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Pneumococcal conjugate vaccine is immunogenic in lung fluid of HIV-infected and immunocompetent adults

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To the Editor

Protein conjugated pneumococcal polysaccharide vaccines have been highly effective (80% to 97%) at preventing vaccine-serotype invasive pneumococcal disease in several trials in the United States, South Africa, and Gambia.¹ Studies reporting all-cause radiologically diagnosed pneumonia similarly showed that vaccine caused a significant reduction in disease in South African and in Gambian children (17% and 37%, respectively). The lower measured efficacy of conjugate vaccination against pneumonia compared with invasive disease is of concern, however, because the burden of pneumonia is much greater than that of invasive disease, particularly in young children, the elderly,² and patients infected with HIV. Possible explanations of the difference include infection with other pathogens³ or a genuine lack of vaccine efficacy at the mucosal surface.

Pulmonary mucosal defense critically depends on alveolar macrophage ingestion of bacteria opsonized by local immunoglobulin.⁴ Failure of local immunoglobulin production in the lung has been associated with increased nosocomial pneumonia in ventilated patients in the intensive care unit, and inhaled delivery of supplementary mucosal immunoglobulin (IgG) has been shown to be protective against bacterial pneumonia in mice.⁵ Nasopharyngeal carriage and pneumococcal disease are immunizing events in human beings,⁶ and mucosal immunization using whole killed pneumococci was more effective than parenteral immunization in preventing pneumonia in animal models.

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We tested the hypothesis that conjugate vaccine offered less protection against pneumonia because of a reduced mucosal response to vaccine compared to vaccine responses measured in serum.

Adult Malawians were initially recruited by advertisement and gave consent to participation in a bronchoscopy study of pulmonary immune responses in HIV infection. Subsequently, a separate study of systemic responses to pneumococcal conjugate vaccine preceeding randomized controlled trial was approved. Individuals in the first study were given the opportunity to participate in the second by using separate written consent. These studies were both approved by the Liverpool School of Tropical Medicine Research Ethics Committee and the College of Medicine Research Ethics Committee of the University of Malawi.

Bronchoscopy with lavage was performed on all subjects as previously described.⁴ Briefly, a flexible bronchoscope was wedged in a subsegmental bronchus of the right middle lobe and 200 mL sterile saline introduced in 4 aliquots. Bronchoalveolar lavage (BAL) obtained by this method typically yielded 120 mL dilute cellular alveolar lung fluid. On the same visit, a venous blood sample was taken for CD4 measurement and serum storage. BAL supernatant and serum were stored at -80°C .

Pneumococcal capsule specific immunoglobulin concentrations were measured as previously described⁷ by ELISA at the Vaccine Immunology Laboratory, Helsinki, Finland. Serum samples were preadsorbed by using cell wall polysaccharide and type 22F polysaccharide adsorption (10 and 30 $\mu\text{g/mL}$ serum diluted 1:100, respectively) steps to remove nonspecific immunoglobulin. BAL samples were diluted 1:2 in a buffer containing 20 and 60 $\mu\text{g/mL}$ cell wall polysaccharide and 22F polysaccharide. Pneumococcal serotype-specific anticapsular IgG and IgA measurements were made for 4 vaccine serotypes (6B, 14, 19F, and 23F) chosen as being common pneumococcal types in our area. Laboratory investigators were blinded as to the HIV status and vaccination status of each subject.

Forty-one subjects (22 HIV-negative and 19 HIV-positive) were recruited to the study. No subject had a history of pneumonia, and all subjects had a normal chest x-ray. There were no significant differences in the clinical details between the vaccine and placebo groups for either HIV-infected or uninfected subjects, as shown in this article's Table E1 in the Online Repository at www.jacionline.org. None of the 19 HIV-infected subjects were being treated with antiretroviral therapy. There were 5 current and 1 cigarette exsmoker, but none had smoked more than 5 pack-years, and none more than 6 cigarettes per day currently. The mean BAL volume was 124 mL (range, 80-150 mL). The mean BAL cell yield was 1.4×10^7 cells (range, 4.4×10^6 to 4×10^7 cells) and was not significantly different between the groups. Minor postprocedure discomfort was reported after 27 of the 123 bronchoalveolar lavages and after vaccine/placebo injection in 17 subjects, but there were no serious side effects. HIV viral load in serum and BAL showed a transient increase after bronchoalveolar lavage in some placebo and vaccine recipients. This change was not statistically significant and is consistent with the known proinflammatory effects of lavage.⁸

Capsular type specific IgG concentrations for 4 serotypes in serum are shown in Table I and in BAL in Table II. There were no significant differences between the groups at baseline (*upper panels*). A significant difference was seen in all 4 vaccine types in both the HIV-negative and HIV-positive vaccine groups in serum and BAL when either 1-month or 6-month samples were compared with baseline. Lower fold increases in serum were seen in HIV-infected compared with uninfected subjects in all 4 serotypes. Pneumococcal capsule specific IgG levels showed that serum and BAL IgG levels had significant correlation at baseline and 1 and 6 months ($P < .0001$). There were no significant changes in immunoglobulin levels in placebo recipients. IgA data from BAL are provided in this article's Table E2 in the Online Repository at www.jacionline.org.

This study showed that 7-conjugate vaccine elicited an IgG response in both serum and lung compartments among both HIV-negative and HIV-positive subjects at 1 and 6 months. The relationship between serum and BAL levels of capsule-specific IgG both at baseline and at each time point after vaccination suggests that circulating IgG is transferred to the lung lining fluid. Our previous study of patients' postpneumococcal infection IgG levels in BAL showed that the pneumococcal capsule specific percentage of total IgG was higher in BAL than in serum, suggesting that local IgG production was important.⁹ This study does not necessarily contradict the previous finding, because the immunoglobulin response at mucosal surfaces is known to be influenced by the route of antigen presentation.¹⁰ We showed no relationship between IgG level and CD4 count in this study. This result is different from that in our larger study in Uganda, in which we have shown lower serum IgG responses to 7-valent vaccine in patients with AIDS.⁷ The relatively small numbers of participants in this study and the high CD4 counts in some of the HIV-infected bronchoscopy volunteers make detailed comparisons based on CD4 count impracticable in this study. The fold-increase in IgG concentration in BAL was less in HIV-infected subjects than uninfected subjects in this study, consistent with previous work.

This study shows an appropriate response to pneumococcal conjugate vaccine in lung fluid, which is encouraging for ongoing trials of pneumococcal conjugate vaccine to prevent pneumonia in adults. An intriguing and unanswered question is whether mucosal antigen presentation in the context of appropriate adjuvant would have an enhanced protective effect.
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Table ISerum IgG by HIV status and intervention group in $\mu\text{g/mL}$ *

Baseline [†]	HIV-negative			<i>P</i> value	HIV-positive			<i>P</i> value
	Placebo N = 12		Vaccine N = 10		Placebo N = 9		Vaccine N = 10	
6b	0.52 (0.16-1.70)		0.24 (0.09-0.68)	.31	0.26 (0.09-0.81)		0.25 (0.08-0.85)	.94
14	1.90 (0.90-4.20)		0.75 (0.23-2.4)	.14	1.28 (0.46-3.55)		0.71 (0.3-1.7)	.32
19f	0.88 (0.35-2.20)		0.65 (0.21-1.98)	.64	0.69 (0.23-2.1)		0.79 (0.21-2.91)	.86
23f	0.50 (0.16-1.53)		0.34 (0.12-0.96)	.58	0.19 (0.05-0.66)		0.49 (0.16-1.48)	.21
	Placebo	<i>P</i> value	Vaccine	<i>P</i> value	Placebo	<i>P</i> value	Vaccine	<i>P</i> value
1 Month [‡]								
6b	0.46 (0.14-1.50)	.29	4.68 (1.50-14.5)	<.0001	0.41 (0.19-0.96)	.12	1.89 (0.24-14.7)	.004
14	1.90 (0.70-4.80)	.97	39.0 (21.8-73.2)	.0001	2.02 (0.93-4.4)	.04	18.9 (7.84-45.7)	<.0001
19f	1.06 (0.43-2.62)	.43	8.30 (2.84-24.0)	.002	0.92 (0.35-2.00)	.03	5.76 (1.50-21.6)	.01
23f	0.65 (0.21-2.00)	.30	13.2 (5.40-32.2)	<.0001	0.26 (0.1-0.7)	.27	8.9 (2.47-32.3)	.0004
6 Month [‡]								
6b	0.62 (0.17-2.27)	.49	2.7 (0.80-9.1)	.0003	0.33 (0.11-0.94)	0.28	2.6 (0.45-14.5)	.0016
14	2.85 (0.96-8.50)	.08	19.9 (11.3-35.1)	.0006	1.76 (0.86-3.6)	0.15	6.6 (1.70-25.3)	.0001
19f	1.06 (0.36-0.18)	.17	5.8 (2.6-12.6)	.0014	0.73 (0.28-3.1)	0.33	5.7 (2.3-14.3)	.0001
23f	1.47 (0.42-5.04)	.02	8.1 (3.6-18.4)	<.0001	0.22 (0.05-0.92)	0.7	4.8 (1.3-17.9)	.0002

* Geometric mean and 95% CI for each value shown.

[†] Comparison of placebo and vaccine groups baseline values by nonpaired *t* test using log-transformed data shown to have a normal distribution.[‡] Comparison of response at 1 and 6 months with baseline values using paired *t* test on log-transformed data shown to have a normal distribution.

Table II

BAL IgG by HIV status and intervention group in ng/mL *

Baseline [†]	HIV-negative			<i>P</i> value	HIV-positive			<i>P</i> value
	Placebo N = 12		Vaccine N = 10		Placebo N = 8		Vaccine N = 10	
6b	0.18 (0.08-0.42)		0.15 (0.059-0.39)	.41	0.13 (0.09-0.19)		0.22 (0.08-0.58)	.47
14	0.28 (0.13-0.6)		0.23 (0.12-0.44)	.72	0.28 (0.1-0.81)		0.2 (0.12-0.33)	.79
19f	0.47 (0.3-0.7)		0.52 (0.21-1.28)	.74	0.40 (0.3-0.5)		0.66 (0.25-1.7)	.52
23f	0.37 (0.18-0.73)		0.22 (0.18-0.27)	.18	0.2 (0.2-0.2)		0.48 (0.17-1.4)	.08
	Placebo	<i>P</i> value	Vaccine	<i>P</i> value	Placebo	<i>P</i> value	Vaccine	<i>P</i> value
1 Month [‡]								
6b	0.22 (0.07-0.68)	.08	1.05 (1.6-7.0)	.017	0.11 (0.08-0.16)	.93	2.0 (0.18-22.6)	.028
14	0.42 (0.2-0.88)	.16	27.8 (6.8-114)	.007	0.34 (0.15-0.8)	.57	39.4 (14.4-108)	.008
19f	0.53 (0.33-0.85)	.56	6.9 (1.4-34.7)	.011	0.44 (0.26-0.78)	.32	5.3 (0.89-31.3)	.012
23f	0.41 (0.16-1.04)	.85	11.9 (2.2-64.8)	.008	0.22 (0.17-0.29)	.32	13.3 (2.6-68.1)	.009
6 Month [‡]								
6b	0.2 (0.05-0.73)	.92	0.38 (0.08-1.9)	.05	0.1 (0.1-0.1)	.16	1.9 (0.18-20.9)	.03
14	0.35 (0.09-1.30)	.16	6.7 (2.6-17.7)	.008	0.47 (0.16-1.39)	.57	5.1 (0.9-27.6)	.014
19f	0.41 (0.28-0.59)	.32	1.99 (0.59-6.7)	.018	0.48 (0.3-0.78)	.47	3.2 (0.88-11.7)	.02
23f	0.76 (0.18-3.13)	.05	4.2 (0.76-22.9)	.012	0.32 (0.15-0.7)	.16	5.5 (0.85-35.9)	.019

* Geometric mean and 95% CI for each value shown.

[†] Comparison of placebo and vaccine groups baseline values by nonpaired *t* test using log-transformed data shown to have a normal distribution.[‡] Comparison of response at 1 and 6 months compared with baseline values using paired *t* test on log-transformed data shown to have a normal distribution.