Responsiveness of Human Immunodeficiency Virus Type 1–Infected Kenyan Women with or without Prior Pneumococcal Disease to Pneumococcal Vaccine

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In East Africa, *Streptococcus pneumoniae* is a common and serious, but potentially preventable, human immunodeficiency virus type 1 (HIV-1)–associated pathogen. For 54 HIV-1–infected women, baseline levels of capsule-specific antibody to 2 of 4 pneumococcal serotypes were lower than levels in 15 seronegative women ($P < .05$). After immunization, specific antibody to all 4 serotypes increased in HIV-1–infected and –uninfected women ($P < .05$). Convalescent levels for 2 of 4 serotypes were greater in seronegative women, but the levels were not different between HIV-1–infected women with ($n = 21$) or without ($n = 33$) prior invasive pneumococcal disease. The baseline functional activity to kill *S. pneumoniae* type 14 was lower in HIV-1–infected than –uninfected women but also rose significantly in all groups after immunization. It is concluded that HIV-1 infection in Kenyan women is associated with decreased levels of natural antibody to selected pneumococcal capsular serotypes, but the vaccine is immunogenic in these patients who are at high risk of invasive pneumococcal disease.

The infections that complicate human immunodeficiency virus type-1 (HIV-1) disease in Africa are quite different than those in the United States and Europe. *Streptococcus pneumoniae* infection rather than *Pneumocystis* infection is prominent, as are *Salmonella* infections other than those due to *S. typhi,* tuberculosis, and herpes zoster [1, 2]. In Kenya, invasive pneumococcal disease affects 4% of HIV-1–infected women per year (compared with 1% in the US) and has a recurrence rate of 26% [2, 3]. Thus, the number of cases of pneumococcal disease in HIV-1–infected persons in Africa is far greater than that in industrialized nations. When a disease is so prevalent and resources are few, as in Africa, preventative measures, particularly immunization, are likely to be cost-effective. Because the polysaccharide capsule of *S. pneumoniae* is its primary virulence factor and because antibodies to the capsule are the primary mechanism of defense against this organism [4], we measured levels of capsule-specific IgG in baseline sera and in response to pneumococcal polysaccharide capsule vaccine in HIV-1–seronegative and –infected women in Kenya, including infected women who had recovered from invasive *S. pneumoniae* infection.

Methods

Study population. We investigated women from a homogeneous, prospectively monitored population of poor commercial sex workers living in the same lower-class suburb in Nairobi [2, 5]. Three groups were examined: 15 HIV-1–seronegative women, 33 HIV-1–infected women without known prior pneumococcal disease, and 21 HIV-1–infected women who had recovered from invasive pneumococcal disease [2]. Pneumococcal disease was defined as radiographically confirmed pneumonia or sinusitis, primary bacteremia, or otitis media, each in association with a credible clinical syndrome and positive culture for *S. pneumoniae.* None of the women who were considered to be free from pneumococcal disease had had symptoms or signs of or therapy for pneumococcal disease within the preceding 2.8 years of observation. Of the women with invasive pneumococcal disease, 10 were bacteremic and 7 had had several episodes. The mean time free from invasive disease until immunization was 10.3 months (range, 2–25). Sera were drawn prior to and 1 month after immunization with pneumococcal vaccine (PNU-immune 23; Lederle [American Cyanamid], Pearl River, NY) containing 25 μg each of 23 capsular polysaccharide serotypes and stored at −70°C until testing.

Capsule-specific IgG by ELISA. Levels of pneumococcal capsule–specific IgG were measured as previously described [6], using purified pneumococcal vaccine polysaccharides (5 μg/mL; American Type Culture Collection, Rockville, MD; serotypes 1, 6B 14, and 19F) as the capture antigen. Consistent with previous results in East African adults, the 4 capsular types tested comprised one-third (22/68) of all invasive disease in the cohort [2]. Type 1 was most common (10 isolates), group 19 second (6 isolates), and types 6 and 14 ranked seventh (both with 3 isolates). Sera were adsorbed with cell wall polysaccharide (50 μg/mL) to remove

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noncapsular immunoglobulin, and 6 serial dilutions were tested in triplicate. Horseradish peroxidase–labeled affinity-purified goat anti-human IgG served as the detector antibody. Standard (CDC/FDA 89SF) and control sera were included on each plate. The coefficient of variation was <12% for all serotypes.

S. pneumoniae killing assay. In a modification of previous methods [7–10], a fixed bacterial inoculum of *S. pneumoniae* type 14 (10^3; ATCC 6314) was incubated in microwells with serial 1:2 dilutions of heat-inactivated human sera (56°C for 30 min.), 0.2 mg of complement (Baby Rabbit; Cedarlane Laboratories, Hornby, Canada), and 4×10^5 phagocytic cells, HL-60, a myelomonocytic cell line, differentiated to a granulocyte line using dimethylformamide (120 m*M*) [11], yielding a granulocyte effector–to–bacterial target ratio of 400:1. After rotation at 37° for 60 min, samples were plated and incubated on blood agar plates. Results are reported as end-point titers showing 50% of the organisms killed, compared with the number of organisms after incubation with fetal calf serum, complement, and effector cells. The mean percent kill of control sera was 70% ± 6% over 15 runs.

Statistics. Differences in levels of capsule-specific IgG and killing activity in serum were compared among clinical groups by analysis of variance (for 3 groups) and unpaired two-tailed *t* test (2-group comparisons) and within individual groups (acute vs. convalescent) by paired two-tailed *t* test, using the Stat-View 4.01 statistical package (Abacus Concepts, Berkeley, CA). Fold rises were determined by dividing each subject's postimmunization levels by their preimmunization levels.

Results

CD4 cell counts and baseline levels of pneumococcal capsule-specific IgG. At the time of immunization, the mean (± SE) CD4 T cell count in HIV-1–seronegative women was 929 ± 68 cells/μL; in the HIV-1–infected women without known prior pneumococcal disease, it was 330 ± 37 cells/μL, and in those with prior pneumococcal infection it was 189 ± 37 cells/μL. Despite the lower number of CD4 T cells in the HIV-1–infected patients with previous pneumococcal disease, baseline levels of capsule-specific IgG were similar to those in women without known prior infection. In contrast, baseline levels of capsule-specific IgG were lower for 2 of 4 common serotypes (14 and 19F) among these 2 groups of HIV-1–infected women compared with those in seronegative women ($P < .05$; table 1).

Response to vaccine. One month after immunization, levels of capsule-specific IgG in serum increased significantly in each group for all serotypes ($P < .05$; table 1). However, these levels were greater for 3 of 4 serotypes (1, 6B, and 14) in the seronegative group than they were in either of the HIV-1–infected groups ($P < .05$). Despite significant differences in CD4 T cell counts between HIV-1–infected patients with and without prior pneumococcal disease, levels of specific IgG were similar after immunization for each serotype.

Serum killing activity. The functional activity of serum antibodies to kill the organism in vitro correlated with levels of capsule-specific IgG measured by ELISA for 1 representative capsular type tested, type 14 ($R^2 = .51$, $P < .02$; figure 1A). Preimmunization levels were significantly lower in both HIV-1–infected groups compared with levels in the seronegative group. Similar to results by ELISA, all groups showed a significant rise from baseline values in killing activity after immunization ($P < .001$; figure 1B), but postimmunization levels were lower in those who had recovered from a pneumococcal disease ($P < .03$).

Discussion

Pneumococcal disease commonly complicates HIV-1 infection in persons in East Africa and worldwide [2, 12]. Recommendations for use of pneumococcal vaccine in this compromised population [13, 14] are predicated on the assumptions that low levels of antibody specific for the common capsular serotypes predispose to infection and that the vaccine elicits significant increases in these antibodies. We have demonstrated that HIV-1 infection in Kenyan women is associated with decreased levels of natural antibody to selected pneumococcal

Table 1. IgG-specific response to pneumococcal capsular polysaccharides in HIV-1–infected and –seronegative women, Nairobi, Kenya, 1989–1992.

	HIV-1–seronegative controls ($n = 15$)			HIV-1–infected controls ($n = 33$)			HIV-1–infected persons after pneumococcal disease ($n = 21$)		
Capsular type	Before immunization	After immunization	Fold rise	Before immunization	After immunization	Fold rise	Before immunization	After immunization	Fold rise
Type 1	4.1	19.9	4.4 (73)	2.9	10.0	3.7 (67)	3.5	6.3*	2.8 (52)
Type 6B	16.1	47.1	2.7 (73)	13.1	22.3*	2.0 (52)	9.8	23.8*	2.0 (67)
Type 14	11.8	48.3	4.9 (80)	4.2*	28.4*	7.0 (79)	2.4*	18.2*	4.2 (71)
Type 19F	13.7	29.4	2.3 (67)	6.5*	22.6	2.9 (70)	8.6	17.9	2.7 (62)

NOTE. Before and after responses are in μg/mL; fold rise responses are median (% ⩾ 2).

For each serotype in each group, capsule-specific IgG levels increased significantly 1 month after immunization compared with preimmunization levels ($P < .05$; paired *t* test).

* $P < .05$ compared with HIV-1–seronegative control subjects.

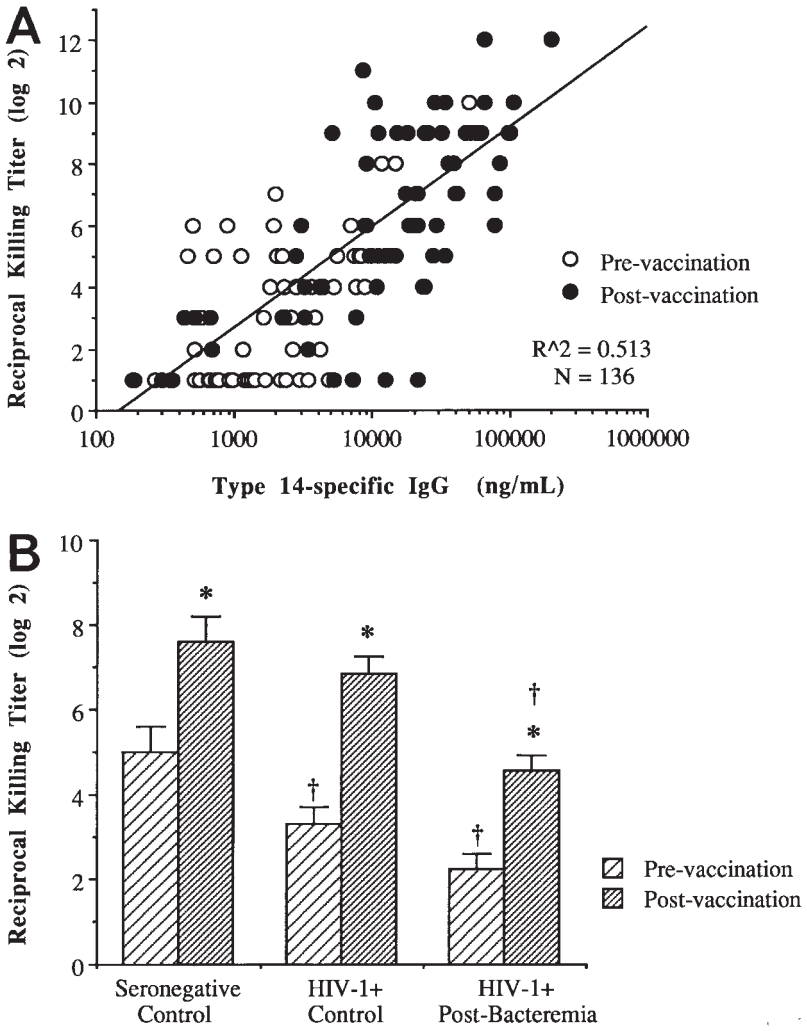

Figure 1. **A**, Relationship between levels of serotype 14 capsule-specific IgG by ELISA and functional activity of sera to kill organism in vitro in presence of standard source of complement and phagocytic cells. Sera were obtained from 54 HIV-1–infected patients and 15 seronegative control subjects prior to and 1 month after immunization with 23-valent pneumococcal vaccine. **B**, Killing activity of sera from HIV-1–seronegative control subjects ($n = 15$), HIV-1–infected patients who had ($n = 21$) and had not ($n = 33$) previously had pneumococcal disease. Results are reciprocal titer (inverse of maximum $\log_2$ dilution) of heat-activated sera that gave >50% kill in presence of standard source of complement and phagocytic cells. *$P < .001$ compared with preimmunization level; †$P < .03$ compared with corresponding values in HIV-1–seronegative subjects.

capsular serotypes. The serotypes tested were those commonly causing disease in Kenya [2]. Other factors likely also contribute to the increased risk of *S. pneumoniae* disease among these HIV-1–infected patients. In this regard, we have recently shown that levels of antibody to pneumolysin, the major pneumococcal toxin associated with tissue invasion and the development of bacteremia, are also depressed during HIV-1 infection [15]. Moreover, defects in mucosal defense, phagocytic cell function, and complement activation, all of which are required for protection against the organism, may also be impaired [12].

Regardless of other host defense mechanisms, antibodies to the bacteria's polysaccharide capsule are considered the principal mechanism of protection against this invasive organism. We have shown that, despite the presence of HIV-1 infection or the development of invasive pneumococcal disease prior to immunization, all groups showed a significant rise in capsule-specific antibodies following immunization. For 1 serotype tested, these antibodies showed functional activity to kill the organism, and this killing increased significantly after immunization in all patient groups. Pneumococcal vaccine may be most appropriate for women with prior *S. pneumoniae* disease, whose risk of recurrent disease is quite high (26% in this population) [2, 3]. Recurrences are most often due to different serotypes, and these patients generate a significant response to immunization that is similar to that of HIV-1–infected women without known prior disease. Determining the duration of these responses will help to establish the vaccine's clinical benefit. Indeed, the critical issue remains whether the vaccine is effective (and cost-effective) in HIV-1–infected patients, a population at increased risk of invasive pneumococcal disease, in East Africa and worldwide.

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References

1. Gilks CF, Brindle RJ, Otieno LS, et al. Life-threatening bacteraemia in HIV-1 seropositive adults admitted to hospital in Nairobi, Kenya. Lancet **1990**;336:545–9.
2. Gilks CF, Ojoo SA, Ojoo JC, et al. Invasive pneumococcal disease in a cohort of predominantly HIV-1 infected female sex-workers in Nairobi, Kenya. Lancet **1996**;347:718–23.
3. Redd SC, Rutherford GW, Sande MA, et al. The role of human immunodeficiency virus in pneumococcal bacteremia in San Francisco residents. J Infect Dis **1990**;162:1012–7.
4. Bruyn GAW, Zegers BJM, van Furth R. Mechanisms of host defense against infection with *Streptococcus pneumoniae*. Clin Infect Dis **1992**; 14:251–62.
5. Simonsen JN, Plummer FA, Ngugi EN, et al. HIV infection among lower socioeconomic strata prostitutes in Nairobi. AIDS **1990**;4:139–44.
6. Carson PJ, Schut RL, Simpson ML, O'Brien J, Janoff EN. Antibody class and subclass responses to pneumococcal polysaccharides following immunization of human immunodeficiency virus–infected patients. J Infect Dis **1995**;172:340–5.
7. Gray BM. Opsonophagocidal activity in sera from infants and children immunized with *Haemophilus influenzae* type b conjugate vaccine (meningococcal protein conjugate). Pediatrics **1990**;85:694–7.
8. Miculek S, Anderson EL, Gray BM. Opsonophagocidal antibodies to pneumococcus group 6 in infants and children given a new polysaccharide conjugate vaccine. Clin Res **1991**;39:842A.
9. Janoff EN, O'Brien J, Thompson P, et al. *Streptococcus pneumoniae* colonization, bacteremia, and immune response among persons with human immunodeficiency virus infection. J Infect Dis **1993**;167:49–56.
10. Romero-Steiner S, Pais L, Holder P, Carlone GM, Keyserling HL. Opsonophagocytosis of *Streptococcus pneumoniae* as an indicator of functional antibody activity in adults vaccinated with a 23-valent polysaccharide vaccine (abstract E42). In: Proceedings of the 95th annual meeting of the American Society for Microbiology (Washington, DC). Washington, DC: ASM, **1995**.
11. Skubitz KM, Zhen Y, August JT. Dexamethasone synergistically induces chemotactic peptide receptor expression in HL-60 cells. Blood **1984**; 59:596–3.
12. Janoff EN, Breiman RF, Daley CL, Hopewell PC. Pneumococcal disease during HIV infection. Epidemiologic, clinical, and immunologic perspectives. Ann Intern Med **1992**;117:314–24.
13. American College of Physicians Task Force on Adult Immunization, Infectious Diseases Society of America. Guide for adult immunization. 3rd ed. Philadelphia: American College of Physicians, **1994**:50–9.
14. Salisbury DM, Begg NT. Pneumococcal vaccination in 1996. Immunisation against infectious diseases. London: Her Majesty's Stationery Office, **1996**:169-72.
15. Amdahl BM, Rubins JB, Daley CL, Gilks CF, Hopewell PC, Janoff EN. Impaired natural immunity to pneumolysin during human immunodeficiency virus infection in the United States and Africa. Am J Respir Crit Care Med **1995**;152:2000–4.