

Elevated levels of soluble adhesion molecules in sera of patients with acute bronchiolitis

Chou-Cheng Lai¹, Hsiao-Yun Tai², Horng-Der Shen², Wen-Ting Chung¹, Ruey-Lung Chung¹, Ren-Bin Tang¹

Departments of ¹Pediatrics and ²Medical Research and Education, Taipei Veterans General Hospital, and National Yang-Ming University, Taipei, Taiwan, ROC

Received: July 11, 2003 Revised: October 6, 2003 Accepted: October 20, 2003

The mechanisms of migration of neutrophils into the airway lumen are crucial in the development of airway injury of acute bronchiolitis and are mediated by adhesion molecules. In this study, we have attempted to evaluate the role of serum concentrations of the soluble form of intercellular adhesion molecule-1 (sICAM-1) in the disease activity in acute bronchiolitis and in respiratory syncytial virus (RSV) infection. Circulating levels of sICAM-1 in sera from 10 normal control subjects, and from 47 hospitalized acute bronchiolitis patients at admission, and from 25 patients on the day of discharge were determined by use of commercially available enzyme-linked immunosorbent assay kits. The mean serum level of sICAM-1 in bronchiolitis patients was significantly higher than in the 10 healthy control infants ($345.8 \pm 99.7 \mu\text{g/mL}$ vs $237.1 \pm 81.7 \mu\text{g/mL}$; $p < 0.05$). However, the mean sICAM-1 concentration was similar between RSV-positive and RSV-negative patients ($337.5 \pm 99.6 \mu\text{g/mL}$ vs $350.9 \pm 101.1 \mu\text{g/mL}$; $p = 0.65$). Although the mean clinical severity score of RSV-positive patients was significantly higher than that of RSV-negative patients (5.94 ± 1.83 vs 3.48 ± 1.70 ; $p < 0.05$). The improvement of clinical severity score was not well correlated with the change of sICAM-1 level ($r = 0.22$). This study provides evidence that serum levels of sICAM-1 are increased in acute bronchiolitis and further confirms the role of adhesion molecules involved in the pathogenesis of the disease. However, the serum concentrations of the soluble adhesion molecules could not reliably reflect the clinical severity of the disease.

Key words: Bronchiolitis, C-reactive protein, intercellular adhesion molecule-1, prognosis, respiratory syncytial virus infection

Bronchiolitis is an acute communicable disease predominantly presenting in infancy and characterized by cough, coryza, fever, expiratory wheezing, grunting, tachypnea, retractions and air-trapping. It clearly has been established that respiratory syncytial virus (RSV) is the major cause of bronchiolitis in infancy, and virtually the only etiological consideration when the disease is epidemic [1]. During epidemics in the colder months, virologic study indicates an RSV etiology in 80% or more of the cases, and especially in severe cases [2]. It has been reported that neutrophils and their products are likely to have an important role in the various clinical and pathological changes occurring in RSV infections of the airways.

The mechanisms of migration of neutrophils into the respiratory tissues and the airway lumen are crucial in the development of airway injury and are mediated

by adhesion molecules and cytokines. However, the process is not well characterized [3,4], especially in RSV bronchiolitis. Given the large number of airway neutrophils in RSV disease, we hypothesized that RSV infection could increase the expression of adhesion molecules on neutrophils and the vascular endothelium, and the subsequent serum levels would be altered according to the disease severity. The main purpose of this study is to investigate the role of soluble intercellular adhesion molecule-1 (sICAM-1) in acute bronchiolitis and whether or not sICAM-1 could be a prognostic indicator of hospitalized acute bronchiolitis children.

Materials and Methods

From October 2001 to June 2002, 47 infants between 1 and 24 months of age (median, 14 months) who were admitted to the pediatric ward of Taipei Veterans General Hospital, and diagnosed with acute bronchiolitis, were enrolled into this study. Eighteen patients were

Corresponding author: Dr. Ren-Bin Tang, Department of Pediatrics, Taipei Veterans General Hospital, 201, Section 2, Shih-Pei Road, Taipei, Taiwan 11217, ROC.
E-mail: rbtang@vghtpe.gov.tw

RSV-positive and 29 patients were RSV-negative. The RSV antigens were detected from an aliquot of nasopharyngeal aspirates (NPAs) by enzyme-linked immunosorbent assay (ELISA). The infants were healthy until the time of infection, and all had a moderate infection with coughing, wheezing, and tachypnea. A healthy control group of 10 infants aged between 3 and 24 months (median, 18 months), who had required blood tests before minor surgery (hernia repair) was selected.

Blood samples were taken on the first day of admission from all patients and the control group, and on the discharge day from 25 patients (13 RSV-negative, 12 RSV-positive), whose parents had consented for blood samples collection. C-reactive protein (CRP) was measured in all patients initially at admission, from blood samples by using commercially available immunonephelometry (Behring, San Jose, CA, USA). Then the remaining sera were stored at -70°C until assay. All patients had their disease severity recorded on the first day and the last day of the hospital stay according to the following parameters: respiratory rate, wheezing, use of accessory muscle, cyanosis. The severity of each symptom was rated on a 4-point scale as follows: 0 = none, 1 = mild, 2 = moderate, 3 = severe, and the sum of each severity item was calculated as the clinical severity score during the study.

Cytokine assays

sICAM-1 found in serum was measured by commercially available sandwich enzyme immunoassay (R&D systems, Minneapolis, MN, USA). The serum samples were diluted 1:20 before the assay was performed. Plates were read at 450 nm in a microplate reader. Duplicate assays were performed for serial dilutions of each sample and the average value was recorded.

Statistical analysis

All results are reported as mean \pm SD. We used Student's *t* test to compare the initial sICAM-1 levels between the groups (RSV-positive, RSV-negative, control). A *p* value less than 0.05 was considered as statistically significant. Besides, we used simple linear correlation method to identify the relationship between initial sICAM-1 and CRP levels, initial sICAM-1 and clinical severity at admission, and compared the changes of sICAM-1 levels and clinical severity between the first day and the last day of the admission. The correlation coefficient was expressed as *r*.

Results

Serum levels of sICAM-1 at admission

The serum levels of sICAM-1 in acute bronchiolitis patients ($345.8 \pm 99.7 \mu\text{g/mL}$) were significantly higher than those in the 10 healthy control infants ($237.1 \pm 81.7 \mu\text{g/mL}$; $p < 0.05$). Both the serum levels of sICAM-1 in RSV-positive bronchiolitis patients ($337.5 \pm 99.6 \mu\text{g/mL}$) and RSV-negative bronchiolitis patients ($350.9 \pm 101.1 \mu\text{g/mL}$) were significantly higher than those in the control infants ($p < 0.05$). However, the mean sICAM-1 concentrations was similar between RSV-positive and RSV-negative patients ($p = 0.65$).

Correlation between serum levels of sICAM-1 and clinical severity

At admission, the serum sICAM-1 level showed a weak negative correlation with the clinical severity score ($r = -0.17$), and the improvement of clinical severity score was not well correlated with the change of sICAM-1 level ($r = 0.22$). In other words, the clinical severity scores in patients were much improved at discharge, but the sICAM-1 level did not have a similar decreased trend corresponding, as shown by the scatter diagram (Fig. 1). Although the clinical severity score of the RSV-positive patients (5.94 ± 1.83) was significantly higher than that of the RSV-negative patients (3.48 ± 1.70 ; $p < 0.05$), the serum sICAM-1 level of the RSV-positive patients was not greater than that of

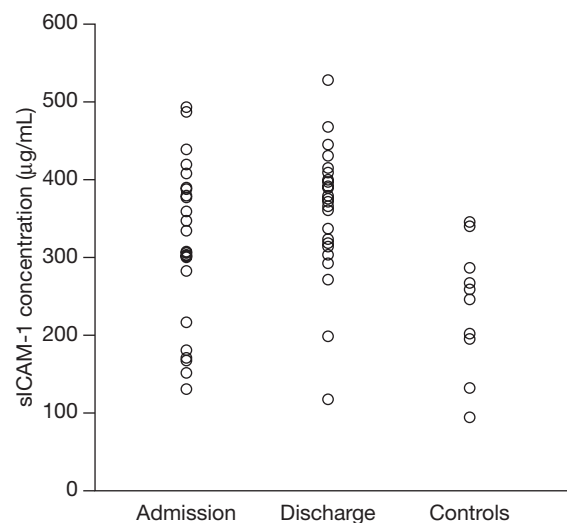


Fig. 1. Scatter of serum concentrations of soluble intercellular adhesion molecule-1 (sICAM-1) in infants or children with an acute bronchiolitis at admission and before discharge, and in 10 healthy control subjects.

Table 1. sICAM-1 serum levels and clinical severity score in RSV-positive and RSV-negative bronchiolitis patients

	sICAM-1 ^a	Clinical severity score
RSV-positive	337.5 ± 99.6	5.94 ± 1.83 ^b
RSV-negative	350.9 ± 101.1	3.48 ± 1.70

Abbreviations: sICAM-1 = soluble intercellular adhesion molecule-1; RSV = respiratory syncytial virus

^aData are expressed as mean ± SD in µg/mL.

^bSignificantly different between RSV-positive and RSV-negative patients ($p < 0.05$).

the RSV-negative patients as mentioned above (Table 1). Therefore, sICAM-1 was not a clinical severity prognostic factor in this study.

Correlation between serum levels of sICAM-1 and CRP

At admission, the serum sICAM-1 level showed weak correlation with the CRP ($r=0.36$). Serum sICAM-1 was not a severity indicator in this study.

Discussion

Our results show that the serum sICAM-1 concentrations of acute bronchiolitis patients were upregulated compared with those of normal control groups. Furthermore, the clinical severity of RSV bronchiolitis is significant greater than non-RSV bronchiolitis. However, there is no significant difference in soluble adhesion molecule expression between RSV-positive and RSV-negative bronchiolitis patients.

sICAM-1 has been reported in serum [5], cerebrospinal fluid [6] and bronchoalveolar lavage [7]. There are few studies showing the relationship between ICAM-1 and bronchiolitis [8-10]. Wang et al [8] presented the first report on the upregulation of murine ICAM-1 (mICAM-1) on neutrophils in viral infection in vivo that mICAM-1, Mac-1 expression on neutrophils from peripheral blood (PB) of RSV-positive infants is increased compared with cells from PB of normal control infants, and mICAM-1, Mac-1 on neutrophils from RSV-positive nasopharyngeal aspirates is upregulated compared with cells from PB of RSV-positive infants. There was also no significant difference in adhesion molecule expression in RSV-positive and RSV-negative PB and NPA groups. The results were similar to this study, except that we used sICAM-1 for our study target.

A recent study [9] showed that neutrophils can augment the epithelial damage and detachment induced by RSV infection. Thus, RSV infection might

enhance the disease activity more than non-RSV infected bronchiolitis. Our data supported the above speculation that there was greater severity in RSV-positive bronchiolitis.

In some studies, serum concentration of sICAM-1 could reflect the disease activity or clinical severity. In asthma studies, serum levels of sICAM-1 were significantly decreased after 6 weeks of montelukast treatment [11] and after 1 week of acute exacerbation [12] in children with mild to moderate atopic asthma. However, there has been no similar report of acute bronchiolitis before. Our data showed that the correlation of clinical severity and sICAM-1 was poor in acute bronchiolitis.

Furthermore, we arbitrary used the sum of 4 items of severity as the clinical severity score. However, each item does not show an equal contribution to clinical severity, indeed. For example, it is more severe in fact if the patient has cyanosis or crackles [13]. Akimoto et al [14] found that strenuous exercise could increase the expression of serum sICAM-1 through the increased expression in myocytes and muscle damage. Irritable crying in some infants could influence to some extent the serum sICAM-1 level.

Saijo et al [15] showed that the concentrations of CRP in RSV lobar pneumonia cases were significantly greater than those in RSV bronchiolitis; there were no significant differences in the white blood cell counts and the CRP concentrations between the bronchiolitis and bronchopneumonia cases and between the RSV-positive and -negative cases. These results suggest that RSV lobar pneumonia cases are coinfecting with some bacterial organisms more heavily than in the RSV bronchiolitis and bronchopneumonia cases. CRP was not correlated with RSV-infected bronchiolitis. Our data showed that serum sICAM-1 levels were not significantly correlated with the CRP ($r=0.36$) or the clinical severity at admission. This implies that the CRP level could not reflect the disease severity in bronchiolitis, nor did the serum sICAM-1 levels in viral bronchiolitis.

In conclusion, we have demonstrated the rise of sICAM-1 serum levels in acute bronchiolitis. This finding confirms the role of adhesion molecules associated with the infection or inflammation stimuli of bronchiolitis in contributing to the pathogenesis of bronchiolitis.

References

1. Robert CW. Bronchiolitis and infectious asthma. In: Feigin RD, Cherry JD, eds. Textbook of pediatric infectious disease. 4th ed. Philadelphia: W.B. Saunders; 1998:249-60.

2. Miller DG, Gabrielson MO, Horstmann DM. Clinical virology and viral surveillance in a pediatric group practice: The use of double-seeded tissue culture tubes for primary virus isolation. *Am J Epidemiol* 1968;88:245-56.
3. Mandi Y, Nagy Z, Ocsofski I, Farkas G. Effects of tumor necrosis factor and pentoxifylline on ICAM-1 expression on human polymorphonuclear granulocytes. *Int Arch Allergy Immunol* 1997;114:329-35.
4. Holtzman MJ, Look DC. Cell adhesion molecules as targets for unraveling the genetic regulation of airway inflammation. *Am J Respir Cell Mol Biol* 1992;7:246-7.
5. Rothlein R, Mainolfi EA, Czajkowski M, Marlin SD. A form of circulating ICAM-1 in human serum. *J Immunol* 1991;147:3788-93.
6. Jander S, Heidenreich F, Stoll G. Serum and CSF levels of soluble intercellular adhesion molecule-1 (ICAM-1) in inflammatory neurologic diseases. *Neurology* 1993;43:1809-13.
7. Shijubo N, Imai K, Shigehara K, Honda Y, Koba H, Tsujisaki M, et al. Soluble intercellular adhesion molecule-1 (ICAM-1) in sera and bronchoalveolar lavage fluid of patients with idiopathic pulmonary fibrosis and pulmonary sarcoidosis. *Clin Exp Immunol* 1994;95:156-61.
8. Wang SZ, Smith PK, Lovejoy M, Bowden JJ, Alpers JH, Forsyth KD. Shedding of L-selectin and PECAM-1 and upregulation of Mac-1 and ICAM-1 on neutrophils in RSV bronchiolitis. *Am J Physiol* 1998;275:L983-9.
9. Wang SZ, Hallsworth PG, Dowling KD, Alpers JH, Bowden JJ, Forsyth KD. Adhesion molecule expression on epithelial cells infected with respiratory syncytial virus. *Eur Respir J* 2000;15:358-66.
10. Oymar K, Bjerknes R. Differential patterns of circulating adhesion molecules in children with bronchial asthma and acute bronchiolitis. *Pediatr Allergy Immunol* 1998;9:73-9.
11. Stelmach I, Jerzynska J, Kuna P. A randomized, double-blind trial of the effect of treatment with montelukast on bronchial hyperresponsiveness and serum eosinophilic cationic protein (ECP), soluble interleukin 2 receptor (sIL-2R), IL-4, and soluble intercellular adhesion molecule 1 (sICAM-1) in children with asthma. *J Allergy Clin Immunol* 2002;109:257-63.
12. Tang RB, Chen SJ, Soong WJ, Chung RL. Circulating adhesion molecules in sera of asthmatic children. *Pediatr Pulmonol* 2002;33:249-54.
13. Mulholland EK, Olinsky A, Shann FA. Clinical findings and severity of acute bronchiolitis. *Lancet* 1990;335:1259-61.
14. Akimoto T, Furudate M, Saitoh M, Sugiura K, Waku T, Akama T, et al. Increased plasma concentrations of intercellular adhesion molecule-1 after strenuous exercise associated with muscle damage. *Eur J Appl Physiol* 2002;86:185-90.
15. Saijo M, Ishii T, Kokubo M, Murono K, Takimoto M, Fujita K. White blood cell count, C-reactive protein and erythrocyte sedimentation rate in respiratory syncytial virus infection of the lower respiratory tract. *Acta Paediatr Jpn* 1996;38:596-600.