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愛滋病毒感染病患肺炎雙球菌疫苗接種後抗體效價反應之前瞻性研究：著重在使用抗愛滋病毒藥物使用後之影響

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A Prospective Study of Anti-capsular Antibody Responses to Pneumococcal Vaccination among HIV-infected Persons: Emphasis on Impact of Highly Active Anti-Retroviral Therapy

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Abstract

A 4-year prospective longitudinal follow-up study was conducted to assess the immunogenicity of 23-valent pneumococcal polysaccharide vaccine (PPV) among 305 HIV-1 infected individuals who received PPV and HAART between June 2000 and June 2004. 89 randomly selected vaccinees were stratified into 3 groups based on their CD4 counts at baseline: Group 1 with CD4 counts less than 100 cells/μl; Group 2 with CD4 counts between 100 and 350 cells/μl; and Group 3 with CD4 counts higher than 350 cells/μl. Antibody responses to 3 capsular antigens (14, 19F, and 23F) were performed using home-made ELISA. Changes in these parameters from baseline to week 4, and every 6 months thereafter were determined and compared using Student's *t-test*. Rates of response to vaccination in different groups of vaccinees were compared using χ^2 test. Vaccinees with more than 2-fold increase in antibody responses were defined as vaccine responders. We found that immunization did not cause any significant changes in CD4 counts or plasma HIV-RNA load between vaccinees of different groups. For type 14, both the percentage of responders (27% v.s. 57% v.s. 54%) and the geometric mean fold-rise of reactive antibody (1.38 v.s. 2.89 v.s. 3.05) were significantly lower for Group 1 compared with Groups 2 and 3. The duration of antibody responses in responders was similar between groups. Among the 24 responders who had been followed for more than 3 years, more than 75% of them remained seropositive for type 14 irrespective of their baseline CD4 counts. Our findings suggest that antibody responses to 23-valent PPV are poorer in HIV-infected vaccinees with baseline CD4 counts less than 100 cells/μl compared with those with baseline CD4 counts higher than 100 cells/μl. The responses may last for more than 3 years in 75% of the vaccine responders who continue to receive HAART regardless of baseline $CD4^+$ counts.

Patients with HIV infection are at higher risk for invasive infections due to *Streptococcus pneumoniae* as compared with those without HIV infection. Rates of invasive pneumococcal infections among AIDS patients may be as high as 100-fold greater than in HIV-seronegative controls [1]. In the era of increasing rates of antimicrobial resistance among isolates of *S. pneumoniae* worldwide as well as an increased risk for developing invasive pneumococcal infections among HIV-infected patients, the US Centers for Disease Control and Prevention (CDC) has strongly recommended that pneumococcal vaccination be given to patients with HIV infection who are aged ≥2 years [2] and a CD4+ count ≥200 cells/μl [3]. In addition, revaccination is recommended when the CD4+ count increases to ≥200 cells/μl in patients with an initial CD4+ count of <200 cells/μl [3].

However, these recommendations are notwithstanding, clinical studies evaluating the benefits of pneumococcal vaccination among HIV-infected patients have yielded

inconsistent results [4-7]. This inconsistency may be due to variations in the study populations or designs, the receipt of antimicrobial prophylaxis or antiretroviral therapy, or study end points and outcome measures. In retrospective studies conducted in the US [4,6,7], vaccination reduced the risk for invasive pneumococcal infections among HIV-infected patients before or after the introduction of highly active antiretroviral therapy (HAART). In Uganda where HIV-infected patients had no access to antiretroviral therapy (ART) [5], immunization with the 23-valent capsular polysaccharide pneumococcal vaccine (PPV) paradoxically increased the incidence of invasive pneumococcal infections and all-cause pneumonia. The reasons for such failure of pneumococcal vaccination to confer protection against invasive pneumococcal infections in Ugandan subjects remains unclear, although destruction of B-cell function resulting from polysaccharide vaccination in those patients without HAART or an increase of plasma HIV load has been proposed [5]. There remain several potential explanations for the failure of the immunity of HIV-infected patients to protect them from pneumococcal infections, including decreased serum opsonic activity against *S. pneumoniae* in HIV-infected patients [8], and a local effect of altered pulmonary immunoglobulin responses to penumococcal infection [9].

In the previous study [10], we have evaluated the impact of vaccination with the 23-valent PPV on HIV-infected patients who were receiving HAART. Our data suggested that vaccination with 23-valent PPV reduced risks for pneumococcal diseases and was associated with a lower risk for death among HIV-1-infected patients receiving HAART. Vaccination did not increase the risks of all-cause community-acquired pneumonia and HIV progression, and CD4+ continued to increase and PVL decrease among HIV-infected patients receiving HAART. Despite the clinical benefits, the serologic responses to the 23-valent PPV has rarely been studied among HIV-infected patients receiving HAART; whether antibody responses increase with immune reconstitution and when the booster vaccination should be administered in HIV-infected patients receiving HAART remain to be studied. In this study, we aim to evaluate the serial changes of anti-capsular antibodies in HIV-infected patients who underwent vaccination with 23-valent PPV and received HAART with increase of CD4+ counts.

Materials and Methods

Patients

Between June 2000 and June 2003, 350 HIV-infected patients who were followed at the National Taiwan University Hospital were immunized with the 23-valent PPV following the recommendations of the CDC of the US. Sequential blood specimens were collected while the patients returned for routine examinations for plasma viral load and CD4+ lymphocyte count every 4-6 months. The blood specimens were stored at -70°C until determinations of anti-capsular antibody titers are performed. As of December 2004, it was estimated that the average number of blood specimens collected from each subject is 9. We will continue to collect blood specimens for routine PVL and CD4+ testing to determine anti-capsular antibody titers over the next one year. In addition, we will give booster vaccination to those whose CD4 count was 100 cells/μl or less at enrollment and to those who responded poorly to pneumococcal vaccination. The study is approved by the Institutional Review Board of the hospital, and every vaccinee gave written informed consent.

Based on the CD4+ lymphocyte count within 3 months of pneumococcal vaccination, three categories of patients are defined: vaccinees with CD4+<100 cells/μl; vaccinees with 100-350 cells/μl; and vaccinees with ≥350 cells/μl. The patients who have been followed for more than 5 years and respond poorly, booster vaccination will be administered. In each category of CD4+ lymphocyte count, serial blood specimens collected from each patient will be subjected to determinations of anti-capsular antibodies. Patients will continue their routine follow-up at the outpatient clinics for antiretroviral therapy. Respiratory or other relevant clinical specimens will be collected for bacteriologic studies if patients present with symptoms suggestive of infections. Radiographic studies and prescription of antibacterials are at the discretion of treating physicians.

Sera

Sera obtained before and after vaccination of HIV infected individuals with 23-valent pneumococcal vaccine will be separated from clotted blood by centrifugation and stored at –70°C. Before ELISA assay, sera will be adsorbed with cell wall polysaccharide (CWPS). Briefly, 1 ml of serum is mixed with 10 μg of CWPS and incubated at room temperature on a rocking platform for 30 min. Sera from patients with AIDS who had no antibody to *S. pneumoniae* serotypes 14, 19F, 23F, or 6B capsules and <1 μg of antibody to CWPS per ml were used as negative controls.

Determinations of anti-capsular antibody titers

The determination of anti-capsular antibody levels among serially collected blood specimens from each enrolled vaccinee will be carried out with ELISA by following the methods described previously with minor modifications [11-13]. Capsular polysaccharide from *S. pneumoniae* serotypes 14, 19F, 23F, or 6B will be obtained from the American Type Culture Collection (ATCC). These capsular polysaccharide or CWPS will be suspended in phosphate-buffered saline (PBS, pH7.4) at concentration of 12.5 μg/ml and used directly to coat wells by incubation at 4°C overnight. After washing, blocking will be done with PBS containing 1% of bovine serum albumin at 4°C overnight. Duplicate samples of sera will be studied in 1:100,

1:300, and 1:900 dilutions, a laboratory reference standard for each serotype that contains known amount of IgG reactive with specific capsular polysaccharide will be included in each plate as a positive control. Following washing, this first antibody incubation will be performed at 37°C for 2h. After thorough washing of unbounded antibodies to wells, Horseradish Peroxidase (HRP)-conjugated goat antibody to human IgG at 1:2000 dilution will be used to detect IgG, and the reaction will be developed 10 min. at dark by addition of K-blue substrate, followed by adding 1N sulfuric acid to stop the reaction. All washings between each incubation will be done with PBS buffer containing 0.05% Tween20. Optical density will be read in an ELISA reader at 450 nm, with subtraction of optical density of the appropriate blank.

Results

Baseline characteristics of the 89 subjects undergoing pneumococcal vaccination and serial determinations of anti-capsular antibody titers are shown in Table 1. Antibody responses to 23-valent PPV are poorer in HIV-infected vaccinees with baseline CD4+ counts of 100 cells/μl or less compared with those with baseline CD4+ counts higher than 100 cells/μl. The percentage of responders to PPS 14 was higher than those to PPS 19F or 23F (52% vs. 25% vs. 10%). (Table 2). For PPS 14, both the percentage of responders (27% vs. 57% vs. 54%) and the geometric mean fold-rise of reactive antibody (1.38 vs. 2.89 vs. 3.05) were significantly lower in Group 1 compared with Groups 2 and 3 (Table 3). The duration of antibody response was similar between groups (Table 4). Among the 24 responders who had been followed up for more than 3 years, more than 75% of them remained seropositive for PPS 14 irrespective of their baseline CD4+ counts. The responses may last for more than 3 years in 75% of the observed responders who continue to receive HAART regardless of baseline CD4+ counts (Table 4).

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Table 1. Baseline characteristics of the randomly selected 89 HIV-infected patients from 350 HIV-infected vaccines between 2000 and 2003

	Group 1	Group 2	Group 3
Patients no.	15	35	
Sex (male %)	87	88	95
Age (median, [range])	39 (27-67)	40 (24-58)	
Risk factors (%)			
Homosex/bisexual	40	44	56
heterosexual	43	56	41
Intravenous drug use	0	0	0
Unknown	0	0	3
CD4, cells/μl (median, [range])	55 (2-99)	203 (100-339)	
PVL, copies/ml (median, [range])	400 [50-589506]	400 [50-750,000]	400 [50-180,000]
Follow up (median, [range]) (d)	877 [207-1,080]	940 [335-1,509]	938 [302-1,499]
Interval between HAART and PPV (month)	6 [2-40]	14 [1-15]	26 [1-56]

Abbreviations: HAART: highly active antiretroviral therapy; PVL: plasma HIV-RNA load by RT-PCR; PPV: pneumococcal vaccination

Table 2. Percentage of responders to 23-valent polysaccharide pneumococcal vaccination

	Group 1 (CD4<100 cells/μl)	Group 2 (CD4, 100-350 cells/μl)	Group 3 (CD4≥350 cells/μl)
Type 14	27	57*	54*
Type 19	20	31	21
Type 23	0	17*	8*
Any	47	54	50

NOTE: Responders are defined as persons who become seropositive for specific anti-capsular IgG or have more than 2-folds increase in antibody titer.

*: statistically significantly different ($p<0.05$) from Group 1 by chi-square test

*: statistically significantly different ($p<0.05$) from Group 1 by chi-square test

Table 4. Duration of antibody responses in responders of different groups

	>6 months	>1 year	>2 years	>3 years	>4 years
Group 1	4/4	4/4	4/4	3/4	NA
Group 2	18/20	18/18	15/16	11/11	3/3
Group 3	20/22	17/19	16/16	8/9	4/4

Numbers of observed responders with reactive antibody responses/numbers of responders observed

NA: not available

Figure 1. Number of subjects undergoing serial determinations of anti-capsular antibody responses following pneumocccal vaccination

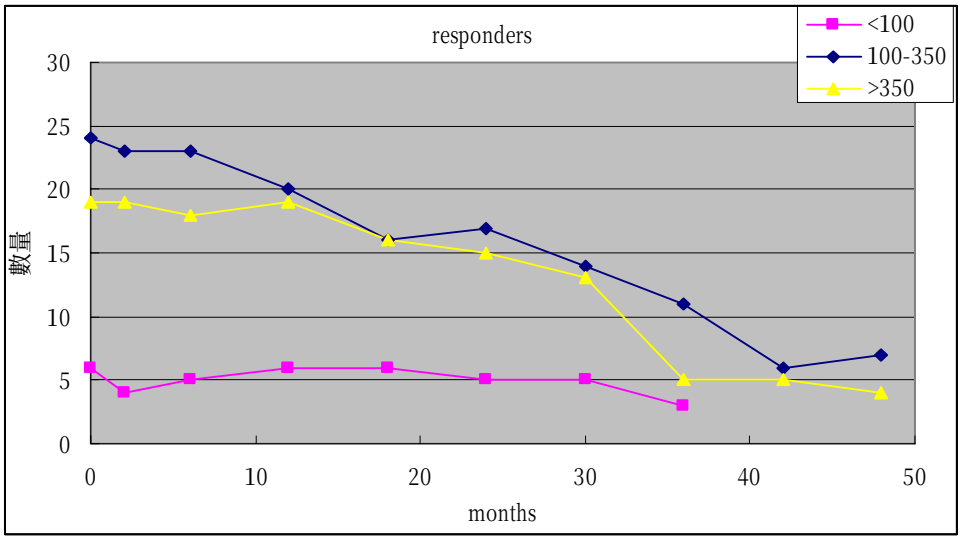

Figure 2. Sequential changes of fold-dilution of anti-capsular antibody following pneumococcal vaccination among vaccine responders with three different categories of CD4 lymphocyte counts.