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Chlorinated pool attendance, airway epithelium defects and the risks of allergic diseases in adolescents: Interrelationships revealed by circulating biomarkers

Alfred Bernard *, Marc Nickmilder, Xavier Dumont

Louvain Centre for Toxicology and Applied Pharmacology, Faculty of Medicine, Catholic University of Louvain, Brussels, Belgium

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ABSTRACT

It has been suggested that allergic diseases might be epithelial disorders driven by various environmental stressors but the epidemiological evidence supporting this concept is limited. In a cross-sectional study of 835 school adolescents (365 boys; mean age, 15.5 yr), we measured the serum concentrations of Club cell protein (CC16), surfactant-associated protein D (SP-D) and of total and aeroallergen-specific IgE. We used the serum CC16/SP-D concentration ratio as an index integrating changes in the permeability (SP-D) and secretory function (CC16) of the airway epithelium. In both sexes, early swimming in chlorinated pools emerged as the most consistent and strongest predictor of low CC16 and CC16/SP-D ratio in serum. Among girls, a low CC16/SP-D ratio was associated with increased odds (lowest vs. highest tertile) for pet sensitization (OR 2.97, 95% CI 1.19–8.22) and for hay fever in subjects sensitized to pollen (OR 4.12, 95% CI 1.28–14.4). Among boys, a low CC16/SP-D ratio was associated with increased odds for house-dust mite (HDM) sensitization (OR 2.01, 95% CI 1.11–3.73), for allergic rhinitis in subjects sensitized to HDM (OR 3.52, 95% CI 1.22–11.1) and for asthma in subjects sensitized to any aeroallergen (OR 3.38, 95% CI 1.17–11.0), HDM (OR 5.20, 95% CI 1.40–24.2) or pollen (OR 5.82, 95% CI 1.51–27.4). Odds for allergic sensitization or rhinitis also increased with increasing SP-D or decreasing CC16 in serum. Our findings support the hypothesis linking the development of allergic diseases to epithelial barrier defects due to host factors or environmental stressors such as early swimming in chlorinated pools.

1. Introduction

Over the recent decades, the prevalences of allergic diseases have increased in a dramatic way affecting now up to 20%–30% of the world population (Pawankar et al., 2013). As allergies have often their onset during childhood, the cause of this allergy epidemic must be linked to changes in the environment or lifestyle during early life. According to the classical hygiene hypothesis, this rise of allergies is due to the decreasing exposure of infants or children to some microbial agents, which would shift the immune system to a Th2 type response (Strachan, 1989). Initially focused on infectious microorganisms, the hygiene hypothesis was over time reformulated, extending the protection against allergies first to non-viable microbial products and now to the decreased biodiversity of commensal and environmental microbiota (Schaub et al., 2006; Haahtela et al., 2013). However, despite more than two decades of intensive research, the hygiene hypothesis and the concepts based on it have not yet resulted in effective prevention measures (Wahn, 2013).

While the hygiene hypothesis regards allergic diseases as allergen-driven disorders, another line of research proposes that allergic diseases primarily arise from functional and structural alterations of the respiratory epithelium caused by environment-related stressors (Holgate, 2011). While facilitating the penetration of harmful agents including allergens and virus, these alterations would create a local microenvironment favoring a sustained Th2-mediated inflammation leading to airways damage and remodeling (Holgate, 2011; Xiao et al., 2011). This hypothesis stems from the evidence that the epithelial barrier of asthmatics presents signs of injury and aberrant repair such as a loss of tight junctions, an increased leakiness or a depletion of Club cells (Holgate, 2011). These changes have been observed in patients with mild asthma, which suggests that they might be involved in the causation of the

Abbreviations: BMI, body mass index; CC16, Club cell protein; IgE, immunoglobulin E; CI, confidence interval; IQR, interquartile range

* Correspondence to: Louvain Centre for Toxicology and Applied Pharmacology, Catholic University of Louvain, Avenue E. Mounier 53.02, B-1200 Brussels, Belgium. Fax: +32 2 7645328.
E-mail address: Alfred.bernard@uclouvain.be (A. Bernard).

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disease and not be simply the consequence of longstanding inflammation (Fedorov et al., 2005). In a recent study among young schoolchildren, we also found evidence of an early implication of epithelium defects in the development of allergic diseases. In these children with no asthma diagnosis, alterations in nasal epithelium as manifested by decreased Club cell protein (CC16) and increased albumin concentrations in nasal lavage fluid, were associated with an higher risk of sensitization to house-dust mite (HDM) (Sardella et al. 2013). Of interest, altered serum levels of CC16 and other lung epithelium biomarkers have been described following exposure to airborne irritants that have been associated with an increased risk of respiratory or allergic diseases, such as tobacco smoke, ambient ozone, strenuous exercise or chlorination products (Bernard et al., 1992a; Broeckaert et al., 2000; Robin et al., 2002; Bernard et al., 2003; Lagerkvist et al., 2004; Bernard et al., 2007; Font-Ribera et al., 2010; Romberg et al., 2011; Jacobs et al., 2012; Fernández-Luna et al., 2013, 2015).

Epidemiological studies investigating the role of respiratory epithelium in allergic processes are hampered by two major difficulties. The first difficulty is to assess in a specific and minimally invasive way the integrity of the lung epithelium, which is particular challenging in non-adult populations. The second difficulty is to determine the direction of causality of associations between epithelium alterations and allergic diseases since the airways epithelium can be damaged by the allergic inflammation or the proteolytic activity of allergens (Vinhas et al., 2011; Wan et al., 1999). To address these issues, we evaluated the integrity of airway epithelium of adolescents by measuring in serum two lung epithelium-specific proteins (pneumoproteins), the Club cell protein (CC16), a marker of Club cell number and the surfactant-associated protein D (SP-D), a marker of the epithelial permeability (Hermans and Bernard, 1998, 1999). We calculated the serum CC16/SP-D ratio as an index integrating the changes in the permeability and secretory function of the airway epithelium. We used multivariate models to assess the associations of these biomarkers with the risks of allergic sensitization or diseases and a wide range of potential stressors related to the lifestyle or environment.

2. Materials and methods

2.1. Subjects

Participants were 835 secondary-school adolescents (470 girls) who were recruited in the frame of an epidemiological study exploring the impact of the environmental pollutants on the various target organs including the respiratory tract. As described previously (Bernard et al., 2008), adolescents were recruited from three secondary schools located in the French-speaking region of Belgium, in the cities of Louvain-la-Neuve, Bastogne and Lessines. Most students attending these schools were living in urban or semi-rural areas. The examination of students, performed in schools between 9:00 am and 4:00 pm, included the measurement of height and body weight and the collection of a blood sample for the determination of pneumoproteins and of total or specific IgE. Information about the adolescent's health and factors likely to influence the risks of allergic or respiratory diseases was obtained by a self-administered questionnaire filled by the parents. The questionnaire also comprised questions intended to calculate the cumulative attendance of chlorinated or non-chlorinated (one pool sanitized only by the copper–silver ionization system) swimming pools over lifetime or before the age of seven years. The initial cohort included 847 subjects (participation rate, 78%) but for the present study we excluded 12 participants with lacking information about the time of asthma diagnosis. Adolescents were examined with their assent and the written informed consent of their parents. The review board of the Catholic University of Louvain approved the study protocol.

2.2. Serum IgE and pneumoproteins

We measured the serum concentrations of total and aeroallergen-specific IgE using the Immulite IgE kits (Diagnostic Products Company, Los Angeles, CA, USA). Participants were tested for sensitization against most common inhalant allergens: house dust mite (HDM) (*Dermatophagoides pteronyssinus*); cat epithelium; dog dander; mould (*Penicilium notatum, Cladosporium herbarum, Aspergillus fumigata, Candida albicans and Alternaria tenui*); tree pollen mixture (*Alnus incana, Betula verrucosa, Corylus avellana, Quercus alba and Salix caprea*); grass pollen mixture (*Antoxanthum odoratum, Sacale cereale, Holcuns lanatus, Lolium perenne and Phleum pretense*); and herbaceous pollen mixture (*Chenopodium album, Solidago virgaurea, Urtica dioca, Artemisia absinthium and Artemisia vulgaris*). Sensitization was defined as a serum concentration of specific IgE > 0.35 kIU/L. We measured CC16 by an automated latex immunoassay, which was validated by comparison with a fluorescence enzyme immunoassay using monoclonal antibodies (Bernard et al., 1992b; Hermans et al., 1998). The serum concentration of SP-D was determined using a commercially available ELISA kit (code no. YSE-7744; Yamasa Corporation, Choshi, Japan). Serum creatinine was quantified by the Beckman Synchron CX5 Delta Clinical System (Beckman Coulter Inc., Fullerton, CA, USA).

2.3. Statistical analyses

Results are presented as percentage for categorical variables and as median with interquartile range for continuous variables. We used log transformation to normalize the distributions of body mass index (BMI) and of the serum concentrations of biomarkers. Boys and girls were compared using the χ^2 test for categorical and the Student's t test for continuous variables. We used backward stepwise regression analyses to identify factors influencing the serum concentrations of pneumoproteins. We tested a total of 33 variables including age, gender, parental allergies, exposure to tobacco smoke, chlorinated pool attendance (CPA) and a variety of other lifestyle- or environment-related potential predictors (see supplementary material). Because there were only 36 subjects who never visited chlorinated pools, we used quintiles to stratify the CPA over lifetime or before the age of seven years. For the attendance of the non-chlorinated pool sanitized only by the copper–silver method, we used as referents subjects who never attended this type of pool ($n=485$) and we stratified the attendees in tertiles for the lifetime attendance and by the median split for the attendance before the age seven years. We optimized the regression models by minimizing the Akaike information criterion. After adjustment for physiological confounders retained by the models, we compared serum pneumoproteins across quintiles of CPA by ANOVA and the Dunett-t post-hoc test. Associations of adjusted serum pneumoproteins with the risks of allergic sensitization or diseases were assessed by logistic regression models in which girls and boys were categorized in tertiles of descending CC16, ascending SP-D or descending CC16/SP-D ratio. We run these models by adjusting odds for parental allergies or asthma and for the total serum IgE concentration of adolescents. Trends in risks of allergic sensitization or diseases across tertiles of serum pneumoproteins were assessed using the Cochran Armitage test. Statistical analyses and graphs were made with the JMP® 10.0.0 from SAS Institute. All p values were two-sided with a level of statistical significance at < 0.05.