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Respiratory Syncytial Virus (RSV): Characteristics of Infection and the Role of Immune Response in the Evolution of Disease

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A B S T R A C T

Infection with RSV is a major cause for hospitalizations of infants in the first year of life in the United States and most other parts of the world. Almost 100% of children are infected with this virus by 2-3 years of age. Normally, the infection is exquisitely restricted to the bronchopulmonary mucosa. However, development of extrapulmonary disease is observed in patients with certain T and B cell immunodeficiency states. The spectrum of illness associated with RSV is diverse and ranges from asymptomatic infection, mild upper airway disease, otitis media, apnea in the early neonatal period, or severe respiratory tract disease associated with bronchiolitis, pneumonia, wheezing or sudden infant death syndrome (SIDS). A small number of children will develop long term abnormalities of pulmonary function, episodes of wheezing or established reactive airway disease after severe disease or bronchiolitis. The immune response to primary infection is modest at best, but reinfection is followed by a robust booster effect with sustained antibody and cell mediated immune responses in both systemic and respiratory mucosal sites. The role of viral specific immune responses such as development of T-helper CD4+ Th1, Th_2; IgE; viral induced cytokines; and suppressor cytotoxic CD8+ T cells, chemokines; leukotrienes and other cellular metabolites; and the importance of viral infectious load in the development and the severity of disease has been studied extensively. Many studies in animal models and a few human investigations have related the pathogenesis and long term outcome of RSV infection to abnormal or increased Th_2 cytokine responses in a manner similar to other Th_2-mediated allergic disorders. However, more recent investigations have also demonstrated that CD8+ T cell responses, non T cell derived pro-inflammatory cytokines and chemokine production, and the magnitude of viral load generated as a result of viral replication in the mucosa may be more important than Th_2 responses in determining the outcome of acute infection and the development of long-term complications. It is still not clear if the association of RSV infection with long term wheezing is a reflection of primary immunologic hyperactivity, underlying genetic predisposition for atopic immune responses or direct effect of viral-induced cytolytic mucosal damage.

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Respiratory syncytial virus (RSV) is the primary cause of hospitalization with lower respiratory tract disease in the first year of life in most parts of the world. It is estimated that in the United States alone, about 100,000 hospital admissions are related to RSV infection, with estimated patient care costs exceeding over 300 million dollars annually. In addition to its disease burden in infants, RSV has also been associated with significant morbidity and mortality in adults and the elderly. As a result, it is not surprising to note that this infection has been studied very extensively and to date, there are over 1,700,000 citations to RSV in the available published database.

This report will be limited to a brief overview of the recent developments in understanding the host immune response in man and its possible role in the outcome of infection. More detailed information about the virus, the disease and other aspects of pathophysiology in non-human and other experimental settings has been extensively reviewed in several recent publications.[1-5]

Spectrum of human infection

Virtually all children are infected at least once with RSV by the time they reach 2 years of age. However, about 50% subjects may experience two infectious episodes by this age. The clinical spectrum of acute infection ranges from mild upper tract infection, otitis media, and lower tract illness characterized by pneumonia or bronchiolitis.

It should be pointed out that only a small minority of RSV infected children develop severe disease. Most children develop asymptomatic or minimal disease with lit-

Table 1. Association of subsequent asthma and other allergic disorders with severe RSV bronchiolitis acquired during early infancy.

Subsequent Clinical Disease	*Age (years)*	*% Subjects*	
		RSV	*Control*
Asthma	1	11	0
	3	23	1
	7.5	23	2
	13	37	5.4
Allergic rhino- conjunctivitis	-	39	15
Positive skin (prick) tests	-	50	28

Sigurs, et al., Am J Respir Crit Care Med 171:137-141, 2005.

tle or no long-term detrimental impact on the child's health.

The disease tends to be more severe in infants 8-30 weeks in age. About 2% of infected subjects require hospitalization, and mechanical ventilatory support may be required in up to 5% of the subjects. The mortality rates associated with RSV infection have been estimated to range from 0.005 to 0.02%.[6,7] Most deaths have been observed in infants with underlying cardiopulmonary disease, immunologic deficiency states, or in bone marrow transplant recipients.

Careful epidemiologic studies carried out during the past two decades have demonstrated that other risk factors for severe RSV infection include prematurity, male sex, presence of multiple siblings in the home or attendance at a day care center, parental smoking, lack of breast feeding and poor socioeconomic living conditions. In addition, exposure to cooking fires and smoke, and absence of sanitary toilets appear to contribute to severe disease in infants in especially poorer countries.[8,9]

The development of severe RSV infection during the first 6 months of life has been associated with transient occurrence of reactive airway disease and wheezing in over 75% of patients. This association may exist for up to 12 years of age. It has been observed that following acute RSV infection, about 10% of infected subjects exhibit recurrent wheezing for the first year, and in about 20% of subjects for up to 7.5 years of age (Table 1). However, no significant epidemiologic association between RSV bronchiolitis and subsequent recurrent wheezing has been demonstrated after 13 years of age.[10,11]

Immune response

RSV infection is followed by the development of virus specific mucosal and systemic antibody and T cell mediated responses in all children. Primary infection in general induces an IgM response in 5 to 10 days, depending on the age of the patient. Lower IgM responses have been observed in patients less than 6 months of age. IgM antibody in general persists, usually, for up to 1 to 3 months. However in a few studies, IgM antibodies were found to remain detectable for as long as 1 year.

Virus specific IgG antibody response is detected in most patients; it reaches peak values in 20 to 30 days after the onset of symptoms. IgG responses occur mainly in IgG1 and IgG3 subclasses, indicating the antigenic nature of the protein moieties of the F and G proteins of the virus. RSV-specific IgG activity appears to decline to low levels one year after primary infection. However, after natural re-infection, a booster effect is noted, with high titers of viral specific IgG detectable within 5 to 7 days of reinfection.[5]

The serum IgA response occurs several days later than IgM and IgG responses. Interestingly, IgA can be found free and cell-bound in nasopharyngeal secretions of patients with RSV infections. Free anti-RSV IgA appears within 2 to 5 days after infection, and peak titers are obtained between 8 and 13 days. The nasopharyngeal IgA response is greater in children older than 6 months. A mucosal immune response to RSV has also been demonstrated by the appearance of RSV specific antibody in vitro, in tonsillar lymphocytes. Most naturally infected subjects develop a virus specific IgE antibody response in the nasopharyngeal mucosa following RSV infection.[3,5]

Several studies have demonstrated specific antibody responses to major RSV structural proteins. Antibody responses to the F protein of are often cross-reactive with both RSV strains tested, whereas antibody responses to the G protein are subgroup specific.[5] Similar findings have been observed for group-specific antibody responses after primary and secondary RSV infections. These observations have suggested that infection with group-A RSV virus induces cross-reactive neutralizing antibody responses to group-B virus

Table 2. Development of immunologic and other viral specific responses after natural or induced infection with RSV.

- Neuropeptides (substance P) and expression of specific receptors (NK-1)
- Th1 and Th_2 cytokines
- IgG, IgA, IgM antibody reactivity
- IgE and homocytotropic antibody
- Proinflammatory cytokines
- CD4, CD8 T cells
- Proinflammatory cytokine-chemokines
- Immunoregulatory cytokines
- Integrins and cell adhesion molecules
- Activation of pathogen recognition receptors (Toll-like receptors, antigen-presenting and processing cells, and expression of major histocompatibility antigens

type.[5] RSV infection induces specific cell-mediated immune responses, as determined by viral specific lymphocyte transformation, cytotoxic T-cell responses, and antibody-dependent cellular cytotoxicity responses.[5] Antibody-mediated immune responses are to a large extent protective. It is clear that IgG, IgA and IgM antibody responses contribute to the development of protection against reinfection. It has also been shown that high levels of maternal antibody are indeed protective against disease in early infancy. However, the magnitude of such protection in the early years is low.[3-5] As pointed out above, virtually all children who get infected with RSV develop virus-specific IgE homocytotropic antibody in the respiratory tract. Such IgE activity is predominantly bound to the epithelial cells of the respiratory tract. In general, there is minimal free (non tissue bound) virus specific IgE antibody detectable in respiratory excretions, unless children are wheezing or they have bronchiolitis or pneumonia. It has been observed that persistent virus-specific IgE response in the respiratory mucosa is an important factor in the development of immunopathology for both RSV and parainfluenza viruses.[3] It seems that most children produce IgE to RSV early in life, but it is the amount, the persistence and the duration of this response which is critical in determining which patients may develop wheezing later in childhood.[3]

During the past decade, there has been considerable interest in the role of cell-mediated immune responses in the mechanisms of immunopathology of viral infections. Both CD4+, Th1 and Th_2 and CD8+ cell types have been implicated in the development of disease during RSV infection.[4]

In addition to viral specific IgE antibody and its interaction with mast cells, and the subsequent release of inflammatory mediators, there is evidence to suggest that the interaction between RSV and the respiratory epithelium also results in the release of a number of other mediators of inflammation. These effector chemicals are very important in mobilizing other cells to the site of disease. Studies conducted by several groups during the past decade have demonstrated the release of inflammatory mediators such as leukotrienes, eosinophil degranulation byproducts and other epithelial cell-derived cytokines and chemokines during the course of RSV infection in *in vitro*, as well as *in vivo* settings.[3-5] Furthermore, during the course of infection, induction of several cell-adhesion molecules and homing ligands (CD11B, ICAM-1, E-selectin) has been demonstrated. These ligands are necessary for inflammatory and immune cells to be mobilized to the site of disease, to rollover, bind, and eventually adhere to the virus-infected tissues. These events have also been associated with expression of antigen-presenting molecules like HLA class I and II. Based on these observations, it is clear there is a complex array of events set in motion by RSV infection in the respiratory epithelium, which results in mobilization of inflammatory and immunoregulatory cells to the site of disease.

Recent studies have described the role of transcription factors in activating certain genes or groups of genes during viral infection. There are a number of nuclear factors, which mobilize gene activation processes and activate many cellular functions. RSV has been shown to induce activation of several transcription factors, including activation of gene promoters for NF-IL-6 and NF-κB. Such transcription factors regulate the synthesis of a variety of cytokines and chemokines and other important immunomodulating proteins. These include TNF-α, IL-1β, IL-2, IL-6, GM-CSF, and G-CSF; adhesion molecules ICAM-1, VICAM-1 and E-selectin; and chemokines, IL-8, MIP-1α, MCP-1, and eotaxin. These molecules are important in initiating the inflammatory cascade and in mobilizing cells to the site of disease.[3-5]

Although a large number of cellular, neuroregulatory and immunologic events are set in motion during RSV infection, it is not clear if RSV disease is a true consequence of all the cellular events identified to date (Table 2) or if these events are set in motion as a result of the activation of different immunoregulatory pathways during the replication of the virus in the mucosa? Clearly, all these events identified to date cannot be the cause of the disease or recurrent wheezing observed after infection with this virus. In order to

provide a unifying approach to explain the multiplicity of phenomena observed during this infection, it is possible that RSV infection may trigger the activation of a *master switch* of genetic control, regulating the expression of one or more cellular functions identified above.

Role of immune response in the outcome of infection

Based on the information summarized above, it is evident that RSV infection is ubiquitous, and almost all children are infected by the virus by the time they reach 2-3 years of age. Most infections are mild and require minimal or no medical care. However, relatively little information is available about the pathogenesis of or the immunologic outcome of human infections associated with minimal or no disease. On the other hand, fatal infections are exclusively confined to those who are severely immunocompromised at the time of acute infection.

The immunologic events observed in subjects with RSV disease include activation of neuropeptides and neural receptors, release of immunoregulatory and pro-inflammatory cytokine and chemokines, activation of T lymphocytes and their subsets, and development of antibody responses in many immunoglobulin isotypes including IgE (Table 2).

The clinical presentations of RSV-induced pulmonary disease appear to be characterized by two distinct pathologic states, 1) acute and sometimes severe pulmonary disease associated with bronchiolitis, or pneumonia, or both, and 2) delayed pulmonary disease, characterized by recurrent wheezing especially in infants infected during the first 6 months of life.[4]

The pathologic features of acute pulmonary disease with RSV are similar to other respiratory viral infections, associated with direct cytotoxicity of bronchopulmonary epithelium due to viral replication. Additional contribution to cytopathology is derived from ciliary damage, mucous secretion, accumulation of inflammatory mononuclear cells and release of pro-inflammatory cytokines and chemokines.

The association of severe RSV disease in infancy and the subsequent development of short term bronchial hyperreactivity and recurrent wheezing is now well accepted. Much of the current investigative effort has focused on defining the mechanisms underlying the development of RSV induced recurrent wheezing. Several important immunologic findings have emerged recently to address this important issue.[4,12]

Immune priming and viral persistence.

In several experimental situations, a sustained increase of IL-2 receptor levels has been observed following RSV infection, suggesting that active inflammation possibly related to persistence of virus may continue well beyond the resolution of acute infection. Although persistence of RSV antigen has been demonstrated in macrophage-like cell lines, in rodents and other experimental infections, little data is available to support the persistence of viral infection in man.[13] In addition, recent studies have failed to demonstrate significant expression of IL-2 after RSV infection in man.

Hyperactive Th_2 response. In contrast to RSV, most viral infections typically induce a Th1 (IFN-γ) CD4+ T cell response. It is interesting to note that infections with type I HSV, varicella-zoster, HHV-6, during the first 7 years of life, or over 2-3 episodes of *common cold* virus infections in the first year of life have been found to significantly reduce the risk of wheezing in childhood.[14] However, a few human studies have demonstrated elevated IL-4 levels or IL-4/IFN-γ ratios in the nasopharyngeal secretions and in the peripheral blood sample during the first week of bronchiolitis with RSV.[15] It has been proposed that primary RSV infection in early infancy is generally associated with poor and short-lived Th1 response and generally weak IL-12 expression. The possibility has been raised that specific IL-4 dependent apoptosis of Th_1 cells may take place in the neonatal environment and thus result in a relative increase in Th_2 (IL-4) type of responses resulting in increased late onset disease expression in a similar manner as with other allergic disease states.[16] On the other hand, preliminary recent evidence has also suggested that severe and even fatal RSV infection in children may conspicuously lack any evidence of Th_1 (IFN-γ) or Th_2 (IL-4) T cell responses.[17]

Viral protein-specific response. A number of recent studies have attributed the severity of RSV disease to the nature of viral replication, viral antigen load generated during primary infection and the possible homology of RSV proteins to chemokine receptors.

In vitro studies of RSV replication have been associated with upregulation of STAT pathway and of IL-8. Polymerism of IL-8 appears to be associated with more severe RSV disease. In additional studies, RSV infection has been shown to upregulate NF-κB activation resulting in increased recruitment of activation of inflammatory cells such as polymorphonuclear leukocytes, activation of other chemokines such as RANTES (CCLS), and upregulation of proapoptic factors.[18]

There is also evidence to suggest a specific relationship between RSV F and G proteins with chemokine and Toll-like (TLR) and other pathogen recognizing receptors (PRR) activation. RSV G protein bears significant homology to fractalkine, a CX3C chemokine. This protein appears to bind to CX3CR1 receptor and induce chemotaxis. Such interaction may also facilitate binding to CX3CR receptor-bearing cells, such as mast cells

or neuronal cells. More recently, RSV F protein has been shown to selectively bind to TLR-4 receptor, a PRR on many antigen processing or presenting cells including dendritic cells.[19,20,21] Such viral protein binding appears to upregulate the surface expression of the TLR and enhance the interaction of endotoxin to the airway epithelium.

The implications of these findings to the severity of acute disease on the development of delayed bronchial reactivity remains to be determined. However, one recent study carried out in allergic Japanese children has suggested that RSV G protein in such RSV infected children is more often associated with Th2 type of responses while non-allergic children exhibit similar Th1 response to both F or G proteins (Shimojo N, Katsuki T, Tateno W, *et al*, personal communication).

Other experimental studies have examined the role of NK, CD8+ T, γδ+, and dendritic cells on clearance of viral antigen, disease enhancement, or re-infection, and shift of immune response toward Th_1 vs Th_2 cells. However, little is known about their role in human disease.[12,21,22]

Finally in a recent multicenter comparative study involving severe or fatal disease associated with RSV or influenza virus infection in infancy, it was observed that severe RSV bronchiolitis was characterized by prolonged viral growth in respiratory mucosa. Significantly, however, T-lymphocyte reactivity was conspicuously absent in fatal cases of RSV disease. In addition, fatal cases of RSV were associated with absence of CD4+, CD8+, CD56+ T cells or specific presence of gramzyme, a marker for apoptosis. However, fatal cases did exhibit appearance of CD16 cells in the lung tissue with extensive apoptotic signaling. Surviving infants exhibited marked paucity of both Th_1 or Th_2 cytokine responses.[17]

Conclusions

The observations summarized above have identified several distinct innate and adaptive immunologic events during the course of RSV infection. Many of these have been proposed as principal mechanisms underlying the development of acute disease as well as the recurrent wheezing in early childhood. It is not possible at this time to determine if any or none of the mechanisms identified to date are causally related to the evolution of the disease. It is, therefore, important to attempt to separate the primary cause of the disease from the subsequent effects of specific cell-virus interaction in the respiratory mucosa during RSV infection. It has been proposed that RSV infection may trigger the activation of *a master switch* of genetic control regulating the expression of one or more cellular functions identified above in a small number of genetically predetermined subjects who will exhibit severe disease in the acute phase initially or, in a delayed manner with recurrent wheezing later in childhood.

The only documented evidence of possible disease enhancement in man after RSV infection has been observed following the use of formalin inactivated RSV candidate vaccine given to a large number of children in early 60's as an intramuscular injection prior to subsequent reexposure during a natural RSV outbreak. Such reinfection in vaccine sensitized subjects resulted in increased hospitalization (80% vs. 5% in controls) and, increased frequency of pneumonia, bronchiolitis and rhinitis in vaccinated subjects. Two vaccinees died after such natural RSV infection. On autopsy these infants exhibited bronchopneumonia with significant peribronchial infiltrates of PMN, mononuclear cells and eosinophils. It is not known if any of these subjects exhibited any evidence of immune priming, enhanced Th_2 cytokine responses or any of the other immunologic parameters which have been since determined in experimental mostly non-human models of RSV infection. On the other hand, recent clinical experience has suggested that passively administered RSV specific antibody may have a significant positive impact on the modification of clinical severity of disease and may even reduce the frequency and severity of late onset wheezing in RSV infected subjects.[25]

Based on the information available, it is clear the immunologic events associated with RSV infection are highly complex. The earlier emphasis on Th_2 mediated mechanisms may be less important than viral induced inflammation, as the principal mechanism underlying the pathogenesis for acute as well as late onset disease with RSV infection.

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