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Review Article

Viral infection and asthma: Respiratory syncytial virus and wheezing illness

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Abstract

A strong link between bronchiolitis and asthma has been indicated. Bronchiolitis that occurs in infants is manifested physiologically by a widespread narrowing of the air passages and, clinically, by asthma-like symptoms.The major cause of bronchiolitis is respiratory syncytial virus infection. While the precise pathophysiologic sequences of infection are incomplete, many observations have suggested that there is an infiltration of eosinophils in the airways. Current studies have shown that the respiratory syncytial virus penetrates the pulmonary defenses and initiates immunologic responses. The histamine and leukotriene mediators that are released produce an inflammatory reaction and the chemotactic factors bring eosinophils to the site of the reaction. Degranulation of eosinophils can release eosinophil cationic protein into the airways. Our finding that chemoattractants for eosinophils, interleukin-8 and RANTES (regulated upon activation, normal T cell expressed and presumably secreted) were detected in nasopharyngeal aspirates of infants with bronchiolitis suggests that such chemokines from epithelial cells may induce an eosinophil infiltration in the airway. Similar allergic inflammatory changes have been observed in asthma and in epithelial cells infected with respiratory viruses. Future investigation of the mechanism by which bronchiolitis can induce asthma will provide benefits in the treatment and prevention of asthma in sensitive individuals.

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Introduction

Today, in industrialized countries, asthma is a leading chronic childhood disease.[1] According to the Dutch hypothesis of the natural history of chronic airway disease, childhood asthma is generally atopic and develops following allergen exposure, but additional factors, such as viral respiratory infections, are usually required for the clinical features of asthma to develop.[2] Epidemiologic or cohort studies have also suggested that viral respiratory infections are the most common stimuli that evoke acute exacerbations of asthma.[3,4] Recent studies have provided new findings on the mechanism of respiratory syncytial virus (RSV) infection in association with asthma. Therefore, this is a critical issue in the prevention of the development of asthma in adulthood.

In the present paper we review RSV infection and the wheezing illness of bronchiolitis in early infancy, as well as asthma.

Viral respiratory infection and wheezing in childhood

Acute respiratory infections are the most common childhood illnesses. Over 90% of these infections are caused by more than 200 viruses. The peak frequency of acute lower respiratory tract infection is approximately 20–30 episodes/1000 children per year in infants and gradually declines to five episodes/1000 children per year by 9 years of age, where it remains throughout adolescence (Fig. 1).[5]

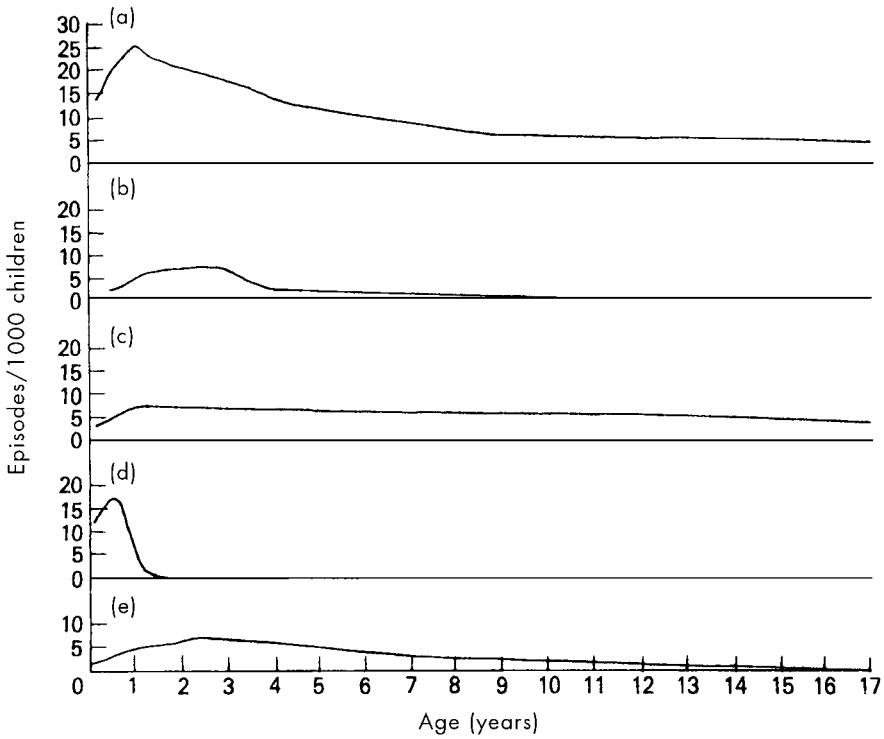

Fig. 1 (a) Total number of episodes of acute lower respiratory infection/1000 children per year compared with age and the number of episodes of (b) acute laryngotracheobronchitis, (c) bronchitis, (d) acute viral bronchiolitis and (e) pneumonia. Reproduced with permission from Phelan *et al*.[5]

Many studies have indicated that there is a strong link between bronchiolitis in infancy and asthmatic symptoms in later childhood.[6–8] In the first year of life, 30% of all infants have at least one episode of wheezing due to a viral respiratory infection. Of these, one-third of infants still wheeze by 6 years of age and manifest childhood asthma.[9] Some studies have demonstrated that children who started to wheeze in early life and who continued to wheeze at 6 years of age were more likely to have mothers with a history of asthma, elevated serum IgE levels and to have normal lung function in the first year of life and diminished values for maximal expiratory flow at functional residual capacity ($\dot{V}_{max}$ FRC) than those who had never wheezed.[10] Respiratory syncytial virus is the major cause of acute lower respiratory tract infection, particularly in infancy, and is responsible for approximately 80% of bronchiolitis, 30% of pneumonia, 15% of bronchitis and 12% of croup cases.[5]

Respiratory syncytial virus infection

General virology of RSV

Respiratory syncytial virus was first isolated in 1956.[11] It belongs to the paramyxovirus family and is medium sized (120–300 nm), with a lipid envelope surrounding RNA. On the surface of the lipid envelope, the fusion (F), attachment (G) and unknown function (SH) glycoproteins are expressed. There are also the M1 (first matrix) and M2 (second matrix or 22 kDa) proteins in the matrix and the nucleoprotein (N), phosphoprotein (P) and large protein (L) in the nucleocapsid (Table 1).[12]

The virus attaches to host cells via a G-protein and penetrates via the district F protein. These structural proteins are important viral antigenic determinants and initiate immunologic responses. Respiratory syncytial virus has two serologic subgroups: (i) the A (long-strain prototype); and (ii) the B (18537 strain prototype). Clinical differences between the two serotypes have been elusive, although some studies indicate that B strains cause less severe disease.

Transmission of RSV

The major mode of transmission of RSV appears to be by larger droplets, like rhinovirus, rather than by small-particle aerosols, as for influenza virus infections. Transmission requires either close contact with infected individuals or contamination of the hands and subsequent contact between fingers and nasal or conjunctival mucus.[13] These transmission patterns may translate into a slower spread of the disease and not be pandemic.

Respiratory syncytial virus replication is initially confined to the nasopharynx, with an incubation period of

4–6 days. Infected cells spread from direct mucosal cell-to-cell contact and form a syncytium. Clinical symptoms of lower respiratory tract infection manifest 1–3 days after the onset of nasopharyngeal infection.

Epidemiology of RSV infection

Approximately 70% of children have received an initial RSV infection by 12 months of age. This virus usually causes lower respiratory tract disease in infancy. The peak incidence of bronchiolitis is between 1 and 6 months of age, while pneumonia occurs throughout the first year of life.[5] Overall, 1–2% of RSV infections result in hospitalization for bronchiolitis and pneumonia. The mortality rate for RSV is less than 1% of all hospitalized patients;[14] however, it rises in high-risk groups[15] such as premature infants and infants with congenital heart disease,[16] chronic lung disease[17] or immunosuppressive diseases.[18] After the age of 3–4 years, RSV usually causes upper respiratory tract infections. Despite long-lasting serum antibodies, reinfection is common.[19,20]

Respiratory syncytial virus and bronchiolitis

The diagnosis of bronchiolitis is made if an infant acquires asthma-like symptoms and it is known that RSV is endemic.[21] Bronchiolitis occurs only in infancy. Because the first infection in infancy cannot exhibit a sufficient immune defense for RSV,[22] infected viruses may easily spread to the lower respiratory tract. In addition, the small conducting airways in infants are more easily obstructed by inflammation than in adults.[23] Moreover, a large area of injured airway epithelial cells may create prolonged hypersensitivity in the airway. Bronchiolitis and asthma are very similar clinical illnesses, with a few differences (Table 2).

Bronchiolitis is characterized histologically by a peribronchiolar mononuclear infiltration with edema of the walls.[24] A recent autopsy study of infants who died of RSV infection has confirmed the shedding of virus-infected epithelial cells into the bronchial lumen.[25]

Treatment and prevention of bronchiolitis

Infants with respiratory distress caused by bronchiolitis should be hospitalized, but generally only supportive treatment is given. β-Adrenoceptor agonists and corticosteroids are used for the treatment of bronchiolitis empirically.[21] However, responses to bronchodilators are unpredictable: some infants show prompt improvement on inhalation, but others resist such treatment and gain no benefit.[26] A number of prospective controlled trials to

Table 2 Differences between childhood bronchiolitis and asthma

	Bronchiolitis	Asthma
Cause	Infection (RSV)	Allergy
Course	Acute	Chronic
Age	Infant (< 6 months)	All
Sex	Male > female	Male > female
Season	Winter	Autumn, spring
Treatment	Ribavirin®	Steroid, β-adrenoceptor agonists
Risk	Immature infant, CLD, CHD, immune deficiency	Atopy

RSV, respiratory syncytial virus; CLD, chronic lung disease; CHD, congenital heart disease.

Table 1 Major proteins of respiratory syncytial virus

Protein	kDa	Glycosylation	Viability	Function
Surface proteins				
G-protein	84	63%, *O*-linked	+ + +	Attachment
F protein	68	5%, *N*-linked	+ +	Fusion
SH	~ 20	50–70%	+	Unknown
Matrix proteins				
M1	26		+	Membrane stability?
M2(22 kDa)	22		(+)	Unknown
Nucleocapsid				
Nucleoprotein	42		(+)	Structural
Phosphoprotein	34		(+)	Polymerase component?
Large protein	250		(?)	RNA polymerase

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evaluate corticosteroids in the treatment of bronchiolitis have been inconclusive.[27] The recent National Institutes of Health (NIH) Guidelines for the Diagnosis and Management of Asthma recommended that inhalation of β-adrenoceptor agonists and corticosteroids was attempted for wheezy infants, because the symptoms of bronchiolitis and asthma cannot be differentiated.[28] Ribavirin® (Viazole, ICN Pharmaceuticals, Coscimes, USA) aerosol, a synthetic nucleoside that acts by limiting viral replication, is currently recommended for use in high-risk patients.[29]

Currently, experimental therapies are available or under investigation for the prevention of RSV infection by active immunization. In the early 1960s, a formalin-inactivated RSV (FI-RSV) vaccine used in trials showed that recipients of the vaccine suffered from more severe bronchiolitis following RSV infection than control infants.[30] Despite current efforts to develop subunit vaccines, such as F protein or live attenuated vaccines, none of these has succeeded to date.[31] Passive immunization in high-risk groups has also been tried. Clinical trials using standard intravenous immunoglobulin failed to demonstrate a reduction in the hospitalization rate.[32] In the late 1980s, infants who received RSV-enriched immunoglobulin achieved sufficiently high neutralizing antibody levels to result in a reduction of the hospitalization rate.[33] However, subgroup infants with congenital heart disease failed to show a reduction in the severity of RSV infection. Immunoglobulin enriched with RSV was licensed by the Food and Drug Administration (USA) and has been available in the US since 1996. There was a recent trial of monoclonal antibodies for prophylaxis against RSV infection because of the possibility of intramuscular injection due to small amounts required.[34]

Airway inflammation in asthma

Asthma has been defined by airway hypersensitivity and reversible airway flow limitation for an extended period.[35] In addition to these symptoms, asthma is defined in the first NIH Guidelines issued in1991 as chronic airway inflammation characterized by an influx of mast cells, lymphocytes and eosinophils.[36] The most important of these may be the influx of eosinophils.

The presence of peripheral eosinophilia in asthmatic patients is well known. It has also been recognized that a number of eosinophils are frequently found in and around the bronchi of patients who have died of asthma.[37] The immunostaining of bronchial tissue from such patients has revealed the existence of a major basic protein, which is one of the granule proteins in the eosinophil, deposited in the airway.[38] Eosinophilia in the airway indicates the allergic inflammation of asthma, in contrast with the neutrophilia observed in common acute inflammation.[39]

According to a recent immunologic concept, inflammation is modulated by T helper (Th) lymphocytes, which are classified into Th1 and Th2 types based on their pattern of cytokine production. The Th1 cells secrete interferon (IFN)-γ, interleukin (IL)-2 and tumor necrosis factor-β, whereas Th2 cells secrete IL-4, IL-5, IL-6, IL-10 and IL-13. The Th1 cells enhance cellular or infectious immune responses, while Th2 cells enhance humoral or allergic immune responses, such as asthma.[40] There is a counterinhibitory link between Th1 and Th2 lymphocytes: Th2 cells produce IL-10, which inhibits the growth and function of Th1 cells, while Th1 cells produce IFN-γ, which inhibits Th2 cell activity (Fig. 2).[41]

The hypothesis based on the Th1 and Th2 theory is able to explain that when allergens such as house dust mites are inhaled, they will interact with airway epithelial cells, Th2 cells and intraepithelial mast cells.This results in the release of chemokines, such as RANTES (regulated upon activation, normal T cell expressed and presumably secreted), or cytokines, such as IL-4 and IL-5, which promote selective eosinophil migration. Eosinophils release granule proteins, which cause epithelial damage, and lipid-derived mediators (e.g. leukotriene), which cause mucus hypersecretion and bronchoconstriction in addition to further supporting eosinophil recruitment.[42]

Effects of viral infection in airway inflammation

It has been shown that the virus affects airway hypersensitivity and air flow limitation, as a result of epithelial disruptions, cholinergic and adrenergic nerve dysfunctions and inactivations of tachykinins such as substance P. In addition to these, it has recently been noted that viral respiratory infections can associate with eosinophil infiltration in the airways (Fig. 3).[43]

T cells

Some studies have shown increased levels of IFN-γ, IL-2 and Th1 cytokines in peripheral blood mononuclear cells infected with respiratory viruses.[44] However, results from other studies have shown that viral infections can induce

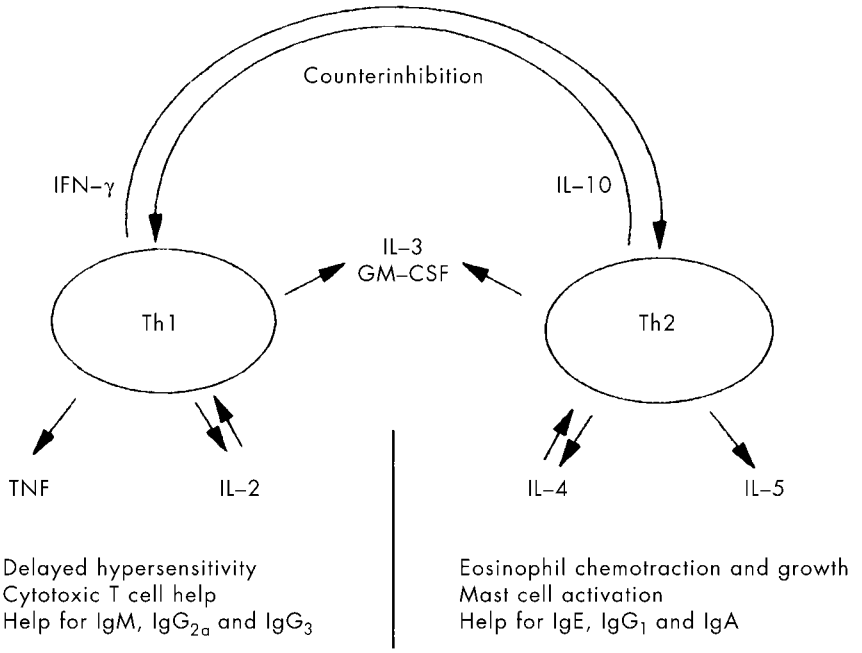

Fig. 2 Counterinhibitory link between T helper cell subsets. T helper (Th)1 cells are typical antiviral immune effectors and are central to the coordination of the classic immune responses to antigens presented by macrophages and dendritic cells. The Th2 cells appear to be specialized for allergic responses in the asthmatic airway. IFN-γ, interferon-γ; GM-CSF, granulocyte–macrophage colony stimulating factor; IL, interleukin; TNF, tumor necrosis factor. Reproduced with permission from Openshaw and O'Donnell.[41]

both Th1- and Th2-type cytokine responses.[45] Viral infections are known to activate $CD4^+$ T cells and produce a Th1-type immune response, however it has not been determined how viral infections can cause an increase in Th2-type cytokines. Recent studies have demonstrated that $CD8^+$ T cells produce both IFN-γ and IL-10 mRNA, with lower levels of IL-2, IL-4 and IL-5 in bronchoalveolar lavage fluid of virus-infected mice.[46,47] These results suggest that $CD8^+$ T cells play a critical role in regulating Th2-driven eosinophilia.

Epithelium

Current studies have demonstrated that many kinds of cytokines and chemokines are generated by airway epithelial cells infected with respiratory viruses. Up-regulation of IL-1β, IL-6, IL-8, IL-11, IFN-γ, tumor necrosis factor (TNF)-α and granulocyte–macrophage colony stimulating factor (GM-CSF) expression has been reported in epithelial cells infected with viruses such as RSV.[48,49] More recent studies have shown that regulated upon activation, normal T cell expressed and presumably secreted (RANTES) is produced from RSV-infected epithelial cells.[50,51] A CC chemokine, RANTES is an effective and selective chemoattractant for eosinophils.[52] Airway epithelial cells infected with RSV may induce the transendothelial migration of human blood eosinophils by RANTES.[53]

Interleukin-8 is a major neutrophil chemoattractant[54] and may play an important role in asthma exacerbation.[55,56] It can also act as a chemoattractant for eosinophils primed by IL-3, IL-5 or GM-CSF.[57] Interleukin-11 is a new pleiotropic cytokine of the IL-6 family. It is known to be a T cell-dependent stimulator of B cell immunoglobulin production and can induce airway hypersensitivity and bronchospasms caused by cholinergic effects and tachykinin production.[58] A recent study has shown an increase in IL-11 concentrations in nasopharyngeal aspirates from children with upper respiratory infections.[59] The GM-CSF also has the ability to activate eosinophils. The roles of pro-inflammatory cytokines, such as IL-1β, IL-6, IFN-γ and TNF-α, in eosinophil infiltration are not clear. These results suggest that some cytokines and chemokines generated from infected airway epithelial cells seem to contribute to eosinophil infiltration.

Respiratory syncytial virus antigen

A massive infiltration of eosinophils has been found in the postmortem lungs of RSV-infected children who had received FI-RSV vaccine and recipients of this vaccine had peripheral eosinophilia at the time of subsequent natural RSV infection.[30]

The mechanisms of eosinophilia that has been induced by the RSV vaccine have been clarified with studies using

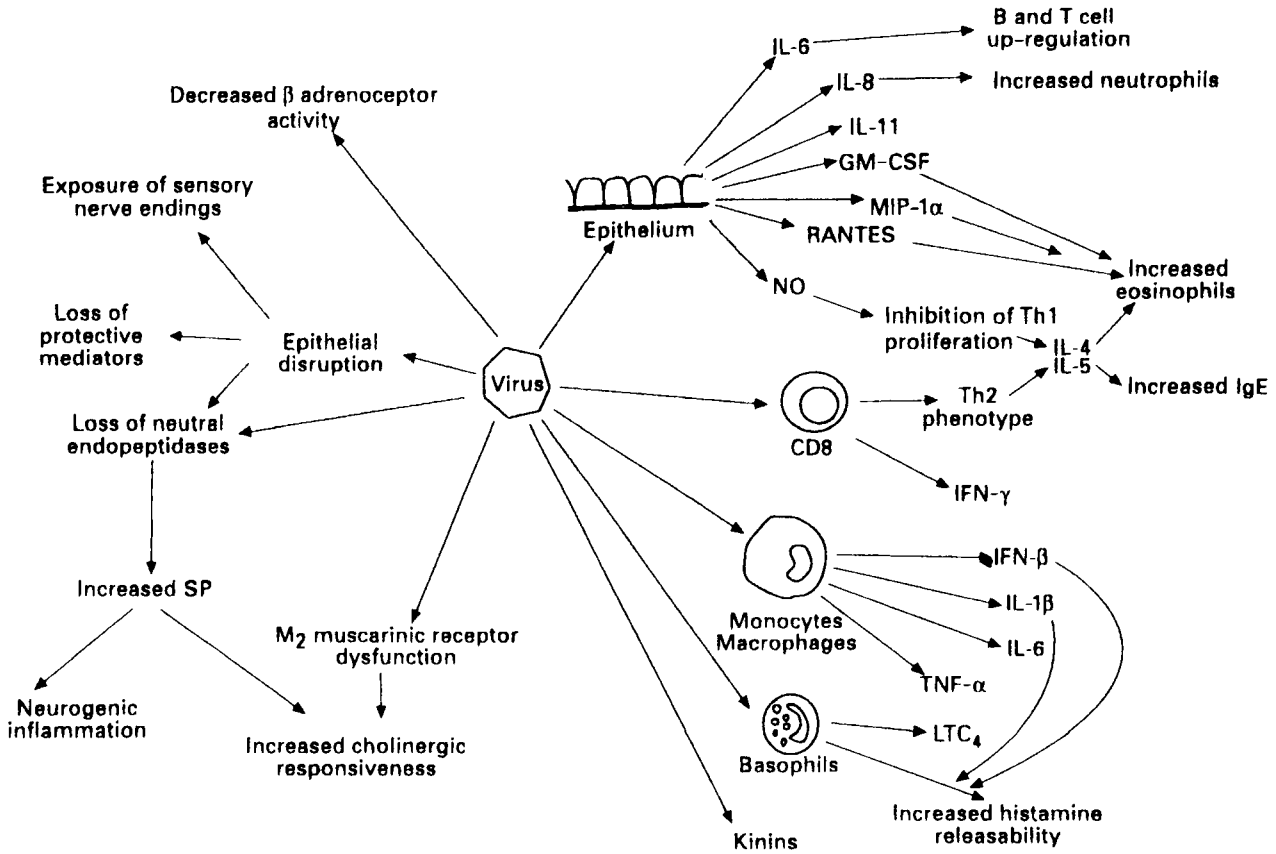

Fig. 3 Summary of the effects of viruses of the upper respiratory tract that may lead to exacerbations of asthma. Viruses (center) have been shown to induce epithelial disruptions, cholinergic and adrenergic nerve dysfunctions and inactivations of tachykinins, such as substance P (SP). These have resulted in the development of neurogenic inflammation. Viruses have also been shown to interact with airway epithelium leading to increases in the production of interleukin (IL)-6, IL-8, IL-11, granulocyte–macrophage colony stimulating factor (GM-CSF) and the chemokines macrophage inflammatory protein (MIP)-1 and regulated upon activation, normal T cell expressed and presumably secreted (RANTES). These induce B and T cell up-regulation and an increased number of eosinophils and neutrophils. This interaction may also lead to increases in nitric oxide (NO) that may inhibit Th1 proliferation. Viruses may act on $CD8^+$ T lymphocytes to cause a shift to a T helper cell (Th) 2-type pattern of cytokine production. They have also been shown to act on monocytes and macrophages, leading to increased production of interferon (IFN)-β, IL-1β, IL-6 and tumor necrosis factor (TNF)-α, and on basophils, leading to increases in basophil histamine releasability and leukotriene (LT)C_4 production. All these factors may contribute to the development of exacerbations of asthma following upper respiratory tract infection. Reproduced with permission from Corne and Holgate.[43]

mice. BALB/c mice inoculated with the FI-RSV vaccine developed a Th2-like pattern of cytokines in the lungs when later challenged with RSV intranasally. In contrast, inoculation with live RSV induced a Th1-like pattern of cytokines.[60] Following immunization with recombinant vaccinia viruses (rVV) expressing individual RSV proteins G and F, clear results were obtained in studies of BALB/c mice sensitized with rVV and challenged 5 days later with live RSV intranasally. Mice sensitized with G-protein produced high levels of IL-4 and -5 in the lung and developed extensive pulmonary eosinophilia, while mice sensitized with F protein produced IL-2 and developed mononuclear cell infiltration.[61] These results indicate that the G-protein can strongly induce a Th2-like response. Further experiments have shown similar results in mice sensitized with individual RSV proteins (Table 3).[62,63]

A recent study has shown that mice treated with IL-12 at various times during immunization by G-protein vaccine and challenged by RSV had reduced vaccine-induced lung eosinophilia but increased total pulmonary lymphocyte infiltration. Cytokine analysis showed that IFN-γ production increased, whereas IL-4 and -5 produc-

Table 3 Summary of the main features of antiviral immunity in respiratory syncytial virus disease in BALB/c mice

	Role in primary infection	Effect of passive transfer on virus	Effect on RSV lung disease	Main proteins seen
B cells/antibody	Virus clearance	Reduced titer or no change	Reduced or no change	F and G
Cytotoxic T cells	Early alveolar response	Reduced titer	Shock lung (PMN and hemorrhage)	M2 and F
Helper T cells				
Th1	Later peribronchial infiltrate	Reduced titer (probably)	Unknown	F (probably)
Th2		Reduced titer	Eosinophilia and shock lung	G

B cell immunity does not enhance disease and sometimes reduces it. T cell immunity is also essential to virus elimination, but can be detrimental if the strength or timing of the response is inappropriate.

RSV, respiratory syncytial virus; PMN, polymorphonuclear neutrophil; G, attachment protein; F, fusion protein; M2, second matrix (22 kDa); Th, T helper cell. Reproduced with permission from Openshaw.[63]

tion decreased,[64] suggesting that IL-12 may be the key cytokine of the Th1 response by producing IFN-γ, which controls the Th2 response in viral infection.[65]

Eosinophil infiltration and bronchiolitis

Children with RSV infection have been shown to develop an RSV-specific IgE response in the airway.[66] The concentrations of RSV IgE in wheezy infants are higher than those in children who do not wheeze after RSV infection.[67] The degree of RSV IgE responsiveness was correlated with the severity of illness by the degree of hypoxia. In addition, the RSV IgE response was correlated with increased concentrations of histamine in the nasopharyngeal aspirates of infants with bronchiolitis.[68] Leukotriene (LT)C_4, which is a potent bronchoconstrictor metabolite of the arachidonic acid cascade in eosinophils, has been detected in the nasopharyngeal aspirates of infants with RSV infection. The concentrations of LTC_4 detected were correlated with those of the RSV IgE.[69] Leukotriene E_4, a selective eosinophil chemotactic factor, was present in the respiratory tract of infants with RSV bronchiolitis. Concentrations of LTB_4, a chemoattractant of inflammatory cells, were significantly correlated with the degree of hypoxia, although LTB_4 has little bronchoconstrictor activity. It has been suggested that LTB_4 levels may reflect the activation of inflammatory cells, such as neutrophils and eosinophils, in the airway.[70]

Significant increases in the levels of eosinophil cationic protein, an indicator of the activity of eosinophils, were observed in nasopharyngeal aspirates from infants with RSV bronchiolitis and from asthmatic school-age children.[71–73] These findings suggest that eosinophil activation occurs in the respiratory tract during RSV bronchiolitis and may play a significant role in the development of virus-induced airway obstruction. With regard to the mechanism of eosinophilia in the airway in bronchiolitis, we have demonstrated that the Th2-type cytokines IL-4 and IL-5 were not detected, whereas the Th1-type cytokines IFN-γ and IL-12 and the chemokines RANTES and IL-8 were detectable in nasopharyngeal aspirates.[74,75] These results suggest that chemokines from epithelial cells may induce eosinophil infiltration in the airway in RSV bronchiolitis, as in asthma.

Summary

A number of causes have been postulated for the increased airway reactivity of asthma. Well-controlled investigations have demonstrated that respiratory viruses are major etiologic factors. In infants, the most important infectious agent is RSV. The mechanism by which RSV induces asthma-like symptoms is unknown, but it is probable that the resulting inflammatory changes in the airway mucosa alter host defenses and make the airway more susceptible to exogenous stimuli. Because a strong link between bronchiolitis and later asthmatic symptoms has been indicated from many studies, it is crucial to elucidate the association between viral infection and asthma. Findings will be useful for the prevention of RSV bronchiolitis, which will result in the prevention of asthma development in sensitive individuals.

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