

THE JOURNAL OF  
**ALLERGY**  
AND  
**CLINICAL IMMUNOLOGY**

VOLUME 100

NUMBER 2

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## Updates on cells and cytokines

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## Viruses in asthma

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### EPIDEMIOLOGY

Viral respiratory tract infections are a major cause of wheezing for infants and adult patients with asthma (Fig. 1). In infancy, infections with respiratory syncytial virus (RSV) provoke episodes of wheezing, which may be recurrent. Martinez et al.<sup>1</sup> have shown that most infants who wheeze during respiratory tract infections have less difficulty as they grow older, and infections with RSV are unlikely to be the sole cause of asthma. In patients with existing asthma, respiratory tract infections with viruses, and not bacteria, are a major cause of exacerbations of wheezing. To more precisely determine the cause of wheezing episodes, Johnston et al.<sup>2</sup> combined the technique of reverse-transcription polymerase chain reaction with culture to detect viruses in association with exacerbations of asthma. These investigators determined that 80% to 85% of school-aged children with wheezing episodes tested positive for virus, and the virus most commonly detected was rhinovirus. Similar techniques have been used to show that about half of asthma exacerbations in adults are caused by viral infections. Thus there is now evidence that in subjects of all ages with asthma, respiratory tract infections, particularly infections caused by rhinovirus, are an important factor in exacerbations of wheezing.

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Grant Support: NIH AI 26609, AI 34891, AI 40685 and HL 44098 Epidemiology.

Received for publication Apr. 14, 1997; revised May 19, 1997; accepted for publication May 20, 1997.

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J Allergy Clin Immunol 1997;100:147-50.

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0091-6749/97 \$5.00 + 0 1/1/83338

### Abbreviation used

RSV: Respiratory syncytial virus

Duff et al.<sup>3</sup> evaluated risk factors for wheezing in infants and children who were seen in the emergency department. In children younger than 2 years of age, RSV infection and cigarette smoke exposure were frequently found in association with acute wheezing. In older children, viral infections, most often caused by rhinovirus, continued to be associated with wheezing, as was respiratory allergy. Children with both allergy and viral infection had an especially high incidence of wheezing, providing evidence for a significant interaction between these two factors.

### MECHANISMS OF VIRUS-PROVOKED ASTHMA

To more fully understand how viral respiratory tract infections influence asthma, investigators have evaluated the effect that respiratory tract infections have on airway symptoms, function, and inflammation. First, to determine whether viral respiratory tract infections alter lower airway responses to allergen, Lemanske et al.<sup>4</sup> identified 10 patients with allergic rhinitis and experimentally infected them with rhinovirus. After virus inoculation, these individuals showed an increase in airway reactivity to both inhaled allergen and histamine. Furthermore, and perhaps providing insight to mechanisms of virus effects on asthma, these individuals had an increased frequency of a late allergic reaction to inhaled antigens, and this persisted for 4 weeks after the viral respiratory tract infection.

These observations have been expanded through the use of bronchoscopy to sample lower airway secretions

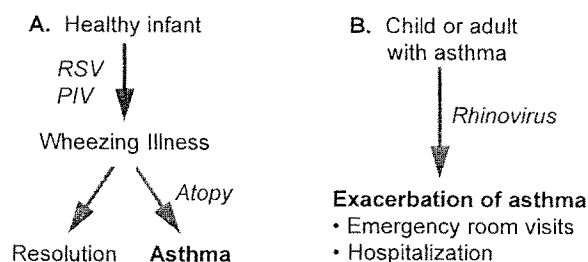


**FIG. 1.** Pattern of response to respiratory viruses in healthy infants (A) and children and adults with existing asthma (B).

and tissues during experimentally induced infections. Calhoun et al.<sup>5</sup> used bronchoscopy to study the inflammatory response to allergen in individual lung segments before, during, and 1 month after rhinovirus infection. Rhinovirus infection enhanced the immediate release of histamine to antigen, presumably from the mast cells, and also increased recruitment of eosinophils to the lung segment after antigen challenge. The increased responses to antigen persisted 4 to 6 weeks after recovery from the experimental viral infection. These observations also indicated that viral respiratory tract infections can enhance both the immediate (i.e., mast cell release of mediators) and late-phase allergic reactions (i.e., bronchial inflammation) and suggest a potential mechanism for the enhanced likelihood of a late-phase inflammatory reaction to antigen during the rhinovirus infection.

To further these findings, Fraenkel et al.<sup>6</sup> evaluated histopathologic samples of the lower airway obtained from volunteers experimentally infected with rhinovirus. In both normal and allergic subjects, lymphocytes increased in the bronchial tissue during the acute rhinovirus infection. In addition, mucosal biopsies during viral respiratory tract infection showed increased mucosal eosinophils, which persisted for 4 weeks beyond the respiratory tract infection, but only in allergic subjects.

Exactly how rhinovirus influences lower airway histopathology is not established. It has been presumed that rhinovirus replication is limited to the upper airway, based on the fact that optimal rhinovirus replication occurs at 33°C, which approximates the upper airway environment. A number of studies have identified rhinovirus in upper airway secretions of children and adults with lower airway symptoms and clinical evidence of bronchitis. To determine whether rhinoviruses invade lower airways, Gern et al.<sup>7</sup> evaluated cells, obtained from the lower airways of subjects infected with rhinovirus, for the presence of rhinovirus RNA by reverse-transcription polymerase chain reaction. In these studies eight subjects were experimentally infected with rhinovirus. Bronchoalveolar lavage was performed before infection and during the acute rhinovirus illness; cell pellets obtained from the lavage fluid during the cold contained rhinovirus RNA, suggesting that rhinovirus was present in the lower airway. Presumably, rhinovirus in the lower airway could influence cell function, induce

cytokine production, and thus promote bronchial inflammation.

Although the mechanisms by which rhinoviruses, and presumably other respiratory viruses, enhance lower airway inflammation are not established, analysis of nasal secretions during respiratory tract infections suggests that cytokines may play a key role in this process. Several cytokines (IL-1, IL-6, IL-8, IL-11, granulocyte-macrophage colony-stimulating factor, and RANTES) are elevated in nasal secretions during naturally acquired or experimentally induced colds, and in some studies nasal cytokine levels were found to correlate with the severity of cold symptoms. These findings suggest that cytokines participate in the pathogenesis of cold symptoms and raise the possibility that cytokines generated by respiratory viruses augment the existing inflammatory response in asthma, and consequently, increase bronchial responsiveness and further compromise airway physiology. The generation of cytokines during rhinovirus infection is undoubtedly a multicellular process, and several cells are of particular interest.

The respiratory epithelial cell is a principal host for rhinovirus replication and is likely to be the first source of cytokines during an acute infection. Studies of epithelial cells, or cell lines, have shown that an *in vitro* inoculation with rhinoviruses or RSV induces generation of granulocyte-macrophage colony-stimulating factor, IL-6, IL-8, RANTES, and IL-11. These cytokines could have a significant effect on resident and recruited inflammatory cells and thus potentiate inflammation in asthma. For example, RANTES is a chemokine and chemoattractant for eosinophils and memory T cells, both of which are important effector cells in the asthmatic airway. Grunberg et al.<sup>8</sup> found that IL-8 levels are increased in nasal secretions during acute rhinovirus infection, correlate with cold symptom scores, and are inversely related to virus-induced changes in airway responsiveness (PC<sub>20</sub>). Furthermore, IL-11 is secreted in large amounts after epithelial cells are infected by rhinovirus *in vivo* and can directly enhance bronchial hyperresponsiveness in mice.<sup>9</sup> Finally, IL-11 levels in nasal secretions were found to be significantly increased in those subjects who had wheezing during their respiratory illness. These data, together with studies that link targeted IL-11 expression in the murine airway with airway inflammation and hyperresponsiveness, suggest that epithelial cell activation and IL-11 secretion are important components in the pathogenesis of wheezing caused by viruses. The cell sources of these cytokines, in addition to epithelial cells, are also of considerable interest.

T cells orchestrate immune responses to both allergens and viruses and regulate effector cells with virucidal and/or proinflammatory effects. Allergens and viral infections generally induce distinct types of T-cell immune responses. Allergens elicit a T<sub>H2</sub>-like response, with secretion of cytokines such as IL-4 and IL-5 that promote IgE production and eosinophil activation, respectively. In contrast, viral respiratory tract infections typi-

cally induce a  $T_{H1}$ -like response, characterized by secretion of interferon- $\gamma$ , which enhances the antiviral activities of effector cells including the macrophage, neutrophil, and cytolytic T cell. These two types of T-cell responses can be mutually antagonistic, and this increases the complexity of potential interactions between allergen- and virus-induced T-cell responses in the asthmatic airway.

It has been suggested that some viruses have evolved mechanisms for manipulating the immune system to hinder antiviral responses and that these mechanisms could adversely affect pulmonary physiology.<sup>10</sup> For example, the attachment protein (G) of RSV induces a  $T_{H2}$ -like response in both mice and human beings. Moreover, passive transfer of protein G-specific T cells into mice, followed by RSV infection, causes pulmonary eosinophilia and increased lung disease. Although these studies demonstrate that virus-specific T cells may contribute to virus-induced lung disease, evidence to define the role of virus-specific T lymphocytes in asthma has not been fully established.

Airway macrophages are also likely to be involved in antiviral immune responses because they bind respiratory viruses *in vitro* and secrete cytokines that have antiviral activity and/or proinflammatory effects. For example, probably as a consequence of its effects on intercellular adhesion molecule-1, tumor necrosis factor- $\alpha$  increases the susceptibility of an epithelial cell line (BEAS-2B) to become infected with a major group rhinovirus.<sup>11</sup> On the other hand, virus-infected macrophages also secrete interferon- $\alpha$ , which has potent antiviral effects. These findings suggest that in addition to contributing to increased airway inflammation during rhinovirus infection, macrophages may also be an important component of the early nonspecific antiviral response.

Finally, the effects of viral infections on mast cells and eosinophils are of great interest in the context of allergy and asthma. The presence of leukotriene  $C_4$ , histamine, and eosinophil granular proteins in the respiratory secretions of virus-infected animals and in infants with wheezing illnesses suggests that these cells are activated during viral infection and participate in causing airway dysfunction. Furthermore, experimental rhinovirus infection enhances histamine release and eosinophil recruitment in response to whole-lung or segmental allergen challenge.<sup>5</sup> It is likely that mast cell and eosinophil recruitment and activation are caused by cytokines (e.g., stem cell factor, eotaxin, RANTES, and granulocyte-macrophage colony-stimulating factor) secreted by epithelial cells or mononuclear cells in response to viral infection, although the precise mechanisms are as yet unknown.

## RESPIRATORY TRACT INFECTION AND THE DEVELOPMENT OF ASTHMA

Although current evidence strongly suggests that respiratory viruses have an important role in causing wheezing in infants and precipitating symptoms of

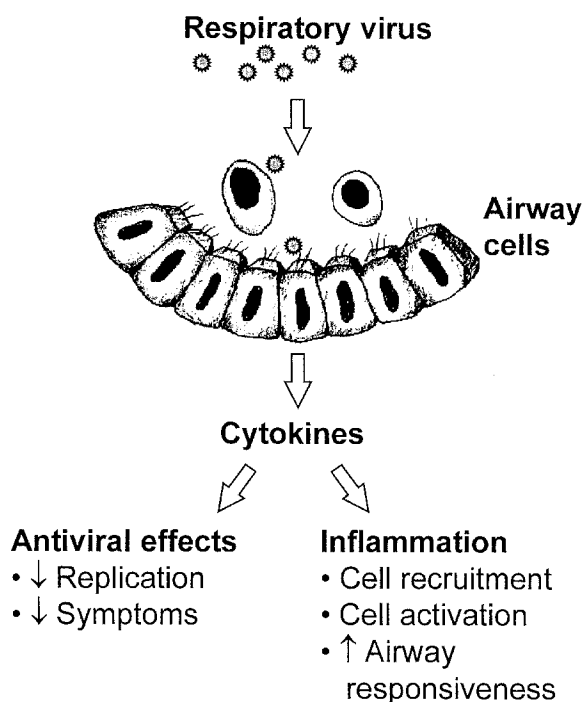


**FIG. 2.** Potential effects of virus-induced cytokines. Respiratory viruses can interact with airway cells (e.g., epithelial cells, macrophages, lymphocytes) and stimulate production of cytokines. These virus-induced cytokines can have antiviral effects plus a capacity to promote inflammation. The net result of viral effects may be determined by existing conditions in the airway (i.e., normal vs asthma).

asthma in those with existing disease, some infections early in life may also have an important immunoregulatory role in the subsequent development of allergy and asthma.<sup>12</sup> For example, several studies have indicated that the odds of allergen sensitization are inversely related to the number of older siblings in the family, which presumably determines the exposures to infectious diseases in early childhood. In addition, some data suggest that early childhood infections with either measles or tuberculosis reduce the risk of allergen sensitization. These data suggest that the generation of a protective immune response, involving the generation of interferon- $\gamma$ , to infections early in life may imprint the developing immune system toward a  $T_{H1}$ -like cytokine profile and thus reduce the subsequent risk of allergic sensitization and asthma.

## SUMMARY

Viral respiratory tract infections make an important contribution to the regulation of airway function. In young children RSV causes episodes of wheezing, which may be recurrent but are largely transient in duration. Although a subsegment of "early wheezers" goes on to have persistent asthma, this transition is found predominantly in infants with elevated levels of IgE and a family history of asthma. In contrast, older children with established asthma are also influenced by respiratory tract

infections, but the virus causing these exacerbations is different: rhinovirus. The common cold virus is no doubt the most frequent precipitant of asthma exacerbations, and this association occurs in patients of all ages.

There is now evidence that viral respiratory tract infections affect existing asthma by increasing the intensity of allergic inflammation. The mechanisms by which these changes occur are not fully established but appear to be associated with the ability of respiratory viruses to generate cytokines, which may have antiviral and/or proinflammatory effects (Fig. 2). In the context of asthma, the net effect of this cytokine response to respiratory viruses is to enhance preexisting airway inflammation, which then leads to increased airway hyperresponsiveness and a tendency to wheeze. Studies are currently underway to more precisely establish the molecular bases to the virus effects on cell function. Because viral infection stimulated the production of many different cytokines that undoubtedly have overlapping effects, it seems unlikely that therapeutic approaches aimed at inhibiting any one particular cytokine or mediator will reverse viral effects on airway function. With this in mind, several teams of investigators are working to define the intracellular mechanisms by which viruses activate cytokine genes, with the hope that viruses may trigger a common signaling pathway that leads to the activation of multiple cytokine genes. If this is true, new therapies for virus-induced asthma could target this pathway.

Another challenge will be to determine why individuals with allergy and lower airway inflammation are so susceptible to the effects of respiratory viruses, such as rhinovirus, which cause mild disease in normal individuals. Is there a fundamental difference in the immune response to viruses in the presence of asthma? Alternatively, are the more severe clinical manifestations of viral respiratory tract infections in asthma a result of the different types of cells, or perhaps a heightened activation state of the cells, in the asthmatic airway? With the answers to these questions will come a better apprecia-

tion of how airway inflammation is regulated in asthma and how to best treat this process when it is augmented by viral infection.

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# Mechanisms of action of intravenous immune serum globulin in autoimmune and inflammatory diseases

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Intravenous immune serum globulin (IVIG) is primarily used as replacement therapy in patients with hypogammaglobulinemia. It has been over 15 years since IVIG was first shown to be effective in the treatment of patients with autoimmune thrombocytopenic purpura. Over the past decade, IVIG has been used in the treatment of a number of autoimmune and systemic inflammatory disorders. Although the use of IVIG in many of these immune-mediated disorders was purely speculative initially, we now have a much better understanding of the mechanisms by which IVIG exerts its effects in these autoimmune diseases. IVIG exhibits a number of immune modulatory activities that are mediated by the Fc portion of IgG through the Fc $\gamma$  receptor on a variety of cell types. Although the major component in IVIG is IgG, other minor components such as solubilized lymphocyte surface membrane determinants and specific antibodies to lymphocyte surface molecules may have important immunoregulatory effects on T- and B-cell immune responses. This review discusses the proposed mechanisms of action of IVIG in autoimmune and inflammatory disorders. (*J Allergy Clin Immunol* 1997;100:151-7.)

**Key words:** Intravenous immune serum globulin (IVIG),  $\gamma$ -globulin, immune therapy, autoimmune disease, immune modulation, cytokines, complement, Fc receptors

The objective of this review is to present the mechanisms by which intravenous immune serum globulin (IVIG) may modulate autoimmune and various inflammatory disease processes. To achieve this objective I will discuss several autoimmune disorders in human beings, and in some cases animal models of disease, which exhibit various immune pathogenic processes, and develop the mechanisms by which IVIG may be acting in modulating these diseases.

## Fc RECEPTOR BLOCKADE AND MODULATION

In 1981 Imbach et al.<sup>1</sup> made a serendipitous observation that the infusion of IVIG in children with immune thrombocytopenia (ITP) produces a marked improvement in platelet counts. ITP was the first autoimmune

### Abbreviations used

|         |                                         |
|---------|-----------------------------------------|
| ANCAs:  | Anti-neutrophil cytoplasmic antibodies  |
| EAE:    | Experimental allergic encephalomyelitis |
| IL-1ra: | IL-1 receptor antagonist                |
| ITP:    | Immune thrombocytopenic purpura         |
| IVIG:   | Intravenous immune serum globulin       |
| KS:     | Kawasaki syndrome                       |
| TNF:    | Tumor necrosis factor                   |

disorder in which IVIG was used as an effective treatment modality. The mechanism by which IVIG increases the platelet count is thought to occur by blockade of the Fc receptors on phagocytic cells, which prevents the removal of sensitized platelets by the reticuloendothelial system in the liver and spleen. This leads to prolonged survival of autoantibody-coated platelets<sup>2,3</sup> and explains the very rapid changes in platelet counts that occur with the administration of IVIG.<sup>4</sup> In a recent study by Debré et al.,<sup>5</sup> 11 of 12 children with acute ITP treated intravenously with purified Fc $\gamma$  fragments prepared from Cohn fraction II  $\gamma$ -globulin showed an increase in platelet counts. These studies with Fc $\gamma$  fragments provide direct support for the hypothesis that Fc $\gamma$  receptor blockade by IVIG is responsible for the improvement of the platelet counts in ITP. In addition, these investigators showed a rise in serum-soluble Fc $\gamma$  RIII (CD16) concentrations, which correlated with the rise in the platelet count. No sFc $\gamma$  RIII was detected in the Fc $\gamma$  fragment preparation, which suggested that the source of these sFc $\gamma$  RIII receptors was the phagocytic cells of the reticuloendothelial system. An additional mechanism postulated by these investigators was that these soluble Fc $\gamma$  receptors could act as competitors with the cell membrane Fc $\gamma$  receptors of the reticuloendothelial system and prevent the clearance of circulating IgG-sensitized platelets.

IVIG is mostly monomeric IgG (>95%) with small amounts of dimeric and aggregated IgG. However, after infusion, some of the monomeric IgG becomes dimeric or complexed to circulating antigens found in the patient. IgG monomers predominantly bind to Fc $\gamma$  RI found mainly on monocytes; whereas Fc $\gamma$  RII and Fc $\gamma$  RIII on monocytes, neutrophils, and other cells interact with polymeric IgG or immune complexes. Fc $\gamma$  R expression and receptor-mediated phagocytosis are decreased in peripheral blood monocytes treated with

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Received for publication Dec. 18, 1996; revised Apr. 8, 1997; accepted for publication Apr. 9, 1997.

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