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Invited Mini Review

Current progress on development of respiratory syncytial virus vaccine

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Human respiratory syncytial virus (HRSV) is a major cause of upper and lower respiratory tract illness in infants and young children worldwide. Despite its importance as a respiratory pathogen, there is currently no licensed vaccine for prophylaxis of HRSV infection. There are several hurdles complicating the development of a RSV vaccine: 1) incomplete immunity to natural RSV infection leading to frequent re-infection, 2) immature immune system and maternal antibodies of newborn infants who are the primary subject population, and 3) imbalanced Th2-biased immune responses to certain vaccine candidates leading to exacerbated pulmonary disease. After the failure of an initial trial featuring formalin-inactivated virus as a RSV vaccine, more careful and deliberate efforts have been made towards the development of safe and effective RSV vaccines without vaccine-enhanced disease. A wide array of RSV vaccine strategies is being developed, including live-attenuated viruses, protein subunit-based, and vector-based candidates. Though licensed vaccines remain to be developed, our great efforts will lead us to reach the goal of attaining safe and effective RSV vaccines in the near future. [BMB reports 2011; 44(4): 232-237]

INTRODUCTION

Since its discovery in 1956, Respiratory Syncytial Virus (RSV) has been recognized as the most important viral pathogen leading to severe respiratory tract diseases in infants and young children worldwide. RSV can also infect and cause diseases in people of all ages, most severely in the elderly and in immuno-compromised individuals. Most children are infected at least once by age 2 and continue to be reinfected throughout life possibly due to incomplete immunity to RSV (1).

Despite the burden of diseases caused by RSV, there is still no licensed vaccine against RSV infection, and currently available prophylactic and therapeutic methods are very limited. Specifically, a humanized monoclonal antibody, palivizumab, is currently licensed only for use in high-risk infants, whereas ribavirin is used to treat RSV infection only in the pediatric population. Due to a tremendous disease burden and limited prophylactic method, the demand for a safe and effective RSV vaccine is now greater than ever.

HRSV is a member of the *Paramyxoviridae* family of non-segmented negative strand RNA viruses. RSV contains 10 genes encoding 11 viral proteins; 9 are structural proteins found in infected cells and in virions, whereas the others are non-structural proteins only present in infected cells. Three envelop glycoproteins, G, F, and SH, are present on the viral membrane and on infected cells. F and G are the only HRSV proteins that induce neutralizing antibodies (2, 3). HRSV can be divided into two antigenic subgroups, A and B, based on reactive patterns to monoclonal antibodies. The highest genetic and antigenic variability between subgroups is present in the G glycoprotein, the sequence of which has only 53% homology between subgroups and even exhibits limited diversity within the same subgroup: differences of ~20% in the HRSV-A subgroup and ~9% in HRSV-B (4).

Immunological balance between protection and pathogenesis

A host infected with RSV induces a broad range of immune responses to clear the infection, but these host responses are also thought to contribute to the clinical manifestations of RSV disease. It is generally thought that the humoral response is sufficient to prevent or restrict the primary infection, as evidenced by palivizumab, a humanized monoclonal antibody currently used for immunoprophylaxis against RSV (5). However, once RSV infection is established, T-cell responses appear to be required to completely eliminate the virus (6). T cells also play important roles in pulmonary inflammation, in which Th2-type cytokines secreted by CD4 T cells provoke severe lung pathology marked by eosinophilia (7).

In the initial trial of RSV vaccine with formalin-inactivated RSV (FI-RSV) during the 1960s, the vaccine proved to be poorly protective and actually enhanced the severity of RSV disease (8). Although the mechanism of this disease enhancement is still not clear, it is likely that FI-RSV induces altered serum an-

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tibody response with little neutralizing activity compared to that induced by natural infection (9). Subsequent studies using experimental models demonstrated that FI-RSV induces imbalanced Th2-type CD4 T-cell responses (10-12). Thus, vaccine-enhanced disease appears to be associated with massive pulmonary inflammation initiated by vaccine-primed Th2-type CD4 T cells (Fig. 1).

G glycoprotein, the major RSV attachment protein (13), is thought to be an important antigen for protection against RSV infection (14). The central region of the G protein is relatively conserved and contains a cystine noose resembling the CX3C chemokine motif as well as several protective B-cell epitopes (15-17). G protein lacks any MHC class I-restricted epitope (18-20) and has not yet been demonstrated to elicit a CTL response in either human or mice (21, 22). Numerous studies have suggested that priming with G protein is associated with induction of the polarized Th2-type response similar to that induced by FI-RSV, which leads to pulmonary eosinophilia upon RSV challenge of G-immunized mice (15, 23-26). On the contrary, it was recently suggested that G-specific immune responses are not solely the basis for vaccine-enhanced illnesses and should not be excluded from potential vaccine strategies (27, 28). Thus, it is likely that the absence of a Th1-promoting effect by RSV-specific CTLs, rather than G protein itself, is responsible for the Th2-biased response. In this regard, it should be emphasized that the fine balance between immune protection and pathology is one of the most important factors to be considered in the development of RSV vaccines.

Current RSV vaccine strategies

The most serious RSV disease occurs between 2-7 months of age, and thus immunization should be started within the first few weeks after birth. However, there are several major hurdles in the development of a pediatric RSV vaccine: first, the immune systems of young infants are relatively immature compared to older children and adults. Secondly, the presence of maternal antibodies might suppress the immune responses elicited by the administered vaccines, especially in the first few weeks of life (29). In addition to these obstacles, the failure of the first RSV vaccine trial with FI-RSV in the 1960s significantly increased the safety concerns surrounding RSV vaccine, which could be associated with such vaccine-enhanced illness. For this reason, evaluation of RSV vaccines should be progressive and careful not to repeat the failure of the FI-RSV trial. The other unusual aspect of RSV infection is that frequent reinfection by the identical or a closely related virus can occur at any age (30, 31). It is likely that natural RSV infection only confers imperfect immunity against subsequent infections. Antigenic variation appears to play only a minor role in this type of immune evasion. The possible mechanisms of immune evasion by RSV are still not clear, but studies suggest that RSV could modulate host responses by secreting soluble G glycoprotein, which might act as an antibody decoy or help shift the CD4 T-cell response from a protective Th1 response to pathogenic Th2 response (32). RSV has been also reported to dysregulate T-cell function by suppressing the CTL response and memory in a murine model (33) and by inhibiting T-cell activation through F protein (34).

Various strategies have been employed to develop a safe and effective RSV vaccine (Table 1). One of the major approaches to developing a RSV vaccine is using live-attenuated RSV strains for intranasal injection. Several live-attenuated RSV vaccine candidates have been developed by conventional cold passage and/or chemical mutagenesis (*cpts* RSV strains) and evaluated in a clinical setting (35, 36). Second-generation live-

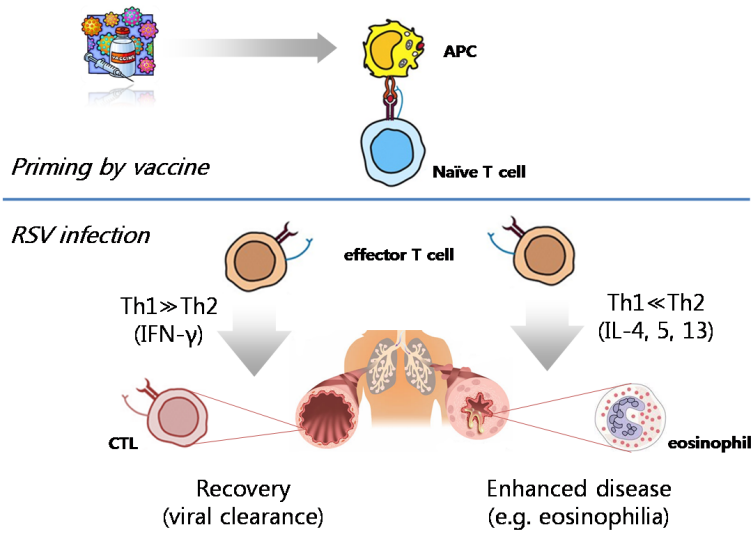

Fig. 1. Possible mechanisms of protective immunity or vaccine-enhanced disease to RSV infection. Natural RSV infection mostly leads to priming Th1-dominated response, but vaccination with FI-RSV or other vaccines results in priming Th2-biased response. Following subsequent RSV infection, primed Th1-type cells induced protective responses such as IFN-γ, CTLs, and IgG2a, which helped clear viral infection, whereas Th2-biased cells produced IL-4, 5, and 13 and triggered immunopathogenesis like IgE production, massive pulmonary inflammation, and eosinophilia.

Table 1. Strategies and status of RSV vaccine candidates

Strategy	Platform	Status
Inactivated virus	formalin-inactivated RSV	Discontinued
Live-attenuated virus	Cold-passage/temperature sensitive	Phase I/IIa
	Genetically engineered by reverse genetics	Phase I/IIa
Protein subunit	Purified F protein (PFP-1, 2, 3)	Phase III-discontinued
	BBG2Na	Phase III-discontinued
	F/G/M mixed formulation	Phase II
	Virus-like particle (F protein)	Phase I
	F/G chimeric protein	Phase I/IIa
	F/G peptides	Pre-clinical
Vectored	Vaccinia virus (F or G protein)	Pre-clinical
	Adenovirus (F or G protein)	Pre-clinical
	PIV3 (F or G protein)	Phase I/IIa
	Alphavirus/Newcastle disease virus (F or G protein)	Pre-clinical
	Staphylococci	Pre-clinical
	DNA	Pre-clinical

attenuated RSV has been also generated by reverse genetics (rA2*cp* strains) and tested in RSV-naïve 1-2 month-old infants, showing that protective immunity could be achieved in a majority of the recipients (37, 38). However, for this live-attenuated RSV approach, it is quite challenging to determine whether or not the balance between over-attenuation and under-attenuation is most appropriate for a safe and effective vaccine.

Due to the potential for disease exacerbation, protein subunit vaccines are considered to be inappropriate for the pediatric population. However, several RSV-derived proteins and their derivatives have been evaluated in preclinical and clinical stages as subunit vaccines. Although purified F and G proteins from RSV-infected cultures and F/G chimeric proteins produced by a baculovirus expression system generate antibody responses similar to those observed upon FI-RSV vaccination in rodent models (39, 40), the appropriate use of adjuvants could improve immunogenicity and diminish the adverse effects of subunit vaccines (41, 42). The purified F protein series (PFP-1, 2 and 3) has been tested in several clinical trials, showing promising results with no obvious adverse events and more than a 4-fold increase in neutralizing antibody titer in the majority of subjects (43-45). However, the preventive efficacy of PFP vaccination was not significantly different between the vaccine and control groups. Another protein vaccine, BBG2Na, containing amino acids 130-230 of RSV A2 G protein fused to the albumin-binding domain of streptococcal protein G, has also been evaluated in preclinical and clinical tests (46, 47). This vaccine was well tolerated in phase II studies, but a further trial had to be stopped due to a limited number of unexpected adverse events. Other forms of subunit vaccines, including short peptides and virus-like particles, are being tested but limited data are available now.

As another platform, live viral vectors, including vaccinia virus (3), adenovirus (48-50), sendai virus (51), and parainfluenza virus (52), have been engineered and applied as vaccine candidates. Most of them exhibit significant immunogenicity or protective immunity in animal models, showing the possibility of further development. Other forms of vaccines using replicon-based non-replicating viral vectors or particles (53, 54), live bacteria (55), and avian virus (56) have been also reported to be immunogenic and protective to varying degrees in mouse challenge studies. Vaccination of DNA plasmids expressing F or G protein has shown a limited degree of protection against RSV challenge (57, 58), but it needs multiple boosting immunizations to overcome a low immunogenicity problem.

CONCLUSION

For many years, development of a RSV vaccine has been a high priority. Numerous vaccine strategies have been tested or are currently tested for further development. However, there are a few factors that must be resolved. First, in order to develop safer and more effective vaccine candidates and satisfy safety concerns for invididual target populations, we must more clearly understand the enigmatic immune evasion and immunopathology of RSV. There also needs to be increased awareness about the social and economical burden of RSV diseases, which are typically taken not seriously. We speculate that our effort in searching for an RSV vaccine will bear a safe and effective vaccine in the near future.

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