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Antibody levels to *Bordetella pertussis* in 10-yr-old children with atopy and atopic asthma

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Suboptimal immune responses to vaccination have been suggested among atopic infants. The aim of this study was to assess the influence of atopy and atopic asthma on the humoral response to *Bordetella pertussis* vaccination. Immunoglobulin (Ig)G and IgA specific antibodies were measured by enzyme linked-immunosorbent assay in 102, 10-yr-old atopic children (66 of them also being asthmatics) and compared with 76 non-atopic and 53 non-atopic non-asthmatic controls of similar age. The levels of antibodies and the percentage of positives to *B. pertussis* were comparable in all groups. Children with a very high total serum immunoglobulin (Ig)E (Percentile (Pct) > 90th) showed higher ($p = 0.01$) IgG pertussis antibodies than children with very low serum IgE (Pct < 10th). In conclusion, we found normal pertussis antibody levels in atopic and in atopic asthmatic children in late childhood, thus overriding any possible suboptimal response during infancy.

Alfredo Blanco-Quiros[1], Luis Garcia-Marcos[2], Jose A. Garrote[1], Antonia E. Martinez-Torres[2] and Alberto Leon[1]

[1]Department of Pediatrics and Molecular Genetics and Biology Institute (IBGM) and Hospital Clinico, University of Valladolid, Valladolid, Spain, [2]Institute of Respiratory Health, University of Murcia, Murcia, Spain

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Luis García-Marcos, Institute of Respiratory Health, University of Murcia, Pabellón Docente HUVA, Campus Ciencias de la Salud, 30120 El Palmar, Murcia, Spain
Tel.: +34 968398129
Fax: +34 968398178
E-mail: lgmarcos@um.es

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The fact that the newborn immune system is skewed to the Th2 arm of the immune response is well established (1). The maturing of this system after birth seems mostly related to building an adequate Th1 response (2). There are some clues indicating that the definite balance between the Th1 and Th2 systems is determined by events taking place during the first months of life (3). On the contrary, T-helper cell activity in neonates and infants genetically predisposed to suffer from atopy is diminished as compared with normal controls, and the maturing of many functions of those cells (specifically those associated to the Th1 response) are slower (4, 5). All those findings suggest that children at risk of atopy may be less respondent to vaccines administered during infancy. However, the only study that tried to establish the possible difference of the immune response to diphteria–tetanus–pertussis vaccine between atopic and non-atopic children concluded that there is no such difference – 6-yr-old atopic children having similar levels of vaccine antigen-specific IgG and equivalent responses of their peripheral blood mononuclear cells to tetanus toxoid, as compared with age-matched controls. The study did not specify whether any of the children (either atopic or non-atopic) were asthmatic.

The aim of the present study was to detemine whether at the age of 10 there is a different level of pertussis-specific IgG and IgA between atopic and non-atopic children, and to determine whether inclusion of the asthmatic condition to the atopic condition has any effect on the levels of the specific immunoglobulins.

Methods

Patients

A total of 178 10-yr-old children (mean 10.0 ± 0.6 yr) were included in the study. Initially 89 children with asthma and 89 children without asthma were selected from the 1472

children who participated in phase II of the International Study of Asthma and Allergies in Childhood (ISAAC) study in Cartagena, Spain. The asthmatic children were all children previously diagnosed of asthma and having shown symptoms during the last year, and the non-asthmatic children were a random sample of the non-asthmatic children of the cohort. All of whom should have also had sera available. From those two groups the following subgroups were analyzed: atopics (n = 102), non-atopics (n = 76), atopic asthmatics (n = 66) and non-atopic non-asthmatics (n = 53). Atopic children fulfilled at least two out of the following three criteria: (i) Total IgE > 100 IU/ml (ii) Positive skin prick test to any allergen included in the prick-test panel and (iii) Phadiatop (Pharmacia, Uppsala, Sweden) >0.35 IU/ml. All children had received a whole cell pertussis vaccine inactivated with formol (4 IU) combined with diphteria (25 Lf) and tetanus (10 Lf) toxoid at 3, 5 and 7 months of age and a diphteria–tetanus (without pertussis) booster dose at 18 months of age.

The ethics committee from the Hospital 12 de Octubre (Madrid) approved the ISAAC phase II study for all the Spanish centers. Parents were informed and agreed that the skin prick test be performed to and that blood be extracted from their children.

Measurements

Skin prick tests. Tests were performed according to the ISAAC phase II protocol in the school setting. The allergen extracts tested (ALK-Abello, Horsholm, Denmark) were: *D. pteronyssinus*, *Dermatophagoides*, *D. farinae*, Cat, *Alternaria*, mixed trees (*Betula verrucosa*, *Alnus glutinosa* and *Corylus avellana*), mixed grasses (*Dactylis glomerata*, *Lolium perennae*, *Festuca pratensis*, *Poa pratensis*, *Phleum pratense* and *Avena eliator*), olive tree and *Parietaria*, plus positive (histamine 10 mg/ml) and negative controls. ALK lancets were used for the prick test. Children with histamine response <3 mm (wheal maximum diameter) were considered non-responders and they were excluded from the study. Skin test-positive subjects were defined as those who had at least one positive reaction (maximum wheal diameter measuring 3 mm or more after subtraction of the control values).

Serum IgE and Phadiatop^{TM}. The measurement of total and specific IgE antibodies was performed by an automatic assay based on the ImmunoCap^{TM} technology with allergens covalently bound to cellulose and activated by cyanide bromide (Pharmacia Diagnostics, Uppsala, Sweden). The Phadiatop^{TM} system is a screening methodology used for detecting IgE antibodies against a panel of the most common inhalant allergens (house dust mite, cat, dog, horse, cockroach, mould spores together with the most prevalent grass and tree pollens). Values >0.35 U/ml were considered positive, according to the manufacturer.

Pertussis antibodies. An enzyme linked-immunosorbent assay (ELISA) commercial assay (Nova-Tec; Immunodiagnostica GMBH, Dietzenbach, Germany), which detects antibodies against pertussis toxin and other pertussis antigens, was used. The optical density (OD) was recorded with a 450 nm length wave reader. Results were measured as OD and expressed as arbitrary units (a.u.) according to the instructions of the manufacturer. The cut-off value was calculated by adding 0.250 absorbance units to the mean OD value of two negative controls. Samples were considered positive if the absorbance was 10% above the cut-off value. The inter-assay and intra-assay coefficient of variation were 4.6% and 4.5%, respectively.

Statistical analysis

Antibody levels were expressed as medians and interquartiles. As the data was not normally distributed, the Mann–Whitney *U*-test was used to compare the differences between groups and the Spearman test to assess the correlation coefficients. The Fisher exact test was applied when assessing the differences between pertussis positive and pertussis negative children. A p-value <0.05 was considered significant (SPSS V12, Chicago, IL, USA).

Results

All four groups had similar concentrations of specific anti-pertussis IgG and IgA (Table 1).

According to the cut-off point established by the assay, 160/178 (89.9%) children were positive for IgG antibodies to *B. pertussis* and 8/178 (4.5%) were positive for IgA antibodies. All IgA positive children were also positive for IgG antibodies. The positivity among the atopic children for IgG and IgA was 93/102 (91.2%) and 5/102 (4.9%) respectively; and among the non-atopic children, the positivity was 73/76 (96.1%) and 7/76 (9.2%) respectively. Positivities among atopic asthmatics and non-topic non-asthmatics were 64/66 (97.0%) and 2/53 (96.2%)

Table 1. Serum immunoglobulin (Ig)G and IgA against *Bordetella pertussis* and total and specific IgE (Phadiatop™) in the different groups of children

Group	n	B.pertussis IgG	B.pertussis IgA	IgE IU/ml	Phadiatop™IU/ml
Atopics	102	17.8 (13.9–29.6)	5.9 (4.7–7.5)	186.0 (83.7–424.7)*	17.5 (3.6–68.1)*
Non-atopics	76	16.4 (13.3–26.6)	5.5 (4.7–7.1)	26.4 (10.4–54.5)	0.35 (0.35–0.35)
Atopic asthmatics	66	19.2 (14.1–31.6)	5.8 (4.7–7.0)	277.0 (114.3–558.5)*	26.4 (8.7–106)*
Non-atopic non-asthmatics	53	16.3 (13.9–27.4)	5.9 (4.8–7.1)	22.9 (8.4–45.1)	0.35 (0.35–0.35)

Median value and interquartiles into brackets.
B. pertussis antibodies are expressed in arbitrary units.
*$p < 0.001$ compared with the non-atopic and non-atopic non-asthmatic groups.

for IgG and 3/66 (4.5%) and 4/53 (7.5%) for IgA respectively. No statistically significant differences were found between these percentages.

The correlations between *B. pertussis* antibodies (IgG and IgA) and total IgE (Fig. 1) and specific antibodies (Phadiatop™) levels were not significant in any of the groups. The Spearman ρ between IgE and *B. pertussis* antibodies was the following: 0.05 ($p = 0.64$) for IgG and −0.05 ($p = 0.64$) for IgA among atopics, 0.22 ($p = 0.057$) and 0.05 ($p = 0.65$) among non-atopics, −0.10 ($p = 0.44$) and 0.01 ($p = 0.91$) among atopic asthmatics, and 0.23 ($p = 0.10$) and 0.05 ($p = 0.73$) among non-atopic non-asthmatics. The corresponding values for Phadiatop™ were 0.03 ($p = 0.77$) and −0.06 ($p = 0.54$) among atopics, and −0.13 ($p = 0.29$) and 0.02 ($p = 0.88$) among atopic asthmatics. All non-atopic children as well as all non-atopic non-asthmatic children had Phadiatop™ values of 0.35 (arbitrary negative value), so no correlations were computed between IgG and IgA and Phadiatop™ in those subgroups. Significant correlations were found between IgG and IgA anti-*B. pertussis* antibodies ($\rho = 0.2$, $p = 0.007$) and between serum total IgE and specific IgE antibodies (Phadiatop™) levels ($\rho = 0.75$, $p < 0.001$) in the whole population of children.

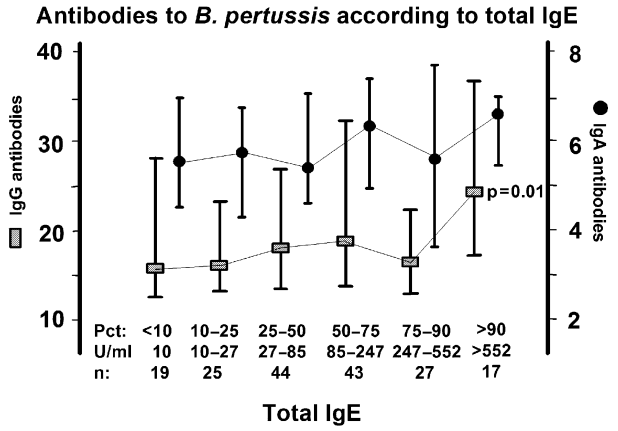

Fig. 1. Children with very high total immunoglobulin (Ig)E levels (Pct > 90) showed higher anti-*Bordetella pertussis* IgG antibodies than children with very low IgE levels (Pct < 10) ($p = 0.01$). No significant differences were found in any other centile. (Serum total IgE levels are expressed as percentiles and IU/ml. Total IgE was not available in three children).

Discussion

In this sample of 10-yr-old children, the levels of IgG against *B. pertussis* is similar in atopics and in atopic-asthmatics as compared with non-atopics and non-atopic non-asthmatics. Although some of the characteristics of the immune system of the child in the newborn and infant period suggest that atopic children might have a limited response to immunizations during the first months of life (4, 5), either this is very small or is well compensated during the following years, as has been previously shown by Prescott et al. (5) in children 5 yr old, and also as ratified by the results of the present study in older children. Repetitive natural contacts with environmental *B. pertussis* could also play a role in the 'updating' of the immnunoglobulin response to pertussis in atopic children. Additionally, no differences were found in IgA anti-pertussis, indicating that atopic children–either asthmatics or not-were not more prone to a recent *B. pertussis* infection.

The possible influence of *B. pertussis* vaccine on the development of atopy has been widely investigated. Previous reports postulated that the *B. pertussis* vaccine constitutes a risk factor for asthma and/or atopy when administered to young infants (6). A recent prospective study (7-yr follow up) did not confirm this association, although the children receiving the two component acellular vaccine showed a higher percentage of positive skin test to a panel of common allergens and a higher prevalence of allergic rhino-conjunctivitis (7, 8). An additional reason to suspect an association between atopy and *B. pertussis* vaccination is the high prevalence of IgE antibodies against *B. pertussis* in immunized children, the levels being higher in atopic children

and the origin of these IgE antibodies being related to the acellular nature of the vaccine, but not to the adjuvant (9). The whole cell vaccine, as the natural whooping cough disease, activates Th1 cells causing a relevant production of interferon-γ and a very low synthesis of interleukin (IL)-5, whereas the first dose of the acellular vaccine induces a mixed response, both Th1 and Th2 (10), causing a clear Th2 response when it is administered as a booster immunization (11).

Looking at this from the opposite angle, the influence of atopy on the response to *B. pertussis* has received much less attention. To the best of our knowledge, the only study yet published addressing this topic is that already quoted, by Holt et al. (4) who studied 25 atopic children 5 yr old. Although the mean value of anti-*B. pertussis* IgG was lower in the atopic than in the control children, the difference was not statistically significant. The immunization schedule of these patients with whole pertussis (2, 4, 6, 18 months and 5 yr of age) was very different to the schedule used on the children of the present study (3, 5 and 7 months). Moreover, the time gap between the immunization and the serological study (1 yr vs. 10 yr) was much higher in our study.

The high percentage (>90%) of children positive to *B. pertussis* found in the present study was striking because they had not received any pertussis dose since they were 7 months old. A much lower rate of positive antibodies against pertussis toxin (38.4%) and a somewhat higher (63.7%) rate against filamentous haemaglutinin has been reported recently in 13 to 19-yr old Spanish adolescents (12). Differences are probably because of technical reasons as the manufacturer establishes an arbitrary cut-off point. Besides, the ELISA method used in the present study detected antibodies against all kind of pertussis antigens. The high positivity rate suggests the existence of an important circulation of *B. pertussis* in our environment and a natural re-infection in children who could not have been totally immunized in the first months of life. Although children with very high IgG antibodies (>32.5 a.u.) (13, 14) or with a positive IgA might have suffered a recent pertussis infection, a relatively high proportion (*c.* 15%) of the children actually had a recent infection.

It would be too speculative to assume any degree of protection against *B. pertussis* in our population as there is no agreement on the levels and specificity of pertussis antibodies needed to achieve it. This objective was not addressed by the present study and a different sampling method, together with a specific study of antibodies against pertussis toxin, pertactin and filamentous haemaglutinin, would be required to clarify this.

In summary, serum IgG and IgA against *B. perussis* are similar in atopic, atopic-asthmatic and non-atopic children 10 yr of age who were immunized with a whole cell vaccine at 3, 5 and 7 months of age without having received any subsequent booster. Moreover, asthmatic children with a very high total IgE showed increased levels of IgG antibodies against *B. pertussis*.

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