

Toll-like receptor 2–positive and Toll-like receptor 4–positive cells in adenoids of children exposed to passive smoking

To the Editor:

The upper airway has a pivotal role in the first encounter of the immune system with antigens and pathogens. In particular, human nasopharyngeal tonsils (adenoids) have an important function in the protection against both bacterial and viral antigens that enter the nasal cavity.^{1,2} Active interaction with innate and adaptive immunity, as well as nonspecific mechanical factors, contribute to the prevention of microorganism invasion. This interaction is partly caused by recognition receptors (Toll-like receptors [TLRs]) that trigger innate immunity, both providing immediate protective responses against pathogens and instructing the adaptive immune response. Although most investigations on TLRs have focused on innate system cells, TLRs have also been described on T and B cells.^{3–5}

Several studies have examined the effect of cigarette smoking on the immune system, underlying the susceptibility of smokers to infections and allergic disease.^{6,7} Indeed, nicotine has been shown to influence cellular and humoral immunity.⁸ Furthermore, it is well recognized that the exposure to environmental tobacco smoke (ETS) in children is associated with an increased incidence of respiratory diseases such as upper and lower respiratory infections and asthma.^{9–11}

In the current study, we evaluate the expression of TLR2 and TLR4 in adenoidal T cells of children exposed to passive smoking.

Adenoids were obtained from 15 children undergoing adenoidectomy or adenotonsillectomy. Six were boys and 9 were girls, with age ranging from 3 to 14 years (median age, 5 years). Informed consent was obtained from patient parents, and the local Ethics Committee approved the study. Adenoid tissue was surgically removed for either adenoidal hyperplasia with secondary nasal obstruction or otitis media with effusion. Eight children were exposed to passive smoking, and 7 were not.

The diagnosis of adenoidal hyperplasia was based on visible obstruction of the nasopharynx with nasal endos-

copy and a history of obstructive symptoms (mouth breathing when awake and snoring during sleep). The diagnosis of otitis media with effusion was based on pneumatic otomicroscopy and a tympanometry showing a flat, Jerger-type B tympanogram. None of the children were treated with inhaled steroids or antihistamines for 6 weeks before surgery. The parents were questioned about the child's exposure to parental tobacco smoke. The subject's medical history, including allergic symptoms and the family history of atopic diseases, was evaluated as well. Adenoids were pressed through a filter, and mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation (Lymphoprep; AXIS-SCHIED, Oslo, Norway). CD4⁺ and CD8⁺ cells were evaluated for the expression of TLR2 and TLR4 by flow cytometry. Phenotypic analyses of the fluorescent stained cells were performed by 3-color flow cytometry using a Becton Dickinson FACScan flow cytometer (Becton Dickinson, Erembodege-Aalst, Belgium). A minimum of 30,000 lymphocyte-gated events was acquired in list mode and analyzed with Cell Quest software (Becton Dickinson; Fig 1). Results are expressed as percentage of CD4⁺ and CD8⁺ cells expressing TLR2 or TLR4. Appropriate isotype-matched controls were included. Tulic et al⁵ recently reported that TLR4 is expressed by CD3⁺ T cells in children's nasal mucosa; in our study, we found the presence of CD8⁺ cells expressing TLR4 in adenoids of children. A statistically significant decrease ($P = .009$) was observed in the percentage of TLR4-positive CD8⁺ cells from children exposed to parental smoke (median, 6; minimum, 4; maximum, 11) compared with children not exposed (median, 8.9; minimum, 7; maximum, 16.7; Fig 2). No difference was observed in the mean fluorescence intensity between the 2 groups. No difference was observed in the percentage of CD4⁺ cells expressing TLR4 and in the percentage of CD4⁺ and CD8⁺ cells expressing TLR2. Our results are in agreement with the data recently published by Tulic et al⁵ reporting that in young children, LPS induces the expression of a T-regulatory cell population via TLR4; this mechanism allows a normal maturation of the immune system. Therefore, the reduced expression of TLR4 that we found on cytotoxic T cells of children exposed to ETS may be one of

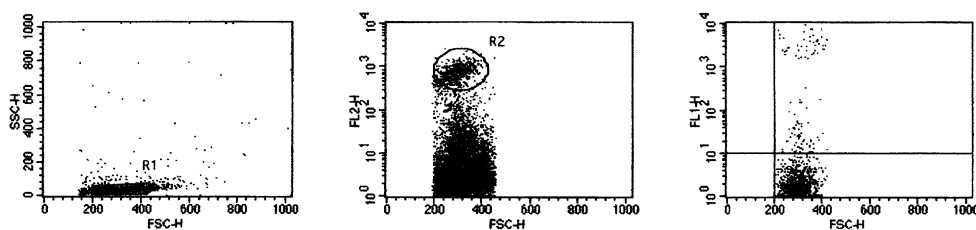


FIG 1. Phenotypic analysis of adenoidal T cells. In the *left panel*, lymphocytes are gated for forward and side scatter (R1). In the *middle panel*, cells are gated for CD8 positiveness (R2) after activation of R1 gate. In the *right panel*, TLR4⁺CD8⁺ cells are reported after activation of R3 = R1 and R2; quadrant markers are defined on appropriate isotype-matched control. All cells in the *upper right quadrant* were considered TLR4⁺CD8⁺. FSC-H, Forward scatter; SSC-H, side scatter; FL, fluorescence.

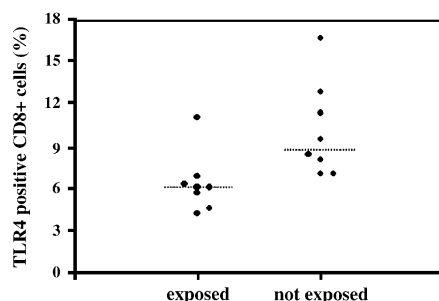


FIG 2. Percentage of TLR4⁺CD8⁺ cells in adenoids of children exposed to parental smoke compared with children not exposed. Medians are reported as horizontal lines. The statistical test used is the Mann-Whitney *U* test; *P* = .009.

the mechanisms involved either in the major susceptibility to upper airway infection or in the atopic risk observed in children exposed to ETS. Although obtained in a small number of subjects, these results suggest the crucial immunological function of TLRs in the upper airway.

Antonio Ricci, MD^a

M. Antonietta Avanzini, PhD^b

Cristina Scaramuzza, MD^a

Anna Maria Castellazzi, PhD^c

Massimo Marconi, PhD^c

Gian Luigi Marseglia, MD^c

^aDepartment of Pediatrics

Istituto di Ricerca a Carattere Scientifico Policlinico S. Matteo

P.le Golgi 19-27100 Pavia

Italy

^bResearch Laboratories

Istituto di Ricerca a Carattere Scientifico Policlinico S. Matteo

^cDepartment of Pediatrics

University of Pavia

Pavia, Italy

REFERENCES

- Brandtzaeg P, Jahnsen FL, Farstad IN. Immune functions and immunopathology of the mucosa of the upper respiratory pathways. *Acta Otolaryngol* 1996;116:149-59.
- Claeys S, de Belder T, Holtappels G, Gevaert P, Verhasselt B, van Cauwenberge P, et al. Human β -defensins and Toll-like receptors in the upper airway. *Allergy* 2003;58:748-53.
- Komai-Koma M, Jones L, Ogg GS, Xu D, Liew FY. TLR2 is expressed on activated T cells as a costimulatory receptor. *Proc Natl Acad Sci U S A* 2004;101:3029-34.
- Matsuguchi T, Takagi K, Musikacharoen T, Yoshikai Y. Gene expression of lipopolysaccharide receptors, Toll-like receptors 2 and 4, are differently regulated in mouse T lymphocytes. *Blood* 2000;95:1378-85.
- Tulic MK, Fiset PO, Manoukian JJ, Frenkel S, Lavigne F, Eidelman DH, et al. Role of Toll-like receptor 4 in protection by bacterial lipopolysaccharide in the nasal mucosa of atopic children but not adults. *Lancet* 2004;363:1689-97.
- Zeidel A, Beilin B, Yardeni I, Mayburd E, Smirnov G, Bessler H. Immune response in asymptomatic smokers. *Acta Anaesthesiol Scand* 2002;46:959-64.
- Abbud RA, Finegan CK, Guay LA, Rich EA. Enhanced production of human immunodeficiency virus type 1 by in vitro-infected alveolar macrophages from otherwise healthy cigarette smokers. *J Infect Dis* 1995;172:859-63.
- Hagiwara E, Takahashi KI, Okubo T, Ohno S, Ueda A, Aoki A, et al. Cigarette smoking depletes cells spontaneously secreting Th1 cytokines in the human airway. *Cytokine* 2001;14:121-6.
- Li JSM, Peat JK, Xuan W, Berry G. Meta-analysis on the association between environmental tobacco smoke (ETS) exposure and the prevalence of lower respiratory tract infection in early childhood. *Pediatr Pulmonol* 1999;27:5-13.
- Difranza JR. Morbidity and mortality in children associated with the use of tobacco products by other people. *Pediatrics* 1996;97:560-8.
- Cook DG, Strachan DP. Summary of effects of parental smoking on the respiratory health of children and implications for research. *Thorax* 1999;54:357-66.

Available online January 31, 2005.
doi:10.1016/j.jaci.2004.11.044

Banana anaphylaxis with a negative commercial skin test

To the Editor:

The diagnosis of food allergy is becoming increasingly complex, but the skin prick test (SPT) is still at the center of the diagnostic work-up. Often the diagnosis of food allergy is based on skin tests alone. Fresh fruit, whether implicated in anaphylaxis or the oral allergy syndrome (OAS), poses many problems for traditional skin testing. As Ortolani et al¹ first reported in 1989, there is significant variability in the response to commercial extracts and fresh fruit applied by the prick-prick method. Other investigators have reported similar findings in food-induced anaphylaxis as well as OAS.² SPT has been compared with food challenge for the diagnosis of food allergy. In one study,³ only 58.8% of patients with a positive challenge had a positive commercial skin test result. However, 91.7% of patients with a positive challenge had a positive skin test result with fresh food.³

Because of the increasing association of banana allergy and latex allergy, the major allergens in banana have been characterized.⁴ Patients not allergic to latex have been confirmed to have anaphylaxis to banana.⁵ The major allergens are 32-kd and 34-kd proteins that have class I chitinase activity.⁴ More than half of patients allergic to banana respond to SPT performed with 1 of these 2 isolated proteins.⁴ Here, we report the case of a young boy with systemic symptoms and negative SPT result to commercial extract. This case is important because it is one of the first reported pediatric cases of systemic anaphylaxis to banana. Banana is generally thought to have low allergenic potential and is often appropriately introduced as part of the early infant diet. As food allergy becomes more prevalent, foods once believed not to cause systemic symptoms will increasingly require more in-depth clinical evaluation.

A 7-month-old boy presented to the allergy and immunology clinic at Duke University for evaluation of atopic dermatitis and possible food allergy. His atopic dermatitis started in the first several months of life. At 3 months of age, he had angioedema and erythema on his face and neck on his first introduction to a cow's milk formula. He was exclusively breast-fed until this point. At 5 to 6 months of age, he was started on solid foods. Approximately 2 hours after an isolated feeding of crushed banana, he awoke from a nap with generalized urticaria, vomiting, and respiratory symptoms, including wheezing. He was immediately taken to the emergency room for