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Immunological goals for respiratory syncytial virus vaccine development

Barney S Graham

Defining the immunological goals for respiratory syncytial virus (RSV) vaccination requires understanding of RSV biology and tropism, mechanisms of cell-to-cell spread and immunity, epidemiology, and transmission dynamics. The immunological goals for a particular vaccine would be product-specific based on antigen selection, delivery approach, and target population. There are many ways to achieve immunity against RSV infection involving innate and adaptive responses, humoral, and cellular effector mechanisms, and mucosal and systemic responses. Both protective and pathological immune response patterns have been demonstrated in animal models and humans. In this short commentary, the entire information matrix that may inform the design of particular vaccine candidates cannot be fully reviewed, but the rationale behind the major vaccine approaches in key target populations will be discussed.

Address
Vaccine Research Center, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892, USA

Corresponding author: Graham, Barney S (bgraham@nih.gov)

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Introduction

RSV is an orthopneumovirus in the Pneumoviridae family. It is a single-stranded, negative-sense RNA virus that encodes 11 known proteins from ten open reading frames [1]. Although RSV can infect most adherent cell lines *in vitro*, human RSV is highly adapted to infect select cells in the human airway *in vivo*. The primary targets of infection are the polarized, differentiated, ciliated epithelial cells and the type 1 alveolar pneumocyte. Virus buds from cells as filamentous projections from cholesterol-rich lipid microdomains at the apical surface, and its life cycle resides in the superficial epithelium and the airway, and it rarely becomes systemic.

There is no known zoonotic reservoir of RSV and while animal models for human RSV have been developed in mice, cotton rats, and African green monkeys, they are all semi-permissive. Even chimpanzees from which the virus was first identified as chimpanzee coryza agent [2] are not known to acquire or transmit RSV in the wild. In part, this is related to the extreme sensitivity RSV has to innate immunity mediated by type 1 interferons (IFN). RSV has devoted the first two 5′ genes to making the non-structural proteins NS1 and NS2. These two proteins have multiple mechanisms to inhibit both the induction and effector mechanisms of IFN and are highly species-specific [3]. This may explain why it has been so difficult to establish animal models of RSV that can be infected nasally with a low dose and reliable spread from the upper to lower airway. It may also explain why only a small fraction of individuals develop severe disease. Roughly 20% of first-time infections in infants result in symptomatic lower respiratory tract infections, about 10% of those (2% overall) require hospitalization. In the frail elderly, about 5–10% are infected each year, about 1–2% have significant medically attended disease and only about 0.1% require hospitalization. Since infection is so ubiquitous, the public health burden imposed by RSV is large and justifies the development of vaccines for prevention.

Vaccine antigens

Apart from using live-attenuated virus vaccines that express all proteins, the major antigenic targets for RSV vaccine development are the surface proteins (Fusion glycoprotein (F), the G glycoprotein, and the Small Hydrophobic protein (SH)) and internal proteins (nucleoprotein (N), matrix protein (M), and nonstructural proteins like M2). The primary goal for vaccines based on surface proteins is to elicit protective antibody responses. However, there are different mechanisms of antibody-mediated protection for each of these targets. Neutralizing activity is the primary endpoint for F-based vaccines, and F antigenicity is conformation-dependent. The prefusion conformation of F (pre-F) has highly neutralization-sensitive antigenic sites that do not exist on the rearranged postfusion conformation of the protein (post-F) [4]. Antibodies to G may induce some neutralizing activity, but one form of G is secreted and known to have immunomodulatory effects [5–7], so antibodies that block this activity could also reduce virulence and improve immune responses during subsequent infections in addition to neutralizing virus [8]. SH is a pentameric ion channel present on the viral membrane. Antibodies to SH do not neutralize RSV, but can mediate Antibody Dependent Cellular Cytotoxicity (ADCC) and perhaps other Fc effector functions [9]. Antibodies to internal proteins are non-neutralizing, but internal proteins are

included in vaccines to induce T-cell responses and can be used alone [10] or in combination with surface proteins [11].

Immunological basis of vaccine-enhanced illness (what to avoid)

The primary goal for RSV vaccine development is protecting antigen-naïve infants less than six months of age, and effective immunization of the frail elderly is an important secondary goal. Immunization of other populations like toddlers, school-age children, or healthy adults would be done largely to prevent transmission and indirectly protect other susceptible populations. It may also be beneficial to immunize individuals preparing for allogenic bone marrow transplantation or lung transplantation because of their susceptibility to often lethal nosocomial RSV infections [12].

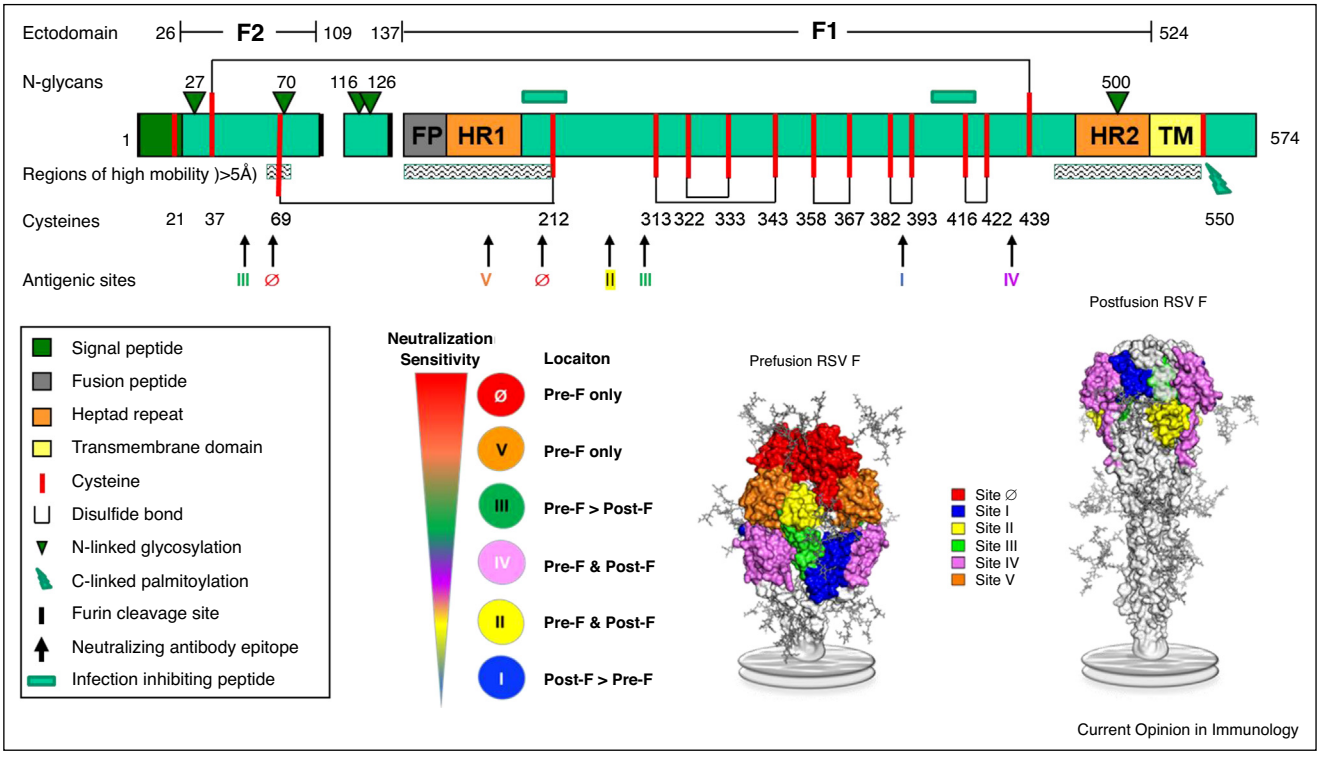
The immunization goals for antigen-naïve infants are complicated by the history of vaccine-enhanced respiratory disease (ERD) that occurred following immunization with a formalin-inactivated virus (FI-RSV) formulated with alum in children in the 1960s. This is the 50-year anniversary of the publications in 1969 describing four age cohorts of children given three doses of the vaccine and the outcomes following infection in the 1966–1967 winter season [13–15,16•]. Within the youngest age cohort, vaccinated children had higher rates of hospitalization (80%) and death (10%) than unvaccinated children [16•]. Subsequent studies of immune response patterns in trial subjects and animal models over the last five decades have identified two major immunological features of the FI-RSV ERD. One is that antibody with poor functional activity was induced. Both the neutralizing and fusion-inhibition activities were weak while binding antibody measured by ELISA and complement fixation titers were substantial [16•,17,18]. This led to immune complex deposition and complement activation in the small airways [19]. Additionally, a Th2-biased CD4 T cell response occurred after infection causing eosinophils in the peribronchiolar infiltrates and neutrophilic alveolitis in the lung histopathology of children who died. A Th2-biased response has been reproduced in mice [20] and alveolitis is a feature of FI-RSV immunization in cotton rats. This pattern of T-cell response has also been demonstrated in bovine models [21] and in nonhuman primates [22]. These types of responses must be avoided when attempting to protect young infants by either active or passive immunization. Observational data have indicated that live-attenuated viral vaccines given nasally or parenterally were not associated with ERD and after priming by natural infection, FI-RSV-induced ERD did not occur [23•]. It has also been demonstrated experimentally that RSV treated in the way the original FI-RSV was prepared, by exposure to 36°C for 72 hours and addition of 0.025% formalin, resulted in all the F glycoprotein being in the postfusion conformation [24].

Antibody-mediated neutralization

F and G are the primary targets for neutralizing antibodies against RSV. F is a class I fusion protein which means after endoproteolytic cleavage and assembly, it is a trimer of heterodimers in a metastable, pre-triggered conformation poised to interact with host cell membranes (Figure 1). When pre-F is triggered by interaction with host cells, the hydrophobic fusion peptide at the N-terminus of F1 anchors the heptad repeat 1 (HR1) adjacent to the host cell membrane. The attraction of HR1 to HR2 adjacent to the transmembrane domain pulls the host and viral membranes together leading to membrane fusion and opening of a pore that allows the viral genome to enter the host cell. This rearrangement is unidirectional and leaves the F protein in antiparallel six-helix bundle postfusion conformation. F is, therefore, required for viral entry making it an important target for neutralizing antibodies. There are five major neutralizing sites (Ø, II, III, IV, and V) on the surface of the pre-F glycoprotein, and the most neutralization sensitive are at the apex (Ø and V). Sites I, II, and IV are preserved on the surfaces of post-F. Sites II and IV are neutralization-sensitive, but site I antibodies are typically non-neutralizing and generally have higher binding affinity for post-F than pre-F. Stabilizing F in the pre-F conformation results in an immunogen that induces about 80-fold higher neutralization activity in Rhesus macaques than a post-F immunogen [25,26••]. The G glycoprotein is another surface protein that is targeted by neutralizing antibodies. The G glycoprotein has two overlapping neutralizing epitopes in the central conserved domain around the cysteine noose [27]. Antibodies to the central conserved domain not only neutralize RSV but can reduce ERD in murine models [28].

Generation of high potency neutralizing antibodies is a primary immunological goal for RSV vaccines for both young infants and the elderly. There is evidence from maternal-infant pairs that neutralizing activity in cord blood correlates with protection from severe disease in infants [29], and passively administered polyclonal immunoglobulin with high neutralizing activity can protect high-risk premature infants from severe disease [30]. Palivizumab is a licensed neutralizing monoclonal antibody directed at site II for protecting premature infants against severe RSV disease [31] and an engineered version of palivizumab with higher potency (motavizumab) has been shown to significantly reduce the risk of RSV disease in full-term Native American children [32]. Newer monoclonal antibodies directed to the apex of pre-F with even greater neutralizing potency and extended half-life mutations are now being tested for efficacy and may allow the use of a single birth dose for protection of infants beyond six months of age [33•]. Even though severe disease in the elderly is multifactorial and complicated by waning T cell immunity and co-existing underlying cardiopulmonary disease, observational studies have

Figure 1



Nomenclature, functional domains, structure, and antigenic topography of RSV fusion (F) glycoprotein.
F is a class I fusion protein that is translated as a 574aa polypeptide with an N-terminal signal peptide cleaved after aa25. During its transit through the secretory pathway it is cleaved between aa109 and 110 and again between aa136 and 137 liberating a 27aa glycopeptide. The protein is folded and glycosylated at aa27, 70, and 500 and palmitoylated at aa550 and is displayed as a trimer of F2-F1 heterodimers on the infected cell or virus membrane with the hydrophobic fusion peptide at the N-terminus of F1 tucked inside the cavity of the head. The 2-dimensional map demonstrates these features and the location of virus-inhibiting peptides [65], regions that undergo significant movement during rearrangement, and major antigenic sites associated with neutralizing antibodies. The antigenic topography of F is conformation-dependent, and the most neutralization sensitive epitopes only exist on the metastable prefusion F (pre-F) as shown. Once F is triggered and undergoes the unidirectional rearrangement into the postfusion form (post-F), it is fixed and stable. Some of the surfaces and thus some of the antigenic sites present on pre-F are preserved on post-F. Therefore, the nonfunctional post-F molecule can induce some antibodies that recognize pre-F and have moderate neutralizing potency. However, the post-F molecule induces a disproportionately large amount of non-neutralizing antibody that can bind the taller post-F on the virion surface and potentially interfere with antibody access to pre-F. As noted in the text, G and SH proteins are also valid surface protein targets for vaccine development and are not shown due to the primary focus on neutralizing activity and space constraints.

suggested that if serum neutralizing activity is high enough, they can also be protected from severe lower airway disease and hospitalization [34]. Thus, the induction of neutralizing antibodies is an important immunological goal for vaccination.

T-cell mediated viral clearance

A primary goal for vaccine-induced T cell responses is to avoid immunopathology and unintended consequences. The T cell response is complex with nuanced effector and regulatory mechanisms, and difficult to control with a single immunization. T cell induction requires special consideration for vaccines targeting antigen-naïve infants where achieving the optimal inductive events is much more difficult than simply boosting memory B cells to produce effective antibodies in older children and adults with pre-existing immunity. Most of what we know about the T-cell response to RSV has come from murine models, but recent work in human challenge models is adding to our understanding of T cells in RSV infection [35,36•]. T cells can produce both favorable and deleterious effects. CD8 T cells were first shown in passive transfer [37] or depletion [38] experiments to be associated with both viral clearance and disease based on death or weight loss. Later, it was found that the specificity and phenotype of the CD8 T-cell response influences the balance of protection or pathology that results from CD8 T cells [39–41], and that the timing of infection and quality of CD8 T cell responses can affect airway hyper-responsiveness [42]. The location of CD8 T cells may also determine the effectiveness of viral clearance without significant disease as suggested by the beneficial impact

of having intraepithelial T resident memory in both mice [43] and humans [36•]. Importantly, inducing CD8 T cells during the initial antigen exposure is also an effective way to avoid the undesirable Th2-biased CD4 T-cell responses associated with ERD. Th2-associated cytokines promote mucus production [44], airway hyperresponsiveness [45], and inhibition of cellular cytolytic activity [46,47]. Nevertheless, vaccine-induced CD4 T cells will be needed to achieve optimal magnitude and duration of antibody responses (CD4 Tfh) [48] and to safely induce effector T cells (CD4 Tregs) [49–52]. The induction of effective T cell responses depends on appropriate antigen uptake, migration, processing, presentation, and co-stimulation by dendritic cells (DC) [53]. DC function is age-dependent [54] and can be affected by RSV proteins either through IFN inhibition or G glycoprotein effects on signaling and activation [3,5,55]. Induction or boosting of a balanced T cell response would improve virus clearance, but it has to be done thoughtfully to achieve cells with the right specificity, phenotype, and location to avoid the potential for immunopathology.

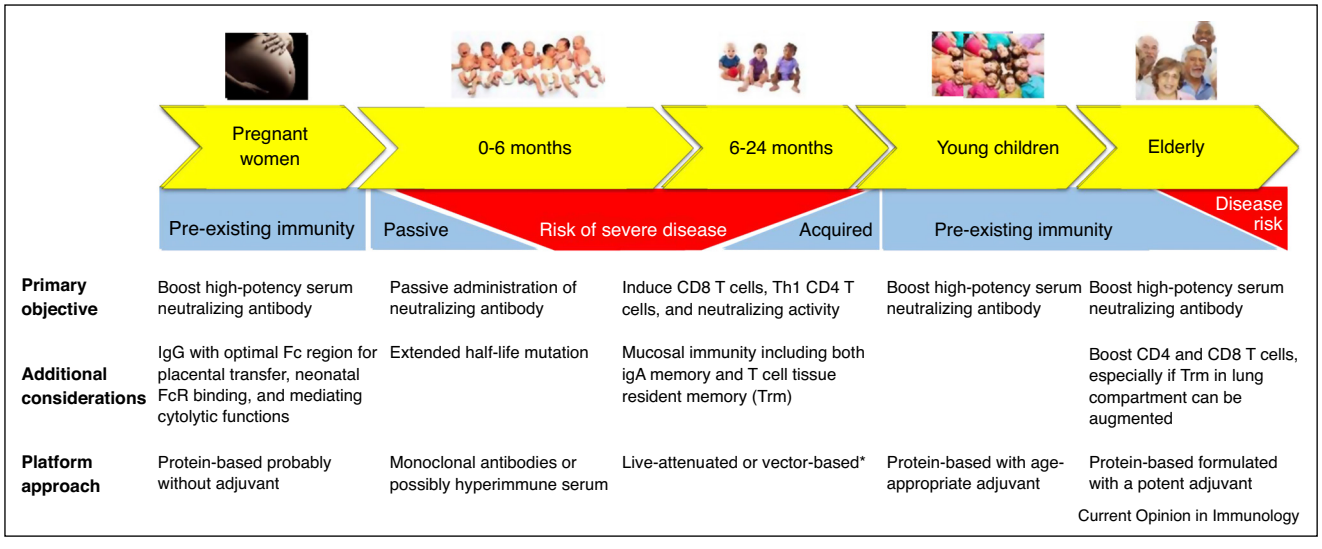
Goals for each major vaccine target population

The primary goal for vaccination is to prevent severe disease defined as medically attended lower respiratory tract disease, hospitalization, and death in the frail elderly and young infants (Figure 2). Another goal is to prevent the secondary consequences of infection in the airway especially in the developing lung in children under six months of age. These include mucus and squamous metaplasia, impaired growth, or promotion of airway hypersensitivity. It is also important to avoid aberrant priming or the establishment of relatively ineffective T and B cell repertoires or memory phenotypes. Limiting the extent and duration of viral shedding may be possible, but prevention of clinical disease is the major goal. Preventing infection altogether may not be feasible or desirable unless the intent is to try for elimination of RSV. If vaccination could result in subclinical RSV infections, re-exposure of the upper airway may be sufficient to maintain immunity throughout life and reduce the frequency of transmission to susceptible populations and the extent of spread through communities each year. Preventing severe disease will require reducing virus spread into the lung and lower airways.

Frail elderly

In this population, immunity may be achieved by boosting and maintaining high neutralizing activity in serum where antibodies have a relatively low barrier or gradient to penetrate and protect the lung and lower airway. Boosting of T cell responses will occur with virtually any vaccine modality but achieving the right phenotype and maintaining those cells in the right location to rapidly clear virus without producing immunopathology is difficult to control. Parenterally administered adjuvanted protein-based immunogens are being pursued for this population. Live-attenuated viruses will not work in this age group because pre-existing immunity will not allow

Figure 2



Target populations for RSV vaccine development.
Proposed target populations for RSV immunoprophylaxis are shown relative to immune status and disease risk. The young infants and the elderly population at higher risk of severe disease would be the primary beneficiaries of each intervention. Maternal immunization would be done to improve passive protection of young infants and would compete with the direct administration of monoclonal or polyclonal antibodies to neonates. Immunization of older children who have been previously infected or adult populations would be to directly or indirectly protect the frail elderly. Immunization of antigen-naïve young infants following the decline of maternally transferred or passively administered antibody would be to protect them from severe disease which remains a risk through about five years of age. *includes live chimeric viruses and nucleic acid.

sufficient replication to be immunogenic. Nucleic acid or gene-based approaches are being explored and are attractive because of their potential to improve CD8 T cell effectors, but these approaches will need to show that the gain of T cell functions does not impact the magnitude or duration of neutralizing activity or impact safety and tolerability. Other approaches that utilize mucosal delivery will need to show clinical benefit because it may be difficult to demonstrate mechanistic correlates of protection in mucosal tissues and secretions, and operationally may prolong development times.

Maternal immunization

Immunizing mothers to passively protect infants during the first six months of life is an approach competing with directly administering a birth dose of neutralizing monoclonal antibodies (mAbs) to infants. The intent of both approaches is to protect the infant airway with serum neutralizing antibodies, and there are geographic, cultural, economic, and logistical reasons why one approach may be preferred over the other. Direct delivery of mAbs provides a known level of neutralizing activity to the child that can be adjusted to achieve protective levels. Maternal immunization has the advantage of providing the infant with polyclonal immunoglobulin and may also contribute to immunoglobulins in breast milk. The goal is to achieve sufficient levels of neutralizing activity from a single immunization late in the second trimester or early third trimester to sustain protective levels of antibody in the newborn through at least six months. There is a biological precedent for this approach based on licensed polyclonal and monoclonal antibody products that have been used for over 20 years in premature and high-risk infants during the RSV season. Active transplacental transport of maternal antibody begins about 28–30 weeks of gestation so vaccination should be timed to achieve peak antibody responses from that time until delivery. The benefit of maternally acquired antibodies may not be available to infants born prematurely, so having monoclonal antibodies for passive prophylaxis of those infants would still be important. The advantage of polyclonal antibodies is that they will more reliably recognize the two circulating subtypes of RSV between which the F proteins have about 25–28 amino acid differences in the ectodomain and many of those occur within key neutralization-sensitive antigenic sites and are sometimes associated with escape from monoclonal antibodies. However, site Ø is a supersite that attracts antibodies with a variety of angles, rotations, and contact residues. Therefore, polyclonal responses to site Ø neutralize both subtype A and B viruses. Even though most neutralizing monoclonal antibodies are effective against both subtypes [56••], mAbs can sometimes be escaped by one subtype of the other [57]. For example, the efficacy trial of a site V mAb reportedly failed because it was effective against subtype A strains, but it was escaped by aa172 and aa173 mutations in the circulating B subtype strains. While maternal immunization does not necessarily require extended durability of serum antibody response, the magnitude and maintenance of response for several weeks will be important for maximal effect. Various factors like glycosylation patterns on antibodies can affect half-life in infants, Fc-mediated effector mechanisms, and possibly transplacental transport that may all contribute to infant immunity 58]. While most current vaccine programs recommend using non-adjuvanted vaccine formulations for maternal immunization, it may be useful for RSV or other neonatal pathogens vaccine candidates to identify vaccine adjuvants or delivery approaches that could optimize antibody transport, durability, and function and avoid interference with other maternal vaccines. In this target population, eliciting T cells to optimize antibody responses would be important, but otherwise induction of effector T cells would not be a major goal since they would not be transferred to the infant.

Seronegative, antigen-naïve infants

Direct immunization of this age group has historically been complicated by enhanced disease as discussed above. Studies using live-attenuated virus vaccines have been safe, but effective immunization has been difficult to achieve in very young infants (<3 months of age). This is in part due to the presence of maternal antibody that can attenuate the replication of live virus vaccines, and likely also a reflection of the regulated immune environment of the neonate and immaturity of neonatal antigen presenting cells [59,60]. While infants can make measurable antibody and T cell responses after an initial vaccination or infection [61,62], the magnitude and durability of serum antibody increases after repeated infections, and yet is still unable to reliably prevent reinfection despite RSV having relatively low levels of antigenic variation. This suggests that the initial inductive event may provide an opportunity through vaccination to establish B and T cell memory phenotypes and repertoires that would be more effective and provide a better lifetime trajectory for RSV immunity. This could potentially be achieved by immunizing after six months of age and relying on passive protection until then. Current live-attenuated vaccine approaches using molecular clones to remove specific virulence factors or change the balance of transcription and viral replication are promising and are in early clinical phase evaluation [63,64••]. Alternative immunization approaches using nucleic acid or vector-based antigen delivery may also allow specific targeting of neutralization-sensitive sites with B cells, induction of more effective T cell responses, and avoidance of responses that may be modulate or dampen immune memory. Immunization of antigen-naïve infants with vaccine approaches that deliver antigens produced intracellularly mimicking live virus is thought to be safe because induction of CD8 T cells will avoid the induction of Th2-biased CD4 T cells.

Conclusions

There is much more to learn about induction and maintenance of protective antibody lineages, and about the importance of mucosal immunity and induction of tissue-resident T cells with phenotypes that can clear infection without immunopathology. There is also a lot unknown about what would happen on a population-wide basis if severe RSV infection could be prevented in early life. Would this diminish the prevalence of childhood asthma; would immunity be established that was more effective against reinfection; would it be possible to eliminate RSV; would there be other viruses that assume the niche in early childhood currently occupied by RSV? These questions may only be answered once there is an effective RSV vaccine available for widespread use.

Conflict of interest statement

The author is an inventor on patents that describe candidate RSV vaccines based on the F protein stabilized in the prefusion conformation.

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- •• of outstanding interest

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