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The Immune Response to Viral Lower Respiratory Tract Infection

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1. Introduction

Viruses are responsible for the majority of respiratory infections in childhood, causing considerable morbidity and mortality. It is estimated that in the United States approximately $ 652 million per year is spent on medical costs for respiratory syncytial virus (RSV) related disease alone (Paramore et al., 2004). Viruses cause a variety of respiratory diseases in children from the common cold to life-threatening pneumonia and bronchiolitis. The host reacts to a viral infection with a combination of innate and adaptive immune mechanisms, usually resulting in the clearance of the virus and clinical recovery. However, there is an accumulating evidence for a number of viral infections that the host immune response actually enhances disease in the course of clearing virus from the infected organs. Interestingly, the effectiveness of the immune response seems to be dependent on the age and probably genetic background of the child. This has important implications for treatment as well as vaccine development.

Viral infections play an important role in both childhood and adult asthma. They might be instrumental in the inception of asthma and are associated with the majority of exacerbations in asthmatic individuals (Johnston et al., 1995; Bont et al., 2000).

In respect to the role of viruses in the pathogenesis of acute and chronic airway disease in children, it is of utmost importance that we gain a proper understanding of the underlying mechanisms involved in order to design effective therapeutic and preventive strategies.

Although viral respiratory tract infections are considered to be mainly pediatric diseases, there is an increasing acknowledgement of their pathogenic potential in the immunocompromised host of all age groups and in the elderly.

2. Epidemiology and Clinical Aspects

Viruses involved in respiratory tract disease in children induce quite similar respiratory illnesses. In many cases, and especially in the ambulatory setting, the

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causative agent is not identified. When an upper respiratory tract infection in an infant progresses to lower respiratory tract disease, bronchiolitis and pneumonia are most common. Both disease entities are hard to differentiate and no clinically relevant differences with regard to outcome have been identified (van Woensel et al., 2003).

2.1. Respiratory Syncytial Virus

2.1.1. The Virus

RSV belongs to the paramyxoviridae family and the genus of Pneumovirus. It is an enveloped unsegmented single-stranded RNA-virus of which two subtypes are known (A and B). A clear relationship between subtype and disease severity has not been established (Kneyber et al., 1996). Since RSV infection does not lead to complete immunity, reinfection is common. Immaturity of the immune system during initial infection seems to be the main cause of incomplete memory-response although an as yet undefined mechanism of partial immune evasion by RSV cannot be ruled out (Bont et al., 2002). Recently, it was suggested that RSV could cause persistent infection or latency (Dakhama et al., 1997; Schwarze et al., 2004), although the significance of this is not clear.

2.1.2. RSV Respiratory Disease

RSV affects 70% of infants in the first year of life, and by the age of two nearly all children have been infected. (Figure 4.1) It is likely that a specific balance and timeframe of changes in air temperature and humidity are responsible for the well-defined yearly winter outbreaks of RSV (Stensballe et al., 2003). In most infants as well as in older children and adults, RSV is the cause of upper respiratory tract infection with mild symptoms. However, in the very young, the infection spreads to the lower respiratory tract in approximately 40% of cases. One to three percent of infants develop bronchiolitis or pneumonia requiring hospitalization, with a considerable number requiring mechanical ventilatory support. Apart from the obvious respiratory symptoms, very young children frequently present with atypical symptoms such as impaired feeding, vomiting, lethargy, and apnea (Kneyber et al., 1998). Several risk factors for more severe disease have been identified, including age less than 6 weeks, prematurity, pre-existent cardiorespiratory disease and immunological impairment (Bont and Kimpen, 2002). Respiratory symptoms are directly related to airway pathology. Necrosis of the airway epithelium is a key phenomenon resulting in sloughing of the epithelial cells. Together with a dramatic influx of inflammatory cells into the airways and increased mucus production, this leads to the formation of copious secretions that block the small airways. Mucosal edema and bronchospasm through irritation of subepithelial nerve endings further compromise airway diameter.

Although antibiotics are prescribed for up to 50% of children with lower respiratory tract infections, proof of bacterial superinfection is only found in a minority of patients and the role of this event in the pathogenesis of severe disease remains controversial (Purcell and Fergie, 2002; Bloomfield et al., 2004). On the other hand,

it has been demonstrated that RSV infection of airway epithelial cells *in vitro* enhances adherence of *S. pneumoniae* (Hament et al., 2004).

2.2. Influenza

2.2.1. The Virus

The influenza virus belongs to the orthomyxoviridae family and is an enveloped segmented single-stranded RNA (ssRNA) virus (Table 4.1). The structural proteins of the virion are encoded by separate gene segments and include three viral RNA polymerases, nucleoprotein, matrix, and the hemagglutinin (HA) and neuraminidase (NA) surface glycoproteins. On the basis of their nucleocapsid and matrix protein antigens, the influenza viruses are divided into three distinct immunological types (A, B, and C). Although all three influenza viruses cause respiratory disease in humans, only A and B are known to cause epidemics.

Induction of a memory-response results in long-lasting immunity and it is the antigenic variation that is responsible for frequent reinfection with the virus. The most antigenic variation is seen in the virus that infects both animals and humans, influenza A. Fourteen subtypes of hemagglutinin (H1–H14) and nine types of neuraminidase (N1–N9) are circulating in nature. The segmentation of the genome makes exchange of genetic material between subtypes possible, resulting in the structural changes observed in influenza, which has caused pandemics in the past. When two different subtypes of influenza A virus infect the same cell, major changes (antigenic shifts) can occur through rearrangement of genetic segments from both infecting viruses. Minor changes (antigenic drifts) in NA and/or HA proteins occur through accumulation of point mutations, and provide a mechanism for the virus to escape protective antibodies and cause respiratory symptoms every year.

Table 4.1. Overview of Respiratory Viruses, Their Family, and the Proteins Used for Infection of Respiratory Epithelium

Virus species	Family	Genus	Viral attachment protein	Host cell receptor (respiratory epithelium)
RSV	Paramyxoviridae	Pneumovirus	G-protein F-protein	CX3CR1 (fractalkine), TLR
Influenza	Orthomyxoviridae	Influenzavirus A, Influenzavirus B, Influenzavirus C	HA-glycoprotein	Neuraminic acid
Parainfluenza	Paramyxoviridae	Paramyxovirus	HN-glycoprotein	Sialic Acid
Adenovirus	Adenoviridae	Mastadenovirus	Fiber-protein	Coxsackie and adenovirus receptor (CAR)
Rhinovirus	Picornaviridae	Major group Minor group		ICAM-1 LDL-R
hMPV	Paramyxoviridae	Pneumovirus	G-protein	
Sars-CoV	Coronaviridae	Coronavirus	Spike (S-) glycoprotein	ACE2

2.2.2. Influenza Respiratory Disease

Influenza virus epidemics are difficult to separate in time from RSV epidemics, and the diseases caused by both viruses can also be difficult to differentiate (Zambon et al., 2001). (Figure 4.1) Compared to other viruses, morbidity caused by influenza is high in all age groups. Children with influenza infection of the respiratory tract are more likely to present with fever. Infants aged less than 6 months and older children with an impaired immune system, or other serious health problems, have a higher risk of hospitalization and mortality. Subclinical infections with influenza in children are common, suggesting children can be an important reservoir and source of transmission.

Yearly updated vaccines are available and effective, and recently it has been proposed to extend the current recommendations to children less than 2 years of age, children with recurrent acute otitis media or respiratory tract infections, and healthy children attending day-care centers or elementary schools (Principi and Esposito, 2004).

2.3. Adenovirus

2.3.1. The Virus

Adenoviruses, belonging to the adenoviridae family and the genus Mastadenovirus, are a group of DNA-viruses of which at least 47 serotypes are known. For lower respiratory disease, subtypes 1, 2, 3, 4, 5, 7, and 21 are most important. It is an icosahedral capsid virus with extruding fiber proteins, which are required for viral entry to epithelial cells (Howitt et al., 2003).

2.3.2. Adenovirus-Induced Respiratory Disease

Although human adenoviruses are ubiquitous, and cause primary infection in the first year of life, there is geographical variation in the distribution of serotypes and in the association of serotypes with different age groups. In Europe, adenovirus is the cause of infection in approximately 5% of hospitalized patients with viral lower respiratory tract disease. However, in some South American and Asian countries, adenovirus is the second most prevalent pathogen for acute lower respiratory tract infection in children after RSV (Carballal et al., 2002). Although adenovirus infections in general occur the whole year round, respiratory adenovirus infections are most common during late winter, spring, and early summer. Adenovirus type 7, acquired by inhalation, has been associated with more severe lower respiratory tract disease (Larranaga et al., 2000). Subtype-specific immunity occurs. However, some types are capable of establishing persistent asymptomatic infections in tonsils, adenoids, and intestines of infected hosts, and shedding can occur for months or years.

2.4. Parainfluenza Virus

Parainfluenza virus (PIV), another pathogen like RSV belonging to the paramyxoviridae, causes a spectrum of disease varying from common cold, croup, and bronchiolitis to pneumonia. Clinically relevant infections are mainly seen in

children under 6-years old. Though it is the causative agent of similar disease entities, hospitalizations occur four times less frequently than for RSV infections (Hall, 2001). Two subtypes are clinically important respiratory pathogens in children, PIV-1 and PIV-3. PIV-1 is the main cause of croup in 2–6-year olds, while PIV-3 is responsible for parainfluenza bronchiolitis in children under 6-months old. PIV-4 only causes mild upper respiratory tract infections. Because of acute narrowing of the subglottic region of the larynx, moderate to severe croup may require emergency management with systemic or inhaled corticosteroids, which are effective in improving stridor in a few hours (Cetinkaya et al., 2004). The hallmark cytopathic effect of acute infection with PIV-3 is comparable to that of RSV with extensive cell fusion resulting in syncytium formation. For fusion to occur, two PIV glycoproteins are required, including the hemagglutinin–neuraminidase (HN) glycoprotein interacting with host cell sialic acid receptor and the viral fusion (F) glycoprotein.

2.5. Rhinovirus

Rhinovirus infections account for the largest number of respiratory tract infections in children. However, rhinovirus infections produce mild symptoms compared to RSV, PIV, and influenzavirus. Most of the symptoms caused by rhinovirus are confined to the upper respiratory tract. Although present in the community the whole year round, rhinovirus infections peak at the onset of fall, which is probably related to schools starting after summer break. Rhinoviruses can cause severe lower respiratory tract infection (Guittet et al., 2003; Papadopoulos, 2004) and in immunocompromised patients, life-threatening pneumonia. Rhinovirus is a positive-stranded RNA-virus belonging to the Picornavirus family and over 100 serotypes exist, making it difficult to develop an effective vaccine. Rhinovirus subtypes have been divided into a major and minor group with respect to the receptor used for cell entry (Table 4.1). Major group rhinoviruses use epithelial intracellular adhesion molecule l (ICAM-1) for cell entry, while minor group viruses bind to the low-density lipoprotein (LDL) receptor. During rhinovirus infection, a predominant granulocyte and monocyte recruitments are observed. While specific antibody production occurs, it is probably not required for viral clearance, although neutralizing antibodies can provide some temporary protection against rhinovirus reinfection (van Kempen et al., 1999). ICAM-1 blocking antibodies have also been utilized and have been shown to decrease inflammation *in vitro*. There are, however, indications that rhinovirus can adapt to this with changes in receptor usage (Reischl et al., 2001).

2.6. Human Metapneumovirus

In 2001, a new respiratory virus was identified in the Netherlands causing infections similar to RSV in children (van den Hoogen et al., 2001). The reported incidence rate of human metapneumovirus (hMPV) infection in children with acute respiratory symptoms varies between 4% and 16%, of which three-quarters occur in children less than 1-year old (Williams et al., 2004). By the age of 5 years, approximately 70% of children have developed antibodies to hMPV. HMPV very much resembles RSV in its clinical spectrum, varying from coryza to bronchiolitis and

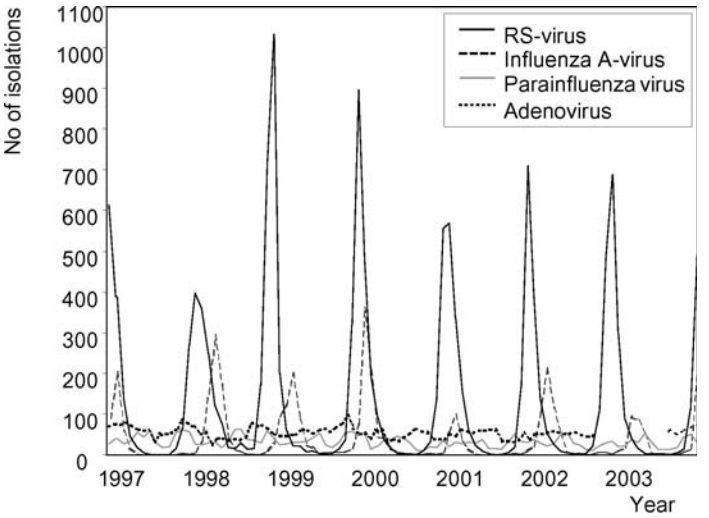

Figure 4.1. Epidemiology of RSV, influenza A, PIV, and adenovirus in the Netherlands. Data derived from the weekly reports of the sentinel system of the Dutch Working Group on Clinical Virology.

pneumonia. However, hMPV is less likely to cause pneumonia than RSV and influenza virus. Children with hMPV infection present less frequently with atypical symptoms such as vomiting, and on physical examination, rales and wheezing are found less often. Co-infection with RSV and hMPV does occur and has been suggested to result in more severe disease (Greensill et al., 2003). There is some evidence that secondary hMPV infection occurs frequently in childhood, probably accompanied only by mild symptoms (Ebihara et al., 2004).

Several investigators have found chemokine profiles during acute infection to be different in children with hMPV infections compared to those with RSV infections, with higher interleukin-8 (IL-8) and lower RANTES concentrations in hMPV patients. However in another study, inflammatory cytokine (IL-8, TNF-α, IL-1β) levels in respiratory secretions were 6-fold lower than in children infected with RSV (Jartti et al., 2002; Laham et al., 2004). Tthe physiological relevance of these observations remains unclear.

2.7. SARS Coronavirus

The outbreak of Severe Acute Respiratory Distress Syndrome (SARS), which started in late 2002 in East Asia, and spread throughout the world during that winter, was found to affect mainly health-care workers and close contacts of diseased individuals. SARS, which was proved to be caused by a new coronavirus, induces an atypical pneumonia with fever, dry cough, and shortness of breath. Many adults also suffer from myalgia, dizziness, chills, and rigors. It was concluded from postmortem examinations that SARS-pathology is primarily caused by immunological damage

to the lungs. The interstitial space of the lungs was mainly filled with mononuclear infiltrates and there was diffuse hemorrhage on the lung surface.

SARS coronavirus (SARS-CoV) spreads mainly via the respiratory route, the epithelial cell being its primary target cell. As for other coronaviruses, the spike proteins, S1 for cell entry and S2 for fusion, also seem to be important for SARS entry of host cells, despite the fact that SARS is only 20–30% homologous to other coronaviruses. Very recently, angiotensine converting enzyme (ACE2) was identified as the host cell receptor for SARS-CoV (Li et al., 2003) and surface expression of ACE2 on alveolar epithelial cells was demonstrated (Hamming et al., 2004).

Transmission of SARS-CoV occurs by droplets and most cases have occurred through close contact exposure. However, recently evidence of airborne transmission has emerged (Yu et al., 2004). Although many individuals were infected in the initial 2 weeks of the epidemic and the disease spread rapidly over several countries, the numbers of infected children stayed relatively low in all regions ($<5\%$). Furthermore, children tend to develop less severe disease. After an incubation period of 5–10 days, similar to adults, infected children developed symptoms of a mild upper respiratory tract infection, clinically indistinguishable from other common colds. None of the 100 pediatric SARS cases in Hong Kong turned out to be fatal and only one adolescent required mechanical ventilation (Leung et al., 2003). Adolescents are more likely to develop severe disease, as observed in adult SARS patients (Leung et al., 2004). A sore throat and a high initial and peak peripheral blood neutrophil count were found to be independent risk factors for severe disease in children with a laboratory-confirmed SARS infection. Furthermore, children seemed to spread the disease less easily to others and there have appeared no reports in the literature demonstrating transmission from children to other individuals. Many children with laboratory-confirmed SARS do not meet the WHO criteria for diagnosis of SARS. As children seem to have a much milder clinical course, the term "SARS" may not represent the disease in children very well.

3. Immunology of Viral Lower Respiratory Tract Infections

3.1. Innate Immunity

The role of the innate immune system in viral lower respiratory tract infection has not been studied intensively until recently. Studies have focused on the adaptive immunity with the goal of developing a vaccine, for example, for RSV. Understanding the mechanisms underlying primary and recurrent viral infection has attracted increased attention. The immunological response against viral invasion of the lower respiratory tract comprises both adaptive and innate immune response mechanisms with both beneficial as well as detrimental characteristics. The innate response occurs in the early phase of the infection and increasing evidence suggests that these early events determine disease course and possibly even long-term outcome (Garofalo and Haeberle, 2000; Tasker et al., 2000). For the rest of the discussion on immunological phenomena, focus will be on RSV as a prototype.

Epithelial cells are key regulators of the innate immune response against viral infections (Garofalo and Haeberle, 2000), producing a number of inflammatory

mediators in response to RSV infection. These include cytokines (interleukin-6, -1, tumor necrosis factor (TNF)-α), several chemokines (IL-8, macrophage inflammatory protein (MIP)-1α, monocyte chemotactic protein (MCP-1), RANTES), type-l interferon (IFN-α/β), and growth factors (GM-CSF, G-CSF). Epithelial-derived levels of chemokines correlate with disease severity (Bont et al., 1999; Smyth et al., 2002). Surfactant proteins produced by epithelial cells (SP-A and SP-D) may also play a role as opsonins for viruses and bacteria. Thus, epithelial cells provide a potential mechanism for serum-independent phagocytosis. Many of these mediators are induced both at the level of secretion and transcription. Interestingly, some mediators (e.g., IL-8 and RANTES) are also upregulated by inactive forms of the virus (Harrison et al., 1999).

RSV uptake by immune and non-immune cells is a receptor-mediated process. Experiments with blocking antibodies against G-protein revealed inhibition of binding of RSV to epithelial cells. The fractalkine receptor, also known as the CX3CR1 chemokine receptor, is involved in G-protein-mediated uptake by epithelial cells (Tripp et al., 2001). Other receptors may very well be involved in uptake by dendritic cells, macrophages, and other cells of the innate immune system (Harris and Werling, 2003), and Toll-like receptors (TLRs), especially TLR-4, are being investigated as possible candidates for mediating viral uptake (Haeberle et al., 2002a, b; Monick et al., 2003).

Several groups have demonstrated activation of the transcription factor NF-kB in RSV-infected epithelial cells (Tian et al., 2002). Many of the exhibited effects observed in epithelial cells can be explained by activation of NF-kB. Several cytokines associated with RSV infection have NF-kB binding sites in their promoter or enhancer regions (Bitko et al., 1997). Epithelial NF-kB activation has also been observed in other viral infections, including parainfluenza, influenza A, and rhinovirus (Pahl and Baeuerle, 1995; Kim et al., 2000; Bose et al., 2003). NF-kB could be an exciting target for therapy development and experiments in which BALB/c mice were treated with perflubron have confirmed this concept. Perflubron has already been shown to be effective in clinical trials of patients with respiratory distress syndrome because of its physical characteristics. Besides the beneficial physical effect in improvement of gas exchange and of lung compliance, this agent was found to have anti-inflammatory effects. RSV-infected BALB/c mice treated with perflubron intranasally showed a reduction in cellular inflammatory infiltrates and decreased chemokine expression in the lung tissue. Both the anti-inflammatory effects were directly linked to interference of perflubron with NF-kB-mediated transcription (Haeberle et al., 2002a, b).

The chemokines produced by epithelial cells attract T-cells, neutrophils, monocytes, and possibly eosinophils to the respiratory tract. Besides induction of secreted products, epithelial cells upregulate expression of adhesion molecules for neutrophils on their surface, allowing neutrophils to adhere firmly to infected cells (Wang and Forsyth, 2000). Furthermore, neutrophils are the dominant cell type found in bronchoalveolar lavage (BAL) fluid of RSV patients (Everard et al., 1994, McNamara et al., 2003). However, their role in fighting viral infection is not as well established as in bacterial infections. Pathological studies of lungs of RSV-infected calves have shown a major influx of neutrophils in the infected airway mucosa,

observed earlier than any other cell type involved. Furthermore, neutrophils are the dominant cell type found in bronchoalveolar lavage (BAL) fluid of RSV patients (Everard et al., 1994, McNamara et al., 2003)"

Several chemokines and cytokines involved in neutrophil activation have been associated with RSV lower respiratory tract infections. Recently, local neutrophil IL-9 production has been linked to RSV bronchiolitis (McNamara et al., 2004). As shown by Wang et al., a major increase in epithelial damage occurs, when RSV-infected epithelial cells are co-cultured with neutrophils (Wang and Forsyth, 2000). This is suggestive of a detrimental role for neutrophil-induced immunopathology in lower respiratory tract infections.

RANTES and MIP-1α are produced by the epithelium in response to RSV infection and these chemoattractants recruit eosinophils to the inflammatory site. The analogy between clinical features of virus-induced wheezing illnesses and asthma has made eosinophils an attractive subject for studies aimed at improving understanding of RSV pathogenesis. However, mainly because of their absence in BAL of RSV patients, their involvement remains controversial. However, eosinophil-derived cationic protein (ECP) has been linked to bronchiolitis and post-bronchiolitic wheezing (Garofalo et al., 1992; Pifferi et al., 2001; Dimova-Yaneva et al., 2004). *In vitro*, eosinophils have also been shown to be susceptible to RSV. Eosinophil priming, superoxide production, and degranulation were induced by incubation with RSV (Garofalo et al., 1992; Kimpen et al., 1992; Olszewska-Pazdrak et al., 1998; Tachibana et al., 2002). Rosenberg and Domachowske (2001) have suggested a beneficial role for eosinophils in RSV bronchiolitis. They identified antiviral properties for the eosinophil based on ribonuclease activity of eosinophil-derived neurotoxin (EDN) and ECP. This enzymatic activity leads to destruction of extracellular ssRNA virions and delayed replication both *in vitro* and *in vivo*.

Recently, there has also been great interest in the involvement of macrophages and dendritic cells in RSV pathogenesis. These cells were already appreciated for their role in antigen presentation, at which dendritic cells are by far superior. Macrophages express phagocyte activity, which may be of importance in clearance of infected epithelial cellular debris. Fascinating new players in host defense against viruses are pattern recognition receptors. Toll-like receptor-4 (TLR-4) and CD14, both present in a complex on these cells, have been found to interact with RSV and receptor-binding results in triggering of the innate immune system. TLR-4 has been shown to activate NF-kB in macrophages of RSV-infected mice (Haeberle et al., 2002a, b) and TLR-4-deficient mice have impaired NK-cell and CD14+ cell trafficking and delayed viral clearance (Haynes et al., 2001). Furthermore, intracellular pattern recognition receptors TLR-3 and -7, may be involved in recognizing double- and single-stranded RNA (dsRNA/ssRNA), respectively (Akira and Hemmi, 2003; Lund et al., 2004). dsRNA is produced during replication of RNA-viruses and is a potent inducer of IFN-α/β. All human cells can produce IFN-α/β in response to viral infection, while only T-cells and NK-cells produce IFN-γ. dsRNA also activates dsRNA-dependent protein kinase R (PKR) and NF-kB via distinct pathways. Transcription of PKR is under control of IFN-α/β. PKR controls enzymes directly involved in protein synthesis, thereby inhibiting cellular and viral protein translation. IFN-α/β-deficient mice as well as PKR−/− mice are extremely sensitive to

influenza infection (Balachandran et al., 2000). Several viruses, including RSV, have evolved mechanisms to escape the interferon system, which will be discussed below.

3.2. Adaptive Immune Response

Respiratory epithelial cells are the principal host cells for viral pathogens in lower respiratory tract disease. The degree of replication and the mechanism of spread along the epithelial layer depend on the virus family characteristics. Through the fusion (F) protein, RSV is capable of syncytium formation, which allows it to replicate and spread relatively undetected by the immune system for a relatively long period. The virus itself is directly responsible for cytopathology and viral envelope proteins are expressed on the surface of infected epithelial cells. Dendritic cells, lining the basal membrane of the respiratory epithelium encounter RSV, pick up viral antigens and migrate to mediastinal lymph nodes where viral antigen is presented to naïve CD4+ T-cells. Antigen presentation and co-stimulatory molecule expression lead to maturation to the T-helper phenotype. This then induces B-cell proliferation with the production of specific antibodies as well as proliferation of virus-specific cytotoxic CD8-cells.

Cellular responses are responsible for controlling and terminating acute infection with RSV. In primary infections, the adaptive cellular immune response develops within 10 days. These CD8+ cells can recognize and eliminate virus-infected epithelial cells resulting in perforin-mediated cytotoxity. Epithelial cells are nonprofessional antigen presenting cells (APC) expressing MHC Class l on the surface (Garofalo et al., 1996). When infected, epithelial cells present viral antigen in association with MHC Class l molecules. MHC Class l restricted antigen presentation to CD8+ cells, among other factors, may determine the strength of the cytotoxic response. In CD8-deficient mice, there is delayed viral clearance; however, these mice also exhibit decreased disease severity (Graham et al., 1991). Therefore, it is conceivable that CD8+ T-cells are crucial in viral clearance while a surplus of cytotoxicity may result in pulmonary injury.

In humans, a cytotoxic T-cell response is elicited against all viral proteins, except the G-(attachment)-protein, which is required for cell entry (Bangham et al., 1986; Hacking and Hull, 2002). It is suggested that a defective response against G-protein is directly associated with enhanced disease. However, G-protein can induce a CD4+ response in mice, which is associated with Th2-cytokine production and eosinophilia both during primary and secondary infection (Openshaw, 1995). The immune response to the F-protein is dominated by IFN-γ production and subsequent polarization toward a Th1-type cellular response, and therefore it has been postulated that responses to the other viral proteins can modulate the strong Th2-response to G-protein (Graham et al., 2000).

A stronger Th1-response seems to induce a more rapid viral clearance and milder disease (Bont et al., 1999; Legg et al., 2003). Besides activated T-cells, NK-cells also produce considerable amounts of IFN-γ (Hussell and Openshaw, 1998). IFN-γ has important antiviral effects and provides a link between adaptive and innate immune system. It can induce expression of TNF-related apoptosis inducing ligand (TRAIL) on immune cells, which has the potential to trigger apoptosis of

virus-infected cells (Sedger et al., 1999). *In vitro* findings suggest that RSV-infected cells *in vivo* are susceptible to killing by immune cells through the TRAIL pathway (Kotelkin et al., 2003). NK-cells are also thought to play a role in activating CD8+ cells, further modulating the degree of cytotoxicity (Hussell and Openshaw, 1998). In summary, in RSV lower respiratory tract infections, cytotoxic CD8+ T-cells are involved in viral clearance while the humoral response is required for the protection against reinfection. However, as has been discussed before, memory is incomplete and repeated infections with RSV are common. Both IgM and IgG as well as secretory IgA against RSV are formed in infants, and a more vigorous antibody response seems to be protective against RSV infections (Meurman et al., 1984; Welliver et al., 1989).

3.3. Immature Immune Response

RSV infections are most severe in the youngest age group, which is the least mature in terms of immunity to infections. Relative deficiencies in both innate and antigen-specific immunity in infancy have been characterized. These include delayed trafficking of immune cells, less-efficient antigen presentation by dendritic cells, and impaired production of IFN-γ by T-cells in response to antigen presentation (Bont and Kimpen, 2002).

The fetus derives maternal IgG-antibodies via the placenta fairly late in gestation. This partly explains why prematurity is an important risk factor for severe disease caused by RSV, as well as the physiological characteristics of the small airways. Antibody titers produced by infants are relatively low compared to older children. Trials with humanized monoclonal antibodies against RSV-F-protein have shown a 50% reduction in RSV lower respiratory tract-related hospitalizations in this high-risk group for severe disease (Impact Study Group, 1998).

The cytokine milieu at the time of infection is another factor possibly contributing to the occurrence of severe RSV bronchiolitis especially in the youngest age group. At birth, there is skewing toward a Th2-phenotype and RSV bronchiolitis was long thought to be a Th2-type disease. This role of Th2-skewing is an attractive concept, because it provides some explanation for the association between RSV bronchiolitis and the development of asthma. Asthma and allergy have long been acknowledged to beTh2-mediated conditions. However, convincing evidence that primary RSV infections are mediated by Th2 cytokines is lacking. Dendritic cells are thought to have an important function in skewing the Th1/Th2-ratio. Viruses may be important in maturation of dendritic cells, which can then drive differentiation of naive T-cells into either a Th2- or a Th1-phenotype. The role of regulatory T-cells that suppress both Th1 and Th2 differentiation has not been studied in RSV bronchiolitis so far.

3.4. Genetic Background

Gene polymorphism studies have been undertaken to identify a genetic background to explain the individual susceptibility to RSV lower respiratory tract infection. Several polymorphisms situated in genes relevant for the adaptive and innate immunity have been found to correlate with occurrence of RSV infection.

Polymorphisms of interleukin-4, IL-4R, and its receptor, have been associated with RSV bronchiolitis, which is consistent with the Th2-hypothesis (Choi et al., 2002; Hoebee et al., 2003). Very recently, a polymorphism of the gene coding for interleukin-10 (IL-10) was found as well (Hoebee et al., 2004). This is particularly interesting since IL-10 is a cytokine produced by T-regulatory cells and monocytes, thought to be primarily involved in development of allergy. Gene polymorphisms involved in innate immunity include surfactant proteins SPA and D (Lahti et al., 2002), the chemokine IL-8 (Hull et al., 2001), TLR-4 (Tal et al., 2004), and the chemokine receptor for RANTES and MIP-1α, CCR5 (Hull et al., 2003).

4. Virus Infection in the Immunocompromised Host

4.1. Immunocompromised Patients

Immunocompromised patients have a higher risk of developing severe disease from viral respiratory tract infections. In particular, the presence of defects in cellular immunity result in an increased duration of viral shedding and enhanced risk of developing severe disease.

Most cellular immunodeficiencies are iatrogenic in nature. An important cause is intensive immunosuppressive treatment. The number of pediatric patients undergoing organ or stem-cell transplantation is increasing and high doses of chemotherapeutic and immunosuppressive agents are often used in the pre- and posttransplant regimens. Immunosuppressive drugs are used in cancer treatment regimens and for a number of inflammatory conditions. Community acquired respiratory viruses such as RSV, rhinovirus, adenovirus, influenza A, influenza B, and the parainfluenza group are frequent causes of respiratory disease in these patients (Soldatou and Davies, 2003). Adenovirus infections have a particularly high risk of adverse outcome, mortality rates are high, and no effective treatment exists. The presence of lower respiratory tract infection and infection in the pre-engraftment phase of HSCT is believed to have a particularly poor prognosis (Khushalani et al., 2001). The risk of severe disease is higher during allogenic HSCT than autologous HSCT. Besides causing increased morbidity and mortality, respiratory tract infections are associated with a greater risk of delayed engraftment (Abdallah et al., 2003). In solid organ transplant patients, respiratory virus infections are also associated with a higher incidence of rejection (Wendt, 1997).

Prolonged shedding of respiratory viruses for weeks or months has been documented in HIV-infected adults and children. This has important implications for infection control in medical facilities. In addition, respiratory viral infection may result in increased HIV replication and, theoretically, HIV disease progression (King, 1997). In HIV-infected children, RSV infections are less limited by season (Madhi et al., 2000). However, generally, the course of RSV infections in HIV patients is not more severe, unless there is profound lymphopenia or pre-existing lung disease (Soldatou and Davies, 2003).

Other viruses may also cause respiratory complications in the immunocompromised patients. In particular herpesviruses, such as cytomegalovirus (CMV) and

varicella zoster virus, can cause severe pneumonia. With a CMV-negative donor and a CMV-positive recipient, there is an especially high risk of reactivation which may lead to severe disease. This reactivation also occurs with Epstein Barr virus (EBV), human herpes virus (HHV)-6, -7, and -8, although these are much less frequent causative agents of pneumonia.

4.2. Impaired Innate Immune System

The innate immune defense to viral respiratory tract infections consists of the mucosal layer, type 1 interferons, activated phagocytes, and NK-cells. The impact of primary defects in the innate immune defense has not been well documented. Phagocyte defects are primarily related to a higher incidence of bacterial infections. One indication that impaired phagocyte function also leads to increased severity of respiratory viral infection can be derived from a report of severe abnormalities on lung-CT-scans of RSV patients with phagocyte defects (Uzel et al., 2000). Interferon-gamma receptor deficiency, which may have implications for both the adaptive and innate immune system, has also been associated with increased susceptibility to viral respiratory pathogens (Dorman et al., 1999).

Chronic lung disease also increases susceptibility to respiratory viruses (Meert et al., 1989; Griffin et al., 2002). Premature patients with bronchopulmonary dysplasia are candidates for RSV-immunoprophylaxis because of their increased risk of developing severe lower respiratory tract infections.

In children with cystic fibrosis (CF), 39% are already hospitalized with respiratory virus infection in their first year of life. Furthermore, there is a correlation between viral infections in infancy and disease progression. Infants with CF suffering from a respiratory virus infection are at significant risk for lower respiratory tract disease, hospitalization, and deterioration in lung function that persists months after the acute illness (Hiatt et al., 1999). CF infants were found to be four times more likely to develop an LRTI compared with controls. It has been shown that CF-derived airway epithelial cells allow a higher degree of PIV replication and have an increased production of pro-inflammatory cytokines (Zheng et al., 2003). CF-derived epithelial cells are also unable to express NO-synthase 2, which results in a decrease production of nitric oxide (NO), which has antiviral capacity, reducing effects on replication. Furthermore, in CF-cells there is no viral induction of 2′5′-oligoadenylate synthetase (OAS), an enzyme that is normally induced by dsRNA and IFN-γ. OAS is involved in inhibition of cellular protein synthesis, thereby inhibiting viral replication.

5. Respiratory Viruses and Asthma

5.1. Virus Infections and Asthma Exacerbations

Respiratory viruses can be isolated from the secretions of approximately 75% of children and of more than half of adults during asthma exacerbations (Johnston et al., 1995; Lemanske, 2003). Recently, COPD exacerbations have also been attributed to viral infection by rhinovirus, RSV, and PIV (Seemungal and Wedzicha,

2003). The underlying mechanisms for this are, however, still a matter of debate. From experimental rhinovirus (RV) infections in humans, it has been shown that RV infection causes increased bronchoconstriction in atopic non-asthmatic and asthmatic individuals, while symptoms in normal individuals are relatively mild. This implies that induction of a wheezing episode requires both RV infection and a pre-existing tendency to develop allergic or asthmatic disease (Message and Johnston, 2001). RV-specific T-cell responses can be activated by either serotype-specific or shared viral epitopes. Cross-reactivity between RV-subtypes could result in vigorous T-cell responses and may amplify allergic inflammation.

Other proposed mechanisms linking viral infections to asthma exacerbation are epithelial dysregulation, airway remodeling, the immune response to virus, and alterations of neural responses (Message and Johnston, 2001; Gern, 2002).

Upregulation of ICAM-1-expression, which is the entry receptor for major group rhinoviruses, has been found in susceptible individuals. This may be one mechanism predisposing atopic individuals to RV-induced exacerbations. Rhinovirus can induce a number of inflammatory mediators (kinins, arachidonic acid) and cytokines (e.g., IL-1, IL-6, IFN-α/β, GM-CSF, TNF-α) that can further enhance inflammation. Th1 cytokines seem to have a general antiviral effect while a predominant Th2-cytokine response leads to enhanced disease, failure to clear the virus, and amplification of allergic inflammation (Message and Johnston, 2001). Eosinophil numbers were found to be increased in bronchial biopsies from both healthy and asthmatic human volunteers after experimental rhinovirus infection. This cell type is associated with allergic inflammation in the lung. In allergic rhinitis patients, the increased level of eosinophils in BAL even persisted for 6 weeks. These data suggest a potential role for eosinophils in virus-induced asthma, which can be either pathogenic or protective.

Virus-induced exacerbations of asthma tend to be resistant to treatment with corticosteroids and may require a different therapeutic approach. *In vitro*, blocking ICAM-1 has been tried with positive results, which may be of particular relevance to rhinovirus infections. The possibility of other immunomodulating drugs is being investigated and may be of significant benefit to future asthma treatment.

5.2. Viral Respiratory Tract Infections and the Inception of Asthma

A causal relationship between viral respiratory tract infections and asthma exacerbations is generally acknowledged. However, the suggestion that respiratory virus infection is a causal determinant in the development of asthma is highly controversial. According to the hygiene hypothesis, viral infection would be expected to have an inhibitory effect on the development of asthma (an allergy), and this is supported by a study from Matricardi et al. (1997), showing an inverse relation between hepatitis A seropositivity and atopy among soldiers. The hygiene hypothesis is based on the theory that the immune system is directed toward a more Th1-skewed immune response with each viral infection. However, this hypothesis is not supported by the observation that RSV infections, severe enough to cause bronchiolitis, are significantly associated with a higher incidence of asthma up to the age of 7–11

years (Stein et al., 1999; Sigurs et al., 2000). These data convincingly show a link between RSV bronchiolitis and recurrent wheezing in childhood. In a recent study, wheezing following RSV lower respiratory tract infection was found to develop independent of atopy (Bont et al., 2004). It may, however, be true that the transmission route, the organs involved, and exposure to microbial products may be important in determining the final effect of a virus infection on the development of asthma and allergy (Gern and Busse, 2002).

The link between RSV infection and atopy is even less clear than the one with recurrent wheezing and asthma, at least in humans. Animal studies have yielded conflicting results. One group found that RSV infection in mice enhances subsequent allergic inflammation (Schwarze et al., 1997), while others reported a decrease in allergic sensitization and BHR after RSV infection (Peebles et al., 2001). No proof exists that severe RSV infections are associated with atopy that persists into adulthood (Peebles, 2004).

A key question is whether the association with the development of asthma is merely an expression of increased susceptibility to both asthma and RSV-induced lower respiratory tract infections or whether true causality is involved.

The prevailing theory on this subject involves maturation effects of the Th1/Th2-balance. The system shifts from a Th2-polarization in fetal life, which is an optimal environment for the placenta, to a more balanced Th1/Th2-phenotype in adulthood. Most viruses are known to induce a Th1 cytokine response (IFN-γ). This theory states that when infections occur early in infancy, there is a reduced ability to react with an appropriate antiviral Th1-response. Low IFN-γ production may result in spread of the virus to the lower respiratory tract. This is in agreement with findings that in children with severe RSV lower respiratory tract infections, lower amounts of IFN-γ are produced (Bont, 2002). The dynamics of this shift toward a more balanced Th1/Th2 immune response may differ between individuals. Both environmental factors and genetic make-up may contribute to a slower maturation of Th1 competence in some individuals. Respiratory virus infections in infancy and an atopic sensitization to aero-allergens, both of which are related to Th2-skewed responses and intermittent wheeze, may than synergistically result in persistent wheeze (Holt and Sly, 2002). It is likely that more links between atopic sensitization and respiratory infections exist, while preventive RSV-IVIG treatment of children results in a decreased sensitization to aeroallergens as well. RSV-prophylaxis may therefore have a long-term benefit in the development of persistent wheeze (Piedimonte and Simoes, 2002; Wenzel et al., 2002).

Another theory linking viral infections in childhood to the development of asthma involves the pathologic effects of viral lower respiratory tract infections on airway physiology. Wall thickening with consequent increased resistance may predispose the airway to more infections and thus influence bronchial hyperreactivity (Bardin et al., 1992). However, it may also be true that small airways predispose to both asthma and airway symptoms from viral infections. Remodeling of the submucosal neural networks by RSV, as observed by Piedimonte et al., is also proposed to result in increased responsiveness to airway irritants (Piedimonte, 2002).

Walter et al. (2002) have proposed that paramyxoviral infection has the ability not only to induce acute hyperresponsiveness, but also to result in long-lasting

changes in airway behavior. From mouse-studies with a PIV (SeV), it has been concluded that viruses cause long-term effects in epithelial cells, associated with airway reactivity and goblet cell hyperplasia. Long-term effects are induced by the virus in the acute phase, and later on, the presence of virus is no longer required for the persistence of symptoms. It is speculated that primary paramyxoviral infection within the proper genetic background may result in chronic dysfunction of epithelial cell behavior. Their results have indicated that different mechanisms are responsible for the induction of the acute and the chronic response (Walter et al., 2002).

Several theories on the induction of asthma have thus been proposed. The current view is that virus infection modulates the development of an asthmatic phenotype in a susceptible host. The relationship is, therefore, not purely causal but certainly requires an intrinsic vulnerability.

6. Viral Evasion of the Immune System

The effectiveness of respiratory virus infections in the host depends partly on the ability to evade the immune system (Figure 4.2). While several viral evasion mechanisms have evolved, not all have been intensively studied in respiratory viruses.

6.1. Viral Entry

Viral entry into host cells is one of the first obstacles viruses have to overcome. Since the cell membrane is in principle impermeable to macromolecules, viruses

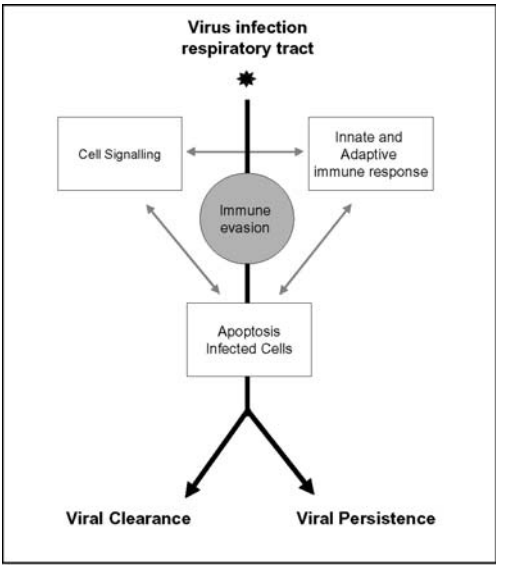

Figure 4.2. Evasion of the host response. The effectiveness of the virus in evading the host response mechanisms by interfering with cell signaling will determine whether the host is able to clear the virus or whether a state of viral persistence is established.

must first have an effective method to attach to the cell membrane. Some viruses bind putative cell surface receptors that do not simply play a role in viral attachment, but also allow viral entry by inducing endocytosis. For some viral pathogens, such as rhinovirus, these receptors have been identified (ICAM-1 and LDL-R), while for others, such as RSV, the receptor that is used for cellular entry has not been unequivocally defined. The CX3CR1-chemokine receptor, also known as fractalkine-receptor, may be involved and TLRs have also been proposed to play a role. Many enveloped viruses have glycosylated proteins, which not only bind to cellular receptors, but also have additional functions as membrane fusion factors, or receptor-destroying enzymatic activity. Membrane fusion factors such as the RSV F-protein also allow cell-to-cell transmission of virus, which keeps it relatively hidden from the cellular immune system (Smith and Helenius, 2004).

6.2. The Interferon Antiviral Response

Escaping the "interferon signaling system" is one of the common mechanisms most viruses have acquired. As mentioned earlier in this chapter, both IFN-α/β and IFN-γ have potent antiviral properties. Both types of interferon regulate transcription of a variety of target genes through activation of interferon inducible transcription factors. IFN-stimulated genes encode a variety of cellular enzymes, including PKR and 2′5 ′-oligoadenylate synthetase, both involved in inhibition of viral protein synthesis. Furthermore, interferons induce cellular apoptosis and upregulate MHC1 expression, targeting cells for CD8+ T-cell-mediated cytotoxicity. Additionally, IFN-γ activates the adaptive cellular immune system.

RSV infection leads to an increase in IFN-α/β, but does not induce IFN-γ from mononuclear and NK-cells as efficiently. Despite the fact that interferons are known for their antiviral properties, intranasal administration of either lFN-α/β or IFN-γ in the airway does not lead to reduction of symptoms of viral respiratory infections (Ramaswamy et al., 2004). This is suggestive of a viral mechanism to evade the host's interferon response.

The Paramyxoviridae family (Figure 4.3), responsible for a large part of children's respiratory infections, consists of two subfamilies based on the structural differences in the gene encoding for the polymerase complex (P-protein). The Paramyxovirinae subfamily members are able to block interferon-mediated promoter activity. These paramyxovirinae, to which parainfluenza-, measles-, and mumps virus belong to, have a P-gene that encodes for additional proteins besides the P-protein, the V-proteins. It is these V-proteins that have been found to be responsible for evading the interferon signaling pathways in this group of viruses. IFN-mediated transcription is predominantly mediated by signal transducers and activators of transcription (STAT). V-proteins have the ability to block interferon-mediated signaling by targeting STATs for proteosomal degradation (Horvath, 2004).

In contrast, RSV, belonging to the Pneumovirinae subfamily, fails to inhibit IFN-induced promoter activity. The pneumovirinae consist of only one genus, the pneumovirus, which also includes hMPV. In this subfamily, P-genes only encode for the P-protein and therefore RSV cannot block interferon-mediated signaling (Young et al., 2000). However, recently it was demonstrated that, although RSV does not

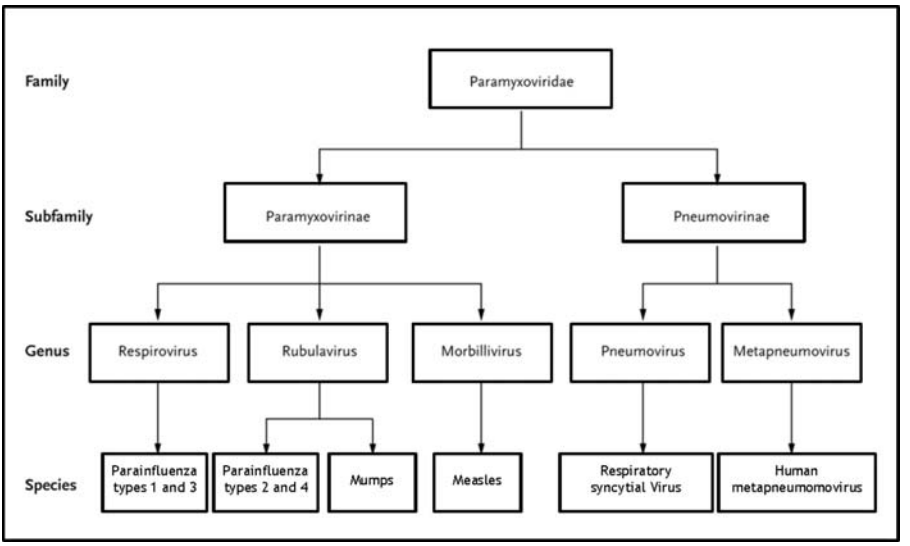

Figure 4.3. Classification of viral pathogens of the Paramyxoviridae family that infect humans. From McIntosh and McAdam (2004).

inhibit interferon-induced promoter activity, RSV replication is still resistant to IFN treatment of infected cells. Apparently, an alternative mechanism to circumvent the interferon antiviral response exists. This has been attributed to additional proteins, characteristic of these pneumoviruses (Spann et al., 2004). These are nonstructural proteins (NS1 and NS2) that have no homologs in the paramyxovirinae. However, the underlying molecular pathway has not yet been elucidated.

6.3. Evasion of Apoptosis

RSV infection of epithelial has been shown to lead to an upregulation of TRAIL-receptor expression on these cells (Kotelkin et al., 2003). Apoptosis of infected cells is an effective way to eliminate intracellular pathogens without damage to the surrounding tissue (Figure 4.4). However, several respiratory viruses have developed mechanisms to inhibit apoptosis. It has been demonstrated that RSV is able to effectively inhibit apoptosis of epithelial cells *in vitro*, in accordance with the limited pathology induced by RSV in epithelial cells during the first few days of the infection (Thomas et al., 2002). Eventually, necrosis is observed when mature viral particles are released from the cells, after 2–3 days. Furthermore, experiments with both adult and cord blood monocytes have shown a prolonged longevity of cells, when cultured in the presence of RSV (Krilov et al., 2000).

6.4. Immune Evasion Techniques by Adenoviruses

Of all respiratory viruses, viral evasion techniques of adenoviruses have been studied most intensively. It appears that approximately a third of the adenovirus

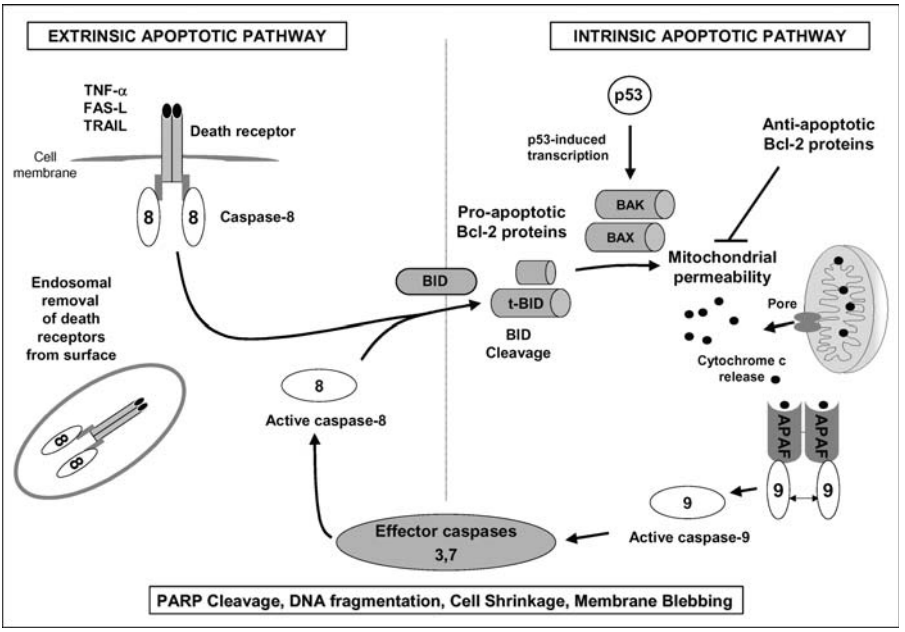

Figure 4.4. Extrinsic and intrinsic signaling pathways of apoptosis. Some viruses such as adenovirus interfere with cellular apoptosis. Cellular apoptosis can normally occur via activation of the extrinsic (death receptor) pathway or the intrinsic pathway. Both lead to activation of effector caspases 3 and 7, which will inevitably result in apoptosis. Via production of external factors, such as TNF-α, Fas-Ligand, and Trail, death-receptors are cross-linked, which leads to apoptosis. Adenovirus E3 proteins can remove these death receptors from the surface, thereby inhibiting extrinsic apoptosis. The intrinsic pathway is regulated by pro- and anti-apoptotic proteins of the Bcl2-family. The transcription factor p53 has anti-carcinogenic activity, induces the intrinsic pathway and promotes the transcription of pro-apoptotic proteins such as Bax and Bak. These proteins induce mitochondrial leakage of cytochrome *c*, which activates caspase 9, finally leading to effector caspase activation and apoptosis. Adenovirus proteins are involved in both inhibition of p53 and the functioning of Bax and Bak.

genome is devoted to counteracting innate and adaptive immune defenses (Burgert et al., 2002). Adenoviruses encode the protein E1A that blocks interferon-induced gene transcription. Through the VA-RNA protein that blocks activation of PKR, they also interfere with the antiviral enzymes that are synthesized under interferon control. Additionally, adenoviruses have developed several mechanisms to inhibit both constitutive and death receptor induced apoptosis (Figure 4.4). The E1B/55K protein inhibits p53-mediated apoptosis, E3/19K interacts with pro-apoptotic proteins Bax and Bak, whereas several E3 proteins are involved in removing Fas and Trail (death) receptors from the cell surface by promoting their degradation in lysosomes (Wold et al., 1999). Finally, adenoviruses interfere with recognition of infected cells by cytotoxic lymphocytes. The adenovirus E3/19K protein inhibits transport of MHC1 molecules to the cell surface, resulting in decreased viral antigen presentation to CD8+ cells.

Future studies may unravel further mechanisms of immune evasion that may also be important in viruses involved in lower respiratory tract disease. This knowledge

is likely to be crucial to the improvement of immunotherapies for prevention and treatment of viral respiratory tract infections.

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