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MINIREVIEW

Respiratory Syncytial Virus: Targeting the G Protein Provides a New Approach for an Old Problem

Ralph A. Tripp,[a] Ultan F. Power,[b] Peter J. M. Openshaw,[c] Lawrence M. Kauvar[d]

[a]University of Georgia, College of Veterinary Medicine, Department of Infectious Diseases, Athens, Georgia, USA

[b]Centre for Experimental Medicine, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast, Northern Ireland, United Kingdom

[c]Imperial College London, London, United Kingdom

[d]Trellis Bioscience, Menlo Park, California, USA

ABSTRACT Respiratory syncytial virus (RSV) is a major cause of lower respiratory tract infection (LRTI) annually affecting >2 million children in the United States <5 years old. In the elderly (>65 years old), RSV results in ~175,000 hospitalizations annually in the United States with a worldwide incidence of ~34 million. There is no approved RSV vaccine, and treatments are limited. Recently, a phase 3 trial in the elderly using a recombinant RSV F protein vaccine failed to meet its efficacy objectives, namely, prevention of moderate-to-severe RSV-associated LRTI and reduced incidence of acute respiratory disease. Moreover, a recent phase 3 trial evaluating suptavumab (REGN2222), an antibody to RSV F protein, did not meet its primary endpoint of preventing medically attended RSV infections in preterm infants. Despite these setbacks, numerous efforts targeting the RSV F protein with vaccines, antibodies, and small molecules continue based on the commercial success of a monoclonal antibody (MAb) against the RSV F protein (palivizumab). As the understanding of RSV biology has improved, the other major coat protein, the RSV G protein, has reemerged as an alternative target reflecting progress in understanding its roles in infecting bronchial epithelial cells and in altering the host immune response. In mouse models, a high-affinity, strain-independent human MAb to the RSV G protein has shown potent direct antiviral activity combined with the alleviation of virus-induced immune system effects that contribute to disease pathology. This MAb, being prepared for clinical trials, provides a qualitatively new approach to managing RSV for populations not eligible for prophylaxis with palivizumab.

KEYWORDS F protein, G protein, RSV, respiratory syncytial virus, monoclonal antibodies, palivizumab

RSV BIOLOGY

The medical need. Respiratory syncytial virus (RSV) is a negative-strand RNA virus in the family *Pneumoviridae* with 10 genes encoding 11 proteins (Fig. 1) that has resisted effective management for >60 years in part because infection does not provide robust immunity. As has been extensively reviewed (1–6), >50% of infants are infected by RSV during their first year, with nearly 5% requiring hospitalization. The only care available for RSV infection is supportive. Preterm infants (gestational age of <29 weeks) have been the focus for prophylaxis with palivizumab, which reduces morbidity but not mortality (1). The RSV F protein is more conserved overall compared to the G protein, and it has been the target for palivizumab and most other pharmacological efforts. However, the G protein has a central conserved domain (CCD) that is nearly invariant across all circulating strains, whose importance has become clearer over the past several years, particularly with regard to the unmet need for a postinfection therapeutic (4).

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Address correspondence to Lawrence M. Kauvar, lkauvar@trellisbio.com.

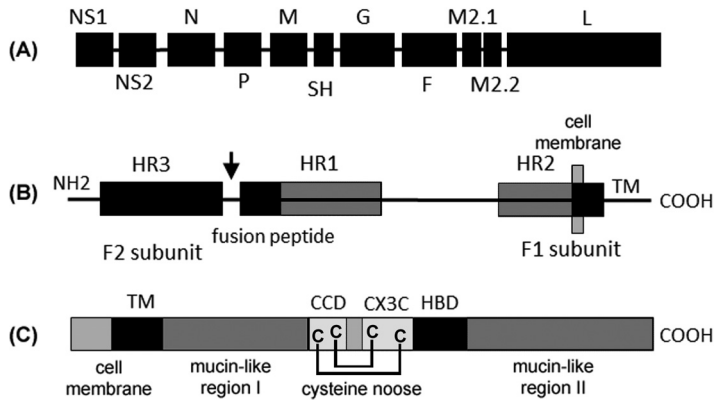

FIG 1 RSV genome. (A) Ten genes produce 11 proteins. The M2-2 open reading frame (ORF) is accessed by ribosomes that reinitiate after exiting the M2-1 ORF. The G protein is produced as both membrane-bound and secreted forms via alternative translation start sites. Two antigenic subgroups (A and B) are defined by the hypervariable mucin-like regions of the G protein. (B) RSV F protein (575 amino acids [aa]) is cleaved by furin (at the arrow) to produce the F1 and F2 domains with a conformational change that promotes fusion with cell membranes; the location of heptad repeats (HR), fusion peptide, and transmembrane domain (TM) are shown. (C) The RSV G protein (298 aa) central conserved domain (CCD) includes a conformationally constrained CX3C motif (182-CWAIC-186) that is implicated in infection of lung epithelial cells through binding to CX3CR1, assisted by a heparin binding domain (HBD). The MAb TRL3D3 binds to an epitope within the CCD.

Vaccine hindrances. For RSV, there are four fundamental vaccination strategies: (i) vaccinate children, (ii) vaccinate adults (19 to 55 years), (iii) vaccinate the elderly, and (iv) vaccinate pregnant woman (2–5). The diminished immune system in the very young and the elderly poses special challenges. Regarding the other vaccine groups, the highly transmissible nature of RSV and the poor immunological memory to natural infection make it difficult to achieve herd immunity. Other obstacles include induction of nonneutralizing antibodies or insufficient titer of neutralizing antibodies, exaggerated Th2-like responses resulting in massive infiltration of inflammatory cells, ineffective priming of CD8 responses, and complement deposition. Moreover, susceptibility varies with factors difficult to manage, such as the level of cocirculating respiratory viruses, including influenza virus and rhinovirus (7). The failure of the Novavax phase 3 vaccine trial (RSV F vaccine) in the elderly (5), despite enrolling nearly 12,000 subjects, is a stark reminder of the obstacles to RSV vaccine development. Novavax is currently in phase 3 testing of the same vaccine in pregnant women. However, persistence of maternal antibodies in the neonate may be too short to achieve reliable protection unless a very high titer of neutralizing antibodies is achieved.

Vaccines that require a cold chain to maintain efficacy have additional obstacles, particularly for global use. Formalin inactivation of whole virus, a technique used to increase stability, caused disease exacerbation upon subsequent natural infection, resulting in two deaths (8), which set back significantly the effort to develop an RSV vaccine for infants. Currently, live-attenuated RSV vaccines remain compelling vaccine candidates for use in infants, while protecting the elderly remains more elusive. Specifically, a structurally stabilized postfusion F protein vaccine has recently failed in phase 2 trials to prevent RSV-associated respiratory illness in the elderly (9). A better understanding of the complex interaction between virus and host, including age-specific factors, is needed for safe and effective RSV vaccine development to proceed (10).

PHARMACOLOGY

Targeting the F protein with a MAb. Given the obstacles to developing a safe and effective RSV vaccine, providing an optimized MAb with strong virus-neutralizing activity is appealing. The F protein promotes fusion of RSV with the host cell membrane and is essential for infectivity both *in vitro* and *in vivo*. Palivizumab, a humanized murine

monoclonal antibody (MAb) that inhibits the fusion process, has become widely used for prophylaxis of premature birth infants at high risk of severe RSV disease (1). Although the MAb reduces the incidence of severe disease from ~10% to less than 5%, widespread use of this costly agent is judged uneconomic (11). Moreover, a phase 2 trial to expand its use to full-term infants as a therapeutic was unsuccessful (12). Although the number of patients enrolled in the study was too small to establish statistical significance ($n = 35$), all three clinical efficacy endpoints measured were inferior to placebo. Motavizumab, a higher-affinity derivative of palivizumab, reduced hospital admissions in Native American full-term infants (13), but the MAb failed to reach FDA approval because of safety concerns (hives and allergic reactions) that were not offset by any clear superiority to palivizumab (4). Further, escape from palivizumab is easily achieved (14) and may be clinically relevant (15). Escape correlates with a reduced on-rate of the MAb (16), which is important since avoiding escape via higher on-rate risks generating increased off-target reactivity as seen in the protein engineering effort leading to motavizumab (17).

Despite the limitations of palivizumab, its commercial success has led to a variety of products that mimic its pharmacological properties (3, 4, 6). REGN2222 is a biosimilar MAb targeting the F protein. In a recently reported double-blind, placebo-controlled phase 3 study ($n = 1,149$), healthy preterm infants (gestational age of <36 weeks and <6 months old at the beginning of the study) were treated with one or two doses at 30 mg/kg of body weight; assessment at day 150 failed to show efficacy for the primary endpoint of medically attended RSV infections (http://investor.regeneron.com/releaseDetail.cfm?releaseid=1037184). Other biosimilar efforts are on hold or still preclinical. MEDI8897 is an anti-F protein MAb engineered for longer serum half-life (18). ALX-0171, a 15-kDa "nanobody" with comparable epitope recognition to palivizumab, is being evaluated as an inhaled formulation for postinfection treatment (19).

Other F protein interventions in development. Vaccine efforts focused on the F protein are continuing, as recently reviewed (2–5), including MEDI-559, a cold-passaged, live-attenuated RSV, and MEDI-534, a human-bovine chimeric parainfluenza virus construct expressing the RSV F protein. GSK3389245A is a vaccine based on an adenovirus vector to stimulate a T cell response. DS-Cav1 and GSK3003891A are stabilized prefusion forms of the F protein (Fig. 1). MEDI-7510 is a recombinant F protein vaccine in the postfusion conformation; it failed to show efficacy in a phase 2 trial in the elderly ($n = 1,894$) despite being immunogenic (9). Preclinical efforts exploring the utility of stabilized postfusion F protein are continuing (4).

Although the first small molecule fusion inhibitor compound, BTA9881, was terminated when the phase 1 clinical results did not meet the desired safety margin (6), several compounds with similar activity are still being pursued, including TMC-353121 (an improved-pharmacokinetics version of JNJ-2408068), AK-0529, RFI-641, and BMS-433771 (4). GS-5806 has shown efficacy in a phase 2a challenge model (attenuated virus in healthy adults); however, it also showed evidence of escape mutations (4).

Targeting intracellular viral proteins. The efficacy of palivizumab is linked to neutralization of RSV replication by blocking cell entry. Other routes to blocking replication have been explored that involve targeting viral proteins expressed intracellularly (3). ALN-RSV01 is an RNA interference (RNAi) construct to the N (nucleoprotein) gene that showed initial signs of efficacy in a live-virus challenge model (Memphis 37 strain) in healthy adults. It was dropped after missing the primary endpoint, namely, a reduction in progressive bronchiolitis obliterans syndrome at 180 days in lung transplant patients with confirmed RSV infection (20). RSV604 is a small molecule targeting the N protein, which reached phase 2 in bone marrow transplant patients but was discontinued due to variability in oral absorption (4). Another approach to blocking replication is inhibition of the viral polymerase (L protein), exemplified by ALS-8174 (4).

Targeting the G protein with a MAb. The G protein is one of two major RSV envelope proteins. The F protein is generally conserved, and its deletion abolishes RSV infectivity; loss of the more variable G protein varies in effect from some inhibition of replication (21) to full prevention of replication (22, 23). The G protein has attracted increasing attention to address the need for RSV prophylaxis in healthy infants through an entire RSV season and for postinfection treatment. Building on a promising vaccine over a decade ago that showed efficacy in mice and immunogenicity in humans (24), three features have emerged that define the G protein as an attractive target. First, there is a small central conserved domain (CCD) that is highly conserved (4). Although variation in the hypervariable domains flanking the CCD increases in response to immune system pressure (25), the CCD itself remains highly conserved. Second, the CCD is essential for infectivity *in vivo* and mediates attachment to airway epithelial cells (26–28). Third, the CCD has a CX3C chemokine motif implicated in alteration of the host immune response (29).

The G protein's role in RSV pathology. Under some circumstances, RSV is known to bias the immune response toward a Th2 phenotype (30). A role for the innate immune response affecting RSV disease severity has also been suggested by observations of increased disease severity in patients with certain polymorphisms of either Toll-like receptor 4 (TLR4) (31) or the CX3C chemokine receptor (32). The G protein modulates neonatal regulatory B lymphocytes (nBreg cells) to produce immunosuppressive interleukin-10 (IL-10), and the frequency of RSV-infected nBreg cells in the neonate respiratory tract is predictive of acute bronchiolitis severity (33). It is worth noting that the cotton rat provides a useful model to study MAbs against the F protein because it is more permissive for viral replication than mice; however, the cotton rat is an imperfect model for understanding the pathological host response in humans (34).

An important unmet need is for a post-RSV treatment since conventional anti-inflammatory agents have failed to provide clinical benefit (35). Nonclinical studies using anti-G protein MAbs targeting the CCD motif have shown efficacy as a postinfection treatment (33, 34, 36, 37). In a mouse model using RSV strain A2, a murine anti-G protein MAb (131-2G) administered at day 3 postinfection reduced the influx of inflammatory cells into the airways, with a pronounced effect at day 5 that was sustained to day 14 (36). A murine anti-F protein MAb (143-6C) had no such effect. In a similar model, using RSV line 19F (known to cause increased airway hyperreactivity and mucus hyperproduction in mice), an anti-G protein MAb improved breath distension of peripheral arteries (pulse oximetry) (37). These studies emphasize the anti-inflammatory activity of MAbs targeting the RSV G protein CCD. Additional studies have boosted this therapeutic rationale by comparison of an anti-G protein IgG to an $F(ab')_2$ construct. Both were able to suppress airway inflammation, but only the intact IgG was able to reduce viral load, consistent with the complement-dependent activity of a different MAb against a similar epitope (38). This experiment established that the anti-inflammatory effect is not just a result of reduced viral load (39).

In normal human bronchial epithelial (NHBE) cells infected by RSV, TLR4 signaling was reduced, an effect linked to increased SOCS3, which suppresses antiviral interferons (IFNs) (40). Treatment with an anti-G protein MAb (131-2G) counteracted the immune-modifying nature of the RSV G protein leading to enhanced IFN, whereas an anti-F protein MAb depressed the IFN response below the mock infection control level. A similar effect was observed in plasmacytoid dendritic cells, where mutation of the G protein CCD prevented IFN suppression with an anti-G protein $F(ab')_2$ antibody, emulating the phenotype of the G protein mutation (41). A finding showing a preponderance of IFN-λ1 (IL-29) in lower airway samples from RSV-infected infants suggests that IFN-λ1 is the principal IFN responding to RSV infection in infants with severe disease (42).

G protein provides a favorable target. Interventions that reduce viral replication are important and have represented the vast majority of RSV control efforts (43).

However, suppression of replication does not specifically address a key feature of RSV, namely, alteration of the host's immune system resulting in airway inflammation (4). An early attempt to target the CCD of the G protein with a recombinant protein vaccine (BBG2Na) showed a moderate ability to induce neutralizing antibodies in healthy, young adults (24). Low immunogenicity of the CCD is a prominent feature of the virus (25). A more direct route to targeting the G protein CCD is use of a MAb. Building on foundational work using murine hybridomas (36), the most advanced preclinical candidate is TRL3D3, a native human antibody that binds the CCD motif with low picomolar affinity (38). As epitope conservation implies essential functionality, escape mutants are less likely than for therapeutics targeting other viral proteins or domains of the G protein.

An important feature of the G protein is an alternative translation initiation site that leads to secretion of ~15% of the protein beginning 6 h following RSV infection and well before the appearance of progeny virus (44). A mutation that prevents production of the soluble G protein improved the efficacy of a polyclonal serum against the F protein (45), and this mutation has been incorporated into a live-attenuated vaccine candidate (46). Neutralization of soluble proteins typically requires higher affinity than for membrane-bound proteins to avoid prolonging the serum half-life that results in increased exposure of tissues to the factor (47). Achieving high affinity uniformly in a diverse population is difficult for a vaccine, and the RSV G protein CCD is particularly difficult to target since it is poorly immunogenic. One approach to achieving a vaccine against both A and B strains has been to create a fusion peptide comprising CCD peptides from both strains, with promising efficacy in mice, although the affinity of the induced MAbs has not yet been studied (23). In another recent study (48), recombinant G protein ectodomain induced protective responses in cotton rats, although the immunodominant epitopes were in the highly variable N- and C-terminal regions (Fig. 1).

The TRL3D3 MAb, whose affinity is high enough to neutralize the soluble G protein, has also shown direct antiviral activity with improved potency over palivizumab in mice (38). Consistent with the *ex vivo* effects of MAbs against RSV F or G proteins on IFN production, treatment with palivizumab was associated with increased airway inflammation in a mouse model, whereas TRL3D3 suppressed it (49). The lack of robust, long-lasting immunity following RSV infection results in frequent reinfection. Early infection, when the immune system is immature predisposes to asthma-like symptoms in childhood (1, 49, 50). To model the effect of infection when the immune system is immature in a mouse entails initial exposure to RSV as a neonate followed by secondary exposure at 6 weeks. Prophylaxis with TRL3D3 at the primary infection provided markedly improved lung function upon secondary infection, whereas palivizumab provided no such improvement (49). Supplemental IFN at the neonate sensitization step substantially reduced perivascular inflammation and mucus hyperproduction upon reinfection (51). Consistent with these results, palivizumab has no effect on reinfection rate or disease severity (3).

SUMMARY

The RSV F protein has historically been favored as a target for vaccine and preventive intervention. As summarized in Table 1, four F protein vaccines and three MAbs or MAb analogs are currently in clinical trials with a corresponding predominance in the catalog of preclinical agents in development (43). However, from an efficacy perspective, the G protein central conserved domain is an increasingly compelling target. Antibodies to this site combine (i) complement-mediated antiviral activity, (ii) blockade of airway epithelial cell infection, and (iii) anti-inflammatory activity. The nearly invariant sequence reduces escape potential, a significant advantage compared to targeting the F protein (14). From a safety perspective, a reduced ability of the G protein to modify the host immune response may provide unique efficacy as a postinfection treatment. The development path for a treatment is more practical than for a prophy-

TABLE 1 RSV interventions in development[a]

Name	Classification	Target	Stage
RSV F Vaccine (Novavax)	Vaccine (nanoparticle)	F protein	Phase 3
MEDI-534	Vaccine (PIV3 vector)	F protein	Phase 2
GSK3389245A	Vaccine (adenovirus vector)	F protein	Phase 2
MEDI-7510	Vaccine	F protein (postfusion)	Phase 2
DPX-RSV	Vaccine	SH protein (epitope)	Phase 1
CX3C-LbL-NP	Vaccine (nanoparticle)	G protein (epitope)	Preclinical
REGN-2222	MAb	F protein	Phase 3
MEDI-8897	MAb	F protein	Phase 2
ALX-0171	Nanobody	F protein	Phase 2
TRL3D3	MAb	G protein	Pre-IND[b]
GS-5806	Small molecule	F protein	Phase 2
JNJ-53,718,678	Small molecule	F protein	Phase 2
BTA-C585	Small molecule	F protein	Phase 2
AK-0529	Small molecule	F protein	Phase 2
AK-0529	Small molecule	F protein	Phase 2
VP-14637	Small molecule	F protein	Phase 1
RFI-641	Small molecule	F protein	Preclinical
TMC-353121	Small molecule	F protein	Preclinical
BMS-433771	Small molecule	F protein	Preclinical
RSV604	Small molecule	N protein	Phase 2
ALN-RSV01	RNAi	N protein	Phase 2
ALS-8176	Small molecule	L protein	Phase 2

[a]See references 2, 3, and 5 for details.
[b]Pre-IND, Master Cell Bank completed, formal toxicology under way.

lactic agent, with RSV detection kits (52) expected to facilitate adoption of novel therapeutic agents.

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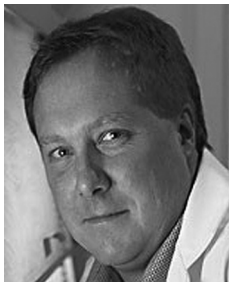
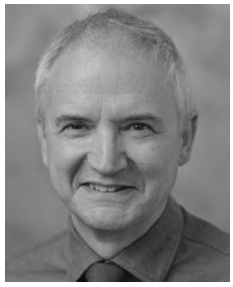
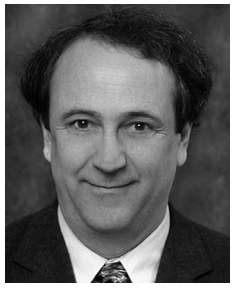


Ralph A. Tripp is Professor and Georgia Research Alliance Chair of Vaccine and Therapeutic Development in the Department of Infectious Diseases at University of Georgia (UGA). He joined UGA in 2004 following 6 years at the U.S. Centers for Disease Control and Prevention. His translational research focus for 30 years has been on interventions to protect humans from established and emerging respiratory viruses. His lab investigates the mechanisms of immunity, inflammation, and disease pathogenesis to understand the functional differences between innate and adaptive immune responses that facilitate vaccine and antiviral therapeutic protocols. Dr. Tripp has published >200 peer-reviewed articles, is an inventor on 12 issued U.S. patents, has written numerous book chapters on virology and/or immunology, and has edited several such books.

Ultan F. Power is a Reader in Molecular Virology at Queen's University Belfast (QUB), Northern Ireland, where he has been a researcher for 13 years. He received his Ph.D. from the National University of Ireland and was a postdoctoral fellow at St Jude Children's Research Hospital. He was previously Head of Virology and Senior Scientist at Institut de Recherche Pierre Fabre, where his RSV vaccine work was awarded the Prix Galien. At QUB, he has developed models of RSV infection based on well-differentiated primary pediatric airway epithelial cell cultures, the primary targets of RSV infection. Using these models has provided novel insights into RSV biology, including tentative identification of gene biomarkers of severe RSV disease in infants. Improved understanding of RSV pathogenesis in humans provides the basis for identifying novel therapeutic targets and for authentic preclinical models to test novel anti-RSV therapeutics. He has published >60 peer-reviewed articles.



Peter J. M. Openshaw is Professor of Experimental Medicine at Imperial College London and an Honorary Physician in the Department of Respiratory Medicine of the Imperial College NHS Trust. He was appointed President of the British Society for Immunology in 2013, the first clinician to lead the organization. He trained at Guy's Hospital, the Brompton and the Royal Postgraduate Medical School before Ph.D. training. His research focus is on the immunology of the lung, viral lung disease, vaccination, and immunopathogenesis of viral disease. He received the Chanock Award for lifetime contribution to RSV research in 2012 and is the inaugural President of the International RSV Society (2017). He is interim Chair of NERVTAG (a DH advisory committee on outbreaks of pandemic potential) and Theme Lead for Infection on Imperial's Biomedical Research Centre. He now leads a network supporting human volunteer challenge to accelerate vaccine development (HIC-Vac, 2017–2021).



Lawrence M. Kauvar is the founder and VP Chief Scientist of Trellis Bioscience, a company focused on discovery and development of native human antibodies against infectious disease and cancer targets. He previously played the same role at Telik, a company focused on small molecules for cancer. He received his B.A. from Harvard and his Ph.D. from Yale and pursued postdoctoral studies at Caltech and UC San Francisco. The primary focus of his industrial research for the past 25 years has been on development of innovative drug discovery assay technologies that combine high-throughput empirical methods with computer intensive data reduction. Dr. Kauvar has published >40 peer-reviewed articles and is an inventor on >60 issued U.S. patents. Trellis monoclonal antibody candidates progressing towards an IND include ones to treat RSV, CMV, influenza, and bacterial drug resistance associated with biofilm formation.