

There were a total of 153 patients between the ages of 4 and 24 months (mean $\pm$ SEM 15.8 $\pm$ 0.5), consisting of 41 (26.8%) patients in group 1 and 112 (73.2%) in group 2. A positive skin reaction to one or more of eight allergens (cow's milk, egg, cacao, mixed grass pollens, DP, DF, wool, cat) was found in 61 of the 102 children (59.8%). Of the 102 children tested, 24.8% were sensitive to house dust mites (DP or DF), 23.8% to cow's milk, 10.9% to mixed grass pollens, 7.9% to egg, 7.9% to wool, 5% to cacao, and 4% to cat antigen. Positive results of the skin tests that were 88.5% in group 2 and 58.5% in group 1 had significant difference (χ^2 10.645; SD 1; P 0.001). Among all possible risk factors only a positive skin test reaction had a significant effect on the increased number of wheezing attacks (P = 0.001). The risk of the children who were skin test positive, of having four or more attacks was 5.36 (OR 5.36; 95% CI 1.97–14.6) compared with the children who had negative skin test results. While 4.2% of the children who had positive skin reaction to cow's milk were included in group 1, the remaining 95.8% of the children were included in group 2 (P = 0.012) (Table 1). Positive reaction to cow's milk skin test was the most prognostic factor for recurrence of wheezing among other risk factors (P = 0.012).

These results suggest that antigen sensitivity, especially cow's milk allergy, in early infancy may be the most important risk factor, which increases the number of wheezing attacks and asthma. Our results also confirm the close relationship between food allergy and respiratory symptoms (3), and between cow's milk allergy and allergic diseases (4).

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Toll-like receptor-4 genotype in children with respiratory infections

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Key words: pneumonia; toll-like receptors; mannose; polymorphism. children.

Respiratory-tract infections are one of the leading causes of morbidity among children

< 5 years old. Susceptibility to infections includes environmental factors and genetically inherited risk

factors such as mannose-binding lectins and pattern recognition receptors (PRR) expressed by cells of innate defense.

Toll-like receptors constitute a distinct family of PRR mostly expressed on the cell surface of leukocytes that trigger cellular responses, including release of pro-inflammatory cytokines, after interaction with molecules of microbial origin (1). The toll-like receptor (TLR) family

member TLR4 mediates the cellular response to lipopolysaccharide (LPS), a component of the outer membrane of Gram bacteria, the most potent activator of innate immune response. Recently, it has been shown that a missense mutation (A896G) in the fourth exon of TLR4 leads to replacement of a conserved aspartic acid residue with glycine at amino acid 299 resulting in hyporesponsiveness of alveolar macrophages to LPS (2).

We aimed to investigate the possible role of TLR4 in susceptibility to pneumonia in Italian patients with a history of recurrent infections.

Between October 2001 and October 2002, 20 children with a history of recurrent infections were referred to the Immunology/Allergology Service of the Children's Hospital in Brescia. Criteria for admission to the study included age younger than 6 years, having presented with at least 12 episodes of ARI in the last year with or without a pneumonia episode, having normal levels of serum immunoglobulins, leukocyte subsets and complement proteins, and having given written informed consent as required by the Ethical Committee of Spedali Civili Hospital in Brescia.

Tlr4 genotyping was performed in 20 patients by polymerase chain reaction amplification of the fourth exon of the gene followed by direct sequencing of the DNA fragments. The mutations in the 299 and 399 codons, in linkage disequilibrium in our cohort, were detected in a heterozygous genotype in four of 20 children (carriage rate of 20%) with a history of recurrent respiratory infections at a higher frequency than observed in a large study on Italian healthy subjects (carriage rate of 6%) (3). Of seven patients with pneumonia, four were carriers of the TLR4 haplotype while none of the remaining 13 subjects with a exclusive history of respiratory infections presented the same polymorphism of the TLR4 gene suggesting that the TLR4 genotype is associated with susceptibility to pneumonia (P < 0.05, chi-squared test).

Recently, the observation that the Asp299Gly/Thr399Ile polymorphisms confer blunted response of epithelial cells and monocytes/macrophages to LPS has been challenged (4). Erridge et al.

reported normal responsiveness of monocytes with heterozygous mutations of TLR4 to endotoxin suggesting that other TLR4 ligands such as heat shock proteins, or defensins may display impaired binding to TLR4. Although the molecular mechanism remains undefined, our observation suggests that the TLR4 polymorphism may confer susceptibility to pneumonia in children.

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Anaphylaxis to boldo infusion, a herbal remedy

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Key words: anaphylaxis; boldo; SDS-PAGE; immunoblot.

Boldo (*Peumus boldus*) is a *Monimiaceae* family tree, native of the Andean regions used in natural medicine as a traditional herbal remedy because of its choleric, cholagogue, diuretic, antipyretic, anti-inflammatory, anthelmintic and hypnotic properties.

We report a case of an anaphylactic reaction (1) after the intake of a boldo infusion. A 30-year-old man with a personal history of allergic rhinoconjunctivitis because of hypersensitivity to grass pollen was attended for an immediate episode of an acute and generalized urticaria, facial angioedema, dysphagia, dysphonia and dyspnea after the intake of a boldo infu-

sion. He required an emergency treatment with subcutaneous adrenaline, parenteral antihistamines and corticosteroids.

Skin prick tests to common aeroallergens were positive to *Phleum* (8 mm wheal diameter) and *Cynodon* (7 mm wheal diameter) with a positive control wheal for histamine of 6 mm. Skin prick test with boldo infusion was negative. Oral administration started with one little spoon; the dose was doubled and at the end the intake to approximately 250 ml. The patient presented a good tolerance initially but 30 min after 250 ml of a boldo infusion, he showed an immediate response with pharyngeal pruritus, hives on neck and thorax and dysphonia, been treated with subcutaneous adrenaline.

We obtained a boldo extract after dialyzing against twice-distilled water, a concentration of 4 g of boldo in phosphate-buffered saline (PBS) 1 : 25 (w/v) for 24 h. It was then sterile-filtered and concentrated by centrifugation. The protein content measured by the Lowry–Biuret method was 15.6 mg/ml. SDS-PAGE as described by Laemmli was used to determine the antigenic profile, showing several proteins with a high molecular weight (Fig. 1). The gel was electrophoretically transferred to a nitrocellulose membrane (BIO-RAD®, Hercules, CA)

A traditional herbal remedy because of its choleric, cholagogue properties.

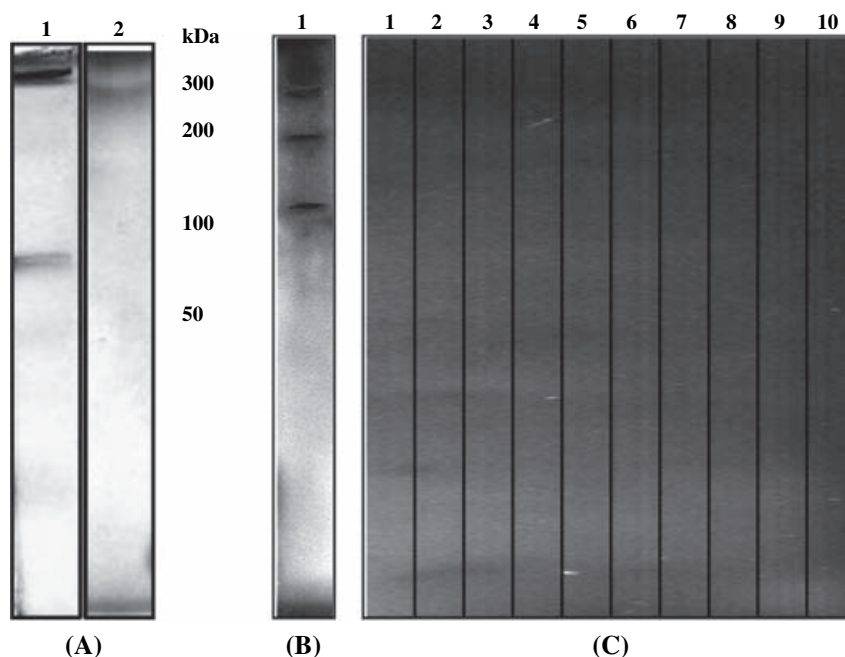


Figure 1. A, SDS-PAGE, silver stain, lane 1 molecular weights, lane 2 boldo extract; B, specific-IgE immunoblot, lane 1 boldo extract; C, negative controls.