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# Clinical Aspects of Bronchial Reactivity and Cell–Virus Interaction

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## Chapter 83

One of the most important aspects of the mucosal immune system in clinical medicine is its role in the development of obstructive airway disease. Although the mucosal immune system is undoubtedly crucial to the survival of humans, certain illnesses are probably a result of an inability to regulate selected components of the immune response occurring at mucosal sites, particularly in the airway. In contrast to recurrent bacterial pneumonia and repeated infections of the sinuses and middle ear cavities, which occur in individuals with immunologic deficits expressed at mucosal sites, wheezing and airway hyperreactivity induced by viral infections occur in individuals who are unable to regulate certain immune pathways but are otherwise immunologically intact. As a result of this dysregulation, viral bronchiolitis in infancy and asthma later in life are associated by common and today well-recognized histopathologic features, including an exuberant inflammation of the lower airway mucosa. Although many inflammatory cells appear to be involved, this inflammatory response is quite unique because the airway mucosa is infiltrated by T lymphocytes of the T helper type 2 (Th2) phenotype and by eosinophils. The composition of this cellular response is dependent upon the discrete target-cell selectivity of chemotactic molecules. Cytokines, chemokines, and colony-stimulating factors (CSFs) generated and released by structural cells of the airways such as epithelial cells regulate the migration and activation of leukocytes and therefore play a crucial role in inflammatory processes of the lung.

In this chapter, we illustrate the clinical importance of virus-induced wheezing and airway hyperreactivity (referred to herein as *infectious asthma*), review the evidence that is in support of the role of the immune system in provoking these illnesses, and summarize experimental data on the inducible expression of epithelial cell cytokines by respiratory viruses.

### EPIDEMIOLOGY OF INFECTIOUS ASTHMA

Wheezing induced by infectious agents occurs with a frequency of 11 cases per 100 children in the first year of life, 6 cases per 100 children in the second year, and 1.3 cases per 100 children in the third year (Denny and Clyde, 1986). The most severe forms of illness are observed in infants between 2 and 3 months of age (Brandt *et al.*, 1973). Illness is both more common and more severe in males until the age of 9 years when the incidence becomes similar between the sexes (Denny and Clyde, 1986). Respiratory syncytial virus (RSV), the parainfluenza viruses, and influenza viruses are probably the most important causes of infectious asthma in infancy, whereas rhinoviruses predominate as a cause of milder outpatient wheezing in older children (**Table 83.1**).

Similar studies have been completed in adults. In a study of 138 asthmatic adults, 89% (223/250) of colds were associated with exacerbations of asthma (Nicholson *et al.*, 1993). In 115 laboratory-confirmed cases of viral or chlamydial

**Table 83.1.** Role of Various Agents in Infectious Asthma—Relative Order of Importance by Age Group

<b>Infants</b>	Respiratory syncytial virus, parainfluenza viruses (especially type 3), influenza viruses
<b>Preschool children</b>	Respiratory syncytial virus, parainfluenza viruses, rhinovirus, coronaviruses
<b>Adolescents</b>	Rhinoviruses, mycoplasma pneumoniae, parainfluenza viruses, respiratory syncytial viruses
<b>Adults</b>	Rhinoviruses, parainfluenza viruses, mycoplasma pneumoniae, chlamydia pneumoniae

infection, 28 (24%) episodes occurred in which peak expiratory flows fell by 50 ml/min or greater. Rhinoviruses are presumed to be the most important etiologic agents in adults.

In the studies mentioned earlier, infection was documented by culture or serologic means. Neither of these techniques is efficient for identification of viral infections, so the actual importance of viral infections may have been underestimated. One study used polymerase chain reaction amplification of nucleic acids to identify infecting agents in children with acute episodes of obstructive airway disease. Viruses or chlamydiae could be detected in 80% of episodes in which children experienced reductions in peak expiratory flow rates (Johnston *et al.*, 1995).

## PRINCIPAL AGENTS RESPONSIBLE FOR INFECTIOUS ASTHMA

An impressive amount of information has been gathered concerning the role of the immune system in RSV, influenza, and rhinovirus infections, with regard to mechanisms of eradication of primary infection, pathogenesis of severe disease, and prevention of reinfection. A review of the immune response to infection with these specific agents provides an overall perspective that can probably be applied to infection with other viral agents.

### Respiratory syncytial virus

#### *Clinical presentation*

RSV is the major cause of serious lower respiratory disease in infancy and early childhood. An estimated 28 episodes of lower respiratory disease caused by RSV occur for every 100 children followed through the first 12 months of life. Reinfections in the second year of life are extremely common and occur with substantial frequency at all ages (Glezen *et al.*, 1986). Therefore, although most individuals develop relatively mild symptoms at the time of RSV infection, certain individuals seem more prone to develop lower respiratory illness (in particular, bronchiolitis) at the time of RSV infection. The fact that bronchiolitis (as well as croup) can be seen with a wide variety of viral agents suggests that the development of these illnesses is not entirely specific to any

feature of RSV but to a similar feature of a variety of viruses as well as to some unique host component.

Inoculation of RSV occurs through the nasal mucosal surface or through the eye (Hall *et al.*, 1981). The incubation period is assumed to be at least 4–5 days, at which time the infected child develops symptoms of rhinorrhea, nasal obstruction, and low-grade fever. In most patients, the illness resolves over 7–10 days, but in others the cough becomes progressive and eventually signs of lower respiratory tract involvement appear. At this point, secretions are profuse and contain  $10^5$ – $10^6$  infectious units of virus per milliliter of secretion (Hall *et al.*, 1976). In cases of pneumonia, descent of virus to the lower respiratory tract probably occurs as a result of aspiration. In the syndrome of bronchiolitis, whether disease is entirely the result of spread of virus to the bronchioles or occurs through stimulation of pathologic immune responses simultaneously is not yet clear. In any case, the child at this point manifests respiratory distress that may be severe. Nasal flaring, dyspnea, and retractions are noted, and auscultation of the chest reveals rhonchi and harsh wheezing. Otherwise, the child remains remarkably free of other symptoms. Fevers are usually not marked, and the child may be quite active despite the presence of moderate hypoxia, again suggesting that severe illness may be mediated, at least in part, by mechanisms other than progressive viral infection.

#### *Histopathology*

The histologic picture of RSV pneumonia reveals mononuclear cells within the alveolar walls and small airways. Engorgement of the capillary bed with edema is also noted. Lymphocytes and plasma cells are recruited into alveolar walls. The alveolar walls may become increasingly thick and filled with proteinaceous material, and intranuclear or cytoplasmic inclusions and giant cells may be observed (Aherne *et al.*, 1970). Bronchiolitis, in contrast, is characterized by necrosis and sloughing of the respiratory epithelium and plugging of the small bronchioles with fibrin and mucus. An intense peribronchiolar infiltration of lymphocytes and plasma cells occurs, with considerable edema. Localized hyperinflation due to airflow obstruction is characteristic, and atelectasis is common.

### *Immune response*

The precise role of RSV-specific antibody in recovery from primary infection is controversial. Local antibody responses, predominantly in the secretory IgA class, appear in the respiratory tract shortly after the onset of primary infection (McIntosh *et al.*, 1979; Kaul *et al.*, 1981). In some studies (McIntosh *et al.*, 1979), viral shedding is terminated at about the time of the appearance of antibody in secretions. However in other studies (Kaul *et al.*, 1981), antibody is present at a time when substantial quantities of virus are still recoverable from the respiratory tract. Thus the role of specific secretory IgA antibody in termination of infection is uncertain. Although repeated infection tends to result in accelerated local antibody responses (Kaul *et al.*, 1981) and reduced severity of illness, whether local antibody is responsible for protection is not clear. In one study of adults undergoing experimental RSV challenge, specific nasal IgA antibody titers did not correlate with protection against infection (Hall *et al.*, 1991). In other investigations (Mills *et al.*, 1971), neutralizing activity in nasal secretions apparently correlated with partial resistance to infection. However, subsequent studies (McIntosh *et al.*, 1979) demonstrated that such neutralizing activity is not necessarily antibody. In summary, RSV-specific secretory IgA antibody present at the time of infection does not prevent reinfection but may reduce the degree of viral replication.

Serum-neutralizing antibody may be more effective in providing immunity. In a study of adults experimentally challenged with RSV, preexisting titers of neutralizing antibody in serum (but not in secretions) provide some degree of protection against infection. A hyperimmune human antiserum against RSV has been prepared by plasmapheresis of donors selected on the basis of having high-serum RSV-neutralizing antibody titers. When infused on a monthly basis to infants at high risk of serious RSV infection, this compound reduced the rate of RSV-related hospitalization by 40% (The PREVENT Study Group, 1997). Therefore, serum antibody, presumably appearing in the respiratory tract by transudation following infection with RSV, appears to be better associated with protection against detectable infection than locally synthesized IgA antibody. Nevertheless, even the highest titers of antibody in serum or respiratory secretions do not seem to provide absolute protection against RSV, because as many as 25% of adults can be infected despite very high antibody titers (Mills *et al.*, 1971; Hall *et al.*, 1991).

Cell-mediated immune mechanisms may also be important in recovery from RSV infection. Individuals with congenital or acquired defects in cell-mediated immune function shed virus for prolonged periods and seem to have a greater frequency of development of pneumonia than immunologically intact individuals (Fishaut *et al.*, 1980; Hall *et al.*, 1986). T lymphocytes expressing cytotoxic activity against RSV-infected cells have been demonstrated in the peripheral blood of human infants and appear at about the time that viral shedding begins to decrease (Issacs *et al.*, 1987; Chiba *et al.*, 1989; Mbawuiké *et al.*, 2001). Cytotoxic cells are CD8+, and cytotoxic activity appears to be MHC restricted in that is

expressed only against cells infected with RSV, and not with other viruses. The cytotoxic activity of these cells correlates directly with their IFN $\gamma$  expression and inversely with IL4 expression. The role of these cells in protection and pathogenesis remains uncertain, and no studies of lymphocytes obtained from the respiratory tract have been completed.

### *Bronchial reactivity*

Potential mechanisms by which certain viruses may induce airway hyperreactivity and obstructive airway disease are included in **Table 83.2**. RSV-specific IgE antibody responses have been documented in respiratory secretions and sera of infants and young children undergoing natural RSV infection (Welliver *et al.*, 1980, 1981; Bui *et al.*, 1987; Russi *et al.*, 1993; Rabatic *et al.*, 1997; Aberle *et al.*, 1999). As with secretory IgA, IgE appears first bound to the surface of RSV-infected cells and later free in secretions (Welliver *et al.*, 1980, 1981). Higher concentrations of RSV-IgE were observed in infants with bronchiolitis in comparison with infants with upper respiratory illness alone (Welliver *et al.*, 1981; Bui *et al.*, 1987). RSV-specific IgE titers in respiratory secretions were also associated with greater degrees of hypoxia at the time of acute infection, suggesting that both the form and severity of illness are partially determined by the magnitude of RSV-IgE responses. Histamine can be detected in both the respiratory secretions and plasma of infants with RSV bronchiolitis, further substantiating the role of immediate hypersensitivity responses in RSV infection. Leukotrienes, particularly LTC<sub>4</sub>, have been identified in the respiratory secretions of infants with RSV infection (Volovitz *et al.*, 1988; Garofalo *et al.*, 1991; van Schaik *et al.*, 1999). Although a direct correlation of the leukotriene content of secretions with severity of illness was not observed, these compounds may nevertheless contribute to the airway obstruction that occurs with RSV infection.

Although autopsy studies of infants with fatal RSV infection do not reveal the presence of eosinophils in the airway, nevertheless eosinophil cationic protein, a component of eosinophil granules that is toxic to respiratory epithelium, has been detected by several investigators in respiratory secretions of infants with RSV infection (Garofalo *et al.*, 1992; Ingram *et al.*, 1995; Garofalo *et al.*, 1994). Eosinophilia in peripheral blood is suppressed at the time of RSV infection, although the suppression is overcome somewhat in subjects with bronchiolitis, particularly males. It is possible that eosinophils present in the respiratory mucosa have degranulated at the time of RSV infection and were not replaced by the time of autopsy in the fatal cases. Thus, although eosinophils certainly degranulate in the respiratory tract during RSV infection, the exact role of eosinophils in RSV infection is uncertain, especially because some preliminary observations suggest that degranulation of eosinophils occurs only after lower airway obstruction has been established (R.C. Welliver, personal communication, 1998). On the other hand, recent studies demonstrate that eosinophils exposed to RSV are able to secrete inflammatory cytokines (Olszewska-Pazdrak *et al.*, 1998a) and, when cultured in the

**Table 83.2.** Potential Mechanisms by which Respiratory Viruses Cause Airway Obstruction

Mechanism	Virus	References <sup>a</sup>
<b>Sloughing of airway epithelium with:</b>		
Exposure of irritant receptors	Parainfluenza, rhinovirus	Empey <i>et al.</i> (1976), Fryer and Jacoby (1991)
Loss of inhibitory mediators and cholinergic overactivity	Parainfluenza, influenza	Vanhoutte <i>et al.</i> (1989), Nadel <i>et al.</i> (1991)
Increased permeability and sensitization to allergens	RSV, parainfluenza	Freihorst <i>et al.</i> (1988), Leibovitz <i>et al.</i> (1989), Riedel <i>et al.</i> (1996), Schwarze <i>et al.</i> (1997)
<b>Antiviral immune response</b>		
Antibody-mediated (antiviral IgE)	RSV, parainfluenza	Welliver <i>et al.</i> (1981), Welliver <i>et al.</i> (1982), Bui <i>et al.</i> (1987), Russi <i>et al.</i> (1993), Rabatic <i>et al.</i> (1997), Aberle <i>et al.</i> (1999)
T cell-mediated (CD4+ and/or CD8+; Th1 and/or Th2)	RSV, rhinovirus, influenza	Openshaw <i>et al.</i> (1995), Corne and Holgate (1997), Neuzil and Graham, (1996)
<b>Airway mucosa inflammation</b>		
Release of mediators (leukotrienes, kinins)	RSV, rhinovirus, influenza	Volovitz <i>et al.</i> (1988), Garofolo <i>et al.</i> (1991), Proud <i>et al.</i> (1990)
Eosinophil and basophil degranulation	RSV, rhinovirus	Garofolo <i>et al.</i> (1992), Ingram <i>et al.</i> (1995), Welliver <i>et al.</i> (1981), Smith and Remingo (1982); Igarshi <i>et al.</i> (1993)
Epithelial-derived cytokines	(See Table 83.3)	
<sup>a</sup> Incomplete list of original and review articles.		

presence of RSV-infected airway epithelial cells, to release eosinophil cationic protein (Olszewska-Pazdrak *et al.*, 1998b).

Evidence also suggests that exaggerated T-cell responses may result in enhanced lung disease following RSV infection. Reconstitution of RSV-infected mice with cytotoxic T cells that have been repeatedly stimulated with RSV antigen clears virus rapidly from lungs but results in enhanced mortality and increases histopathologic changes in the lungs (Cannon *et al.*, 1988). Whether similar events occur in humans is not known, but infants with RSV bronchiolitis have been reported to have peripheral blood lymphocytes that are more responsive to RSV antigen than similar cells from infants with upper respiratory infection alone because of RSV (Welliver *et al.*, 1979). In addition, recipients of an inactivated RSV vaccine developed evidence of cell-mediated hypersensitivity to RSV after vaccination and subsequently developed enhanced lung disease when naturally infected with RSV (Kim *et al.*, 1976).

In asthma, certain evidence suggests that airway disease is a result of overexpression of T-helper type 2 (Th2) cytokines such as IL4, IL5, and IL13, especially because these cytokines are important in the synthesis of IgE antibody and

in migration and activation of eosinophils, the predominant airway inflammatory cell in asthma. In mice previously vaccinated with the formalin-inactivated vaccine that enhanced disease in humans, RSV infection causes increased expression of mRNA for IL5 and IL13 (Waris *et al.*, 1996). In humans, polymorphisms of IL4 genes have been associated with more severe forms of RSV-related illness (Choi *et al.*, 2002; Hoebee *et al.*, 2003). However, stimulation of lymphocytes from RSV-infected humans causes the expression of mRNA for the Th1 cytokine IFN $\gamma$  primarily, little IL4 expression, and essentially no increase in IL5 or IL13 (Anderson *et al.*, 1994; Mbawuike *et al.*, 2001; Tripp *et al.*, 2002). This pattern is reflected in studies that measured cytokine protein in secretions of RSV-infected infants. In these studies, IFN $\gamma$  predominated, whereas smaller amounts of IL4 were detected, and IL5 and IL13 were only rarely detected in infants with bronchiolitis (van Schaik *et al.*, 1999; Garofalo *et al.*, 2001). Although IFN $\gamma$  is the predominant cytokine present in bronchiolitis, there is some evidence that infants with the most severe forms of illness have lesser IFN $\gamma$  responses than those with milder forms of bronchiolitis (Aberle *et al.*, 1999; Bont *et al.*, 1999; Garofalo *et al.*, 2001; Johnston *et al.*, 2002).

Although there are little data to support a role for the IL5 and IL13 in bronchiolitis, certain chemokines that have the same biologic effects are present in RSV-infected infants (Sheeran *et al.*, 1999). The chemokines RANTES (regulated on activation, normal T-lymphocyte expressed and secreted) and MIP (macrophage inflammatory protein) 1-alpha and 1-beta can attract lymphocytes and eosinophils, and activate mast cells and basophils (Kuna *et al.*, 1998). Alternatively, they may be synthesized by Th1 lymphocytes (Schrum *et al.*, 1996). RSV infection induces expression of mRNA for these chemokines, as well as their appearance in the respiratory tract (Garofalo *et al.*, 2001; Tripp *et al.*, 2002). Overexpression of these chemokines may then result in more severe forms of bronchiolitis (Garofalo *et al.*, 2001).

### Influenza virus

#### *Clinical presentation*

Influenza viruses are responsible for yearly outbreaks of respiratory illness among all age groups. The highest mortality rates are observed in infants and in the elderly. Characteristic “flulike” symptoms include fever, headache, intense myalgia, and prolonged malaise. Cough may or may not be a prominent finding, especially early in the illness. The principal sites of involvement in most influenza virus infections are the trachea and the bronchi. As with RSV infections, lower respiratory tract illnesses such as pneumonia or wheezing are not as common in otherwise healthy persons. Nevertheless, croup and bronchiolitis may occur in infancy and pneumonia may occur at any age.

#### *Histopathology*

Influenza infection of the respiratory tract results in swelling and desquamation of infected ciliated cells. Airway edema and infiltration by mononuclear and polymorphonuclear cells follows, with extensive sloughing to the layer of the basal cells. Influenza pneumonia is characterized by hemorrhagic alveolar infiltrates, formation of hyaline membranes, and marked lymphocytic infiltration of the interstitium and alveolar walls (Kilbourne, 1987).

In contrast to infection with RSV and most other viruses, influenza infection is accompanied by an increased frequency of bacterial superinfection that may be the result of reduced mucociliary clearance following influenza virus infection (Camner *et al.*, 1973), greater damage to the mucosa with resultant diminished resistance to bacterial invasion (Kilbourne, 1987), or suppression of activity of phagocytic cells (Casali *et al.*, 1984; Hartshorn and Tauber, 1988).

#### *Protective immune response*

Initial infection with one strain of influenza virus provides partial immunity to reinfection with the same strain. Such homotypic reinfections are generally asymptomatic (Frank *et al.*, 1979; Sonoguchi *et al.*, 1986). Resistance to reinfection has been attributed to the presence of antibody in serum or respiratory secretions; high titers of antibody at either site are capable of conferring resistance (Clements *et al.*, 1986). Whether local or serum antibody is most efficient

in providing protection against reinfection in humans is uncertain.

Antibody to the hemagglutinin of influenza appears to contribute to immunity by preventing attachment of the virus to the respiratory epithelium, whereas antibody to the neuraminidase antigen inhibits cleavage of preformed virus from the cell membrane (Ogra *et al.*, 1977). Virtually no data are available on the role of serum or secretory antibody in recovery from primary influenza virus infection. Minor changes in hemagglutinin structure by variation in amino acid sequences (“antigenic drift”) or major changes *via* genetic reassortment (“antigenic shift”) enable the virus to infect previously exposed individuals.

The role of cell-mediated immunity in influenza virus infection has been extensively investigated in mice and, to a lesser extent, in humans. Transfer of primary or secondary influenza-immune splenic cells to mice infected intranasally with influenza virus results in significant clearance of virus from the lung and protection against death in the recipients. T lymphocytes represent the cell population primarily responsible for this immunity (Yap and Ada, 1978). Evidence suggests that the pathogenesis of influenza virus infection may be a reflection of the cell-mediated immune response in the lung. First, influenza virus replicates in and is released from the cells without causing cell death. Second, mice without functioning T cells develop extensive replication of virus in the lung with dissemination to other organs but develop minimal pulmonary infiltration following influenza virus challenge (Wyde *et al.*, 1977). In contrast, cytotoxic T cells appear in the lungs of immunocompetent mice at the peak of respiratory symptoms and when histopathology in the lung is most prominent (Ennis *et al.*, 1977).

Although such definitive studies have not been repeated in humans, cytotoxic T-cell responses develop in humans after influenza virus infection. Maximal responses occur in the second week after infection (Greenberg *et al.*, 1978). These cytotoxic cells apparently recognize all influenza type A strains but not influenza viruses of other types or noninfluenza viruses. These cytotoxic cells appear to be HLA restricted and are apparently capable of providing protection against subsequent influenza virus infection, even when influenza-specific serum antibody is undetectable in serum or secretions. These cytotoxic responses are apparently short lived, because only 30% of individuals exposed to a given strain of influenza virus had inducible cytotoxic T-cell activity when tested 5 years later (McMichael *et al.*, 1983). The majority of the cytotoxic T-cell responses appear to be mediated by CD8+ cells with major histocompatibility complex (MHC) restriction class I (Fleischer *et al.*, 1985; Yamada *et al.*, 1986).

In addition to specific cytotoxic T cells, nonspecific natural killer cell activity also increases after influenza virus infection in humans. However, the same increase in natural killer activity is observed in humans who continue to shed virus as in those individuals who do not continue to shed virus (Ennis *et al.*, 1981). A sufficiently large viral inoculum may be able to overcome natural killer cell resistance.

Therefore, although data on mucosal cell-mediated immune responses are lacking, cytotoxic T cells circulating in the blood appear to be important in restricting primary viral infection as a result of RSV and influenza and probably other viruses. These cytotoxic cells may play some role in protection against reinfection and are almost undoubtedly responsible for much of the histopathology seen with viral infections of the lung. There is some evidence that exaggerated T-cell responses may be responsible for unusually severe forms of infection due to both RSV and influenza virus.

#### *Bronchial reactivity*

Increased bronchial reactivity is a frequent complication of influenza infection (Little *et al.*, 1978; Laitinen and Kava, 1980). The increase in bronchial reactivity is associated with loss of the epithelium and may be due to either exposure of airway irritant receptors (Empey *et al.*, 1976) or a loss of natural inhibitors of airway reactivity (Jacoby *et al.*, 1988). The roles of influenza-specific IgE responses, other inflammatory responses, and mediator release have not been extensively evaluated for a possible role in inducing bronchial hyperreactivity, presumably because the principal morbidity and mortality resulting from influenza virus infection is not manifested by airway obstruction. Nevertheless, MIP1a is required for induction of inflammatory responses in animal models of influenza virus infection (Cook *et al.*, 1995), and influenza infection of epithelial cells and monocytes results in the release of many of the chemokines described previously for RSV infection (Matsukura *et al.*, 1996, 1998; Sprenger *et al.*, 1996).

#### **Rhinoviruses**

##### *Clinical presentations*

Rhinoviruses are the most frequently identified agents causing the common cold. Attack rates in the general population are in the range of 0.42–0.64 infections per person per year, with higher rates in children. Infections occur more commonly in the spring and autumn but can be documented year-round (Fox *et al.*, 1985). Although most infections are trivial, nevertheless, rhinoviruses are recognized as the most frequent causes of asthma exacerbations in school-aged children and adults (Nicholson *et al.*, 1993; Johnston *et al.*, 1995).

##### *Histopathology*

Because of the mild nature of rhinovirus infections, autopsy materials are limited. Nasal biopsies have been performed in a small number of individuals with natural or experimental rhinovirus infection. These reports describe minimal damage to epithelial cells in rhinovirus colds (Winther *et al.*, 1986) and detection of only a few infected cells using nuclear probe techniques (de Arruda *et al.*, 1991). This implies either that very few cells become infected or that infected cells exfoliate quickly and are rapidly replaced. Nasal biopsies also reveal minimum to no changes in the number of inflammatory cells in tissue from infected individuals in comparison to preinfection samples (Fraenkel *et al.*, 1994). In contrast to these tissue samples, neutrophils are abundant in nasal lavage fluids

obtained from infected subjects. The degree of upper respiratory symptoms correlates with the number of invading neutrophils, and with the release of kinins in nasal secretions (Naclerio *et al.*, 1988). However, it remains uncertain whether the magnitude of the neutrophil and kinin responses simply reflects the degree of inflammation or is actually responsible for the manifestations of illness. Mucosal biopsies from individuals with rhinovirus colds show modest infiltration with lymphocytes and eosinophils (Fraenkel *et al.*, 1995).

##### *Immune response*

Rhinovirus-specific antibody responses occur both in the respiratory tract and in serum following rhinovirus infection, but the nature of these responses has not been investigated extensively. In experimental infection, responses become detectable 2 to 3 weeks after inoculation (Barclay *et al.*, 1989). Serum antibody responses are greater and more prolonged in adults than in children, suggesting that reinfection occurs and gradually induces solid immunity (Fox *et al.*, 1985).

Cell-mediated responses also occur following infection. Tonsillar lymphocytes proliferate in response to stimulation with rhinovirus antigens if antigen-presenting cells are present (Wimalasundera *et al.*, 1997). The responding cells are CD4+ and produce interleukin-2 (IL-2) and interferon gamma, but not IL-4. In peripheral blood, the number of CD4+ cells is reduced, suggesting they migrate to the respiratory tract in response to infection (Levandowski *et al.*, 1986). Upon restimulation *in vitro*, peripheral blood cells release IL-2, and the magnitude of this response correlates inversely with virus shedding and with nasal mucus scores. These cells also release interferon gamma, but the quantity of this cytokine produced did not correlate with virologic or clinical parameters (Hsia *et al.*, 1990). In two other studies, the amount of IFN $\gamma$  present either before (Parry *et al.*, 2000) or during (Gern *et al.*, 2000) rhinovirus infection was related to the peak titer and duration of rhinovirus shedding.

Studies of Th1 and Th2 cytokines are limited to date. However, peripheral blood lymphocytes recovered from rhinovirus-infected subjects and stimulated with rhinovirus release IFN $\gamma$  (before and after infection) but not IL5 (Parry *et al.*, 2000). Ratios of IFN $\gamma$ /IL5 do not change markedly over the course of experimental rhinovirus colds (Gern *et al.*, 2000). Higher IFN $\gamma$ /IL5 ratios were associated with reduced cold symptoms in this study.

##### *Airway reactivity*

The mechanism by which rhinoviruses result in lower respiratory disease is still unclear. Rhinovirus infection of nonallergic individuals does not alter forced expiratory flows (FEV1) nor increase airway reactivity to histamine (Fraenkel *et al.*, 1995; Halperin *et al.*, 1983; Lemanske *et al.*, 1989; Calhoun *et al.*, 1991, 1994). Even in subjects with allergic rhinitis or mild asthma, rhinovirus infection apparently does not alter lung function (Fraenkel *et al.*, 1995; Lemanske *et al.*, 1989; Calhoun *et al.*, 1991, 1994), indicating that simple infection with this virus is insufficient to cause airway obstruction and wheezing.

The question of whether rhinovirus infection induces changes in airway reactivity in atopic individuals has been extensively investigated. Most studies have determined that airway responsiveness to histamine and to allergens is increased in atopic subjects (Lemanske *et al.*, 1989; Grunberg *et al.*, 1997a, 1997b; Gern *et al.*, 1997). This suggests that rhinovirus infection might provoke wheezing only in those individuals who are atopic. This would presumably occur by increasing the reactivity of the airway, so that the degree of obstruction following allergen exposure would be enhanced during acute rhinovirus infection. Rhinovirus infection does not apparently increase the reactivity of the airway to bradykinin, which is a potent stimulus of sensory nerves. Therefore, the increase in airway reactivity induced by rhinovirus infection is probably not due to an increased exposure of sensory nerves (Grunberg *et al.*, 1997a).

Even though rhinovirus infections may increase airway reactivity in atopic individuals, it cannot be concluded that this is sufficient to result in airway obstruction. Indeed, subjects with allergic rhinitis (but not asthma) develop increases in airway reactivity following rhinovirus infection but do not wheeze or manifest other evidence of airway obstruction. Symptomatic airway obstruction following rhinovirus infection must have another basis.

Pertinent data in this regard have been generated (Lemanske *et al.*, 1989; Calhoun *et al.*, 1991, 1994), in which both normal individuals and those with ragweed-induced allergic rhinitis are experimentally infected with rhinovirus or given a placebo. Each group is then challenged with ragweed antigen E by instilling the antigen directly into the lung *via* an endoscope. No change in pulmonary function (FEV1 or FVC) occurs in these individuals. However, the group infected with rhinovirus and challenged with allergen develops increased airway reactivity. More importantly, late phase asthmatic responses (LARs), defined as a fall in FEV1 occurring at 48 hours after allergen challenge, developed in the majority of individuals infected with rhinovirus, but not in the uninfected group (Lemanske *et al.*, 1989; Calhoun *et al.*, 1991). These results indicate that rhinovirus infection may not result directly in airway obstruction. However, they do suggest that atopic individuals are more likely to develop airway obstruction if they encounter an allergen to which they are sensitive during acute rhinovirus infection.

Further studies from the same group attempted to determine the mechanism by which rhinovirus infection enhances the potential for allergen-induced airway obstruction. The results of these studies indicate that histamine release in the airway and in the blood is increased following allergen challenge in atopic individuals infected with rhinovirus (Calhoun *et al.*, 1991, 1994). The degree of histamine release is greater than that accounted for by the allergen challenge itself (Calhoun *et al.*, 1991), suggesting that rhinovirus infection enhances the capability of inflammatory cells to release mediators following allergen exposure. Because the release of histamine, but not tryptase, is enhanced in these subjects, it appears that the primary effect of rhinovirus infection is on basophils recruited to the lung rather than mast cells resid-

ing in the lung. Again, rhinovirus infection in normal individuals did not result in abnormal histamine release in the airway or in the blood.

Rhinovirus was infrequently recovered from lung lavage fluids in these experiments, even though it was readily recovered from the nasopharynx of study participants. This implies that infection with rhinovirus that is limited to the upper respiratory tract may still cause enhanced reflex release of mediators of airway obstruction in the lower respiratory tract. These are important observations with regard to attempts to develop rhinovirus vaccines. For vaccines to be effective in preventing asthma induced by rhinoviruses, they may need to be capable of preventing rhinovirus infections absolutely rather than simply restricting them to the upper respiratory tract.

In summary, available data suggest that rhinovirus infections are important causes of lower respiratory disease primarily in individuals with underlying asthma. Even then, the principal effect of rhinovirus infections may be in enhancing the response to allergens to which the individual is sensitive. The precise mechanism appears to involve increased degranulation of basophils, and perhaps eosinophils. In support of this are the autopsy findings in a child with fatal rhinovirus infection (Las Heras and Swanson, 1983). This 11-month-old infant had symptoms of asthma for 3 months before dying unexpectedly. Rhinovirus type 37 was recovered from both the lung and the blood. Lung findings at autopsy were consistent with acute asthma, including mucus plugging and eosinophil infiltration of the airways.

## AIRWAY MUCOSA INFLAMMATION IN RESPIRATORY VIRAL INFECTIONS

### Human and animal models of RSV-associated disease

In addition to the clinical and epidemiologic relationship between bronchiolitis in infancy and asthma later in life (Hall *et al.*, 1984; Sigurs *et al.*, 1995), the two diseases are linked by common histopathologic features characterized by profound inflammation of the airway mucosa. As mentioned earlier in this chapter, necrosis of the bronchial epithelium associated with peribronchial and perivascular mononuclear cell infiltration is considered a hallmark of RSV infection both in human (Aherne *et al.*, 1970) and in animal models (Prince *et al.*, 1986; Graham *et al.*, 1988). Moreover, the presence of cell-specific inflammatory mediators in nasopharyngeal secretions of children with bronchiolitis suggests that RSV infection triggers the migration to the airways and local activation of eosinophil and basophil leukocytes (Welliver *et al.*, 1981; Garofalo *et al.*, 1992, 1994; Sigurs *et al.*, 1994; Colocho Zelaya *et al.*, 1994; Ingram *et al.*, 1995).

### Epithelial cytokines

The specific antiviral immune response appears to play a role in the pathogenesis of RSV-induced airway inflammation. However, several studies suggest that other mechanisms may also trigger and sustain lung inflammation following viral



infection. In this regard, although the defense against foreign organisms, such as viruses or bacteria, is mediated by innate and by specific acquired immunity, the effector phases of both natural and specific immunity are largely mediated by cytokines. These molecules operate in networks and produce a cascade of effects that contribute to the orchestration, development, and functions of the immune system. Cytokines such as IL-1 $\alpha$ / $\alpha$ , IL-6, and tumor necrosis factor (TNF)- $\alpha$ , mediate and regulate inflammatory responses that are often associated with the immune response (reviewed in Arai *et al.*, [1990]). IL-1, as an example, can induce cytokine genes, protein synthesis, and secretion in a wide variety of cell types and increase the expression of adhesion molecules on endothelial cells and modulate other cell surface molecules that are critical for cell-cell interaction and leukocyte migration (reviewed in Dinarello [1992]).

In addition to the pivotal role of inflammatory cytokines, much of the cellular response at sites of tissue inflammation is controlled by gradients of chemotactic factors that direct leukocyte transendothelial migration and movement through the extracellular matrix. The composition of this cellular response is dependent on the discrete target-cell selectivity of chemotactic molecules. Chemokines, a newly identified family of small chemotactic cytokines, regulate the migration and activation of leukocytes and therefore play a key role in inflammatory processes of the lung (reviewed in Oppenheim *et al.*, [1991]). Two subfamilies, the CXC and the CC chemokines, are defined by the splicing of the conserved cysteine residues that are either separated by one amino acid (CXC chemokines) or are adjacent (CC chemokines). IL-8 and growth-related peptide (GRO)- $\alpha$  belong to the CXC family and are mainly chemotactic factors for neutrophils. However, members of the CC branch of the chemokine family such as RANTES (regulated upon activation, normal T cell expressed and presumably secreted), macrophage inflammatory protein-1 $\alpha$  (MIP-1 $\alpha$ ), monocyte chemotactic protein (MCP)-1, MCP-3, MCP-4, and eotaxin are chemotactic factors for monocytes, basophils, and eosinophils, with virtually no activity on neutrophils. With regard to human T lymphocytes, RANTES, MIP-1 $\alpha$ , and MCP-1 have been shown to be specifically chemotactic for CD4<sup>+</sup> T cells of the CD45RO<sup>+</sup> memory phenotype (reviewed in Baggiolini *et al.* [1997]). The chemokine receptor CCR3, which binds eotaxin, RANTES, MCP-3, and MCP-4, has been found to be expressed by Th2 cells, but not by Th1 cells (Sallusto *et al.*, 1997). The expression of CCR3 by Th2 cells is also functional because both eotaxin and RANTES-induced intracellular Ca<sup>2+</sup> increase and chemotaxis by CCR3<sup>+</sup> Th2 clones.

Colony-stimulating factors (CSFs) are growth factors that control proliferation of stem cells and differentiation along the granulocyte pathway. The CSFs are further defined by the types of granulocyte colonies they induce in culture and include neutrophil colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF), and granulocyte-macrophage colony-stimulating factors (GM-CSFs). GM-CSF is recognized as an important factor for stimulating hematopoiesis and prolonging eosinophil survival (Ruef

and Coleman, 1990). It also enhances LTC<sub>4</sub> release (Silberstein *et al.*, 1986) and eosinophil cytotoxicity (Lopez *et al.*, 1986). At high concentrations (produced locally during inflammation), GM-CSF can act as a true chemotaxin for eosinophils and can directly mediate eosinophil migration (Warringa *et al.*, 1991).

The respiratory epithelium represents the principal cellular barrier between the environment and the internal milieu of the airways. Epithelial damage and loss of integrity are a recognized feature of asthma (Jeffery *et al.*, 1989) and are thought to be associated with the increased bronchial hyper-responsiveness that is characteristic of the disease (Boushey *et al.*, 1980). In addition, during the last few years it has become clear that the epithelium, after contact with exogenous stimuli such as microbial infections, can actively modulate the local inflammatory and immune responses by releasing a variety of mediators and cytokines. In this regard, human airway epithelial cells appear to be a dominant source of CC chemokines including RANTES (Stellato *et al.*, 1995), MCP-1 (Standiford *et al.*, 1991), MCP-4 (Stellato *et al.*, 1997), and eotaxin (Lilly *et al.*, 1997), and, among the CSFs, of GM-CSF (Ohtoshi *et al.*, 1991; Cromwell *et al.*, 1992).

#### **Epithelial cytokines and respiratory viruses (*in vitro*)**

Respiratory epithelial cells are the primary target of viruses that enter the airways. RSV infection results from inhalation or self-inoculation of the virus into the nasal mucosa followed by infection of the local respiratory epithelium. Spreading along the respiratory tract occurs mainly by cell-to-cell transfer of the virus along the intracytoplasmic bridges (Hall *et al.*, 1981). This has led to our current view that recruitment and functional activation of eosinophils, basophils, and mononuclear cells in the airway mucosa play a key role in protective immunity as well as in the development of virus-associated wheezing during naturally acquired RSV infection. Active research during the past 5 years has led to the discovery of a number of cytokines, chemokines, and CSFs that are produced *in vitro* by virus-infected respiratory epithelial cells in both cell lines and primary cultures (Table 83.3). IL-8 was the first cytokine that was demonstrated in the supernatant of influenza- and RSV-infected nasal, bronchial, and lung type II epithelial cells. Synthesis of IL-8 appears to be dose and time dependent, to require replicating virus, and to be primarily transcriptionally induced (Arnold *et al.*, 1994; Garofalo, *et al.*, 1996a). Concurrently with the discovery of IL-8, a number of different laboratories have reported the presence of IL-6 in the supernatant of airway epithelial cells infected by RSV, rhinovirus, and influenza virus (Table 83.3). The mechanism of IL-6 production in RSV-infected lung epithelial cells appears to be regulated by the autocrine release of IL-1 $\alpha$  and, to less extent, IL-1 $\beta$  and TNF- $\alpha$  (Jiang *et al.*, 1998), cytokines with potent bioactivity in infected epithelial cell supernatant. In this regard, IL-1 $\alpha$  has been shown to enhance the expression of the intercellular adhesion molecule-1 (ICAM-1) on infected pulmonary epithelial cells (Patel *et al.*, 1995).

**Table 83.3.** Cytokines Produced by Airway Epithelial Cells Infected with Respiratory Viruses

Cytokines	Viral Agents	References
<b>Inflammatory cytokines</b>		
IL-1 $\alpha$ , IL- $\beta$ , TNF- $\alpha$	RSV	Patel <i>et al.</i> (1995)
IL-6	RSV, rhinovirus, influenza	Noah and Becker (1993), Arnold <i>et al.</i> (1994), Jiang <i>et al.</i> (1998), Subauste <i>et al.</i> (1995), Zhu <i>et al.</i> (1996), Matsukura <i>et al.</i> (1996)
IL-11	RSV, rhinovirus, parainfluenza	Einarsson <i>et al.</i> (1996), Bitko <i>et al.</i> (1997)
IFN- $\beta$	RSV	Garofalo <i>et al.</i> (1996b)
<b>CXC chemokines</b>		
GRO- $\alpha$	RSV	Garofalo and Ogra (1996)
IL-8	RSV, rhinovirus, influenza	Noah and Becker (1993), Becker <i>et al.</i> (1993), Arnold <i>et al.</i> (1994), Mastronarde <i>et al.</i> (1995), Fielder <i>et al.</i> (1995), Garofalo <i>et al.</i> (1995), Garofalo <i>et al.</i> (1996a), Subauste (1995), Choi and Jacoby (1992), Matsukura <i>et al.</i> (1996)
<b>CC chemokines</b>		
MCP-1	RSV	Garofalo and Ogra (1996), Olszewska-Pazdrak <i>et al.</i> (1998a)
MIP-1 $\alpha$	RSV	Garofalo and Ogra (1996), Olszewska-Pazdrak <i>et al.</i> (1998a)
RANTES	RSV, influenza	Garofalo and Ogra (1996), Saito <i>et al.</i> (1997), Becker <i>et al.</i> (1997), Olszewska-Pazdrak <i>et al.</i> (1998a), Matsukura <i>et al.</i> (1996)
<b>Colony-stimulating factors</b>		
G-CSF	RSV	Garofalo and Ogra (1996)
GM-CSF	RSV, rhinovirus	Noah <i>et al.</i> (1993), Garofalo and Ogra (1996), Subauste <i>et al.</i> (1995)

Moreover, we have demonstrated that RSV infection elicits interferon- $\beta$  (IFN- $\beta$ ) production by a number of respiratory epithelial cells, which in turn leads to an increase in their synthesis of class I MHC molecules (Garofalo *et al.*, 1996b). Because mice infected with RSV develop a lymphocytosis in the bronchoalveolar lavage (BAL) fluid, which is predominantly CD8+ T cells (Kimpen *et al.*, 1992; Openshaw, 1989), and adoptive transfer of CD8+ CTL causes hemorrhagic pneumonitis (Cannon *et al.*, 1988), the increased expression of class I MHC by respiratory epithelial cells during RSV infection marks the infected cells, and possibly neighboring cells that passively acquire released RSV peptides, for CTL-mediated lysis and associated immunopathology. Thus, increased class I MHC mediated by IFN- $\beta$  and other inflammatory cytokines released in infected airway mucosa may be considered an important component of the immunopathologic response to RSV. However, IFN- $\beta$  may play an equally important role in the protective immune response to RSV by inhibiting or restricting viral infection (Merolla *et al.*, 1995) and by enhancing expression of class I MHC on the infected cells and their lysis by RSV-specific CTL. IL-11, an IL-6-

type cytokine, has been shown to be produced by A549 (type II lung cells) infected with RSV, parainfluenza type 3, and rhinovirus 14 (Einarsson *et al.*, 1996).

A quite impressive list of different pharmaceutical and chemical agents has been shown to modulate *in vitro* virus-induced cytokine production, including dexamethasone, ribavirin, sodium salicylate, aspirin, dimethyl sulfoxide, and amiloride (Jiang *et al.*, 1998; Bitko *et al.*, 1997; Mastronarde *et al.*, 1995, 1996a). Although the effect of some of these compounds on virus-induced cytokine may be due to their well-known antiviral or generically anti-inflammatory properties, the exact mechanism of activity of most of the other compounds used in those studies is poorly understood.

We have investigated the expression and release of the CC chemokines RANTES, MCP-1, and MIP-1 $\alpha$  in response to RSV in primary cultures of human nose- and adenoid-derived epithelial cells, epithelial cells derived from the large bronchi (NHBE) or from the terminal bronchioles (SAE), and A549 cells, using (RT-PCR), Northern blot and specific ELISA. The profile and concentrations of the CC chemokines produced by the respiratory epithelium were

characteristically cell specific. RSV infection induced the release of RANTES by epithelial cells isolated from the upper respiratory airways (Saito *et al.*, 1997) and from all the segments of the lower respiratory tract (Garofalo and Ogra, 1996; Olszewska-Pazdrak *et al.*, 1998a). Similar findings using a bronchial cell line have been reported by another group (Becker *et al.*, 1997). With regard to the protein concentration, RSV-infected epithelial cells obtained from the distal portion of the bronchial tree (SAE) and type II cells (A549) produced significantly greater levels of RANTES than those from the major bronchi and the upper airways. In contrast to the widespread expression of RANTES in the infected respiratory epithelium, RSV induced the release of MCP-1 only from A549 cells. Furthermore, our results demonstrate for the first time that when infected human epithelial cells, both the A549 cell line and the normal SAE cells, are able to express and secrete MIP-1 $\alpha$  (Fig. 83.1). Thus, the release of MCP-1 and MIP-1 $\alpha$  by the airway epithelial cells appears to be regionalized in the distal segments of the bronchial tree and in the lung, a site where RSV-mediated necrosis of the epithelium and peribronchial cellular infiltration are associated with greater pathologic changes, particularly in infected young infants. Moreover, because the recently discovered CC chemokine eotaxin appears to be a potent and specific chemoattractant for eosinophils and Th2 cells, we investigated by Northern blot the expression of eotaxin mRNA in RSV-infected A549 cells. As shown in Figure 83.2, RSV infection of A549 cells induced the accumulation of eotaxin mRNA with maximal accumulation at 36 hours postinfection (R.P. Garofalo, personal communication, 1998). With regard to other cytokines with known activity on eosinophils, it is of interest that both RSV and rhinovirus appear to be strong inducers of GM-CSF by bronchial and lung epithelial cells (Noah and Becker, 1993; Garofalo and Ogra, 1996; Subauste *et al.*, 1995). These findings have important implications for the activation and enhanced survival of human eosinophils in the airway mucosa.

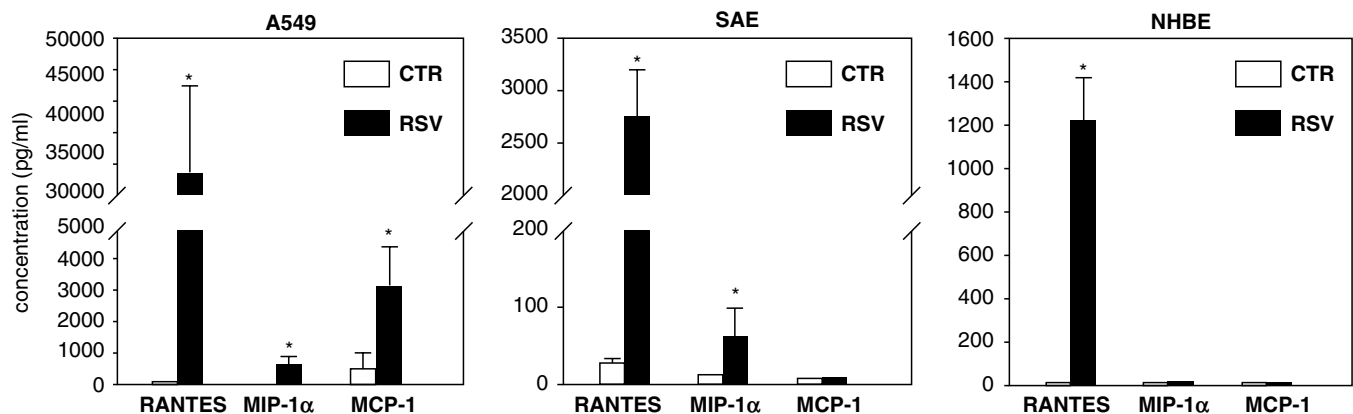


Fig. 83.1. RANTES, MIP-1 $\alpha$ , and MCP-1 production by uninfected (Ctr) or RSV-infected (RSV) A549, normal human bronchial epithelial cells (NHBEs), and epithelial cells from the terminal bronchioles (SAE) cells, as detected by ELISA in the 48-hours culture supernatant (mean  $\pm$  SD,  $n=5$ ;  $p=0.01$  for RSV-infected versus control).

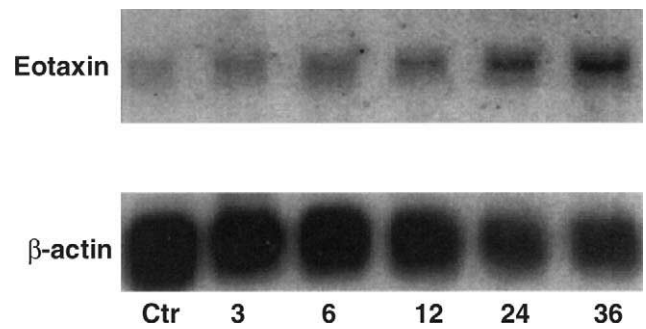


Fig. 83.2. Expression of eotaxin and  $\beta\beta$ -actin mRNA in uninfected (Ctr) or RSV-infected A549 cells (for 3–36 hours), by Northern blot. Total cellular RNA was extracted, fractionated by electrophoresis on 1.2% agarose-formaldehyde gel, and transferred to a nitrocellulose membrane. The membrane was hybridized with  $^{32}$ P-labeled human eotaxin cDNA probe generated by polymerase chain reaction (PCR), at 37°C overnight.

#### Epithelial cytokines and respiratory viruses (*in vivo*)

Still limited are the human studies that have appeared in the literature addressing the presence, concentration, and clinical correlate of cytokines in biologic samples of subjects with respiratory viral infections. Increased levels of IL-1 have been measured in nasal secretions of volunteers during experimental rhinovirus colds (Proud *et al.*, 1994). IL-6 has been detected in the respiratory secretions and middle ear effusions during naturally acquired RSV infection (Matsuda *et al.*, 1995; Noah *et al.*, 1995; Okamoto *et al.*, 1993) and in experimental infection with rhinovirus (Zhu *et al.*, 1996). IL-8 has been demonstrated in nasal lavage fluids of children with upper viral infections (Noah *et al.*, 1995) and in middle ear effusions of children with antigen-proven viral otitis media (Chonmaitree *et al.*, 1996). mRNA for IL-1 $\beta$  and TNF- $\alpha$  have also been found in middle ear effusions positive for RSV genome (Okamoto *et al.*, 1993). In addition, IL-11 can be detected *in vivo* during viral upper respiratory infections (Einarsson *et al.*, 1996). Levels of IL-6,

IL-8, TNF- $\alpha$ , and IL-1 $\beta$  appear to be high in nasal washes of children with RSV lower respiratory tract disease and decrease during convalescence (Neuzil and Graham, 1996). High amounts of the CC chemokines RANTES and MIP-1 $\alpha$  have been demonstrated in the nasopharyngeal secretions of infants with different forms of RSV respiratory disease (Becker *et al.*, 1997; R.P. Garofalo and R.C. Welliver, personal communication, 1998).

#### **Transcriptional activation of epithelial cytokines by respiratory viruses**

Inducible genes such as those that encode for cytokines, chemokines, and CSFs are regulated by transcription factors that are activated by diversified extracellular stimuli and bind to the promoter region of the genes to increase their rate of transcription. Although many transcription factors have been identified and are involved in the regulation of these genes, two, nuclear factor (NF) NF-IL-6 and NF- $\kappa$ B, appear to be of particular importance. The identification of factors that control switches in epithelial cells is central to the understanding of virus-host interaction.

NF-IL-6, a human basic domain-leucine zipper-containing transcription factor, is an important transcription factor participating in inducible gene expression in acute infectious and inflammatory responses (Akira *et al.*, 1990; Brasier and Kumar, 1994). Originally identified as a transcription factor that bound to the inducible enhancer of the IL-6 gene, NF-IL6 has been shown to regulate the promoter activity of other genes, including IL-8 and GM-CSF (Dunn *et al.*, 1994; Stein and Baldwin, 1993) and acute-phase reactants angiotensinogen and serum amyloid A (Akira *et al.*, 1990). The expression of NF-IL-6 is controlled in a tissue-type-dependent fashion. In the many tissues that express NF-IL-6 mRNA, such as the liver and brain, the protein is also produced. In the lung, however, a discrepancy has been noted (Descombes *et al.*, 1990). Although normal lung tissue is, in fact, one of the most productive sources of NF-IL-6 mRNA, the NF-IL-6 protein is almost undetectable. This observation suggests that tissue-specific factors are involved in the control of posttranscriptional expression of NF-IL-6. However, data from Brasier's group indicate that human alveolar type II epithelial cells synthesize the NF-IL-6 transcription factor in response to RSV replication (Jamaluddin *et al.*, 1996). RSV infection stimulates the translational synthesis of a single NF-IL-6 isoform comigrating with recombinant NF-IL-6 (amino acids 24 to 345). The induction of NF-IL-6 is independent of changes in NF-IL-6 mRNA and is associated with an increased rate of NF-IL-6 synthesis. Thus, the accumulation of NF-IL-6 after RSV infection is due to increased translation of the preformed NF-IL-6 mRNA. The mechanisms for enhanced translation of preformed mRNA used by RSV are contrasted with an effect of influenza virus infection in HeLa cells (Wada *et al.*, 1995). Influenza virus infection increased, rapidly and transiently (over 2–4 hours), the DNA-binding activity of preformed NF-IL-6 protein without changing its steady-state levels. That this effect could be reproduced by double-stranded

RNA left the suggestion that double-stranded RNA kinase could be an important virus-inducible activator for post-translational activation of NF-IL-6 DNA-binding activity. Thus, NF-IL-6 activity appears to be inducible by several distinct posttranslational mechanisms by viral infection within the same epithelial cell type.

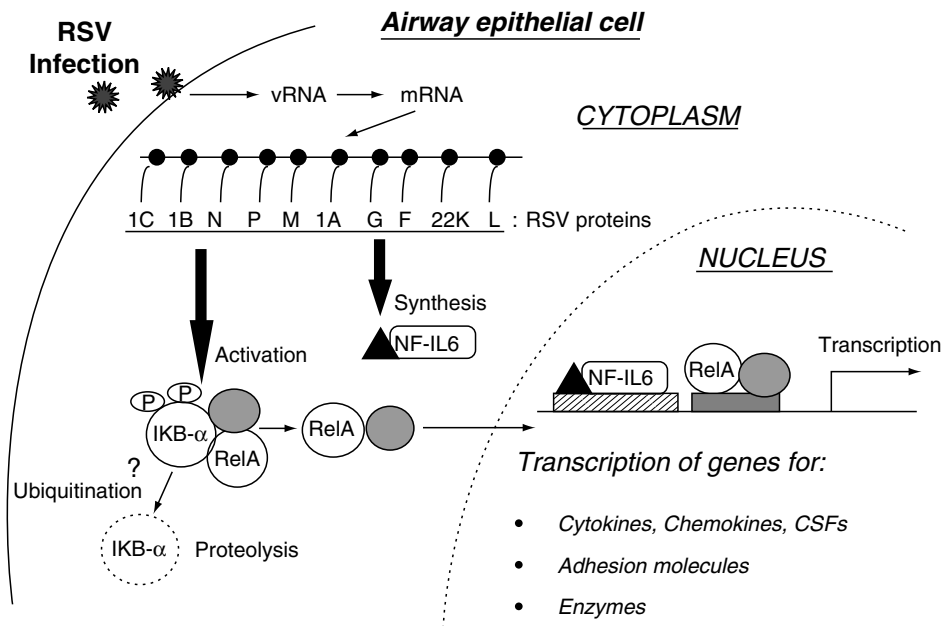
NF- $\kappa$ B is an inducible transcriptional activator implicated in the inducible expression of viral, cytokine, and acute-phase reactant genes by binding to regulatory sites within the promoters of these genes (reviewed in Baeuerle [1991] and Thanos and Maniatis [1995]). Of relevance to pulmonary inflammation, NF- $\kappa$ B activates the expression of IL-8, and many other inflammatory gene products, such as IL-6, IL-11, GM-CSF, TNF- $\alpha$ , and ICAM-1, whose expression is known to be enhanced by RSV infection (see Table 83.3 and Patel *et al.*, [1995]). Therefore, NF- $\kappa$ B activation may be responsible for activating gene networks of inflammatory molecules and cell surface receptors required for immune activation in the bronchial mucosa.

As shown previously (Table 83.3), *in vitro* studies have shown a dramatic production of IL-8 by airway epithelial cells on RSV infection through an incompletely characterized mechanism involving enhanced gene expression. Thus, using the model of lung type II alveolar epithelial cells (A549) to study the initial events in viral-induced regulation of gene expression, we have investigated the mechanism for IL-8 induction by RSV infection. Infection with sucrose purified RSV produced a time-dependent increase in transcriptional initiation rate of the IL-8 gene. Transient transfection of the human IL-8 promoter mutated in the binding site for NF- $\kappa$ B demonstrated that this sequence is essential for RSV-activated transcription (Garofalo *et al.*, 1996a). Gel mobility shift assays (EMSAs) demonstrated RSV induction of sequence-specific NF- $\kappa$ B binding complexes. NF- $\kappa$ B is a transcription factor superfamily composed of NF- $\kappa$ B1, NF- $\kappa$ B2, Rel A-, and c-Rel proteins that heterodimerize in cells expressing them, producing complexes with various transcriptional activity and subtle sequence-specific binding preferences. For this reason, we also tested whether the inducible complexes cross-reacted with specific antibodies for various NF- $\kappa$ B subunits in the EMSA. These complexes were supershifted only by antibodies directed to the potent transactivating subunit Rel A. Both Western immunoblot and indirect immunofluorescence assays showed that cytoplasmic Rel A in uninfected cells localized to the nucleus after RSV infection. Rel A activation requires replicating RSV because neither viral conditioned medium nor UV-in-activated RSV were able to stimulate its translocation. Other laboratories have demonstrated an increase in NF- $\kappa$ B DNA-binding activity following RSV infection (Bitko *et al.*, 1997; Fielder *et al.*, 1996; Mastronarde *et al.*, 1996b), and a simultaneous increase in the nuclear abundance of the potent transcriptional activator RelA (Bitko *et al.*, 1997). This event appears to be essential not only for IL-8 but also for the activity of a genetic network including the cytokines IL-1, IL-6, and IL-11 in RSV-infected epithelium (Bitko *et al.*, 1997). Moreover, NF- $\kappa$ B activation has been reported as a

consequence of Sendai virus infection of fibroblastic and renal carcinoma cells (Garoufalidis *et al.*, 1994) and rhinovirus infection of alveolar epithelial cells (Zhu *et al.*, 1996). The kinetics of Rel A activation in response to RSV infection occurs slowly, requiring 3–12 hours before significantly detectable NF- $\kappa$ B protein can accumulate in the nucleus (Garofalo *et al.*, 1996a). This contrasts with an extremely rapid NF- $\kappa$ B activation in response to hormone receptors (such as TNF- $\alpha$ ) where NF- $\kappa$ B is activated within minutes and the intermediate activation observed during rhinovirus infection (Zhu *et al.*, 1996). Other investigators, using non-purified viral preparations, have shown a biphasic effect for NF- $\kappa$ B activation (Fielder *et al.*, 1996; Mastrorarde *et al.*, 1996b), where an initial transient, replication-independent increase in NF- $\kappa$ B binding is seen 30–90 minutes postinfection, followed by a nadir and a gradual reaccumulation after 24 hours. These differences in NF- $\kappa$ B kinetics, as well as in the pattern of cytokine and other inflammatory gene expression, may be due to the contamination of the nonpurified viral preparations with NF- $\kappa$ B activating cytokine activity such as IL-1, known to be produced by human epithelial cells routinely used for RSV and other respiratory virus growth. Our observations imply that although NF- $\kappa$ B activation may be a final common pathway for both infectious and inflammatory signaling, this event is mediated by distinct intracellular signaling mechanisms. During RSV infection, we note that the kinetics of Rel A activation coincide with the production of viral antigens. This time course and the obser-

vation that both NF- $\kappa$ B and IL-8 production do not occur in response to exposure to RSV-conditioned medium or to UV-inactivated virus argues that the process of active viral replication may be necessary to trigger NF- $\kappa$ B binding and subsequent IL-8 synthesis. Indeed, viral products of replication such as free radicals (Schreck *et al.*, 1991) and double-stranded RNA (Visvanathan and Goodbourn, 1989) are potent activators of NF- $\kappa$ B in certain cell types. It is interesting to speculate that the expression of one or more viral gene products may be responsible for activating Rel A translocation (Fig. 83.3).

Inducible NF- $\kappa$ B subunits interact with cytoplasmic inhibitors, collectively known as I $\kappa$ Bs, through motifs contained within a conserved NH<sub>2</sub>-terminal Rel homology domain. I $\kappa$ B subunits, responsible for cytoplasmic retention and inactivation of NF- $\kappa$ B DNA-binding activity, consist predominantly of four isoforms, I $\kappa$ B $\alpha$ , I $\kappa$ B $\beta$ , I $\kappa$ B $\gamma$ , and the NF- $\kappa$ B1 precursor that are expressed and regulated in a cell-specific fashion (Beg and Baldwin, 1993). NF- $\kappa$ B-inducing signals control its cytoplasmic-nuclear abundance by a mechanism involving proteolytic degradation of the I $\kappa$ B inhibitor. Once liberated, free cytoplasmic NF- $\kappa$ B passes through the nuclear pore complex and enters the nucleus to bind and activate target genes. Stimulation of cells with TNF- $\alpha$  results in rapid serine phosphorylation of the I $\kappa$ B $\alpha$  NH<sub>2</sub> terminus, an event coupled to the rapid polyubiquitination and proteolysis of phospho-I $\kappa$ B through the 26S proteasome (DiDonato *et al.*, 1996). Brasier's group has



**Fig. 83.3.** Infection of alveolar type II epithelial cells by RSV results in the rapid synthesis (within 3 hours) of NF-IL6, a basic domain-leucine zipper-containing transcription factor. In addition, RSV infection results in the activation of NF- $\kappa$ B (*i.e.*, Rel A DNA binding) that begins at 3 hours and peaks at 24 hours and that involves proteolysis of the I $\kappa$ B $\alpha$  inhibitor *via* a still unknown and (largely) proteasome-independent pathway. Cytoplasmic-nuclear translocation of RelA and binding of RelA and NF-IL-6 to regulatory consensus sites within the promoters of cytokine, adhesion molecules, or other inflammatory genes are responsible for their transcription. The role of specific RSV protein in the translational regulation of NF-IL-6 and in the activation of NF- $\kappa$ B is currently unknown. vRNA, viral RNA; 1C, 1B, N, P, M, 1A, G, F, 22K, L identify the 10 RSV proteins.

systematically investigated the mechanism and kinetics of NF- $\kappa$ B activation in RSV-infected airway epithelial cells in comparison to TNF- $\alpha$  (Jamaluddin *et al.*, 1998). In contrast to the rapid activation of Rel A produced by TNF- $\alpha$ , RSV infection produces a gradual increase in Rel A binding peaking at 24 hours. For both activators TNF- $\alpha$  and RSV, I $\kappa$ B $\alpha$  and I $\kappa$ B $\beta$  proteolysis occurred in parallel with the increases in Rel A DNA-binding activity. Specific proteasome inhibitors lactacystin, MG-132, and ZLLF-CHO significantly blocked 26S protease activity and I $\kappa$ B $\alpha$  proteolysis induced by TNF- $\alpha$ . However, although total proteasome activity in RSV-infected cells increased by twofold and its activity was almost completely inhibited by the proteasome inhibitors, they did not prevent I $\kappa$ B $\alpha$  proteolysis by RSV infection. These data indicate a major component of I $\kappa$ B $\alpha$  proteolysis occurs via a proteasome-independent mechanism in viral-infected epithelium.

## SUMMARY

RSV, the most common etiologic agent worldwide to induce lower respiratory tract infections in infants and young children, has been linked to the development of asthma, airway hyperresponsiveness, and sensitization to environmental allergens. The mechanisms of RSV-induced airway disease and its long-term consequences are largely unknown, but the delicate balance between immunopathology and immunoprotection in the airway mucosa may be altered by an exuberant and unwanted local inflammatory response. Massive infiltration of mononuclear cells and activation of eosinophil and basophil leukocytes has been shown to correlate with the severity of acute RSV disease in both human and animal models. Inflammatory cytokines and CC chemokines with discrete target-cell selectivity for eosinophils and Th2 cells are strongly induced to express in RSV-infected respiratory epithelium. We propose a cohesive hypothesis that links RSV, and perhaps other respiratory viral infections, to the development of respiratory allergic disease, in which the inducible production of CC chemokines by RSV is regulated by intracellular pathways and signaling mechanisms characterized by the increased rate of NF-IL-6 synthesis, cytoplasmic-nuclear translocation of the potent transcription factor NF- $\kappa$ B leading to the transcriptional induction of CC chemokine genes. The secretion of CC chemokines, including RANTES and eotaxin by respiratory epithelial cells, in combination with Th2-derived cytokines, is the central pathogenetic event in airway mucosa inflammation, sensitization to bystander antigens, and augmentation of allergen-induced Th2 cell recruitment. Moreover, pattern and absolute levels as well as relative concentrations, together with the kinetics of release of epithelial cell chemokines in different areas of the respiratory tract, are all factors that may significantly contribute to the various histologic and inflammatory features of RSV-induced airway disease. Selective production of MIP-1 $\alpha$ , MCP-1, and the severalfold higher amount of RANTES released by epithelial

cells of the small bronchioles and lung in comparison to bronchial or upper airway epithelium, along with autocrine and paracrine mechanisms of amplification may largely explain the findings of mononuclear cell infiltration, eosinophil and basophil migration, and activation. Future studies, both in children with different forms of RSV-induced airway disease and in animal models, are needed to correlate the different parameters of histopathology, inflammation, and immune response with the expression and secretion of cytokines, chemokines, and CSFs in airway mucosa.

## ACKNOWLEDGMENTS

We thank the current and former fellows of Drs. Brasier's, Garofalo's and Patel's laboratories, including Antonella Casola, Barbara Olszewska-Pazdrak, Mohammad Jamaluddin, Zili Jiang, Masaru Kunimoto, Monica Manganaro, Mona Sabry, and Tadahito Saito, who greatly contributed to the published and unpublished work discussed in this chapter. We greatly appreciate the help of Judith Maurino and Virginia Reimer in the preparation of this publication. This work was supported in parts by grants A115939 and A140218, from the National Institutes of Health.

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