)	128/153 (83.7)	.18
3 strains	34/151 (22.5)	25/153 (16.3)	
For renal transplant recipients			
⩽2 strains	30/37 (81.1)	33/37 (89.2)	.5
3 strains	7/37 (18.9)	4/37 (10.8)	

Table 2. ***(Continued.)***

Vaccine characteristic	Subunit vaccine group ($n = 151$)	Virosomal vaccine group ($n = 153$)	P
In patients who received long-term dialysis			
⩽2 strains	17/21 (81.0)	19/26 (73.1)	.7
3 strains	4/21 (19.0)	7/26 (26.9)	
In patients with rheumatologic disease			
⩽2 strains	20/28 (71.4)	18/19 (94.7)	.06
3 strains	8/28 (28.6)	1/19 (5.3)	
In patients with HIV infection			
⩽2 strains	49/63 (77.8)	55/68 (80.9)	.6
3 strains	14/63 (22.2)	13/68 (19.1)	

NOTE. Data are proportion (%) of immunosuppressed patients, unless otherwise indicated. CI, confidence interval; HA, hemagglutinin; HI, hemagglutination inhibition; HIV, human immunodeficiency virus.

[a] Seroconversion is defined as an increase in anti-HA antibody titers of ⩾4 in comparison to baseline.

RESULTS

Study subjects. A total of 304 subjects were enrolled: 151 were randomized to receive the subunit vaccine, and 153 were administered the virosomal vaccine. Patients' characteristics are presented in table 1.

Most of the patients with rheumatologic disease received tumor necrosis factor (TNF)–α blockers (i.e., 21 [75%] of 28 patients who received the subunit vaccine and 13 [68%] of 19 patients who received the virosomal vaccine), mostly in combination with prednisone or methotrexate. Almost one-half of patients with rheumatologic disease had never received an influenza vaccine before.

The immunosuppressive regimens of renal transplant recipients were based on prednisone and calcineurine inhibitors, either alone or in combination with azathioprine or mycophenolate. The percentage of patients who had never been vaccinated against influenza was higher in the virosomal vaccine group than in the subunit vaccine group (9 [13.6%] of 66 patients vs. 1 [1.7%] of 60 patients; $P = .015$).

The HIV-infected patients in the 2 vaccine groups were at similar stages of HIV infection, according to the definition of the Centers for Disease Control and Prevention (table 1). At least two-thirds of the patients were receiving highly active antiretroviral therapy (HAART) including at least 3 antiretroviral drugs. Accordingly, approximately two-thirds of the patients had HIV viremia below the threshold of detection (<50 HIV RNA copies/mL). Of the 304 patients, 267 (88%) had their follow-up serum samples obtained before the end of the local influenza epidemic during the 2005–2006 influenza season (i.e., before the middle of March 2006) [17].

Immunogenicity. The mean duration between obtaining baseline serum samples and obtaining follow-up serum samples was comparable for the 2 vaccine groups (~50 days) (table 1). Baseline geometric mean anti-HA antibody titers were similar for all 3 vaccine strains and increased significantly ($P < .001$) after vaccination for all 3 vaccine strains (table 2). Postvaccination anti-HA antibody titers were highest for the A1 California strain (followed by the A1 Caledonia strain) and lowest for the B Shanghai strain.

At baseline, protective anti–hemagglutination inhibition (HI) antibody titers against the A1 California strain and the A1 Caledonia strain were observed in approximately one-fourth of patients from both vaccine groups, but protective anti-HI antibody titers against the B Shanghai strain were observed in a fewer number of patients from both vaccine groups (table 2). After vaccination, the number of patients from both vaccine groups with protective anti-HI titers against all 3 strains increased significantly ($P < .001$): 109 (72%) of 151 patients from the subunit vaccine group and 116 (76%) of 153 patients from the virosomal vaccine group had a protective anti-HA antibody titer against the A1 California strain; 99 (66%) of 151 patients from the subunit vaccine group and 97 (63%) of 153 patients from the virosomal vaccine group had a protective anti-HA antibody titer against the A1 Caledonia strain; and 74 (49%) of 151 patients from the subunit vaccine group and 59 (39%) of 153 patients from the virosomal vaccine group had a protective anti-HA antibody titer against the B Shanghai strain. The 2 vaccines did not differ in terms of vaccine response against the A1 California strain and the A1 Caledonia strain. There was a trend toward a better vaccine response against the B Shanghai strain among patients who received the subunit vaccine, compared with patients who received the virosomal vaccine (74 [49%] of 151 patients vs. 59 [39%] of 153 patients; $P = .06$). However, baseline rates of protective anti-HA titers were lower in the virosomal vaccine group than in the subunit vaccine group (21 [14%] of 153 patients vs. 31 [21%] of 151

Table 3. Adverse effects of a subunit influenza vaccine and a virosomal influenza vaccine in immunosuppressed patients.

Adverse effect in patient	Subunit vaccine group (n = 151)	Virosomal vaccine group (n = 153)	P
Immediate reactions			
Any	14/151 (9.3)	15/153 (9.8)	.8
Local pain	6/14 (42.9)	9/15 (60.0)	.5
Local redness	1/14 (7.1)	1/15 (6.7)	
Vasovagale syncope	2/14 (14.3)	0/15 (0.0)	
Anaphylaxia	0/14 (0.0)	0/15 (0.0)	
Local symptoms during the first 7 days after vaccination			
Pain	38/129 (29.5)	55/139 (39.6)	.3
Duration of pain, days	0.8 ± 1.4	1.2 ± 1.6	.2
Redness	17/125 (13.6)	13/136 (9.6)	.2
Diameter of redness, cm	0.7 ± 1.0	1.1 ± 1.4	.2
Warmth	7/122 (5.7)	11/132 (8.3)	.4
Swelling	14/125 (11.2)	13/133 (9.8)	.7
Diameter of swelling, cm	1.8 ± 1.1	2.6 ± 1.1	.1
Duration of swelling, days	0.7 ± 1.5	0.9 ± 1.7	.7
Systemic reactions during the first 7 days after vaccination			
Shivering	12/124 (9.7)	6/133 (4.5)	.1
Temperature ⩾37.8°C	4/125 (3.2)	4/125 (3.2)	.9
Fatigue	30/126 (23.8)	36/136 (26.5)	.5
Malaise	19/123 (15.4)	13/129 (10.1)	.2
Headache	27/124 (21.8)	27/133 (20.3)	.7
Muscle pain	16/124 (12.9)	17/133 (12.8)	.9
Arthralgia	14/123 (11.4)	17/133 (12.8)	.7
Duration of arthralgia, days	1.3 ± 1.7	1.3 ± 1.6	.7
Consultation with a physician	3/125 (2.4)	0/136 (0.0)	.1
Consumption of additional drugs	9/125 (7.2)	8/137 (5.8)	.4
Not willing to receive influenza vaccine next year	1/119 (0.8)	7/129 (5.4)	.04

NOTE. Data are proportion (%) of patients or mean values ± standard deviation.

patients). The seroprotection rates between the 2 vaccine groups did not differ among patients with nonprotective baseline titers against the 3 individual vaccine strains.

The percentage of patients with protection against all 3 vaccine strains was significantly higher after vaccination for the subunit vaccine group than for the virosomal vaccine group (62 [41%] of 151 patients vs. 46 [30%] of 153 patients; P = .03). A similar trend was seen for subgroups of patients with HIV infection, with rheumatologic disease, or who received long-term dialysis, but the trend did not reach statistical significance.

Reactogenicity. Overall, the subunit and virosomal vaccines were comparable with regard to the frequency of adverse effects (table 3). Pain was by far the most frequent local reaction, with 38 (30%) of 129 patients in the subunit vaccine group and 55 (40%) of 139 patients in the virosomal vaccine group having reported pain at the site of injection.

Fatigue was the most frequently reported systemic reaction, occurring in approximately one-fourth of patients. The disease-specific scores for the patients in both vaccine groups with rheumatologic disease were comparable; scores either decreased 4 weeks after vaccination or remained stable (table 4).

Factors influencing vaccine response. For the analysis of the factors influencing vaccine response, patients from both vaccine groups were pooled. Vaccine response was defined as a protective anti-HA antibody titer against all 3 vaccine strains in the postvaccination (i.e., follow-up) serum sample. Among renal transplant recipients, vaccine response was not statistically significantly associated (by use of univariate analysis) with age, history of past opportunistic infections, leukocyte or lymphocyte count at baseline or during the 3 months preceding vaccination, subcutaneous administration of the vaccine, immunosuppressive treatment, or the time since transplantation (table 5). However, in the multivariate analysis, which included time since transplantation, treatment with mycophenolate or purinantagonists, and number of immunosuppressive drugs,

Table 4. Disease activity in patients with chronic rheumatologic disease at baseline and after vaccination with a subunit or virosomal vaccine.

Patients, disease-specific score	Subunit vaccine	Virosomal vaccine	*P*
DAS of 24 patients with rheumatoid arthritis, mean ± SD			
Baseline	3.6 ± 1.3	3.0 ± 1.3	.3
Follow-up	3.2 ± 1.2	2.1 ± 1.2	.05
BASDAI of 13 patients with ankylosing spondylitis, mean ± SD			
Baseline	2.9 ± 1.7	3.3 ± 1.1	.08
Follow-up	2.0 ± 1.1	2.4 ± 1.4	.4
ECLAM of 3 patients with lupus erythematodes, mean			
Baseline	1.5	1	.1
Follow-up	1.5	1	.1
DEI of 2 patients with Wegener granulomatosis, mean			
Baseline	4	4	>.99
Follow-up	2	4	.3

NOTE. BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; DAS, Disease Activity Score; DEI, Disease Extend Index; ECLAM, European Consensus Lupus Activity Measurement; SD, standard deviation.

treatment with mycophenolate was the only statistically significant risk factor associated with nonresponse to vaccination (odds ratio [OR], 0.2; 95% confidence interval [CI], 0.04–0.93; $P = .04$).

For HIV-infected patients, a CD4 cell count of >200 cells/μL or a CD4 cell percentage of >14%, the time since a CD4 cell percentage of >14%, receipt of HAART, and undetectable HIV RNA in plasma at time of vaccination were statistically significantly associated with a protective vaccine response in the univariate analysis (table 6). In the multivariate analysis, only an undetectable HIV plasma RNA level <50 copies/mL was a statistically significant predictor of vaccine response (OR,

Table 5. Factors influencing immune response to influenza vaccination in 73 renal transplant recipients.

Factor	Protective anti-HA antibody titers against: ⩽2 strains ($n = 54$)	Protective anti-HA antibody titers against: 3 strains ($n = 19$)	*P*
Age, years	55.6 ± 10.2	52.0 ± 13.6	.4
History of opportunistic infections	21 (38.9)	4 (21.1)	.2
Lymphocyte count at time of vaccination, g/L	1.0 ± 0.57	1.7 ± 0.3	.08
Nadir of lymphocyte count 3 months before vaccination, g/L	0.8 ± 0.7	1.1 ± 0.7	.3
Leukocyte count at time of vaccination, g/L	8.4 ± 2.7	7.8 ± 2.0	.3
Nadir of leukocyte count 3 months before vaccination, g/L	7.0 ± 2.1	6.1 ± 1.5	.1
Subcutaneous administration of vaccine	44 (81.5)	18 (94.7)	.2
Patients who received immunosuppressive drug treatment at time of vaccination			
With prednisone	50 (92.6)	19 (100)	.3
With sirolimus	6 (11.1)	3 (15.8)	.6
With calcineurin inhibitors	46 (85.2)	16 (84.2)	.99
With azathioprine	14 (25.9)	7 (36.8)	.3
With mycophenolate	27 (50.0)	5 (26.3)	.1
Patients who received treatment			
With 1 immunosuppressive drug	1 (1.8)	0 (0.0)	.3
With 2 immunosuppressive drugs	18 (33.3)	8 (42.1)	
With ⩾3 immunosuppressive drug	36 (66.6)	11 (57.9)	
Time since transplantation, years	7.5 ± 6.5	7.6 ± 5.6	.9

NOTE. Data are no. (%) of patients or mean values ± standard deviation. HA, hemagglutinin.

Table 6. Factors influencing vaccine response in 131 patients infected with human immunodeficiency virus (HIV).

Factor	Protective anti-HA antibody titers against		*P*
	⩽2 strains	3 strains	
Absolute CD4 cell count, cells/μL	437.5 ± 234.8	495.9 ± 237.1	.1
Nadir of absolute CD4 cell count, cells/μL	234.9 ± 199.4	202.6 ± 151.9	.3
Time since nadir of absolute CD4 cell count, days	1789 ± 4491	1580 ± 1279	.7
Patients with CD4 cell counts			.02
⩾200 cells/μL	62/74 (83.8)	55/57 (96.5)	
<200 cells/μL	12/74 (16.2)	2/57 (3.5)	
Time since CD4 cell count ⩾200 cells/μL, years	2.2 ± 3.7	5.4 ± 3.7	.1
Relative CD4 cell count, %	23.5 ± 9.3	26.0 ± 7.9	.1
CD4/CD8 cell count ratio, median (range)	0.46 (0.1–2.0)	0.5 (01–2.1)	.1
Patients with CD4 cell percentage of >14%	64/74 (86.5)	54/57 (94.7)	.04
Time since CD4 cells >14%, median years (range)	3.7 (0.1–14.7)	3.9 (0.1–15.6)	.8
Patients who received HAART	53/74 (71.6)	48/57 (84.2)	.04
Plasma $\log_{10}$ HIV RNA at time of vaccination, median (range)	3.5 (0–11.2)	1.6 (0–11.8)	.006

NOTE. Data are proportion (%) of patients or mean values ± standard deviation, unless otherwise indicated. HA, hemagglutinin; HAART, highly active antiretroviral therapy.

3.7; 95% CI, 1.63–8.46; $P = .002$). For patients who received long-term hemodialysis, only advanced age was predictive of achieving protective titers against all 3 vaccine strains (median, 58 vs. 71 years; OR, 1.05; 95% CI, 1.0–1.1; $P = .03$). For patients with rheumatologic disease, neither the characteristics of the disease nor the patients' treatments were predictive of response against all 3 vaccine strains (table 7).

DISCUSSION

Our study compared the immunogenicity and reactogenicity of a virosomal influenza vaccine with the immunogenicity and reactogenicity of a subunit influenza vaccine in adult patients with immunosuppression due to long-term dialysis, renal transplantation, rheumatic disease, or HIV infection. To our knowledge, such an analysis has not been performed before.

The subunit vaccine was slightly more immunogenic than the virosomal vaccine in terms of the percentage of patients who reached a protective anti-HA antibody titer against all 3 vaccine strains (41% vs. 30%). This trend was also observed for subgroups of patients with HIV infection, with rheumatologic disease, or who received long-term dialysis but not for renal transplant recipients.

The overall response rate to all 3 vaccine strains was low (i.e., <50% of patients had protective anti-HA antibody titers against all 3 vaccine strains after vaccination). Among subgroups of patients, however, patients with HIV infection and patients who received dialysis showed higher response rates (~50% of patients) than renal transplant recipients and patients with rheumatologic disease (~25% of patients). This difference is probably attributable to the degree of immunosuppression in the different subgroups of patients; most patients with HIV infection received efficacious antiretroviral therapy (i.e., HAART), and most patients who received dialysis received no additional immunosuppressive treatment.

The vaccine response rate observed among patients who received long-term dialysis was comparable to earlier findings [1, 4, 18] that showed a lower vaccine response rate, compared with healthy subjects [1, 4]. In our study, patients who received dialysis had a lower response rate than the patients studied by Song et al. [19], who also showed that the vaccine response rate seems to decrease the longer the duration of dialysis. Our patients received dialysis for an average of 5 years before the start of our study, which is considerably longer than the duration of dialysis among the patients in the study by Song et al. [19] and may explain the lower response rates in our study.

A comparison of vaccine immunogenicity in HIV-infected patients observed in our study with that found in the literature is hampered by differences between the study populations. Most of the earlier studies were conducted before the introduction of HAART, and the stratification of patient groups according to surrogates of immunosuppression (such as CD4 cell count) varies between studies [3, 8, 20–22]. However, among adult patients with HIV infection, the vaccine response rate is generally higher among patients who received antiretroviral treatment [3]. Most studies emphasized the importance of a high CD4 cell count to a higher vaccine response rate [8, 21]. These studies were performed before or at the beginning of the HAART era, when therapy had no long-term suppressive effect on the HIV RNA level in a patient. In our study, only the HAART-associated undetectable plasma HIV RNA level, not

Table 7. Factors influencing immune response to influenza vaccination in 47 patients with rheumatologic disease.

Factor	Protection against ≤2 strains (n = 34)	Protection against all 3 strains (n = 13)	P
Age, years	51.2 ± 12.5	48.4 ± 14.5	.5
Male sex	20 (58.8)	7 (53.8)	.7
Type of heumatologic disease			
Autoimmune connectivitis	2 (5.9)	1 (7.7)	.4
Chronic idiopathic polyarthritis	21 (61.8)	7 (53.8)	
Sarcoidosis	0 (0.0)	1 (7.7)	
Spondarthritis	10 (29.4)	3 (23.1)	
Vasculitis	1 (2.9)	1 (7.7)	
Duration of rheumatologic disease, years	10.3 ± 7.8	9.6 ± 8.2	.7
Lymphocyte count at time of vaccination, g/L	1.9 ± 0.8	1.6 ± 0.7	.2
Nadir of lymphocyte count 3 months before vaccination, median (range), g/L	1.3 (0.4–7.7)	1.3 (0.2–2.7)	.7
Leukocyte count at time of vaccination, g/L	7.7 ± 2.1	6.7 ± 1.8	.1
Smoker	7 (20.6)	2 (15.4)	.99
Elevated transaminase levels	1 (2.9)	1 (7.7)	.4
Liver cirrhosis	1 (2.9)	1 (7.7)	.4
Charlson comorbidity index, median score (range)	1 (1–3)	1 (1–3)	.2
Diabetes	2 (5.9)	1 (7.7)	.99
First influenza vaccination ever	17 (50.0)	6 (46.2)	.5
Received subcutaneous vaccination	2 (5.9)	1 (7.7)	.99
No. of immunosuppressive drugs received	1.9 ± 0.8	1.9 ± 0.7	.8
Received TNF-α blockers	25 (73.5)	9 (69.2)	.99
Received methotrexate	20 (58.8)	7 (53.8)	.7
Duration of treatment, years	8.3 ± 8.4	9.2 ± 9.3	.7
Type of drug used for treatment			
Prednisone	14 (41.2)	3 (23.1)	.2
Purinantagonists	3 (8.8)	1 (7.7)	.99
Azathioprin	1 (2.9)	1 (7.7)	.4
Calcineurinantagonist	2 (5.9)	2 (15.4)	.3

NOTE. Data are no. (%) of patients or mean values ± standard deviation, unless otherwise indicated. TNF, tumor necrosis factor.

the CD4 cell count, was an independent predictor of a higher vaccine response rate. This finding is in accordance with that of Yamanaka et al. [23], who showed an association between cellular response against the H1N1 influenza antigen and HIV RNA level but not CD4 cell count. Therefore, suppression of HIV replication may be more important than the CD4 cell count for predicting immune response against influenza vaccine, and recommendations against influenza vaccination for patients with CD4 cell counts of <100 cells/μL should be questioned. We argue that patients who are responding to HAART (on the basis of viral load) should be considered for influenza vaccination pending further studies.

Both our study and the studies of others demonstrated low vaccine response rates among renal transplant recipients [4, 24, 25]. The immunosuppressive treatment regimen seems to play an important role. Significant increases in anti-HA antibody titers were seen in patients who received prednisone, cyclosporine, and azathioprine [4, 25]. However, treatment with mycophenolate mofetil was associated with a lower vaccine response rate in our study and the studies of others [25, 26].

As a group, patients with rheumatic disease who underwent immunosuppressive treatment had a low vaccine response rate (19% of these patients were protected against all 3 vaccine strains). However, two-thirds of our patients received at least 2 immunosuppressive drugs, and one-half of them were treated with TNF-α inhibitors, which may explain the poor vaccine response. Our study population was too small for an analysis of individual risk factors. However, a poor vaccine response associated with treatment with TNF-α inhibitors alone or in combination with methotrexate was found in a study of patients with rheumatoid arthritis who had been treated for a median of 0.7–1 year before vaccination [2]. Also, treatment with azathioprine has been associated with low seroconversion rates, compared with treatment with hydroxychloroquine and prednisone, among patients with systemic lupus erythematodes [27].

For both vaccines, the rate of immunogenicity was found to be different for each of the 3 vaccine strains. The vaccine response rate was highest against the A1 California vaccine strain and lowest against the B Shangai strain. A lower rate of immunogenicity for B type strains has been found in some but not all studies that used the same or different vaccine strains for immunosuppressed patients [24, 25, 28]. The immune response to different vaccine strains seems to be more balanced in immunocompetent populations [1, 3, 4, 8, 22, 25]. Subcutaneous vaccination did not affect vaccine response in our study, as has been shown for subcutaneous vaccination against hepatitis B [29].

Split and virosomal vaccines were equally well tolerated by patients in our study. Furthermore, there were no signs or symptoms for the activation of the underlying disease in HIV-infected patients and patients with rheumatologic disease during postvaccination surveillance. This finding is in accordance with the findings of other studies, in which influenza vaccines were found to be safe for immunosuppressed patients [8, 27, 28, 30]. We did not evaluate postvaccination dynamics in HIV viral load. Two other studies did not find an increase in viral load after vaccination, although they chose a relatively high (400 copies/mL) cutoff level [8, 21]. Günthard et al. [31] demonstrated a slight but transient increase in HIV RNA levels in patients with HIV RNA levels of <50 copies/mL during the first 2 weeks after vaccination.

In conclusion, a subunit influenza vaccine was found to be slightly more immunogenic than a virosomal vaccine in immunosuppressed patients with various underlying diseases. Vaccine response was mostly influenced by the degree of immunosuppression, with higher immunogenicity in patients who received long-term dialysis and HIV-infected patients receiving efficacious antiretroviral treatment than in renal transplant recipients and patients receiving immunosuppressive treatment for chronic rheumatologic diseases. The viral load in plasma rather than the CD4 cell count predicted the immunogenicity of the influenza vaccine in HIV-infected patients. We argue that HIV-infected patients who receive efficacious antiretroviral treatment should be offered influenza vaccination regardless of their CD4 cell count.

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