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SHORT REPORT

CYTOKINES IN THE NASAL WASHES OF CHILDREN WITH RESPIRATORY SYNCYTIAL VIRUS BRONCHIOLITIS

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Although respiratory syncytial (RS) virus is the major cause of bronchiolitis and pneumonia in young children, the factors that regulate the associated lung inflammation have not been defined. The levels of interleukin (IL)10, IL-12, and interferon (IFN) were determined in the nasal wash samples from 20 infants with a clinical diagnosis of bronchiolitis, seven with confirmed RS virus infections and 9 control children without respiratory illnesses. IL-10 levels were significantly higher in acute nasal wash samples (1-4 d post-hospitalization) from RS virus- infected infants than in convalescent samples from these children (14-21 d post-hospitalization), from children with other forms of bronchiolitis and from control children. In contrast, only one RS virus-infected infant had detectable IL-12 in an acute nasal wash sample. IFN activity was not detected in any samples from RS virus-infected children. RS virus infection stimulates IL-10 expression but not IL-12 and IFN, possibly contributing to an ineffective cell-mediated immune response.

Respiratory syncytial (RS) virus is the major cause of bronchiolitis and pneumonia in normal or immunocompromised infants (1). A formalin-inactivated vaccine for RS virus proved to be deleterious (2). RS virus causes repeated infections indicating that natural infection does not induce effective immunity. Although virus-specific IgE is rapidly detectable in infected children (3), virus-specific IgG, IgA and cell-mediated immunity are often detectable only after the acute infection has resolved (4). These results suggest that RS virus may induce an imbalance in the local immune response delaying specific antiviral immunity thus permitting efficient viral replication. It has been demonstrated that RS virus suppresses human alveolar macrophage expression of early immunoregulatory and anti-viral cytokines through induction of IL-10, a potent cytokine synthesis inhibitor (5). IL-10 also inhibits cell-mediated immunity by decreasing expression of IFN, tumor necrosis factor, and IL-12 (6).

To examine whether RS virus altered expression of IL-10, IL-12, and IFN, the levels of these cytokines were measured in nasal wash samples from children with confirmed RS virus-induced bronchiolitis and

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children with bronchiolitis from other causes, as well as normal children.

MATERIALS AND METHODS

Patients

This study included 29 infants who were seen in the Pediatric Clinic of the University of Rome "La Sapienza" during the winter season of 1999-2000. Twenty patients were diagnosed with bronchiolitis (mean age 3.6 months; range 1-15 months) based on clinical examination, chest radiographs, pulse oximetry and complete virological and bacteriological cultures. Nine control infants without respiratory disease (mean age 10.4 months; range 2-24 months) were examined.

Nasal Washes

One to four days after hospitalization all patients and controls underwent nasal lavage (acute samples). Nasal washes were performed by instilling two aliquots of sterile saline (3 ml per aliquot) into each nostril via a syringe then recovering fluid by gentle aspiration. Returned volume of nasal lavage fluid did not differ significantly between acute RS virus-infected and non-infected children, convalescent children and control children. Aliquots of nasal lavages were immediately cultured for pathogenic bacteria, Chlamydia trachomatis, and Mycoplasma pneumonia by standard techniques. Separate aliquots were cytocentrifuged, fixed in ice-cold methanol [acetone (3:1 v/v)] and analyzed for virus protein expression by indirect immunofluorescence for Adenovirus, Influenza A and B, Parainfluenza virus 1,2,3 and RS virus (Baxter Immunodiagnostics, West Sacramento CA). Parallel aliquots were frozen at -80°C until analyzed by specific ELISA for IL-10 (Bender, Medsystems, Vienna, Austria) and IL-12 (R&D Systems, Minneapolis, MN). There was no obvious correlation between IL-10 levels collected in nasal lavages on day 1 compared to day 4. IFN activity was determined by inhibition of RS virus replication in Hep-2 cells (2 IU/ml lower limit of detection) using the methods previously described. Clinical samples were assessed for IL-10, IL-12 and interferon recovery by adding recombinant cytokines to parallel aliquots. Recovery of all cytokines exceeded 80%. The lower limit of sensitivity for IL-10 was 2 pg/ml and for IL-12 it was 0.5 pg/ml.

Statistics

Statistical analysis was performed using non-parametric Wilcoxon rank-sum tests for RS virus-infected acute and convalescent samples. Mann-Whitney tests were used for comparison between RS virus-infected children and children with RS virus-negative bronchiolitis and normal children. Results are presented as mean ± SEM, and P values less than 0.05 were considered significant.

RESULTS

RS virus was detected by immunofluorescence in 7 of 20 children with bronchiolitis (35%) who had been infected during a winter outbreak of RS virus. None of these children had pneumonia on chest radiographs. No other viruses were detected in the nasal samples from children with bronchiolitis or the 9 control children. None of the nasal wash samples contained detectable pathogenic bacteria including Chlamydia trachomatis or Mycoplasma pneumoniae.

IL-10 levels were significantly higher in nasal samples from children with acute RS virus infection than in convalescent samples (46.1 ± 9.6 versus 22.4 ± 4.5 pg/ml; $P<0.03$) (Fig. 1). In addition, IL-10 levels were significantly higher in samples from children with acute RS virus infection than in children with bronchiolitis of unknown cause (46.1 ± 9.6 versus 20.9 ± 2; $P<0.006$) or control children without respiratory disease (23.8 ± 4.4; $P<0.02$) (Fig. 1). IL-10 levels in convalescent samples from RS virus-infected children did not differ significantly from children with bronchiolitis of unknown cause or controls. In contrast, IL-12 levels, in parallel aliquots from these children, were undetectable except for one RS virus-infected child whose acute lavage sample contained IL-12 at 72 pg/ml with IL-10 present at 54 pg/ml.

Although children with bronchiolitis of unknown cause who were negative for viral antigens by immunofluorescence may have had a preceding viral infection, their undetectable levels of viral antigens indicated that viral replication was not active at the time of examination. IFN activity was not detectable in any RS virus-infected samples, but was detectable in four children with bronchiolitis of unknown cause (28 ± 24 IU/ml; range 6-72).

DISCUSSION

Although nasal wash samples from RS virus-infected infants (7) contain increased levels of IL-8, no studies have yet determined the levels of other

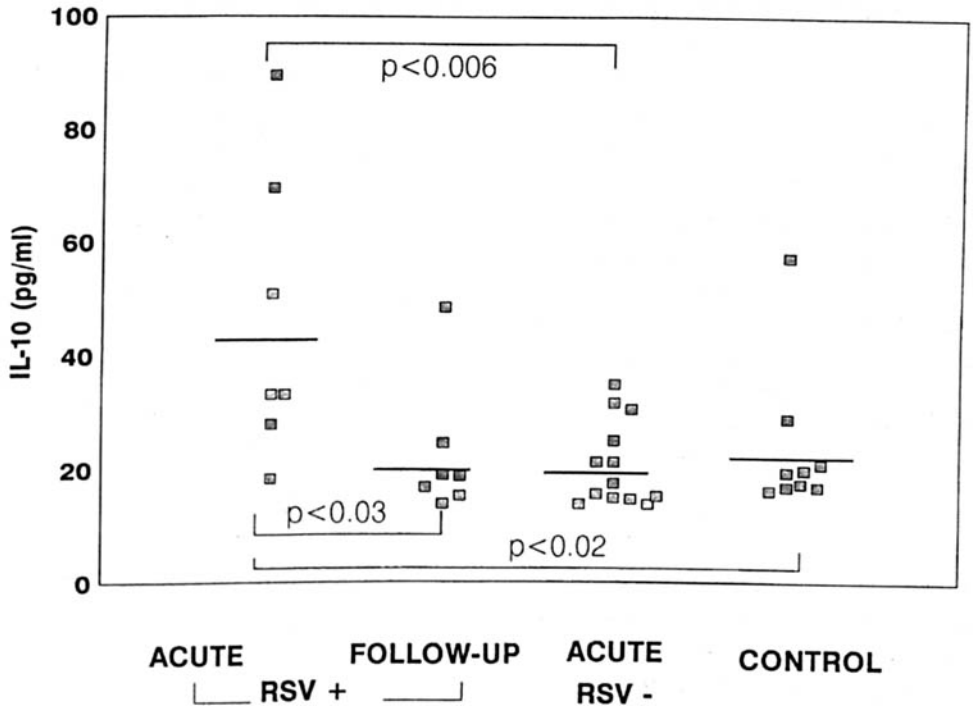

Fig. 1. *IL-10 levels (pg/ml) in nasal lavage samples obtained from children during acute or convalescent respiratory syncytial (RS) virus infections, bronchiolitis of undetermined cause, or control children without lung infection are indicated. Values shown are mean values from duplicate aliquots. Horizontal lines represent mean values for each group. Brackets indicate P values determined between groups analyzed by Wilcoxon tests (acute and convalescent RS virus-positive samples) or Mann-Whitney tests for RS virus-positive versus RS virus-negative or control groups.*

important immunoregulatory cytokines. The results presented here extend prior studies (8-9) and indicate that acute nasal wash samples from RS virus-infected children contain markedly elevated levels of IL-10. After the infection resolves, IL-10 decreases to levels found in control children (non-respiratory illnesses). In contrast, none of the parallel aliquots from any of the children we studied, except from one RS virus-infected child, contained detectable IL-12 or IFN activity. The lack of IL-12 or IFN in nasal lavage samples from RS virus-infected children is consistent with prior studies demonstrating that RS virus is a poor inducer of IFN and often does not induce cellular immunity until virus shedding has ceased (10-12). Elevated levels of IL-10 in nasal wash samples from RS virus-infected children may serve to suppress the expression of IL-12 and IFN, which can activate anti-viral pathways and drive cell-mediated immune responses (12). Il-10 also promotes the production of IgE which has been previously found to be rapidly induced in RS virus-infected children and associated with more severe disease (3). Thus,

RS virus induction of IL-10 may facilitate efficient viral replication and interfere with cell-mediated elimination of virus-infected cells.

Although IFN activates several anti-viral pathways (14) we found no evidence of IFN activity in the nasal washes of acute or convalescent samples from RS virus-infected children. Nasal lavage samples containing increased IL-10 uniformly lacked detectable IFN activity, suggesting, in line with *in vitro* studies, that IL-10 may inhibit IFN expression (15-16). IL-10 promotes T_H2 cell expansion and cytokine expression, thereby directly promoting IgE production (17). In contrast, IFN and IL-12 promote T_H1 cell-mediated immune responses (17). The exclusive presence of IL-10 in nasal washes from acutely infected RS infants indicates that this cytokine may have a direct role in promoting IgE production and delaying the expression of virus-specific neutralizing antibodies and cell-mediated immunity.

It is possible that children with symptoms of bronchiolitis, yet lacking detectable RS virus, might have been infected with other lung tropic viruses or have harbored RS virus at levels below the detection sensitivity of the assays used. IFN activity was detected in 4 of 13 patients with bronchiolitis of unknown cause. Perhaps children with undetectable RS virus lack detectable IL-10 while those with detectable RS virus have elevated IL-10. If this hypothesis is true, then we could envisage a dose-response relationship between RS virus burden and IL-10 expression. Increased viral burden and elevated IL-10 levels may correlate with decreased IL-12 and IFN. IL-10 was detectable in the nasal lavages of all children, including those without known respiratory disease. Thus IL-10 may serve to regulate expression of pro-inflammatory cytokines after RS virus infection, but it may also have a role in homeostasis perhaps to inhibit expression of pro-inflammatory cytokines (17). Although IL-10 levels were elevated in acute samples of children with RS virus bronchiolitis, it will be important to study larger groups of children to determine the kinetics of cytokine expression and to assess whether cytokine levels correlate with disease severity.

IL-10, but not IL-12 and IFN, were present at significantly elevated levels only in children with acute RS virus-induced bronchiolitis and at higher levels in this group than in children with non-RS virus-induced bronchiolitis or control children. Increased IL-10 may account for the delayed induction of virus-specific neutralizing antibodies and cell-mediated immunity.

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