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A comparison of cytokine responses in respiratory syncytial virus and influenza A infections in infants

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Abstract  Respiratory syncytial virus (RSV) infection is a major cause of bronchiolitis in infants while influenza A infection usually manifests as upper respiratory tract infection. We hypothesised that the immunological responses of infants to RSV infection and influenza A infection are different. This prospective study was undertaken to compare the cytokine responses during RSV and influenza A infection. Sera and nasopharyngeal aspirates (NPA) were collected from infants with a coryzal illness with or without wheeze who were admitted to the paediatric wards during 1998. Cytokines, adhesion molecules, RANTES, IgE and eosinophil cationic protein (ECP) were measured by enzyme linked immunosorbent assay or fluorescence enzyme immunoassay. The diagnosis of RSV and influenza infections was based on direct immunofluorescence and viral culture. Of the 39 infants studied, RSV infection was confirmed in 11 patients and Influenza A in 10 patients. All RSV patients and one influenza A patient had wheeze during the infection. The serum concentrations of interleukin (IL)-4 and IL-5, regulated upon activation normal T cell expressed and secreted (RANTES) and soluble intercellular adhesion molecule 1 (sICAM-1) in infants with RSV infection were significantly higher than those with influenza A infection (all \( P < 0.02 \)). The concentration of tumour necrosis factor-\( \alpha \) (TNF-\( \alpha \)) in NPA was significantly lower in infants with RSV infection (\( P < 0.01 \)).

Conclusion  A predominant T helper cell type 2 cytokine and related immunological response was observed in infants with respiratory syncytial virus infection whereas a predominant pro-inflammatory cytokine response was observed in infants with influenza A infection. This may explain the different clinical manifestations of the two viral infections in infants.

Key words  Cell adhesion molecule · Cytokine · Influenza A · Respiratory syncytial virus

Abbreviations  ECP eosinophil cationic protein · IL interleukin · INF-\( \gamma \) interferon-\( \gamma \) · NPA nasopharyngeal aspirates · RANTES regulated upon activation normal T-cell expressed and secreted · RSV respiratory syncytial virus · sICAM-1 soluble intercellular adhesion molecule 1 · sVCAM-1 soluble vascular cell adhesion molecule 1 · Th-1 T helper cell type 1 · Th-2 T helper cell type 2 · TNF-\( \alpha \) tumour necrosis factor-\( \alpha \)
Introduction

Respiratory syncytial virus (RSV) infection is an important cause of early wheezing in infants. Other respiratory viruses, notably parainfluenza, influenza and adenovirus may also cause wheezing in infancy but are considerably less frequent [4, 6, 20].

The pathogenesis of RSV disease and its relation to early wheezing has yet to be elucidated. There is partial obstruction of small airways due to lymphocytic infiltration, mucosal oedema, sloughing of necrotic epithelial debris, and secretion of mucus. Bronchospasm may play a role in early wheezing as some infants responded to inhalational bronchodilator therapy [8, 18]. Recruitment of inflammatory cells by cytokines, cell adhesion molecules and other immune mediators may be important in the development of airway disease.

Studies on cell cultures have shown that RSV infection enhanced neutrophil and eosinophil adhesion to respiratory epithelial cells by the induction of adhesion molecules [19]. Matsuzaki et al. [11] observed that adhesion of phytohaemagglutinin-activated tonsillar lymphocytes to RSV-infected epithelial cells caused a significant increase in T helper cell type 2 (Th-2) cytokine and interleukins (IL) 4 and 5, but no increase of T helper cell type 1 (Th-1) cytokine IL-2 or interferon-gamma (INF-\(\gamma\)), suggesting that RSV-infected epithelial cells induce Th-2-like cytokines by mucosal lymphocytes [11]. They further reported that regulated upon activation normal T-cell expressed and secreted (RANTES) was strongly induced by RSV infection and proposed that RANTES regulates the recruitment and activation of eosinophils and basophils in airway mucosa following RSV infection [14]. Coincidentally, Roman and colleagues [13] also proposed a predominant in vitro Th-2 cell response to RSV infection. They demonstrated significant increase in the IL-4/INF-\(\gamma\) ratio in peripheral blood mononuclear cell cultures from RSV-infected infants.

The results of in vivo studies on human subjects, however, are much more variable. Smyth et al. [17] provided evidence of lymphocyte and eosinophil involvement in the peripheral blood of infants with RSV bronchiolitis. They observed increased levels of soluble IL-2 receptor and soluble intercellular adhesion molecule 1 (sICAM-1), but were unable to detect any significant change of IL-4. Using a different approach, Everard et al. [3] have shown that in infants with RSV bronchiolitis, neutrophils accounted for 93% of the cells obtained from nasopharyngeal aspirates (NPA) and suggested that neutrophils may play a major role in causing symptoms in RSV bronchiolitis. Although preliminary results by Khan et al. [9] had shown that IL-4, IL-5, INF-\(\gamma\) and IL-8 were significantly elevated in the nasal secretions of RSV bronchiolitis, only IL-5 was significantly elevated in the serum. It was believed that cytokine responses in RSV bronchiolitis were compartmentalised to the lung and not polarised to the Th-2 pathway [9].

Compared to RSV infection, the cell mediated immunological responses of influenza A infection have attracted less attention. It has been shown in vitro that only monocytes and macrophages are highly susceptible to influenza A infection and increased levels of tumour necrosis factor-\(\alpha\) (TNF-\(\alpha\)), IL-1, IL-6 were found to be released from macrophage during influenza A infection [7]. It has also been shown that influenza virus infection induced expression of RANTES by normal human bronchial and nasal epithelial cells [10]. Despite the scanty data available, it is apparent that the immunological responses of RSV and influenza A virus infection are quite different. This prospective study was undertaken to compare the concentrations of cells, cytokines, cell adhesion molecules, RANTES and other related proteins in sera and NPA during RSV and influenza A infections. We hypothesised that there are more Th-2 related cytokine responses in RSV infection in comparison with influenza A infection.

Patients and methods

Patients and samples

April to October is the usual RSV season in Hong Kong, but in 1998 there was also an influenza A outbreak during this period. We recruited 39 infants less than 18 months of age who were admitted to the paediatric unit at the Prince of Wales Hospital from March to October 1998 manifesting a coryzal illness with or without wheeze. Patients with underlying conditions such as bronchopulmonary dysplasia or congenital heart disease were excluded.

A blood specimen and two NPA samples were collected from each patient on admission. The NPA samples were obtained by inserting a polyethylene catheter into the nasopharynx via the nostril, followed by aspiration using a suction pump. The catheters were then flushed with 2 ml of normal saline or virus transport medium respectively for immunological tests or viral diagnosis based on direct immunofluorescence antigen test and viral culture.

Preparation of NPA samples

The NPA samples for immunological study were agitated to break up mucus and centrifuged at 4 °C for 300 × g for 10 min. The supernatants were promptly stored at −85 °C until analysis, and cell deposits were re-suspended in saline for total and differential cell counts. Viability of cells was assessed by trypan blue exclusion and cytospin of cells was prepared after adjusting cell concentration to 3–5 × 10^6 per 100 μl saline (Shandon-Elliot cytocentrifuge, Shandon Scientific Ltd., Cheshire, England). The cytospin slides were air dried, fixed with absolute ethanol and stained with Giemsa for differential cell count (Olympus BH2 microscope, Olympus Optical Co. Ltd., Tokyo, Japan).

Immunological study

Cytokines and adhesion molecules IL-2, IL-4, IL-5, RANTES, sICAM-1, soluble vascular cell adhesion molecule 1 (sVCAM-1) and TNF-\(\alpha\) in serum and NPA supernatant were measured by enzyme-linked immunosorbent assay (R & D Systems, Minn., USA), and both eosinophil cation protein (ECP) and total IgE by fluorescence enzyme immunoassay (Pharcacia Diagnostics AB, Uppsala, Sweden). Measured concentrations in NPA were standardised to a protein concentration of 1 mg/ml of fluid using Bradford’s dye-binding reagent (Bio-Rad Laboratories, Calif., USA).