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Immune Response to *Haemophilus influenzae* Type b Vaccination in Patients with Chronic Renal Failure

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Adult chronic renal failure patients undergoing hemodialysis are at an increased risk of invasive *Haemophilus influenzae* type b (Hib) disease due to the lack of functionally active anti-Hib antibodies. The pediatric Hib polysaccharide-protein conjugate vaccine is highly immunogenic in these patients and can provide protection against invasive Hib infection for at least 1 year.

Haemophilus influenzae type b (Hib) was the major cause of bacterial meningitis in children worldwide before the introduction of the conjugate Hib vaccine in the late 1980s. Since then, the incidence of invasive Hib disease has dramatically declined in all countries where this vaccine was included into public immunization programs (13). The vaccine stimulates production of anti-Hib capsular polysaccharide antibodies, which trigger complement-dependent killing and phagocytosis of bacteria (17). In unvaccinated individuals, protection against Hib is mediated by natural antibodies induced by Hib carriage and exposure to cross-reactive bacteria (8, 12).

Chronic renal failure (CRF), i.e., the end-stage kidney disease requiring renal replacement therapy, is characterized by high mortality rates, especially in the elderly (6). Such patients have profound immune dysfunction due to both uremia and the dialysis procedure (10); infections are the second leading cause of death following cardiovascular disease (2, 14). Immunization with pneumococcal polysaccharide, influenza, hepatitis B virus (HBV), and varicella vaccines is recommended for adults undergoing dialysis; however, such patients are not routinely immunized against Hib (4). It is unknown whether adults with CRF are able to adequately respond to Hib vaccination, but previous studies found their poor response to HBV, even to increased vaccine doses (5, 9). When immunized with pneumococcal polysaccharide vaccine, such patients lose protective antibodies within 6 months postimmunization (4).

Thirty-four CRF patients undergoing hemodialysis (29 to 91 years old, median age of 65) and 19 healthy controls (24 to 80 years old, median age of 54) were given one dose of pediatric Hib conjugate vaccine (Act-Hib; Sanofi Pasteur). Blood was taken immediately before immunization and 4 weeks and 6, 9, and 12 months after. Serum antipolyribosylribitol phosphate (PRP) IgG antibody concentrations were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit (The Binding Site, Birmingham, United Kingdom). Serum anti-PRP IgM was quantified using a Hib IgG ELISA kit (IBL International, Hamburg, Germany) with the following modifications. Serum IgG was depleted using IgG/RF stripper (The Binding Site, Birmingham, United Kingdom), and goat anti-human IgM secondary antibody (SouthernBiotech, Birmingham, AL) was used in place of anti-human IgG. Antibody functional activity was measured with a serum bactericidal assay (SBA) as previously described (16). For statistical analysis, SBA titers below the low detection limit (of 8) were reported as 4. Geometric mean antibody concentrations (GMC), SBA geometric mean titers (GMT), and 95% confidence intervals (CI) were calculated and data were analyzed using the Mann-Whitney rank sum test, Wilcoxon matched pairs test, and Spearman's correlation.

In compliance with previous studies, we considered serum IgG anti-PRP levels as the major indicator of protection against Hib invasive disease (11). As antibody detected by ELISA may have different functional capabilities due to their specific chemical and genetic characteristics, the functional activity of anti-Hib antibody was measured using the SBA. Although present at lower concentrations than IgG, IgM has more potent complement-binding abilities and may account for a potential discordance between IgG antibody levels and SBA scores (18). Preimmunization, less than half of the patients (47%) had anti-Hib IgG antibody concentrations of $\geq$1.0 μg/ml and 82% had concentrations of $>$0.15 μg/ml; 29% of the group had detectable serum bactericidal activity (Table 1). Four weeks postimmunization, GMC of IgG antibody in both CRF patients and controls increased greater than 23-fold. As a result, 97% of patients and 95% of controls displayed IgG antibody concentrations of $\geq$1.0 μg/ml, with no statistical difference between the groups (Tables 1 and 2). Four weeks postimmunization, in CRF patients and healthy controls, a significant increase in SBA GMT (greater than 23- and 60-fold, respectively), as well as in a proportion of individuals with detectable serum bactericidal activity ($P < 0.0001$ and $P = 0.01$, respectively), was found, with higher GMT in controls ($P = 0.005$; Tables 1 and 2).

Although the baseline IgG antibody levels did not correlate with SBA titers, a strong correlation was detected between IgM antibody and SBA in CRF patients ($r = 0.651$, $P < 0.0001$), suggesting that in unvaccinated adults, IgM rather than IgG is largely responsible for serum bactericidal activity. In contrast, we de-

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TABLE 1 Anti-PRP antibody concentration and bactericidal activity following immunization with Act-HIB in patients with chronic renal failure[a]

		Anti-PRP IgG				SBA			Anti-PRP IgM	
Time since immunization	No. of subjects with chronic renal failure	GMC (μg/ml)	95% CI (μg/ml)	No. (%) of subjects with concn of >0.15 μg/ml	No. (%) of subjects with concn of ≥1.0 μg/ml	GMT (μg/ml)	95% CI (μg/ml)	No. (%) of subjects with ≥MDA	GMC (μg/ml)	95% CI (μg/ml)
Baseline	34	0.64	0.38–1.10	28 (82.4)	16 (47.1)	13.59	6.85–26.95	10 (29.4)	0.09	0.05–0.15
Four wk	32	15.2	8.77–26.37	31 (96.9)	31 (96.9)	324.9	160.8–656.2	29 (90.6)		
P value		<0.0001				<0.0001				
Six mo	31	6.45	3.60–11.56	30 (96.8)	28 (90.3)	114.5	53.83–243.4	24 (77.4)		
P value		<0.0001				<0.0001				
Nine mo	30	4.51	2.78–7.32	30 (100)	26 (86.7)	55.72	23.62–131.4	19 (63.3)		
P value		<0.0001				0.0079				
One yr	26	6.31	3.50–11.38	26 (100)	24 (92.3)	124.6	57.95–268	22 (84.6)		
P value		<0.0001				0.0003				

[a] GMC, geometric mean concentration; CI, confidence interval; SBA, serum bactericidal assay; GMT, geometric mean titer; MDA, minimum detectable activity. *P* values are compared to baseline.

tected a correlation between postimmunization IgG antibody and SBA in CRF patients ($r = 0.426$, $P = 0.0076$) and controls ($r = 0.435$, $P = 0.035$), emphasizing the importance of IgG antibodies generated as a result of isotype switching, in protection of vaccinated individuals against Hib (3).

Following a peak at 4 weeks postimmunization, anti-PRP IgG concentrations declined by 6 months ($P < 0.0001$) but then remained at similar levels at 9 and 12 months (Table 1). One year postimmunization, IgG antibody levels were still significantly higher than baseline GMC ($P < 0.0001$), and 92% of patients had ≥1.0 μg/ml of anti-PRP IgG (Table 1). Similar to IgG antibody, SBA titers also reached their peak 4 weeks postvaccination, and their largest decline occurred between 4 weeks and 6 months ($P = 0.0021$). Nevertheless, until the end of observation, SBA GMT was still significantly higher than the baseline ($P = 0.0003$), and bactericidal activity was detectable in almost 85% of patients (Table 1). The decline in anti-Hib postimmunization antibody levels was not as dramatic as those reported in CRF patients following HBV and pneumococcal polysaccharide vaccination (9, 19). Apparently, the response of CRF patients to Hib conjugate vaccine was superior to that of other immunizations. This could be due to the capacity of protein-conjugated vaccines to recruit T helper cells that provide costimulatory signals to B cells producing anticapsular polysaccharide antibodies (1).

Both IgG antibody levels and their functional activity detected 1 year after vaccination strongly correlated with the corresponding parameters at 4 weeks postimmunization (IgG, $r = 0.906$ and $P < 0.0001$; SBA, $r = 0.771$ and $P < 0.0001$), suggesting that the magnitude of the peak vaccine response may predict its longevity. While a further decline in antibody levels beyond 1 year of observation is possible, a postimmunization decrease in circulating anti-Hib antibodies may not necessarily indicate a loss of clinical protection, because conjugate vaccines induce immunological memory, ensuring long-lasting protection (1). Previous studies suggested the retention of clinical protection against invasive Hib in infants despite a decline in antibody levels following vaccination (7). In conclusion, adult CRF patients undergoing hemodialysis are capable of developing protective antibody response to one dose of pediatric conjugate Hib vaccine comparable to that of healthy adults. Considering that Hib is still present in countries with universal pediatric anti-Hib immunization (15), this study

TABLE 2 Anti-PRP antibody concentration and bactericidal activity following immunization with Act-HIB in control group[a]

		Anti-PRP IgG				SBA			Anti-PRP IgM	
Time since immunization	No. of subjects in the control group	GMC (μg/ml)	95% CI (μg/ml)	No. (%) of subjects with concn of >0.15 μg/ml	No. (%) of subjects with concn of ≥1.0 μg/ml	GMT (μg/ml)	95% CI (μg/ml)	No. (%) of subjects with ≥MDA	GMC (μg/ml)	95% CI (μg/ml)
Baseline	19	0.9	0.49–1.66	17 (89.5)	10 (52.6)	22.22	7.80–63.30	8 (42.1)	0.12	0.07–0.23
Four wk	19	21.28	10.71–42.26	19 (100)	18 (94.7)	1,341[b]	741.1–2,426	19 (100)		
P value		<0.0001				0.0002				

[a] GMC, geometric mean concentration; CI, confidence interval; SBA, serum bactericidal assay; GMT, geometric mean titer; MDA, minimum detectable activity. *P* values are compared to baseline.

[b] One outlier was removed from this calculation: SBA titer of 16,284.

provides a rationale for immunization of adult CRF patients with conjugate Hib vaccine to achieve protective immunity.

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