Immunoglobulin G Avidities in Infants in Mexico after Primary Immunization with Three Doses of Polysorbylribitol Phosphate-Tetanus Toxoid Haemophilus influenzae Type b Vaccine

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Serum immunoglobulin G concentrations and avidities specific to Haemophilus influenzae type b (Hib) were measured in 208 children living in Guadalajara and Mexico City. Protective concentrations were found in 98.9% and 100.0% of participants, respectively. Geometric mean concentrations differed between both populations and/or among age groups. Mean avidities differed only among the 7- to 12-month-old children. Diphtheria–tetanus–whole-cell pertussis–hepatitis B–Hib primary vaccination seems to induce protection in Mexican children.

Immune response to Haemophilus influenzae type b (Hib) conjugate vaccines is usually evaluated by measuring serum anti-polysorbylribitol phosphate (PRP) antibodies and/or avidity (2, 12). PRP conjugates elicit high-avidity antibodies, with increased functional activity (2, 12, 26).

Avidity of anti-PRP immunoglobulin G (IgG) has been assessed by enzyme-linked immunosorbent assay (ELISA)-based elution assays (1, 12, 22, 24–26), radioisotopic antiglobulin binding assay, and competitive inhibition (21, 27).

Although anti-PRP IgG antibodies show heterogeneous avidities among vaccinees (1), in vitro antibody functionality correlates directly with avidity (25, 27); however, the relationship with long-term protection is unclear (18).

The diphtheria–tetanus–whole-cell pertussis–hepatitis B–Hib vaccine was introduced into the Mexican infant immunization program in 1999. There is no information on the qualitative features of IgG antibodies in Mexican children after vaccination. The aim of this study was to assess the avidity of anti-PRP IgG antibodies in vaccinated Mexican children.

Sera from 208 healthy Mexican children of ages from 7 to 180 months were collected after signed informed consent. All children were formerly immunized with the DTP-HB/Hib vaccine (GlaxoSmithKline Beecham, Middlesex, United Kingdom) at 2, 4, and 6 months of age. At the sampling time, 115 children resided in Mexico City and 93 in Guadalajara. Children were arbitrarily stratified into four age groups: 7 to 12, 13 to 24, 25 to 48, and 49 to 180 months.

Concentrations and avidities of anti-PRP IgG were assessed on serially diluted sera by using ELISA and ELISA-based elution assays with 0.15 M sodium thiocyanate (NaSCN) (2, 13, 26), PSAB-90 serum was used as a quality control. Data for IgG concentrations from Mexico City sera have been previously described (13).

Concentrations were calculated against the FDA-1983 standard curve and log transformed for comparisons by parametric tests. The significance level was set at a P value of <0.05. Almost all children had protective IgG levels against Hib disease (16). Children from Guadalajara had a higher geometric mean concentration (GMC) than those from Mexico City (Table 1). Such difference was an age-related effect.

The overall GMC observed for the 7- to 12-month-old group decreased nearly twofold in the 13- to 24-month-old group and remained at this level until the fourth year of life. An increase of 2.5-fold in GMC was observed for the 49- to 180-month-old group (Fig. 1). A similar trend was seen for age-stratified sera from Mexico City (13). In contrast, the GMCs of sera from Guadalajara increased in an age-dependent manner (Table 2).

For avidity estimations, parallelism between curves of NaSCN-treated (NaSCN+) and untreated (NaSCN−) series was verified (3, 23). IgG concentrations were determined and relative avidity (RA) values were calculated by the following equation: RA = (IgG concentration NaSCN+/IgG concentration NaSCN−) × 100. The RA represents the remaining IgG concentration in the presence of thiocyanate. These data were contrasted by using nonparametric tests. Seventeen data points for the FDA-1983 serum (mean ± 2 standard deviations) were used to define the moderate-avidity range. Samples showing RA values of <68.1%, between 68.1 and 75.8%, or >75.8% were categorized as low-, moderate-, or high-avidity sera, respectively.
The RA data ranged between 45.2 and 99.4%. No correlation between anti-PRP IgG concentrations and RAs was found (Spearman's rho 0.091). Nearly 80% of sera had moderate- to high-avidity antibodies. Mean RAs were similar in sera from both populations, i.e., from Mexico City and Guadalajara (Table 1).

Minor increments in mean RA up to 48 months were observed for the age-stratified whole population (Fig. 1). Sera from children living in Mexico City showed an increase in mean RAs with increasing age (P < 0.01) (Table 2). In contrast, children aged 25 months or older from Guadalajara displayed mean RAs with a nonsignificant increasing trend (Table 2). There was a significant (P < 0.01) (Table 2) difference in the mean RAs for the youngest age group (7 to 12 months).

For comparison purposes, optical density (OD)-based RA values (RAOD, RAOD = [OD NaSCN−/OD NaSCN−] × 100) were calculated. A high linear correlation between pairwise RA and RAOD data was observed (r = 0.965; P < 0.01), although RAOD data were significantly higher (P < 0.01, Wilcoxon signed-rank test).

Analyses were performed with Office Excel 2003 (Microsoft Corp.), SPSS 10.0 (SPSS Inc.; Plover, WI), and/or Ascent 2.6 (Thermo Electron Corp., Vantaa, Finland) software.

Early development of an adequate quantity and quality of antibody responses to Hib during infancy is more critical in countries or ethnic groups in which the highest attack rates occur at earlier ages (17, 20, 28, 31). The vaccinees of this study had protective concentrations of anti-PRP IgG antibodies, though no booster dose had been given at 18 months of age and in some instances up to 174 months had elapsed since their primary vaccination. The overall GMC observed in this study was similar to the postprimary GMCs observed in the United States and Germany (24).

The differing GMCs between children from Mexico City and those from Guadalajara could be attributed to differences in natural boosting, given the discrepancy in the average age of the two study populations (4, 17, 19). Besides, the immunoepidemiologies of Hib can differ among the subpopulations of a country (10).

Although this was a cross-sectional study, the GMC decline observed after the first year of age was comparable to the responses seen in previous studies assessing PRP or other bacterial polysaccharide conjugates (5, 9, 19, 29, 30). The increasing IgG concentration after the fourth year of age could be related to the maximum carriage rates of Hib (4, 6).

Although antibody concentrations per se do not substantiate

### Table 1. Antibody concentrations and avidities of anti-Hib polysaccharide in vaccinated Mexican children

| Population group (no. of children) | Anti-PRP IgG concn (µg/ml) | No. (%) with concn of: | Relative avidity (%) | No. (%) with indicated avidity |
|------------------------------------|-----------------------------|------------------------|----------------------|------------------------------|
|                                   | Range GMC (CI95%)            | ≥0.15                 | ≥1.0                 | ≥5.0                         |
| Mexico City (115)                 | 0.24–54.64                  | 115 (100.0)           | 80 (69.6)            | 16 (13.9)                    |
| Guadalajara (93)                  | 0.13–40.16                  | 92 (98.9)             | 86 (92.5)            | 37 (39.8)                    |
| Total (208)                       | 0.13–54.6                   | 207 (99.5)            | 166 (79.8)           | 53 (25.5)                    |

a Sera were collected at Instituto Nacional de Pediatria in Mexico City and at Centro Medico de Occidente of Guadalajara.
b Student’s t test contrasting IgG data from Mexico City and from Guadalajara. P < 0.01.
c Fisher’s exact or χ2 tests comparing serum proportions between Mexico City and Guadalajara. P value, not significant (P > 0.05).
d Fisher’s exact or χ2 tests comparing serum proportions between Mexico City and Guadalajara. P < 0.01.
e Mann-Whitney U test contrasting the antibody avidities from Mexico City and Guadalajara. P value, not significant (P > 0.05).

FIG. 1. Avidities and concentrations of IgG anti-PRP antibodies in sera from 208 age-stratified children. *no significant avidity differences among age groups as assessed by the Kruskal-Wallis test; †concentrations of the 13- to 24- and 25- to 48-month-old groups were significantly lower than the concentration of the 49- to 180-month-old group (P < 0.05 by the Bonferroni test in one-way analysis of variance). Bars are the 95% level confidence interval for the mean (CI95%). mo, month.
TABLE 2. Concentration and avidity of antibodies by population and age groups

| Age group (mo) and location | Avg age (mo) | No. of subjects | Anti-PRP IgG concn (µg/ml) | Relative avidity (%) |
|-----------------------------|-------------|----------------|---------------------------|----------------------|
|                             | GMC         | CI95%          | Range                     | Mean                 |
|                             |             |                |                           | CI95%                |
| 7–12                        | Mexico City | 9              | 4.05                      | 1.19–13.79           | 0.26–33.98           | 64.08 | 56.95–73.20 | 45.22–80.08 |
|                             | Guadalajara | 14             | 2.50                      | 1.13–5.50            | 0.13–33.80           | 80.15 | 73.25–87.06 | 60.77–97.63 |
| 13–24                       | Mexico City | 34             | 1.55                      | 1.12–2.15            | 0.28–11.11           | 74.95 | 71.30–78.59 | 50.83–93.81 |
|                             | Guadalajara | 8              | 2.76                      | 1.58–4.83            | 0.87–6.73            | 83.08 | 73.92–92.23 | 66.51–94.66 |
| 25–48                       | Mexico City | 50             | 1.44                      | 1.12–1.84            | 0.24–54.64           | 79.70 | 76.70–82.71 | 49.41–95.57 |
|                             | Guadalajara | 15             | 3.09                      | 1.86–5.16            | 0.48–10.65           | 78.77 | 72.19–85.36 | 58.32–97.67 |
| 49–180                      | Mexico City | 56             | 4.96                      | 3.89–6.32            | 0.61–40.16           | 80.93 | 76.37–85.49 | 53.19–93.74 |
|                             | Guadalajara | 22             | 2.45                      | 1.49–4.04            | 0.43–29.34           | 78.52 | 75.47–81.56 | 50.41–99.42 |

a Sera were arbitrarily stratified according to ages of children.
b P values for the comparison (Mann-Whitney U test) between values for subjects from Mexico City and those from Guadalajara were as follows: for the 7- to 12-month age group, <0.05; for the 13- to 24-month age group, <0.05; for the 25- to 48-month age group, not significant (NS); for the 49- to 180-month age group, <0.01.

* P values for the comparison (Student’s t test contrasting log-transformed data) between values for subjects from Mexico City and those from Guadalajara were as follows: for the 7- to 12-month age group, NS; for the 13- to 24-month age group, NS; for the 25- to 48-month age group, <0.01; for the 25- to 48-month age group, <0.05.

** P values for the comparison (Mann-Whitney U test) between values for subjects from Mexico City and those from Guadalajara were as follows: for the 7- to 12-month age group, <0.05; for the 13- to 24-month age group, NS; for the 25- to 48-month age group, not significant (NS); for the 25- to 48-month age group, not significant (NS); for the 49- to 180-month age group, not significant (NS).

Such an avidity increment could be ascribed to vaccine reaction of earlier colonization-induced memory B cells (15). In fact, postprimary avidity maturation has been described (12, 22), although IgG concentrations tend to wane over time (14).

Notwithstanding the fact that schemes including booster doses are recommended for other countries (especially if DTaP is used, e.g., as in England), our findings support the use of the DTwP-HB/Hib primary scheme alone. Moreover, these data provide a reference framework for other developing countries, where higher rates of colonization are likely to occur. Until now, Hib vaccination has proved to be highly efficacious in controlling invasive disease in third world countries (www.HibAction.org). Whether widespread vaccination reduces the background incidence of Hib disease and/or carriage in this setting remains to be determined.

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