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Feasibility of nasal epithelial brushing for the study of airway epithelial functions in CF infants☆

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Abstract

Background: For a better understanding of the early stages of cystic fibrosis (CF), it is of major interest to study respiratory epithelial cells obtained as early as possible. Although bronchoalveolar lavage has been proposed for this purpose, nasal brushing, which is a much less invasive technique, has seldom been used in CF infants. The aim of the present study was to examine in a few infants the feasibility of a nasal brushing technique for studies of airway epithelial functions in very young CF infants.
Methods: In 5 CF (median age 12, range 1–18 months) and 10 control infants (median age 5, range 1–17 months), a nasal brushing was performed by means of a soft sterile cytology brush, after premedication with oral paracetamol (15 mg/kg body weight) and rectal midazolam (0.2 mg/kg body weight). Samples were used for microbiological, cytological and functional studies.
Results: The procedure was well tolerated. Number of cells collected was similar in CF and non-CF patients (CF: median 230×10^3, range 42×10^3–900×10^3; non-CF: median 340×10^3, range 140×10^3–900×10^3). Median number of viable cells was 67% (range 31–84%). Freshly obtained samples were successfully used for studies of ciliary beating frequency and cAMP-dependent chloride efflux. In 7 out of 17 cell cultures, confluence was obtained (CF: 2 out of 7; non-CF: 5 out of 10). The feasibility of studying protein release and mRNA expression of IL-8, IL-6 and TNF-α, under basal conditions and after stimulation by *Pseudomonas aeruginosa*, was demonstrated.
Conclusions: By means of a simple nasal brushing technique easily performed and well tolerated, it is feasible, in infants, to harvest respiratory cells in sufficient amounts to study the airway epithelium using a broad range of techniques including cell culture.

Keywords: Cystic fibrosis; Cell physiology; Cell culture

1. Introduction

Cystic fibrosis (CF), the most common lethal genetic disease in the Caucasian population, is characterized by airway inflammation and infection leading to progressive destruction of lungs. Nevertheless, the events that occur very early during the progression of the disease at the airway level in infants are not known.

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One of the most important abnormalities in CF is an abnormal processing of the mutated CFTR protein through the endoplasmic reticulum that causes abnormal location or even absence of the protein at the apical plasma membrane of airway epithelial cells [2]. This abnormality results in a defective cAMP-regulated chloride transport associated with a marked dehydration of the airway surface fluid, decreased mucus transport and airway obstruction. In CF adult patients with the most common mutation (homozygous ΔF508), it has been shown that the clinical manifestations may vary considerably and that mild phenotype could be partly related to a residual CFTR which could explain a normal cAMP-dependent chloride conductance [3,4]. At cellular level, we have also reported that the CFTR expression and localization could be related to the differentiation state of the airway epithelium [5]. In CF infants, such studies are still lacking.

It has been suggested that in CF patients, inflammation may precede infection [6,7]. Whether the CFTR defect is directly linked to an enhanced inflammatory process beginning before the first infection [8], or the airway inflammation is only a consequence of infection due to the impaired bacteria clearance [9,10] is still debated. In bronchoalveolar lavages (BAL) of CF infants with negative bacterial and viral cultures, Khan et al. [11] found that the number of neutrophils and the interleukin 8 (IL-8) levels were increased as compared with healthy controls. In contrast, in similar studies, Armstrong et al. [12,13] could not confirm these results. However, while BAL is the standard method to obtain material for studies of the respiratory epithelium, it is an invasive technique which cannot be easily performed in asymptomatic infants. In addition, the lavages are not only the reflection of the only epithelium because they contain also inflammatory cells and their products. Thus, studies are scarce, and the situation in the CF infant lung before the first infection remains an open question.

Taking into account the recent initiation of systematic neonatal CF screening, our objective was to find a simple and repeatable minimally invasive technique to analyze respiratory epithelial cells as early as possible in order to obtain information on the state of inflammation, infection, cAMP-dependent chloride conductance and histological characteristics of the airway epithelial sample. Interestingly, it has been recently reported that the use of non-bronchoscopic brushing to study the paediatric airways for clinical and research purposes was safe and easy to perform [14,15]. However, this technique was applied to healthy and asthmatic children prior to a surgical treatment that is under general anaesthesia and after tracheal intubation. Therefore, this technique does not appear appropriate to study asymptomatic babies. It is generally admitted that nasal epithelium characteristics and functions well reflect those of the lower airway epithelium [16,17,18] although some contradictory results about inflammation levels in CF and control patients have been reported. Noah et al. reported that nasal lavage fluid and BAL IL-8 levels are not significantly correlated [7]. However, Pitrez et al. have found that nasal IL-8 values well reflect lower airway levels [19], and Bergoin et al. that IL-8 concentrations measured in nasal lavages are significantly higher in CF patients than in healthy controls, similarly to what is found when using BAL [20]. Although several previous studies have shown that nasal brushing is safe and useful in adults and in children [21–28] this technique has been rarely used in CF [21,29] and only one study included CF infants [30].

In the present report, we describe a nasal brushing technique that can be easily performed in very young CF and non-CF infants and gives the ability to analyze airway cells and their functional properties, from native cell sheets and primary cell cultures. We believe this technique might be useful for further studies including a larger number of infants, in order to compare the airway epithelial functions between CF and non-CF infants.

2. Materials and methods

2.1. Patients

The present study was approved by the Regional Ethics Committee (Comité de Protection des Personnes de Champagne-Ardenne) and written informed consent was obtained from the parents of the infants prior to sampling. CF infants were recruited from the outpatient clinics devoted to CF care (Centre de Ressources et de Compétences pour la Mucoviscidose) of the American Memorial Hospital, Reims, France. Non-CF infants were recruited in the same hospital, among patients needing mild sedation for diagnostic investigation or minor surgery. At the time of nasal brushing, all non-CF patients were free of clinical signs of respiratory infection, and CF patients had no exacerbation and no or only mild respiratory symptoms (Table 1).

Nasal brushings were performed in 5 CF (median age 12 months, range 1–18 months) and 10 non-CF control infants (median age 5 months, range 1–17 months). Four out of the 5 CF patients were diagnosed by neonatal screening; in the other one, neonatal screening was falsely negative and diagnosis was done because he had chronic diarrhea. Four CF infants had ΔF508/508 homozygous genotype, the other one was compound heterozygous ΔF508/G542X. In one CF infant, nasal brushing was repeated 3 times, at ages 8, 13 and 17 months. The respiratory status (symptoms, bacterial colonization and medication) of all the infants are summarized in Table 1.

2.2. Nasal brushing technique in infants

Nasal brushing was performed on an in-patient basis. The infants were given a premedication with oral paracetamol (15 mg/kg of body weight) and rectal midazolam (0.2 mg/kg of body weight) 20 min before nasal brushing. Nasal brushing was performed by means of a soft sterile brush (Scrinet Diam.5.5, Laboratoire C.C.D., Paris, France). After nasal lavage with physiological saline in order to remove mucus, a