Induction of Opsonophagocytic Killing Activity with Pneumococcal Conjugate Vaccine in Human Immunodeficiency Virus-Infected Ugandan Adults

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Abstract

The levels of IgG determined by ELISA may have limited relevance in human immunodeficiency virus (HIV)-infected adults because of nonfunctional antibodies. 58 HIV-1-infected and 29 HIV-uninfected Ugandan adults were immunized with conjugate vaccine (CV) followed by polysaccharide vaccine (PV) after a two-month interval, and the opsonophagocytic killing (OPK) titers against serotype 4 or 14 pneumococcal strains as well as the levels of serotype-specific IgG in sera were determined. Significant increases were found in the OPK titers and IgG levels for both serotypes after CV vaccination irrespective of HIV status. Increases in IgG levels and OPK titers were largely dependent on the $CD4^+$ cell counts, except for increases in the IgG levels for serotype 4. The proportions with serum OPK titer equal to or greater than 8 were 0-4.3% for serotype 4 and 26.7-42.9% for serotype 14 before vaccination, but the proportions increased up to 43.3-86.2% for serotype 4 and 63.3-96.6% for serotype 14 in all three groups 2 months after CV vaccination. The serum OPK titers remained at levels higher than the pre-vaccination level for at least 8 months after CV vaccination. A single dose of CV could afford some protective immunity in HIV-infected African adults before the introduction of antiretroviral therapy.

Key words: Pneumococcal conjugate vaccine, pneumococcal polysaccharide vaccine, serotype-specific IgG, opsonophagocytic killing, HIV-infected adults

Introduction

More than 60% of the 40 million people living with HIV in the world are in sub-Saharan Africa [1]. HIV infection is, therefore, a major health concern facing adults in this area. The authors' previous study of community-acquired pneumonia (CAP) among adults in Uganda revealed a high prevalence of HIV-1 infection among these patients [2]. The majority of HIV-1 infected patients with CAP have peripheral CD4 counts below 400/μl. *Streptococcus pneumoniae (S. pneumoniae)* was found to be a common pathogen, which was isolated in approximately 40% of bacterial CAP. The host defense against *S. pneumoniae* depends largely on opsonophagocytic killing antibodies to capsular polysaccharide (CPS) [3,4], although an antibody-independent, $CD4^+$ T cell-dependent mechanism of protection has been reported in mouse model [5,6]. Low opsonic activity in the sera of HIV-infected African adults, therefore, predisposes them to a serious risk of invasive pneumococcal infections [7-9].

Approaches to prevent bacterial pneumonia in adults with HIV infection involve antimicrobial prophylaxis, highly active antiretroviral therapy (ART) and vaccination [10]. Although a significant decrease in the rates of bacterial pneumonia in HIV-infected adults after antimicrobial prophylaxis with co-trimoxazole has been reported in Cote, d'Ivoire [11], further studies are required to confirm its effects in preventing bacterial pneumonia even in areas where penicillin-resistant pneumococci are highly prevalent in the population [12]. ART can prevent bacterial pneumonia in HIV-infected adults in developed countries, but this has not been confirmed in developing countries [13]. Although the WHO recommends that HIV-infected adults and adolescents, who are

either in WHO clinical staging 4 or have a CD4 cell count below 200/μl start ART [1], an access to ART is limited in sub-Saharan Africa including Uganda [14]. The WHO estimates that only 20% of the estimated 6.5 million people in low and middle-income countries who are in urgent need of ART were receiving ART at the end of 2005 [15].

Previous studies reported the effects of pneumococcal polysaccharide vaccine (PV) in HIV-infected African adults [16, 17]. A clinical study of PV in HIV-infected Ugandans, however, has demonstrated an increased risk of invasive pneumococcal infection and no protection against all-cause pneumonia [18], and the same group subsequently reported a 16% reduction in all-cause mortality after PV vaccination [19]. The use of PV in HIV-infected African adults is therefore inconclusive. On the other hand, previous studies in the Netherlands, US and Uganda reported promising effects of pneumococcal conjugate vaccine (CV), which induced high levels of serotype-specific IgG among HIV-infected adults [20-22]. Furthermore, an enhanced antibody response to certain serotypes has been shown in HIV-infected adults following immunization with PV after prior immunization with CV [20,23]. The concentration of serotype-specific IgG determined by ELISA, however, may have limited relevance in HIV-infected adults and children because of nonfunctional anti-CPS IgG [6,7,24,25]. Analysis of serum opsonophagocytic killing (OPK) activity is, therefore, required to study the immunogenicity of CV in HIV-infected adults. This study was designed to determine whether immunization with CV followed by PV could increase the levels of serotype-specific IgG and OPK titers against *S. pneumoniae* in sera of HIV-infected African adults before the introduction of ART.

2. Materials and methods

2.1 Subjects

After providing written informed consent, HIV-1-infected and HIV-uninfected asymptomatic adults were enrolled by two physicians at the Joint Clinical Research Centre (JCRC), Kampala between February and June 2005. HIV-1 infection was determined by a screening EIA (HIV1&2 Rapid Serotest, Healthcare Technologies Ltd., Ashdod, Israel) and a second EIA for confirmation (Vironostika HIV Uni-FormII Ag/Ab, bioMétrieux, Marcy l'Etoile, France). Study subjects were classified into three subgroups: group I (HIV-1-infected adults with peripheral $CD4^+$ cell count: 200-499/μl; n=30), group II (HIV-1-infected adults with peripheral $CD4^+$ cell count: ≧ 500/μl; n=28) and group III (HIV-uninfected adults; n=29)(Table 1). Plasma HIV-1 RNA loads were quantified as described elsewhere for the total of 58 HIV-infected adults [26]. Enrolling 90 subjects in three study groups would have given the study 70% power at an alpha level of 0.05 to detect a significant increase in the percentage of persons responding with a ≧ 2-fold rise in the serum levels of serotype-specific IgG from 42% to 74% between groups of HIV-infected and HIV-uninfected adults [27].

2.2 Immunization of study subjects with pneumococcal vaccines

7-valent CV (Prevenar^R, Wyeth-Lederle), containing 2 μg of six serotypes (4, 9V, 14, 18C, 19F and 23F) and 4 μg of CPS from 6B covalently linked to a CRM_{197}, non-toxic mutant diphtheria toxin, was given as an intradeltoid injection to all of the study subjects. Two months after CV vaccination, 23-valent PV (Pneumovax, Merck-Banyu),

which contains 25 μg of each of 23 serotypes was given as an intradeltoid injection to all of the study subjects. All of the study subjects were requested to visit the outpatient department of JCRC, and be examined by two physicians immediately before and 2, 3 and 8 months after CV vaccination. Serum samples were also obtained at the time of the scheduled visits throughout the study, and stored at -80℃ until use. When they had new symptoms, the participants were asked to visit the outpatient department of the JCRC for medical examination by the physicians. At 8 months post-CV vaccination, the peripheral $CD4^+$ cell count was reexamined in HIV-infected adults. While all of HIV-infected subjects were successfully followed for the study period, six of the HIV-uninfected healthy control subjects were lost by the time of follow-up. Sera were subsequently collected from 23 HIV-uninfected subjects 8 months after CV vaccination.

2.3 Determination of serotype-specific IgG levels

Since preabsorption of serum with both cell wall polysaccharide (PS) and 22F PS is necessary to remove non-specific antibodies in HIV-infected adults and HIV-uninfected adults, the concentrations of serotype-specific IgG were measured using the US reference pneumococcal antiserum (89 SF-3), courtesy of Dr. Carl Frasch, as previously described [28,29]. Among 29 pneumococcal isolates known as etiologic agents of CAP in Ugandan adults [2], 5 strains of serotype 4 and 3 strains of serotype 14 were identified (unpublished data). Therefore, we decided to use ELISA to determine the IgG levels in sera for serotypes 4 and 14 CPS, which are major serotypes in Uganda.

2.4 Differentiation of HL-60 cells and OPK assay

Undifferentiated HL-60 cells (Cell number; JCRB0085, Health Science Research Resources Bank, Japan) were cultured in RPMI-1640 medium with 10% fetal calf serum (FCS) (Hyclone, South Logan, UT). HL-60 cells were differentiated into granulocytes in the presence of 1 μM all-*trans*-retinoic acid (Sigma Chemical Co., St. Louis, MO) in RPMI-1640 medium with 20% FCS for 3 days [30, 31]. Cells were washed twice by centrifugation, and the supernatant was removed. 20 μl of PE-conjugated mouse anti-human CD11b monoclonal IgG_1 (BD PharMingen, San Diego, CA) or PE-conjugated mouse monoclonal IgG_1 isotype control (Dakocytomation AS, Glostrup, Denmark) were added to each tube and incubated at 4ºC for 30 min. Samples were washed twice and resuspended in PBS containing 1% paraformaldehyde, and analyzed by flowcytometry (FACSCalibur, BD Biosciences, San Jose, CA). While no expression of CD11b was found in the undifferentiated cells, increased expression of CD11b was found in the differentiated cells by retinoic acid (data not shown). The expression of CD11b was specific because no increase was found in the fluorescent intensity of the differentiated cells stained with PE-conjugated control antibody.

OPK titer against *S. pneumoniae* was measured as described elsewhere with some modification [32]. Briefly, the differentiated HL-60 cells were used at an effector/ target cell ratio of 400/1. 10 μ1 of serially diluted serum sample was aliquoted into each well of 96-well microtiter plate. *S. pneumoniae* serotype 4 (strain P-03-106) or serotype 14 (strain P-03-170), both isolated from patients with CAP, were used for this assay. In addition, we also employed two reference strains (DS2382-94 for serotype 4 and

DS2214-94 for serotype 14) as standard quality control strains for this assay [32]. These reference strains were gifts from Dr. Moon H. Nahm, University of Alabama at Birmingham. 20 μl of bacterial suspension (~10^3 cfu) was added to each well. Then the plate was allowed to incubate at 37ºC in a 5% CO_2 atmosphere for 15 min. Following this incubation period, 10 μl of rabbit complement (Dynal Biotech Inc, Lake Success, NY) was added to each well. Then, 40 μl of washed cells (4×10^5 cells) was immediately added to each well. The assay plate was incubated at 37ºC for 45 min with horizontal shaking (220 rpm). A 5 μl aliquot from each well was plated onto an agar medium plate. Plates were incubated overnight and viable colonies were counted for each well. Typically, 10 to 60 colonies were counted. The OPK titers were expressed as the reciprocal of the serum dilution with ≥ 50% killing compared with the growth in the complement control wells. Serum samples with titers of < 8 were reported as a titer of 4 for analysis of the levels of serotype-specific IgG, but excluded for analysis of the correlation between the levels of IgG or HIV-1 viral load and the serum OPK titers. Quality control sera (sera with a known titer) were added to each plate and the blinded test samples for OPK titer were examined only when the titers of quality control sera were identical. Functional activity of serotype-specific IgG was expressed as a concentration of IgG required for 50% killing of pneumococcal strain by dividing the IgG concentration of a test sample by OPK titer.

2.4 Statistical analysis

The subjects' ages, $CD4^+$ cell count and levels of HIV-1 viral load were compared by 1-way analysis of variance and by multiple comparison methods. Differences in

geometric mean concentrations (GMCs) of serotype-specific IgG, OPK titers and the ratios of OPK titer to serotype-specific IgG were assessed using the Friedman and Steel-Dwass tests [33]. The significance of the correlation was estimated using Spearman's rank correlation. Data were considered significant when $P < 0.05$.

3. Results

No difference was found in age between groups I and II, but differences in age were significant between either of groups I or II and the group III (P<0.001, Table 1). Differences in $CD4^+$ cell count and the level of HIV-1 viral load were significant between groups I and II (P<0.001), which is in agreement with our previous report [26]. No significant decrease in the $CD4^+$ cell count was found in either group I or II during the 8 months post-CV vaccination. ART was initiated in one case (32 year old, male) in the group I at 7 months post-CV vaccination because of recurrent pneumonia. The $CD4^+$ cell count and plasma $\log_{10}$ HIV viral load of this case were 212/ μl and 5.78, respectively, at the time of enrollment.

No significant difference was found in the GMCs of IgG specific to either serotype 4 or 14 in sera among the three groups before vaccination (Table 2). Two months after CV vaccination, significant increases in the GMCs of IgG specific to both serotypes 4 and 14 were found for all three groups, compared to those before vaccination (P <0.01).

No significant difference was found in the GMCs of IgG specific to serotype 4 among the three groups two months after CV vaccination (P=0.23). In contrast, the GMCs of IgG specific for serotype 14 were highest in group III, followed by group II, with group I having the lowest GMC two months after CV vaccination, and the difference between groups I and III was significant at this time point (P< 0.05). One month post-vaccination with PV (three months after CV vaccination), however, no significant increase was found in the GMCs of IgG specific to either serotypes 4 or 14 compared to those at 2 months after vaccination with CV in each group. Although the GMCs of IgG

specific to serotype 14 largely declined at 8 months post-vaccination with CV, no significant decrease in the GMCs of IgG specific to serotypes 4 was found in any group between 2 months after CV vaccination and 8 months after CV vaccination. The GMCs of IgG specific to both serotypes 4 and 14 were still higher 8 months after CV vaccination than before vaccination. In addition, no correlation was found between the fold increase of specific IgG to either serotypes 4 or 14 after CV vaccination and the levels of HIV-1 viral load in HIV-infected adults.

The concentration of 0.35 μg/ml for serotype-specific IgG has been suggested by world health organization (WHO) working group as a putative measure of protection against invasive disease in infants at a population level after immunization with pneumococcal conjugate vaccine [34]. This working group reported that antibody concentrations of 0.2-0.35 μg/ml correlated best with an OPK titer of 8 [34], which in turn correlated best with protective efficacy in infants. The proportions of subjects with IgG specific to serotype 4 of ≧ 0.35 μg/ml were 100% in group I, 85.7% in group II and 79.3% in group III before vaccination and 100% for all groups 2 months after CV vaccination in this study. The proportions of subjects with IgG specific to serotype 14 of ≧ 0.35 μg/ml were 100% in groups I and II and 96.4% in the group III, before vaccination and 100% for all groups 2 months after CV vaccination.

The geometric means of serum OPK titers for the serotypes 4 and 14 were similarly low in sera of all three groups before vaccination (Table 3). The differences among the three groups were not statistically significant. Two months after CV vaccination, significant increases in the serum OPK titers for both serotype strains 4 and 14

compared to pre-vaccination levels were found in all three groups (P<0.01). The serum OPK titers for both serotypes 4 and 14 were highest in group III followed by group II, with group I having the lowest titers 2 months after CV vaccination. Serum OPK titers for both serotypes 4 and 14 differed significantly between groups I and III at 2, 3 and 8 months after vaccination with CV (P<0.01). Group II had significantly higher OPK titers than group I for serotype 4 at 3 and 8 months post-vaccination with CV, and for serotype 14 at 8 months post-vaccination with CV (P<0.05). In additional experiments, the OPK titers of nine serum samples (three from each group) were determined using strains of DS2382-94 and DS2214-94 which are widely used as target strains for serotypes 4 and 14, respectively. The values of serum OPK titer were identical or close to those determined using the non-reference strains (data not shown).

The proportions of subjects with a serum OPK titer of ≧ 8 were very low (0-4.3%) for serotype 4 in all three groups before vaccination (Fig.1). On the other hand, the proportion was lower in group I (26.7%) than those in group II (42.9%) or group III (41.4%) for serotype 14 before vaccination. In contrast, marked increases in the proportion with a serum OPK titer of ≧ 8 were found for both serotypes in all three groups two months after CV vaccination. The proportion of the serum OPK titer of ≧ 8 was highest in group III, followed by group II and group I for both serotypes at 2 and 3 months post-CV vaccination. Eight months after CV vaccination, the proportions of subjects with a serum OPK titer of ≧ 8 decreased in groups I and II for both serotypes.

Since few serum samples contained detectable OPK titers for serotype 4 before

vaccination (1 in group I, none in group II and 3 in group III), correlations were not assessed between the levels of serotype-specific IgG and the serum OPK titers. On the other hand, the concentrations of serotype-specific IgG significantly correlated with OPK titers for serotype 14 before vaccination in groups II (r=0.61, P<0.05, n=14) and III (r=0.61, P<0.05), but not in group I (r=0.56, P=0.08, n=11). Two months after CV vaccination, significant correlations between two parameters were found for serotype 4 in groups I (r=0.74, P< 0.01, n=15) and III (r=0.67, P< 0.001, n=26), but not in group II (r=0.27, P=0.2, n=23) and for serotype 14 in groups II (r=0.85, P< 0.001, n=23) and III (r=0.38, P=0.05, n=28), but not in group I (r=0.44, P=0.06, n=11).

The GMCs of serotype-specific IgG required for 50% killing ranged 136-601 ng/ml in the Group I, 79-377 ng/ml in the Group II and 72-316 ng/ml in the Group III, although few data are available for serotype 4-specific IgG before vaccination (Table 4). No significant difference was found in the GMCs of serotype-specific IgG required for 50% killing in sera among the three groups before and after vaccination. No significant difference was also found in the GMCs of serotype-specific IgG required for 50% killing of each group among different time points, with a exception of a significant difference in those of Group I between 3 months after CV vaccination and 8 months after CV vaccination (P< 0.05). No significant effect of vaccination with PV following CV was found in the serum OPK titers for either serotypes 4 or 14 in any group. Eight months after CV vaccination, the serum OPK titers for serotypes 4 and 14 largely declined compared to those 2 months after CV vaccination. The OPK titers for serotype 4 and 14 in sera of the three groups,

however, remained higher than pre-vaccination levels at 8 months post-vaccination with CV. In addition, no correlation was found between the increase in serum OPK titers for both serotypes 4 and 14 after CV vaccination and the levels of HIV-1 viral load in HIV-infected adults.

4. Discussion

In this study, it has been demonstrated that a single dose of CV can increase serum OPK titers against pneumococcal strains as well as the levels of serotype-specific IgG, in HIV-uninfected and HIV-infected Ugandan adults with peripheral $CD4^+$ cell counts of ≧ 200/μl for at least 8 months after CV vaccination. HIV-infected adults with peripheral $CD4^+$ cell counts less than 200/μl who were at risk for pneumococcal infections were not involved in this study, because they are recommended to receive ART [1] and are known to be poorly responsive to CV [20,27]. Serotype 14-specific IgG response to a single dose of CV was dependent on CD 4^+ cell counts in HIV-infected adults, which was in agreement with previous reports [20,22], but the magnitude of IgG response to CV in our study was higher than that shown in a previous study [22]. In contrast, serotype 4-specific IgG response to a single dose of CV was independent of $CD4^+$ cell counts in our study: this finding was also consistent with a previous study [22].

The major limitation in this study may be the lack of an unvaccinated control group. Pneumococcal pneumonia or nasopharyngeal colonization may increase the levels of serotype-specific IgG in sera from subjects, in addition to the immunological effects by vaccination, during the study period [35]. Since only five episodes of pneumonia with unknown etiology were identified in the HIV-infected and HIV-uninfected groups (2 episodes in group I, 1 episode in group II and 2 episodes in group III) during the 8 months (data not shown), we speculate that the possibility of active immunization by pneumococcal pneumonia among study participants was low in this study. Since the

nasopharyngeal carriage of pneumococcal strains was not examined in this study, the influence of nasopharyngeal carriage on the levels of IgG and OPK titers in serum cannot be dismissed.

Although a previous study reported an immunological effect of a CV primed PV booster with a 4-weeks interval in HIV-infected adults [23], no effects of PV following CV vaccination were found in the levels of serotype 4 or 14-specific IgG in our study. The lack of additional immune response after PV following CV could be explained by the differences in races and ages of the participants and in the timing of the PV boost 2 months following CV in this study [23]. Another reason for the lack of additional immune response by PV might be the increased levels of serotype 4 or 14 specific IgG achieved by CV, because a recent study similarly demonstrated that elderly subjects with a serotype-specific IgG higher than 5 μg/ml in sera before vaccination tended to respond to PV at a lower magnitude [36]. The undiminished persistence of the levels of OPK titers and of IgG shown in this study up to 8 months after CV vaccination may, in part, be explained by the boosting effect of nasopharyngeal carriage of pneumococcal strains [37].

The general consensus on measuring the serum OPK titers encouraged the authors to develop this assay for the evaluation of immunogenicity in this study [24]. Although an OPK assay for the measurement of functional antibody activity has been available [32], there were difficulties in achieving differentiation of HL-60 cells into granulocytes in the presence of dimethylformamide as a previous study indicated [38]. In this study, it has been shown that differentiated HL-60 cells by the use of retinoic acid can be

applicable for an OPK assay against *S. pneumoniae* for evaluation of the immunogenicity of CV.

While the proportions of subjects with serotype-specific IgG of ≧ 0.35 μg/ml were higher than 80% and 95% for serotypes 4 and 14 among HIV-infected and HIV-uninfected Ugandan adults in this study, a previous study demonstrated that the proportions of subjects with serotype-specific IgG were less than 10% for serotype 4 and less than 30% for serotype 14 among unvaccinated HIV-infected African children [25]. A relatively low proportion of subjects, however, exhibited OPK titers of ≧8 for serotypes 4 and 14 in both HIV-infected and -uninfected Ugandan adults before vaccination in this study. Although WHO working group reported that 0.2-0.35 μg/ml for the levels of serotype-specific IgG correlate with an OPK titer of 8 in infants [34], this threshold may not be applicable for unvaccinated adults.

In this study, we determined the levels of serotype-specific IgG, but not IgM or IgA. While a poor correlation was found between the opsonic activity and the levels of either CPS-specific IgM or IgA, the serum opsonic activity correlated best with the levels of CPS-specific IgG in healthy adults following CV vaccination [39,40]. The levels of IgG significantly correlated with the OPK titers for serotype 4, except for group II, and for serotype 14, except for group I, 2 months after CV vaccination in this study. Furthermore, the GMCs of serotype-specific IgG required for 50% killing ranged 79-601 ng/ml in HIV-infected adults and 72-315 ng/ml in HIV-uninfected adults after a single dose of CV vaccination in this study. In contrast, the GMCs of serotype-specific IgG required for 50% killing ranged 2-119 ng/ml in infant

populations after CV vaccination [41-43]. Collectively, our present data suggest that opsonic function of serotype-specific IgG after CV vaccination is much decreased in Ugandan adults, irrespective of HIV infection, than those in infants.

In conclusion, this study document the immunogenicity of a single dose of CV on the OPK titer and the levels of serotype-specific IgG in sera of HIV-infected Ugandan adults with $CD4^+$ cell count higher than 200/μl. A single dose of CV increased the serum OPK activity as well as the levels of IgG, and maintained them above their pre-vaccination levels for at least 8 months after CV vaccination. A single dose of CV could increase protective immunity in HIV-infected African adults with $CD4^+$ cell count higher than 200/μl before the introduction of ART. A clinical trial of the efficacy of CV in this population is needed in sub-Saharan Africa.

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Figure legends

Fig. 1. The proportion of subjects with opsonophagocytic killing (OPK) titers equal to or greater than 8 for serotype 4 (upper panel) and serotype 14 (lower panel) in groups I (n=30, closed bar), II (n= 28, grey bar), and III (n= 29, open bar) before and 2 months, 3 months and 8 months after vaccination with pneumococcal conjugate vaccine (CV). *n=23 in group III at 8 months after CV vaccination.

Fig.1

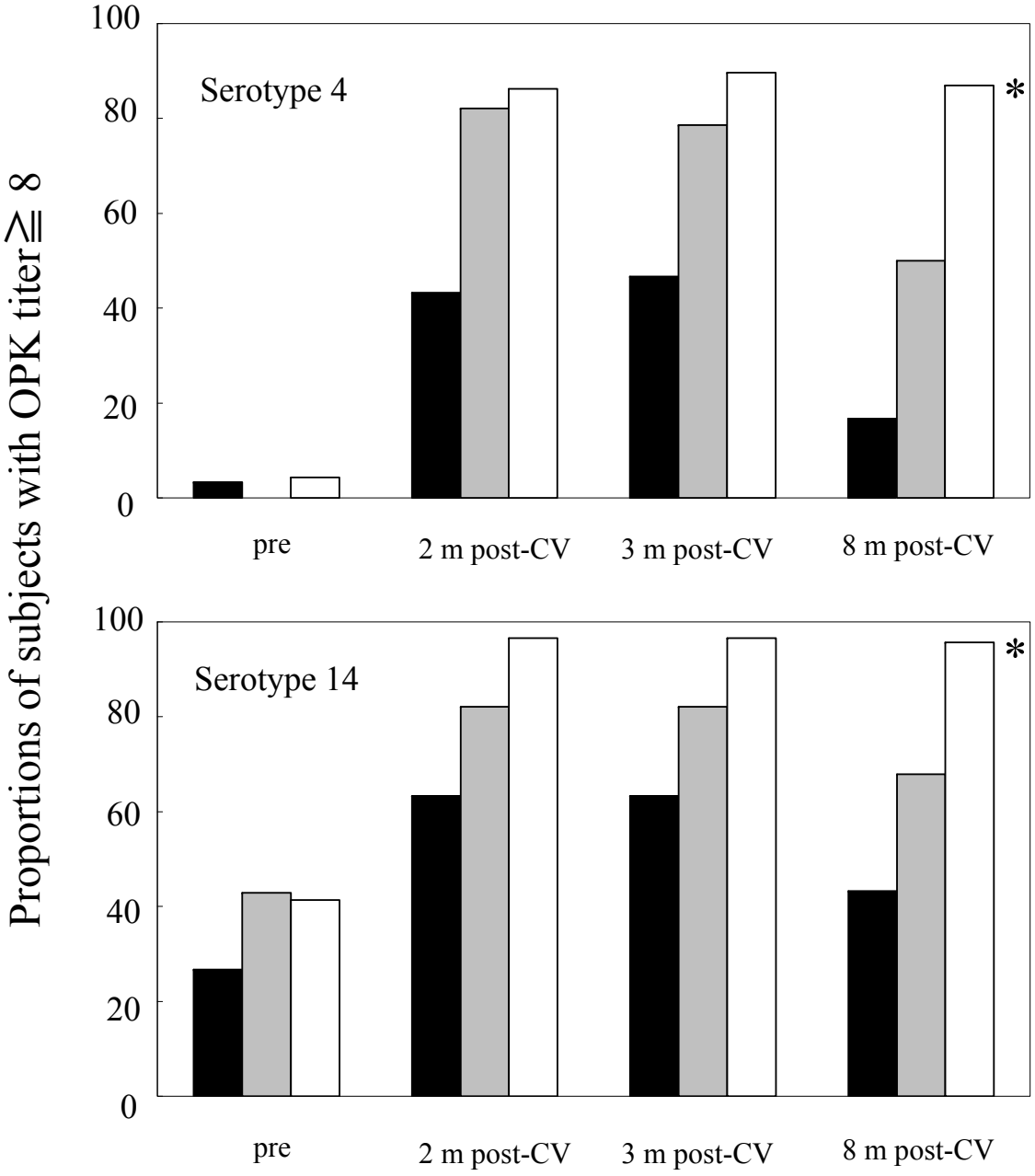
Proportions of subjects with OPK titer ≧ 8
100
80
60
40
20
0
Serotype 4
*
pre
2 m post-CV
3 m post-CV
8 m post-CV
100
80
60
40
20
0
Serotype 14
*
pre
2 m post-CV
3 m post-CV
8 m post-CV

Table 1. Clinical characteristics and laboratory data for 58 HIV-1-infected and 29 HIV-uninfected adults.

Characteristic	Time point	Group I (n = 30) HIV-1+, CD4 :200-499/μl	Group II (n = 28) HIV-1+, CD4 ≧ 500/μl	Group III (n = 29) HIV-
Age, mean years ± SD	Pre	36.77 ± 7.45 [a]	37.11± 7.63 [a]	26.83 ± 7.70
Peripheral CD4+ cell count, mean cell/ μl ± SD	Pre	352.00 ± 80.71 [a,b]	720.25 ± 170.69 [c]	882.45 ± 257.45
Log_{10} plasma HIV RNA load copies/ ml ± SD	Pre	4.81 ± 0.85 [b]	3.84 ± 0.83	ND
Peripheral CD4+ cell count, mean cell/ μl ± SD	8 m post-CV	362.41 ± 155.82 [b]	707.39 ± 213.79	ND

[a]$P < 0.001$ (vs group III), [b]$P < 0.001$ (vs group II), [c]$P < 0.01$ (vs group III). ND: Not done.

Table 2. Comparison of geometric mean concentrations of serotype-specific IgG in sera from HIV-1-infected patients and HIV-uninfected adults before vaccination and after vaccination.

Serotype	Time point	GMC of IgG (μg/ ml) (95% CI) in sera from:		
		Group I (n = 30) HIV-1+, CD4 :200-499/μl	Group II (n = 28) HIV-1+, CD4 ≧ 500/μl	Group III (n = 29) HIV-
4	Pre	1.55 (1.15~2.08)	0.82 (0.58~1.18)	0.83 (0.59~1.16)
	2 m post-CV	4.17 (3.03~5.73) [a]	4 (2.78~5.74) [a]	6.75 (4.78~9.52) [a]
	3 m post-CV (1 m post-PV)	4.56 (3.51~5.92) [a]	3.88 (2.73~5.52) [a]	6.87 (4.89~9.66) [a]
	8 m post-CV	5.52 (4.41~6.9) [a]	4.07 (3.19~5.19) [a]	4.64 (3.60~5.98) [a,d]
14	Pre	5.27 (3.76~7.37)	5.1 (3.40~7.66)	4.67 (2.75~7.94)
	2 m post-CV	25.31 (16.79~38.14) [a,b]	42.44 (23.41~76.95) [a]	52.11 (31.41~86.45) [a]
	3 m post-CV (1 m post-PV)	23.14 (15.96~33.55) [a,c]	42.76 (23.98~76.24) [a]	53.86 (34.11~85.05) [a]
	8 m post-CV	17.73 (12.68~24.79) [a,c]	28.66 (17.18~47.81) [a]	37.8 (24.99~57.18) [a,d]

CI: confidence interval, Pre: prevaccination. CV: pneumococcal conjugate vaccine. PV: pneumococcal polysaccharide vaccine. [a] $P < 0.01$ (vs Prevaccination), [b] $P < 0.05$ (vs group III), [c] $P < 0.01$ (vs group III), [d] n=23 in group III.

Table 3. Comparison of geometric mean opsonophagocytic killing (OPK) titers of sera from HIV-1-infected and HIV-uninfected adults before vaccination and after vaccination.

Serotype	Time point	GM OPK titer (95% CI) of sera from: Group I (n = 30) HIV-1+, CD4 :200-499/μl	Group II (n = 28) HIV-1+, CD4 ≧ 500/μl	Group III (n = 29) HIV-
4	Pre	4.29 (3.72~4.94)	4 (4~4)	4.62 (3.76~5.66)
	2 m post-CV	13.93 (7.78~24.94) [a,b]	32 (19.08~53.68) [a,c]	75.66 (45.15~126.77) [a]
	3 m post-CV (1 m post-PV)	13.61 (7.91~23.41) [a,b,e]	37.12 (22.21~62.07) [a]	73.87 (45.71~119.36) [a]
	8 m post-CV	6.81 (4.61~10.04) [b,d,e]	18.11 (10.18~32.22) [a]	44.58 (26.55~74.84) [a,g]
14	Pre	7.29 (5.10~10.43)	12.8 (7.52~21.82)	12.01 (6.94~20.78)
	2 m post-CV	41.26 (19.57~87.01) [a,c]	107.63 (51.49~225.02) [a]	275.03 (173.88~435.03) [a]
	3 m post-CV (1 m post-PV)	43.21 (20.42~91.46) [a,b]	118.84 (57.32~246.38) [a]	281.68 (181.69~436.72) [a]
	8 m post-CV	13.61 (7.62~24.3) [b,e]	47.55 (22.52~100.42) [d]	116.94 (69.96~195.45) [a,f,g]

CI: confidence interval, Pre: prevaccination, CV: pneumococcal conjugate vaccine, PV:pneumococcal polysaccharide vaccine. [a] $P < 0.01$ (vs prevaccination), [b] $P < 0.01$ (vs group III), [c] $P < 0.05$ (vs group III), [d] $P < 0.05$ (vs prevaccination), [e] $P < 0.05$(vs group II), [f] $P < 0.05$(vs 2 months and 3 months post-CV), [g] n=23 in group III.

Table 4. Comparison of geometric mean concentration (GMC) of serotype-specific IgG required for 50% killing (OPK) titers of sera from HIV-1-infected and HIV-uninfected adults before vaccination and after vaccination.

Serotype	Time point	GMC of IgG (ng/ml) required for 50% killing (95% CI) [no. of subjects evaluated] in sera from:		
		Group I (n = 30) HIV-1+, CD4 :200-499/μl	Group II (n = 28) HIV-1+, CD4 ≧ 500/μl	Group III (n = 29) HIV-
4	Pre	284 (NA) [1]	NA (NA) [0]	179 (9~3492) [3]
	2 m post-CV	136 (80~234) [14]	102 (68~155) [23]	72 (52~100) [26]
	3 m post-CV (1 m post-PV)	137 (80~234) [15]	79 (56~111) [23]	76 (55~106) [26]
	8 m post-CV	201 (57~706) [7]	86 (46~161) [16]	96 (56~163) [22] [a]
14	Pre	441 (236~825) [11]	248 (139~441) [14]	307 (149~631) [15]
	2 m post-CV	302 (182~501) [19]	279 (204~382) [23]	176 (101~305) [28]
	3 m post-CV (1 m post-PV)	229 (142~372) [19]	246 (163~369) [23]	174 (106~288) [28]
	8 m post-CV	601 (364~992) [14] [b]	377(269~529) [18]	316 (207~481) [22] [a]

CI: confidence interval, Pre: prevaccination, CV: pneumococcal conjugate vaccine, PV:pneumococcal polysaccharide vaccine, NA: Not available. [a] n=23 in group III, [b] $P < 0.05$ (vs 3 m post-CV).