Respiratory syncytial virus (RSV) vaccines—Two steps back for one leap forward

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Abstract

Respiratory viruses are among the most important causes of morbidity and mortality worldwide. From a vaccine viewpoint, such viruses may be divided into two principle groups—those where infection results in long-term immunity and whose continued survival requires constant mutation, and those where infection induces incomplete immunity and repeated infections are common, even with little or no mutation. Influenza virus and respiratory syncytial virus (RSV) typify the former and latter groups, respectively. Importantly, successful vaccines have been developed against influenza virus. However, this is not the case for RSV, despite many decades of research and several vaccine approaches. Similar to natural infection, the principle limitation of candidate RSV vaccines in humans is limited immunogenicity, characterised in part by short-term RSV-specific adaptive immunity. The specific reasons why natural RSV infection is insufficiently immunogenic in humans are unknown but circumvention of innate and adaptive immune responses are likely causes. Fundamental questions concerning RSV/host interactions remain to be addressed at both the innate and adaptive immune levels in humans in order to elucidate mechanisms of immune response circumvention. Taking the necessary steps back to generate such knowledge will provide the means to leap forward in our quest for a successful RSV vaccine. Recent developments relating to some of these questions are discussed.

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1. Introduction

From an immunological viewpoint, respiratory virus infections can be divided into two broad categories—those resulting in life-long immunity upon recovery to the infecting strain (or close relatives) and those in which recurrent infection is evident, even with the same strain, because of poor immunological memory. Although several problems remain to be resolved, efficacious vaccines were successfully developed for the former group, which is typified by influenza virus (Edwards et al., 1994). In contrast, no vaccines are available for the latter group, of which RSV is characteristic. RSV is the leading cause of severe respiratory disease in infants and young children, in particular causing bronchiolitis and pneumonia (Hall, 2001). The elderly and immunocompromised individuals constitute other groups susceptible to severe disease and death, while asthma, cystic fibrosis (CF) and chronic obstructive airway disease (COPD) may be severely exacerbated by RSV infection (Meissner, 2003; Thompson et al., 2003; Wilkinson et al., 2006). Furthermore, RSV infection is associated with the subsequent development of wheeze and asthma in children (Sigurs et al., 2000; Stein et al., 1999). Thus, there is a compelling unmet medical need for a safe and efficacious vaccine and/or therapeutic against RSV. Despite over 50 years of research, no such vaccines or therapeutics exist. Palivizumab (Synagis™), a humanised monoclonal antibody against the RSV F protein, is the only RSV-specific product on the market and is used prophylactically. Its use is restricted to infants considered at high risk of severe respiratory disease (Meissner and Long, 2003). These include pre-term infants (<32 weeks gestation), those with chronic lung disease and congenital heart disease. However, controversies remain about the cost-benefit ratios of this product and over 50% of children hospitalised with RSV do not have any apparent underlying risk factors (Harkensee et al., 2006; Prais et al., 2003; Reeve et al., 2006).

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Many strategies have been tried with a view to an RSV vaccine, including formalin inactivated whole virus (FI-RSV), live attenuated viruses and subunit vaccines but none have been successful to date (Alwan and Openshaw, 1993; Crowe et al., 1993; Kim et al., 1969; Murphy et al., 1989; Power et al., 1997). Indeed, rather than protecting, FI-RSV formulated in aluminium salts was an infamous vaccine candidate that primed young infants for exacerbated disease upon exposure to natural RSV infection and two of the vaccinated infants died (Kim et al., 1969). The prolonged failure to develop a safe and efficacious RSV vaccine, despite many hundreds of millions of dollars invested and strong collaborations between industry, public health and research agencies and academia, has led to a considerable lethargy and scepticism in industrial circles about the possibilities of success. Rather than exhaustively reviewing the literature on RSV vaccine strategies, for which there are many excellent publications, and as an alternative approach, this overview will discuss the biology of RSV in relation to infection and immune responses in humans and animal models, along with comparisons with other viral infections, in an attempt to identify questions whose answers may re-ignite the search for efficient vaccines.

2. RSV classification and structure

Human RSV is a member of the family Paramyxoviridae, subfamily Pneumovirinae and genus Pneumovirus (Collins et al., 2001). It has counterparts in cattle (bovine RSV) and sheep (ovine RSV) and is closely related to the recently identified human metapneumovirus (hMPV). RSV is furthered divided into two subgroups, A and B, differentiated primarily on the variability of the G gene and encoded protein. It is also related to other members of the Paramyxoviridae, such as Sendai virus (murine parainfluenza virus type 1) and measles virus. It is an enveloped virus characterised by a single stranded negative sense RNA genome encoding at least 11 proteins. The genome is encapsidated in a nucleocapsid composed primarily of the nucleoprotein (N). The nucleocapsid is also composed of the RNA-dependent RNA polymerase complex of the large (L) and phospho (P) proteins and transcription/replication factors including the matrix 2 proteins M2-1 and M2-2. The matrix (M) protein surrounds the nucleocapsid and provides the link between it and the surface glycoproteins that are inserted into the cell-derived bi-lipid virion membrane. The surface glycoproteins include the G, fusion (F) and the small hydrophobic (SH) proteins. The G and F proteins are responsible for attachment and membrane fusion, respectively, although other functions have also been attributed to these proteins, as will be discussed below. These proteins are also the principle targets of adoptive immune responses following infection and constitute the most promising antigens for subunit vaccines. The function of the SH protein is unclear, although recent evidence suggests that it is implicated in blocking TNF-α-dependent apoptosis of infected cells (Fuentes et al., 2007).

The genome also encodes non-structural proteins, designated NS1 and NS2, which are implicated in circumventing innate immune responses to RSV infection (Elliott et al., 2007; Lo et al., 2005; Ramaswamy et al., 2006; Spann et al., 2004).

3. RSV infection/vaccine models

Several animal models have been developed to study RSV infection, pathogenesis and potential vaccines. The most common small animal models include BALB/c mice and cotton rats, while non-human primate models include African green monkeys, macaques, bonnet monkeys and chimpanzees (Byrd and Prince, 1997; Prince et al., 1978; Prince et al., 1979). Although genetically very close to humans, chimpanzees are a protected species. Therefore, their use in RSV experiments is highly restricted and, for most laboratories, prohibitively expensive. Some general principles may be derived from RSV infection or vaccination of these animal models compared with humans that will be important for the discussion below.

First, the small animal models are semi-permissive for RSV and, unless unnaturally high titres are used, infection does not cause symptomatic disease (Graham et al., 1988; Power et al., 1997; Taylor et al., 1984). Non-human primates are more permissive, but only the chimpanzee exhibits overt symptoms following RSV infection, which are restricted to rhinorrhea (Crowe et al., 1993). This is similar to mild disease in humans. In contrast to humans, there is no evidence of lower respiratory tract disease in these primates.

Second, a single RSV infection of mice, cotton rats or African green monkeys is sufficient to induce strong RSV-specific cellular and humoral responses that prevent detectable virus replication upon subsequent infection (Jin et al., 2003; Power et al., 1997). In mice and cotton rats, at least, this protection is very long lasting (Power et al., 1997; Power, unpublished observations). This is not the case in humans, where primary infection induces weak immune responses that are of short duration (Crowe, 1999; Murphy et al., 1986). Furthermore, an initial infection does not protect against a subsequent infection, even with the same strain of virus (Beem, 1967; Glezen et al., 1986; Hall et al., 1991; Henderson et al., 1979; Parveen et al., 2006).

Third, several RSV vaccine candidates were shown to be highly immunogenic in mice and cotton rats and less so in non-human primates (Bukreyev et al., 1997; Collins et al., 1990; Olmsted et al., 1986; Whitehead et al., 1998). As indicated above, it could be argued that wild-type RSV infection provided as much or better protection against repeat infections than any vaccine candidates studied to date in these models. In contrast, for those vaccine candidates that entered clinical trials, modest immunogenicity of short duration was often observed in humans, with little or no evidence so far of efficacy against natural RSV infection (reviewed by Piedra, 2003). This is similar to the poor immune responses induced following human infection, as discussed above.
Taken together, these points indicate that RSV induces species-specific immune responses and that animal models do not accurately reflect RSV infection and immune responses thereto in humans. Furthermore, they are consistent with the capacity of RSV to efficiently circumvent human immune responses but not those from other species. Is it possible that the circumvention of one or more immune responses might help explain the poor memory responses to RSV in humans and thereby provide the rationale for an effective vaccine? If so, which one(s)?

4. RSV NS proteins block type I interferon (IFN) responses

Type I interferons (alpha interferon [IFN-α] and IFN-β) are important components of the innate immune system that stimulate potent antiviral responses (Decker et al., 2005; Platanias, 2005). This is achieved by signalling through the JAK/STAT signalling pathway. Briefly, type I IFNs bind to their cellular receptor, consisting of two subunits IFNAR1 and IFNAR2. The receptor subunits are associated with the Janus kinases JAK1 and TYK2, respectively (Platanias et al., 1994). When activated, these tyrosine kinases phosphorylate signal transducer and activator of transcription 2 (STAT2) and STAT1. The activated STAT1 and 2 heterodimer then associates with interferon regulatory factor 9 (IRF-9) to form the transcriptional activator complex interferon-stimulated gene factor 3 (ISGF-3). This complex translocates to the nucleus and binds IFN-stimulated response elements (ISRE) to initiate gene transcription and thereby antiviral immunity (Horvath et al., 1996).

Circumvention of the IFN-induced antiviral immunity is essential for a virus to productively infect its host. Indeed, the literature describing the various mechanisms employed by a range of viruses to block these responses is expanding rapidly. Importantly, the ability (or not) to block type I IFN responses helps explain, in part at least, the host restriction of some viruses. This is the case with RSV, where Bossert and Conzelmann (2002) demonstrated that the NS1 and NS2 proteins from human and bovine RSV, respectively, preferentially blocked IFN responses in a species-specific manner, consistent with their host-specific capacity to induce disease. We and others have also recently demonstrated that RSV is capable of blocking type I IFN responses following infection of human cells in vitro (Elliott et al., 2007; Lo et al., 2005; Ramaswamy et al., 2004, 2006). This NS1- and NS2-mediated block occurs through proteasomal degradation of STAT2, a key component of the JAK/STAT signalling pathway. Both proteins are independently capable of down regulating STAT2 expression, although the level of degradation is highest when both proteins are present. Evidence suggests that NS1 acts as a component of an E3 ligase in association with cullin 2 and the RING finger containing protein Rbx1, resulting in the polyubiquitylation of STAT2 followed by its degradation (Elliott et al., 2007). However, the mechanism by which NS2 effects STAT2 degradation remains to be elucidated, as does the reason why both NS1 and NS2 together are more efficient than either protein alone.

As blocking type I IFN responses is important for RSV to productively infect hosts, it was reasonable to assume that deleting the NS1 and/or NS2 genes will attenuate resultant recombinant viruses and thereby provide a possible basis for live attenuated vaccines. This was confirmed by Whitehead et al. (1998) and Teng et al. (2000) for NS2 and NS1, respectively, whereby deletions in either gene resulted in significant attenuation and promising protection data in chimpanzees. Furthermore, a recombinant live attenuated vaccine candidate with an NS2 gene deletion introduced within the genomic backbone of a previously described RSV vaccine candidate demonstrated interesting properties in recent clinical trials (Wright et al., 2006).

As indicate above, however, blocking type I IFN responses are not unique to RSV. Indeed, Kochs et al. (2007) recently demonstrated multiple anti-IFN actions of the influenza virus NS1 protein. Similarly, Shaffer et al. (2003) demonstrated that the measles virus (MeV) non-structural protein C also blocked type I IFN responses. As both influenza virus and MeV induce life-long immunity in humans following infection but RSV does not, it is reasonable to suggest that the anti-IFN properties of the RSV NS proteins are not implicated in the induction of the poor long-term protective immune responses following infection.

5. RSV G protein has multiple putative immune modulation functions

Two forms of G protein are derived from the RSV G gene—a membrane-bound (Gm) and secreted form (Gs) (Hendricks et al., 1987; Wertz et al., 1985). These are derived from alternative translation initiation at the first and second 5’ proximal start codons on the G mRNA. Gm is a type II glycoprotein, while both forms are mucin-like proteins with the extra-cellular domain consisting of two variable highly glycosylated domains separated by a central conserved domain containing a double disulphide bridge cysteine noose. The native G protein is an important protective immunogen, although induction of protective immunity is subgroup-specific and it has been associated with the induction of enhanced pathology in a BALB/c mouse model (Alwan et al., 1994; Elango et al., 1986; Johnson et al., 1998; Sullender et al., 1990). However, a recombinant non-glycosylated subunit vaccine candidate, consisting in part of the central conserved domain of a subgroup A G protein, is capable of inducing protective immunity against both subgroups in rodents without evidence of enhanced pathology following RSV challenge (Plotnick-Gilquin et al., 1999; Power et al., 2003, 1997). Indeed, the latter vaccine candidate, designated BBG2Na, successfully underwent phase I and II clinical trials, although infrequent serious adverse events in phase II and III precluded...
further development (Bouveret Le Cam et al., 2000; Power et al., 2001).

Several recent studies illustrate the immune modulating potential of the RSV G protein. Langedijk et al. (1998) demonstrated homology between the cysteine noose domain of the RSV G protein and the fourth subdomain of the 55-kDa tumor necrosis factor receptor (TNFr). Although the biological significance of this homology is unknown, it suggests that RSV-G may somehow modulate the activity of TNF or another unknown ligand of the 55-kDa TNF. This cysteine noose domain also contains a CX3C-like chemokine motif that is able to bind the fractalkine receptor CX3CR1 and adversely affect CX3CR1+ T cell responses (Harcourt et al., 2006; Tripp et al., 2001). Furthermore, Polack et al. (2005) demonstrated, both in vitro in human monocytes and in vivo in C57Bl/6 mice, that this cysteine-rich domain of the RSV G protein was a powerful inhibitor of innate immune responses to RSV, including IL-6, IL-1β, IL-10 and TLR-2, 4- and 9-mediated responses. This inhibition was mediated by Gs and not Gm and appeared to be independent of the CX3C-like properties of the G protein. Arnold et al. (2004) similarly reported that a spontaneous RSV mutant lacking only Gs-induced higher IL-8, RANTES and ICAM-1 expression and NF-κB activation in the human type II pneumocyte-derived A549 cell line than the wild-type parental strain.

However, as similar Gs immune modulation seems to occur in both mouse and human cells, and a single RSV infection is sufficient for long-term protective immunity in mice, a role for the Gs in blocking long-term memory responses in humans is questionable.

6. RSV F protein and immunosuppression

RSV F is a homotrimer type I glycoprotein that is synthesized as an F0 precursor. Intracellular proteases cleave it at two furin-like motifs to release F1 and F2 subunits and a small 27-mer peptide of unknown function (Gonzalez-Reyes et al., 2001; Zimmer et al., 2001). F1 and F2 are joined by disulphide bridges to form the active molecule, whose primary role is considered to be virus/cell membrane fusion but F is also capable of directly mediating virus attachment to cells (Collins et al., 2001; Techaoopunkul et al., 2002). With regard to immune modulation, RSV F was shown to block proliferation of mitogen-stimulated peripheral blood lymphocytes (PBLs) in a contact-dependent manner (Schlender et al., 2002). This is consistent with data from Heidema et al. (2004) in which RSV infection blocked antigen-stimulated human CD8+ T cell proliferation. It is also similar to other members of the Paramyxoviridae, most notably the morbilliviruses, in which the respective F proteins were implicated in suppression of mitogen-stimulate leukocytes (Heaney et al., 2002; Schlender et al., 1996). However, in contrast to RSV F, morbillivirus-mediated immunosuppression also required co-expression of the corresponding haemagglutinin–neuraminidase protein.

Interestingly, Schlender et al. (2002) provided evidence to suggest that the RSV F-mediated suppression was species-specific. Human RSV F efficiently blocked proliferation of human PBLs but not bovine PBLs, while the opposite was the case for bovine RSV F. This species-specific immunosuppression contrasts with the morbilliviruses, in which a range of different human and animal viruses were equally efficient at blocking mitogen-stimulated proliferation of the human B lymphoblast cell line BJAB (Heaney et al., 2002). As recovery from MeV (a morbillivirus) results in life-long protection, it is evident that the acute immunosuppression following infection does not prevent the induction of long-term protective immune memory responses against this virus. However, the modalities of immunosuppression for both RSV and MeV appear to be sufficiently different, such that immune responses to MeV infection may not be predictive of RSV-induced long-term immune responses.

Thus, a number of important questions remain to be answered in relation to this phenomenon. For example, does RSV F-induced immunosuppression lead to poor effector and/or central memory T cell responses? Does it lead to poor helper T cell responses and, thereby, limited B cell maturation and antibody responses? Is the immunosuppression a consequence of inducing regulatory T cells that result in general suppression of the immune responses to infection? What are the mechanisms of RSV F-induced suppression? Are there other types of RSV-induced immunosuppression that are independent of the F protein? Recent evidence by Chi et al. (2006) indicates that this is indeed the case. They showed that IFN-α and λ expressed from RSV-infected human monocyte-derived dendritic cells were implicated in the efficient block of proliferation of human CD4+ T cells in a contact-independent manner.

7. RSV/human cell interaction studies—the importance of ex vivo models

As RSV-induced immunosuppression and pathogenicity is in large part species-specific, it seems imperative that outstanding questions are addressed either directly in humans or in appropriate human tissues ex vivo. The recent paper by Welliver et al. (2007), in which the immunopathogenesis of fatal RSV infection in infants was described, is very timely in this regard. The authors observed that fatal RSV disease is associated with insufficient adaptive immune responses, in contradiction of the current thinking that excessive adaptive responses are implicated.

The principle targets of RSV infection are airway epithelial cells. Therefore, we and others have developed the means to culture well-differentiated primary human airway epithelial cells at an air/liquid interface as a means to study virus/host interactions (Doherty et al., 2003; Wright et al., 2005; Zhang et al., 2002). Electrophysiological, confocal and electron microscopic evidence suggests that these pseudostratified cultures are physiologically and morphologically
authentic. This technology provides a highly relevant platform with which to address respiratory virus/host interactions at cellular and molecular levels. The importance of the choice of infection model is emphasised by our recent observations that the same strain of RSV grows and behaves differently in undifferentiated monolayer primary bronchial epithelial cells compared to their well-differentiated cell counterparts (Power and colleagues, unpublished observations).

Evidently, well-differentiated primary airway epithelial cell models of RSV infection preclude interactions with the adaptive immune system. As poor long-term immunity and inactivated vaccine-induced enhanced disease are hallmarks of RSV biology, developing models to study human adaptive immunity to RSV infection ex vivo is also important. Indeed, Matthews et al. (2007) recently described such a model, in which primary and secondary neonatal CD4+ T cell responses to a subunit RSV vaccine candidate were studied following presentation by monocyte-derived dendritic cells. Both cell types were initially derived from cord blood and the study demonstrated the functionality of neonatal CD4+ T cells and dendritic cells.

In my view, the continued development of human ex vivo models of RSV infection, in parallel with judicious clinical studies of RSV pathogenesis will provide opportunities to study RSV immunopathogenesis at a level that has not been possible heretofore.

8. Concluding remarks

The mechanisms by which RSV causes disease and is capable of repeated infection in humans remain enigmas. Animal models have played undisputed roles in our understanding of RSV pathogenesis and immunity in these models. With the exception of palivizumab, however, this understanding has not translated into vaccines and/or therapeutics against RSV. There remain large gaps in our understanding of how RSV interacts with humans to cause disease, circumvent innate immune responses and inhibit the development of long-term protective immunity. Research is urgently needed to bridge this gap. A better understanding of RSV/human interactions is essential to identify host pathogenicity factors, virus components triggering these factors and virus components of immune evasion and suppression. This information will be invaluable in facilitating the design of safe and effective vaccines against what remains a major human pathogen.

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