Soluble intercellular adhesion molecule-1 in the bronchoalveolar lavage fluid of normal children exposed to parental cigarette smoke

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Soluble intercellular adhesion molecule-1 in the bronchoalveolar lavage fluid of normal children exposed to parental cigarette smoke. J. Grigg, J. Riedler, C.F. Robertson. ©ERS Journals Ltd 1999.

ABSTRACT: This study sought to test the hypothesis that normal children exposed to parental cigarette smoke have increased bronchoalveolar lavage (BAL) fluid levels of soluble intercellular adhesion molecule (sICAM)-1.

Cells and solutes from the lower airway of normal children were obtained by nonbronchoscopic BAL using three aliquots of 1 mL/kg body weight¹ normal saline, prior to elective orthopaedic surgery. Children with evidence of recent or ongoing infection, atopic disease, previous history of wheeze, and chronic respiratory symptoms were excluded. Twelve children with parents who smoked (group 1) were paired with 12 age- and weight-matched controls with self-reported nonsmoking parents (group 2).

There was no significant difference (group 1 versus 2) in the volume of BAL fluid recovered (median 29.0 versus 28.7 mL), the percentage of alveolar macrophages (92.5 versus 91.8%), neutrophils (1.1 versus 2.1%), lymphocytes (5.3 versus 5.6%) and eosinophils (0 versus 0%), and the total BAL fluid leukocyte concentration (80 versus 61 × 10³ cells·mL⁻¹). BAL fluid albumin concentration was similar between the two groups (0.033 versus 0.020 mg·mL⁻¹). sICAM-1 was detected in all BAL fluid samples, and was significantly increased in group 1 (39.2 versus 22.5 ng·mL⁻¹, *p* < 0.01).

It was concluded that exposure of children to parental cigarette smoke is associated with increased soluble intercellular adhesion molecule-1 concentrations in the bronchoalveolar lavage fluid and this may reflect an altered activation of pulmonary immune cells.

Eur Respir J 1999; 13: 810–813.

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Keywords: Bronchoalveolar lavage
children
smoking
soluble intercellular adhesion molecule-1

Received: September 3 1998
Accepted after revision January 11 1999

Postnatal exposure of children to parental cigarette smoke is strongly associated with an increased prevalence of acute lower respiratory symptoms in infants [1], and wheeze, cough, phlegm and breathlessness in school-age children [2]. The mechanisms whereby parental cigarette smoke increases the vulnerability of the normal lung to respiratory disease are unknown. There have been two studies of the lower respiratory tract milieu of children exposed to environmental cigarette smoke. HEANEY *et al.* [3] performed nonbronchoscopic bronchoalveolar lavage (BAL) on children prior to elective surgery, and found no alteration in the BAL fluid leukocyte differential in association with parent-reported cigarette smoking. Using fiberoptic BAL of normal children prior to surgery, RATJEN *et al.* [4] also found no change in the BAL fluid leukocyte differential or leukocyte concentration with parental smoking. The majority of BAL studies in adults found that smokers had an increased alveolar macrophage (AM) differential [5, 6]. Active smoking also influences markers of pulmonary cell function. These include increased AM expression of β_2 integrins CD11a, CD11b, CD11c/18 [7], and an altered pattern of cytokines in the BAL fluid [8]. Similarly, passive inhalation of parental cigarette smoke could be associated with pulmonary cell activation, without a concomitant change in the alveolar leukocyte profile.

Soluble intercellular adhesion molecule (sICAM)-1 in the alveolar epithelial lining fluid is a nonspecific marker of immune cell activation within the pulmonary micro-environment, and is increased in a variety of immune diseases including idiopathic pulmonary fibrosis and allergic inflammation [9–11]. It is formed by shedding from ICAM-1 expressing cells [12], including alveolar leukocytes [13]. Upregulation of pulmonary tissue ICAM-1 is therefore associated with increased sICAM-1 levels in the BAL fluid [14]. sICAM-1 levels are increased in the serum of adult smokers [15], suggesting that it is an important marker of cellular activation induced by cigarette smoke. In the present study, nonbronchoscopic BAL was used to recover alveolar epithelial lining fluid and cells from normal children undergoing elective surgery, in order to determine whether pulmonary sICAM-1 is related to parent-reported cigarette smoking.

Methods

Subjects

Children between 6 months and 18 yrs of age, intubated for elective orthopaedic surgery, were eligible for BAL.

The exclusion criteria were premature birth, chronic respiratory pathology, chronic systemic disease, atopic symptoms, acute respiratory symptoms in the preceding 8 weeks, history of wheeze, and the isolation of a bacterial or viral pathogen from the BAL fluid. Exposure of the child to parental cigarette smoke was assessed by asking the caregiver(s), "do either you or your partner smoke?". To reduce false negative reporting, no further information about parental smoking was obtained. Children of smoking parents (group 1) were paired with age- and weight-matched children of self-reported nonsmoking parents (group 2). The study was approved by the Ethics in Human Research Committee of the Royal Children's Hospital and required written parental consent.

Bronchoalveolar lavage

BAL was performed before surgery by a single operator (J. Grigg) using a nonbronchoscopic BAL technique [16, 17]. Prior to elective surgery, children did not receive anticholinergic premedication. Anaesthesia was performed with isoflurane, intravenous thiopentone and pancuronium. Immediately after intubation, and with the child's head turned to the left, a straight 60 cm end-hole suction catheter (Vygon S.A., Écouen, France) was inserted into the endotracheal tube (ET) through a right-angle swivel bronchoscope connector (Mallinckrodt Medical Pty. Ltd., Mount Waverley, Australia). Catheter size was adjusted to the ET diameter (6 F catheter for ≤ 3.5 cm ET, 7 F catheter for 3.5 cm ET, 8 F catheter for 3.5–5.0 cm ET, 10 F catheter for ≥ 5.5 cm ET). The catheter was advanced until wedged, 1 mL/kg body weight⁻¹ of saline at room temperature was instilled, and BAL fluid immediately aspirated into a suction trap using 20 kPa negative pressure and a three-way tap. With the catheter remaining wedged *in situ*, two further aliquots of 1 mL/kg body weight⁻¹ saline were instilled and aspirated (*i.e.* a total instilled volume of 3 mL/kg body weight⁻¹). Pooled BAL fluid was placed on ice and 0.5 mL aliquots cultured for respiratory viruses, aerobic and anaerobic bacteria. All potential bacterial pathogens and commensals of the upper respiratory tract were identified and reported. Viral immunofluorescence was performed on cells concentrated by centrifugation. Immediately after collection, the concentration of leukocytes was determined by haemocytometer and viability determined by exclusion of a 0.04% solution of trypan blue. Ciliated epithelial cells in the haemocytometer chamber were not counted. Unfiltered BAL fluid was cytocentrifuged at $91 \times g$ for 5 min (Cytospin 2, Shandon Products Ltd, Runcorn, UK), stained with Wilson's reagents and examined under oil microscopy ($\times 1,000$ magnification). BAL fluid leukocyte differentials were determined by counting ≥ 300 leukocytes. Particulate-free BAL fluid supernatant was obtained by centrifugation of unfiltered BAL fluid at $1,000 \times g$ for 10 min (4°C) and $8,000 \times g$ for a further 3 min (4°C). Aliquots of BAL fluid were stored at -70°C for analysis of albumin and sICAM-1.

BAL fluid albumin and sICAM-1

The albumin assay was performed in duplicate samples of BAL fluid using a Roche Cobas centrifugal analyser

(Roche Diagnostics, Basel, Switzerland). Albumin was determined by immunoturbidimetry with an assay linear dynamic range of 0.005–0.8 mg·mL⁻¹. The interassay coefficient of variation was <9%. sICAM-1 was determined in duplicate BAL fluid samples using an enzyme-linked immunosorbent assay (ELISA) kit (Predicta® ICAM-1, Genzyme Diagnostics, Cambridge, MA, USA) with 1:10 and 1:50 dilutions of particulate-free BAL fluid. The assay sensitivity was 0.09 ng·mL⁻¹, with a linear dynamic range of 0.5–9.0 ng·mL⁻¹. The intra-assay coefficient of variation was 6.9%.

Statistical analysis

Data from groups 1 and 2 were compared by Wilcoxon matched-pairs signed ranks test, and are summarized as median (25th, 75th percentile). Spearman's rank correlation (coefficient r_s) was used for univariate correlations. A value of $p < 0.05$ was considered significant throughout. Analysis was performed using a statistical package for microcomputers (SPSS® for Windows, SPSS Inc, Chicago, IL, USA).

Results

Twelve children with parents who smoked (group 1) were matched with 12 children of nonsmoking parents (group 2). No child had respiratory symptoms in the preceding 8 weeks. There was no significant difference in age, weight, volume of saline instilled, and the volume of BAL fluid recovered between the two groups (table 1). BAL fluid leukocyte viability was >95%, and the proportion of nucleated cells that were ciliated epithelial cells was <3%. There were no differences between groups 1 and 2 for BAL fluid leukocyte differentials and concentrations, and for BAL fluid albumin concentration (table 2). sICAM-1 was detected in all BAL fluid samples, and in both groups the BAL fluid sICAM-1 concentration decreased with age ($r_s = -0.65$, $p < 0.05$, group 1; and $r_s = -0.53$, $p = 0.06$, group 2). BAL fluid sICAM-1 was significantly greater in group 1 ($p = 0.007$, table 2, fig. 1).

Discussion

In the present study, the concentration of sICAM-1 in the BAL fluid of children exposed to cigarette smoke was

Table 1. – Clinical details and bronchoalveolar lavage dynamics in children with exposure to parental cigarette smoke (group 1) and with no exposure (group 2)

	Group 1 (n=12)	Group 2 (n=12)
Age months	73.7 (32, 104)	62.8 (34, 103)
Weight kg	21.5 (13, 25)	18.3 (16, 30)
Saline instilled mL	62.7 (40, 75)	54 (40, 81)
BALF aspirated mL	29.0 (22, 44)	28.7 (20, 43)

Values are shown as median (25th, 75th percentiles). BALF: bronchoalveolar lavage fluid. Statistical assessment by Wilcoxon signed-ranks test. There was no significant difference between group 1 and group 2 for any variable.

Table 2. – Bronchoalveolar lavage (BAL) fluid parameters in children with exposure to parental cigarette smoke (group 1) and nonexposed children (group 2)

BAL fluid parameter	Group 1 (n=12)	Group 2 (n=12)
Differential %		
Alveolar macrophage	92.5 (89, 94)	91.8 (90, 94)
Neutrophil	1.1 (0.7, 2.6)	2.1 (1.0, 2.8)
Lymphocyte	5.3 (4, 7)	5.6 (4, 8)
Eosinophil	0 (0, 0.8)	0 (0, 0.3)
Cell concentration $\times 10^3$ cells·mL ⁻¹		
Total leukocyte	80 (52, 140)	61 (42, 100)
Alveolar macrophage	74.4 (46, 129)	53 (38, 94)
Neutrophil	1.2 (0.8, 2.0)	1.1 (0.3, 1.4)
Lymphocyte	5.5 (3, 6)	2.9 (2, 6)
Eosinophil	0 (0, 0.5)	0 (0, 0.1)
Albumin mg·mL ⁻¹	0.033 (0.02, 0.04)	0.020 (0.01, 0.03)
sICAM-1 ng·mL ⁻¹	39.2 (27, 46)**	22.5 (20, 28)

Values are shown as median (25th, 75th percentiles). sICAM-1: soluble intercellular adhesion molecule-1. Statistical assessment by Wilcoxon signed-ranked test. **: $p < 0.01$ versus group 2.

shown to be increased compared to children of nonsmoking parents. There is no accepted way of correcting for the dilution of alveolar epithelial lining by instilled saline, and the possibility that this observation is a technical artefact cannot be completely excluded. However, differences in dilution caused by differences in the dynamics of the instilled saline were minimized by using age- and weight-matched controls, and a weight-adjusted lavage volume. The use of age-matched controls was also important since sICAM-1 in other tissue compartments falls significantly with age [18]. The similar volume of BAL fluid recovered, and the similar BAL fluid cell and albumin concentrations in groups 1 and 2, suggest that the dynamics of the instilled saline were equally matched, and that the dilution of epithelial lining fluid was equivalent. Furthermore, the lack of influence of parental smoking on the paediatric alveolar leukocyte profile in the present study is compatible with the previous data from normal children [3, 4].

Fig. 1. – Concentrations of soluble intercellular adhesion molecule (sICAM)-1 in the bronchoalveolar lavage fluid (BALF) of children exposed to parental cigarette smoke (group 1) and age- and weight-matched controls (group 2). Bars represent median. **: $p < 0.01$ (Wilcoxon signed-ranks test).

An "objective" marker of environmental cigarette smoke exposure was not used in this study. It is unlikely that parents had misreported their smoking habit for two reasons. Firstly, the smoker(s) were not identified by name, which reduced the pressure for misreporting. Secondly, there is good agreement between parent-reported smoking and the urinary cotinine level in their children [19, 20], and in some circumstances, parent-reported smoking may be a better marker of passive exposure than urinary cotinine [21].

Could another variable account for the increased BAL fluid sICAM-1 seen in smoke-exposed children? With the small numbers of subjects available, it was not possible to test the independent effect of smoking in a multivariate model. Potential confounding variables such as ongoing and recent viral infection and atopic disease were controlled for by excluding these children. However, it was not known how many of the study children will develop chronic pulmonary pathology in the future. For example, asthma is associated with increased concentrations of alveolar sICAM-1 [11], and the levels might also be abnormal in the "pre-asthmatic" paediatric lung.

Both the cellular mechanisms for the increased levels of sICAM-1 in the alveoli of smoke-exposed children, and its effect on pulmonary immunocompetence, are unclear. Accumulation of sICAM-1 could either result from increased shedding of ICAM-1 from pulmonary cells, and/or delayed clearance of sICAM-1 from the alveolar space. Upregulation of cell surface ICAM-1 and increased formation of its soluble form is compatible with the observation that cigarette smoke condensate upregulates ICAM-1 on human endothelial cells *via* the activation of protein kinase C [22]. Although sICAM-1 is present in the BAL fluid of normal adults [23], and the present study found detectable levels in the normal paediatric lung, its function remains unknown. In general, sICAM-1 *per se* is a poor inhibitor of ICAM-1 dependent interactions [24], although it does have some immunomodulatory capacity. For example, microspheres coated with recombinant sICAM-1 are capable of specifically inhibiting ICAM-1- β_2 -integrin adhesion [24], and pretreatment of endothelial cells with recombinant sICAM-1 reduces leukocyte adherence [25]. Perhaps more importantly, ICAM-1 expression on pulmonary cells is critical to a range of immunological processes, including T-cell activation and neutrophil recruitment [26]. The accumulation of alveolar sICAM-1 in smoke-exposed children may therefore be a marker of upregulation of ICAM-1 on immunocompetent cells within the alveolar milieu.

In conclusion, the present study supports the hypothesis that cigarette smoking by parents is associated with subtle changes in the alveolar microenvironment of their children. Whether the increased levels of soluble intercellular adhesion molecule-1 seen in this study are associated with other changes in pulmonary immune cell function remains to be determined.

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