Vaccination against RSV

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ABSTRACT

Respiratory syncytial virus (RSV) causes infection throughout life, with infants, adults who are severely immunocompromised and the elderly at special risk of developing severe lower respiratory tract disease, hospitalisation and death. The burden of severe disease in the elderly is comparable to seasonal influenza and there remains no effective anti-viral drugs or vaccine for any target population. The development of a vaccine to confer immunity against severe disease is a major global health priority. A multitude of safe and immunogenic vaccine candidates have failed to induce the protective immunity needed for licensure and in recent years this has included the largest clinical trials of RSV vaccines in history. The obstacles to vaccine development in elderly populations include an incomplete understanding of the immune responses needed for protection, the effect of aging on induction and maintenance of immunity (natural and vaccine induced immunity), and the high rate of co-morbid disease in older adults. Recent advances in structural biology, new biological platforms for antigen delivery, and insights from experimental challenge models mark the latest developments in over 50-years of research. This continues to be an active and evolving field of scientific discovery with renewed hope for a vaccine in the future.
BACKGROUND

“Chimpanzee coryza agent”, which is now known as respiratory syncytial virus (RSV), was first isolated in October 1955 from a colony of healthy chimpanzees after an acute respiratory illness at the Walter Reed Army Institute of Research. At around the same time, clinical isolates obtained from sick infants reproduced the cytopathic effects of this newly discovered virus from chimpanzees, and RSV soon gained recognition as a major human pathogen responsible for mild to severe respiratory disease in infants. An important early observation was that sera from infants convalescing after a recent severe respiratory illness demonstrated significant increases in hemagglutination-inhibition (HI), complement fixation and neutralising antibody, whereas HI titres of 1:10 were found in only a quarter of infants aged 2-34 months without recent disease and in 90% of adults. The conclusion from this, and supported by observations made from other studies, was that primary RSV infection occurred early in life and the immunity that followed conferred protection to developing severe disease in adulthood. It was known that healthy younger adults were not immune from infection, from both observational studies and the first RSV challenge studies in the 1960’s, but infection was either asymptomatic or resulted in mild, self-limiting disease. The first indications that the elderly were another population at risk of hospitalisation and death from RSV were made years later from observational studies in long-term elderly care facilities in the 1970’s[1, 2]. Although RSV vaccine development in infants began in the mid-1960s it would not be until 30-years later that RSV vaccine candidates were first tested in an older adult population. Although there
have been several great advances in the understanding of the genetics, structure and biology of the virus, and detailed characterisations of the human RSV-specific immune repertoire, there remains no licensed vaccine or effective treatment. Infants, children and younger adults with severe immunocompromise, and the elderly in particular, remain at risk of developing severe disease, hospitalisation and death from RSV until a vaccine is developed.

Human RSV belongs to the genus *Orthopneumovirus* (family *Pneumoviridae*, sub-family *Paramyxovirinae*, species *Human Orthopneumovirus*), and the genus includes bovine RSV with 70% sequence homology to human RSV. There are two subtypes of RSV, designated RSVA and RSVB, which were identified by differential monoclonal antibody binding to the surface expressed G (attachment) protein, and different subtypes and genotypes of RSV can circulate in different years or simultaneously to cause clinically indistinguishable disease. The viral genome consists of 10 genes that encode 11 proteins, as illustrated in the figure below, with the overlapping open reading frames in the M2 gene resulting in two different matrix proteins. The resulting viral structure is a single, linear, negative-stranded, enveloped, non-segmented RNA virus. The viral envelope contains three surface proteins designated the F (fusion) glycoprotein, attachment (G) glycoprotein and the small hydrophobic (SH) protein. Within the virus particle are six internal proteins concerned with nucleocapsid construction and RNA replication. Two non-structural proteins (NS1 and NS2) complete the RSV protein complement and serve to inhibit the host immune response to infection.
Schematic representation of the human RSV genome, protein products and viral structure.

The 10 genes are ordered from 3′ to 5′ as top to bottom. The size of each block is proportional to the size of each gene (total 15’191 base pairs).

The 11 protein products are annotated next to each gene; the M2 gene contains two open-reading frames (ORFs) that generate two protein products (M2-1 and M2-2). The nucleocapsid forms filamentous viral particles up to 100-350nm in length, with the lipid envelope derived from the host cell.

The sequencing of historical RSV isolates has demonstrated that the viral genome, with the exception of the G gene, has generally remained
stable over time. The temporal genetic changes in the G gene do not appear to be associated with significant losses in G-specific antibody neutralisation. In nature, RSV is essentially a human restricted pathogen, although non-human primates, sheep, and cattle can be infected. The selective pressure for RSV is the human host immunity derived from repeated seasonal exposure, with only low rates (<10%) of escape virus observed following palivizumab use in infants at risk of developing severe RSV disease[3]. The fact that RSV causes repeated infections throughout life with little/no antigenic variation is a powerful reflection of the potency of the virus for immune suppression and evasion. Of particular interest are the non-structural proteins, NS1 and NS2, whose genes are located from the 3’ promotor end. These proteins appear early after host cell invasion and function to inhibit host innate and adaptive immune responses through the interaction with over 200 host proteins (in the case of NS1). The effects of NS1/2 include the inhibition of type I and type III interferon production (IFNα, β and λ) and the response to interferon signalling from other cells (through the interferon α/β receptor), the suppression of dendritic cell maturation, and to extend the life of an infected cell by inhibition of early apoptosis.

THE LIFETIME BURDEN OF RSV DISEASE

RSV causes infection throughout life. The virus is spread from person-to-person by droplet transmission or from contact with infected surfaces and hands. In temperate and many tropical regions of the world the
annual peaks of transmission occur in winter months concurrent with many other respiratory viruses, such as influenza, and co-infections are not uncommon.

RSV infection begins with attachment of free virus and entry of the viral RNA into airway epithelial cells that line the respiratory tract, although the specific cell receptors needed for this remains unknown (nucleolin, heparin sulfate and CX3CR1 are possible candidates). The symptoms of clinical disease arising from the infection are a combination of both the direct effects of the virus (ciliary dysmotility, loss of airway epithelial cells, occlusion of small airways by necrotic and inflammatory cell debris) and an over-exuberant innate inflammatory response recruited to control and clear the infection (cytokines, inflammation and adaptive immune responses). The severity of this disease can be seen on a spectrum, in all ages, with the majority of infections being either asymptomatic or causing mild disease restricted to the upper respiratory tract (rhinorrhoea, nasal congestion, coryza and cough). Observational studies consistently demonstrate that a significant proportion of infants, adults who are severely immunocompromised and the elderly progress to developing lower-respiratory tract involvement needing hospitalisation and are at risk of death.

The annual infection (or attack) rate in the first year of life ranges from 30-70%, and approximately 2-3% of primary infections require admission to hospital (and 2-6% of these admissions require management on paediatric intensive care units), making RSV-bronchiolitis the single greatest burden to paediatric hospital resources each year and second only
to malaria in all-cause infant mortality between one and 12-months of age[4-6]. The risk of developing severe disease declines dramatically by 12-months of age, but repeated exposure does not confer protection from re-infection and annual attack rates of asymptomatic or mild disease in the adult population range from 9-11%[7, 8]. Healthy younger adults rarely develop significant RSV disease, but severe pathogenic and/or iatrogenic immune suppression can re-establish the risk of developing severe disease and death, and this has been especially noteworthy in haematopoietic stem cell transplant (HSCT) patients, with associated mortality risks of 20-55%[9-13].

As early as 1967 the annual incidence of RSV infection in the elderly was estimated at 7%, and several later studies have measured variable seasonal RSV attack rates for the elderly living in the community and care homes between 5 to 10% each year[1, 2, 14-16]. The attack rate also gradually rises with increasing age (6.1% in adults aged 65-69 years, 7.1% aged 70-74 years, 8.9% aged 75-79 years and 8.5% aged ≥80 years). Overall, the infection rate in the elderly appears comparable to the rates observed from younger adults, but from as early as the 1970s it was clear that where the two populations differ is with the risk of disease progression and subsequent respiratory failure (8-13%) and death (2-5%)[17, 18]. Prospective studies have identified RSV infection as being responsible for hospitalisations in the elderly for pneumonia (10.6%), exacerbations of chronic obstructive pulmonary disease (11.4%) and asthma (7.2%), and worsening of congestive cardiac failure (5.4%) with all-cause 30-day and 60-day mortality rates of 9% and 12% respectively [19-23]. The overall burden
of emergency hospital admissions and mortality from RSV in the elderly may be comparable to seasonal influenza[19, 22, 24-27]. Furthermore, the increasingly older population common to many parts of the world indicate that RSV infection in the elderly is set to become a greater burden to healthcare resources. By 2050 an estimated 2 billion of the human population will be 65-years or older and represent 25% of the population in high income countries.

IMMUNITY TO RSV, AGING AND ADDRESSING THE BURDEN OF DISEASE IN THE ELDERLY THROUGH VACCINATION

The development of a safe and effective vaccine for the elderly requires an understanding of the age-related immunological factors that confer a loss of protection from severe lower-respiratory tract disease, and how these protective mechanisms might be restored through vaccination. Critically, immune correlates of protection from RSV for any age group remain unknown. Instead, a detailed comparison with the humoral and cellular immune responses from younger healthy adults who do not develop severe disease can serve as a benchmark for desirable vaccine immune responses in the elderly, and identify where vaccine-induced immunity might achieve some effectiveness in reducing the burden of hospitalisations and death from the disease.

Serum antibody targets free virus and neutralising antibodies are generated against the surfaced-expressed F and G proteins (the SH protein is poorly immunogenic). The RSV F (fusion) protein is responsible for merging
the virus and host cell membranes and is essential for infection to occur. Furthermore, the F protein is also highly conserved between RSVA and RSVB strains and a reliable target for protective antibody following exposure to different strains and subtypes of RSV in different years. Conversely the G protein is not essential for *in vitro* infection, neutralising antibodies are subtype specific, and the production of a soluble truncated form of the G protein acts as a decoy antigen in natural infection.

Serum F-specific antibody alone can confer protection from severe disease in the lower respiratory tract in infants, as has been elegantly demonstrated with the use of F-specific monoclonal antibody (Palivizumab, MedImmune). In infants at high risk of developing severe disease the use of palivizumab prophylaxis can reduce hospitalisations by 55% (4.8% palivizumab vs 10.6% placebo) [28]. Serum neutralising antibodies to the F-protein develop early in life and reach titres in early childhood which are comparable to those observed in adults [29]. Unlike infants, healthy adults can be safely challenged (experimentally infected) with RSV for a closer examination of the baseline immune responses associated with protection from infection and the development of mild disease. Here, along with several observational studies, a positive correlation has been observed with titres of serum neutralising antibody (a combination of F- and G-specific neutralising antibody activity) and the risk of disease, indicating a continued role for antibody-mediated protection[30-32]. Sera from the elderly do not show any significant quantitative or qualitative loss of antibody-mediated immunity to RSV, or measures of B-cell frequencies in blood that underpin the production and maintenance of antibody. However, serum neutralising
antibody titres vary widely in younger and older adults, and relatively lower titres remain associated with the development of disease in the elderly[32-36]. Age-related differences have been observed in the antibody response following natural infection, where sera from the elderly showed greater fold-changes in antibody response without any changes in neutralising antibody titres following infection, suggesting that older age may be associated with a loss of antibody regulation and/or an accumulation of non-neutralising antibody[33]. A vaccine that boosts serum neutralising antibody titres could reduce the risk of developing severe disease in the elderly. However, the fact many of the elderly develop severe disease despite having levels of serum neutralising antibody similar to levels in younger adults, means that a more complex explanation is needed. A vaccine that targeted the induction of antibody alone may have limited effectiveness. Another important observation is that the depletion of lymphocytes, not antibody, is associated with RSV disease progression in HSCT patients and the development of severe disease with significant risks of mortality[37].

Antibody is maintained and produced in response to infection by long-lived plasma cells resident within the bone marrow. F-specific IgG memory B-cells in peripheral circulation form a stable population in healthy adults that persists into old age [Green et al, manuscript in preparation]. In contrast, observations from natural infection, experimental challenge in younger adults and from vaccine responses in younger and older adults highlight an absence of any comparable F-specific IgA memory B-cell population in peripheral circulation[29, 38]. RSV infection (or vaccination) results in the transient appearance of both F-specific IgA and IgG B-cells in
the form of antibody secreting cells in the blood, but does not result in the maintenance of a population of F-specific IgA memory B-cells in peripheral circulation\[39, 40\]. The relevance of this in relation to the risk of re-infection and/or development of severe disease in the elderly remains unknown, but RSV-specific IgA plays an important role in protection from RSV infection at the respiratory mucosa, and the biology of memory IgA cells remains the subject of ongoing investigation.

RSV-specific CD8+ effector (T-cell) epitopes have been identified on several RSV proteins (including F and the internal proteins N, M, M2 and NS2) and play a critical role in controlling and ultimately terminating infection. Severe disease in infants has been characterised, in some studies, by a relative skew from protective Th1-associated cytokines (IFN\(\gamma\) and IL-2) towards Th2/Th17-associated cytokines, and extended periods of viral shedding is common in infants with underlying T-cell disorders. The T-cell immune response to RSV infection in adults is more clearly Th1-dominated. Immune senescence in respect to the RSV-specific CD4+ and CD8+ T-cell populations in peripheral circulation in the elderly is characterised by a contraction of the memory T-cell population and an expansion of suppressive regulatory T-cells, without significant changes in central and effector memory T-cell populations\[36, 41, 42\]. A future RSV vaccine for the elderly might, in addition to boosting serum neutralising antibody, aim to restore these changes in T-cell immunity.

The immune response at the respiratory mucosa, where RSV initiates infection, is the first opportunity to protect the host from spread of disease and to signal the recall of protective systemic immune responses
from previous exposure. Observations in adults following natural exposure and experimental RSV challenge have depicted low-levels of respiratory mucosal antibody (RSV-specific nasal IgA) as conferring an important predisposition to developing RSV infection independent of systemic immunity[38, 43, 44]. Nasal RSV-specific IgA appears to have biological significance in determining whether infection becomes established in the host, but may not have a similar impact on the risk of disease progression. It remains possible that measures of mucosal and systemic immunity might be independent measures of immunity to infection and severity respectively[43]. Several RSV vaccine candidates have been, and continue to be, intra-nasally administered to target the induction of RSV-specific nasal IgA antibody. Selective accumulation of memory T-cells in the lung against respiratory viral pathogens has also been described in humans and pulmonary T-cells may contribute substantially to protection afforded by mucosal immunisation.

OBSTACLES AND OPPORTUNITIES FOR AN EFFECTIVE RSV VACCINE FOR THE ELDERLY

At present there are 43 RSV vaccines and four RSV monoclonal antibody candidates in development, of which 19 are at clinical stage evaluation with seven of these under assessment for safety and effectiveness in the elderly (reviewed in detail here[45] and continually monitored by the Program for Appropriate Technology in Health, or PATH website).
The first RSV vaccine candidate, a formalin-inactivated RSV whole-virus vaccine (FI-RSV), was tested in infants and children approximately 10-years after the discovery of the virus in the mid-1960’s, with disastrous consequences. The approach of whole-virus formalin-inactivation was also used as vaccines for polio, measles, influenza and para-influenza. However, in the case of FI-RSV this primed the naïve infant immune system towards a harmful Th2-biased immune response on subsequent encounter to natural infection, termed enhanced respiratory disease (ERD), that was responsible for a 16-fold increase in hospital admissions and two fatalities in FI-RSV vaccinated infants. The advent of a semi-permissive animal models capable of reproducing pulmonary histopathological features of FI-RSV following RSV challenge, principally the Sigmodon hispidus cotton rat in 1986, was a significant step forward that allowed for a level of pre-clinical screening for this potentially fatal immune response to vaccination in future RSV vaccine candidates. Although concerns for FI-RSV related ERD in pre-clinical animal models has resulted in the termination of the development of subunit vaccines for use in seronegative infants, a plethora of RSV vaccine designs remain in consideration for development as a vaccine for use in the elderly. It was not until the 1990’s, however, that RSV vaccines were first evaluated in this population. Historical RSV vaccines used in the elderly have been safe and immunogenic, and have included live-attenuated, subunit, viralvectored and nanoparticle designs, but none have progressed beyond clinical trials and the obstacles in generating durable protective immunity have been too difficult to overcome. Recent examples include the largest clinical trials in RSV vaccine history, such as a phase 3 trial of a promising RSV
F-nanoparticle vaccine performed across 60 sites in the United States and enrolling nearly 12,000 adults 60 years and older, and another phase 2b trial of a subunit vaccine with a TLR4 agonist adjuvant tested over 61 sites in seven countries and enrolling nearly 2000 adults 60 years and older. Despite promising safety and induction of desirable immune responses by both of these vaccines in earlier clinical trials, both failed to meet their trial endpoints and demonstrate protective efficacy in the elderly (data unpublished)[45].

Critically important obstacles remain with there being an incomplete understanding of what immune responses are needed to confer protection from severe disease, overcoming the negative effects of pre-existing immune responses on vaccine immunogenicity, defining clinical outcome measures in phase 3 trials, and the challenge of maintaining immune responses. Many confounding factors cannot be addressed through vaccination, such as advanced age-related thymic involution and waning bone marrow lymphopoiesis, the low seasonal attack rate and the effect this has on the trial sizes needed to prove efficacy, and in having a target population where 90% of RSV-related hospital admissions are associated with one or more co-morbid diseases[22]. Without any known immune correlates of protection, arguably the optimal response to vaccination in the elderly would be to restore immune responses similar to those observed in younger adults when the risk of disease progression, hospitalisation and death were minimal. RSV disease is propagated within the host by free virus (targets of neutralising antibody) produced from infected cells (targets of cellular immunity), and advanced age is associated with losses in both these
host effector arms. The design of a future RSV vaccine for the elderly might therefore consider the need for the parallel restoration of humoral and cellular RSV-specific immunity.

Younger adults can be experimentally re-infected with the same RSV strain within months, and a significant proportion of confirmed RSV infections in the elderly with COPD are not associated with changes in serum or nasal antibody (15% of infections, 73% of whom were symptomatic)\[46]. In these examples of absent or short-lived protection following natural infection, the virus has employed potent mechanisms for immune subversion and evasion, such as through the effects of the non-structural proteins and soluble G protein. However, following vaccination with candidates that used other RSV proteins, we continue to observe short-lived vaccine immunogenicity that suggests other host factors are also important, and these are not well understood. It remains unknown whether a future RSV vaccine could confer protection over more than one season, and the frequency of vaccination needed for protection in the elderly could create logistical obstacles and, in the case of viralvectored vaccines, the generation of vector-specific antibody that could abrogate immunogenicity in later years.

The challenge of restoring age-related losses of humoral and cellular immunity is substantial, but there have been several major advances in recent years that could help to achieve this goal. Examples include having a better understanding of the structural biology of the F protein and superior antibody neutralisation, the use of experimental challenge models that can provide early insight into which vaccines might induce protective immunity,
several new biological platforms for antigen delivery that induce both antibody and T-cell responses, and learning more about the epidemiology of disease in infants and toddlers that could identify indirect vaccination strategies to reduce burden of severe disease in the elderly. Of particular importance has been the stabilisation of the RSV F protein in the conformational shape needed to bring the virus into close proximity with the host cell and facilitate infection. This shape, called prefusion F (or pre-F) rapidly transforms to a more stable postfusion (or post-F) form. The pre-F protein exposes uniquely potent epitopes such as antigenic site zero (or ø) for antibodies that are 10- to 100-fold more potent at neutralising virus than the humanised mouse monoclonal palivizumab[47-49]. RSV vaccines to date have used F protein antigen in the more stable post-F confirmation, whose epitopes do not include antigenic site ø and generate less potent neutralising antibody. There are currently several vaccine candidates using stabilised pre-F protein as antigen in clinical stage evaluation and the results of these are eagerly awaited[45].

Another major advance has come from the use of experimental human challenge models of RSV infection, which began in the 1970s, which to date have only involved healthy younger adults. The data from these studies have provided valuable insights into the baseline immune factors that can be related to the risk of infection and mild symptomatic disease, and with sampling directly from the lung by bronchoscopy we have begun to understand immunity to severe RSV disease in ways that cannot be appreciated from blood or nasal sampling. Resident RSV-specific T-cell populations (CD8+ CD69+ CD103+) in the lung appear to correlate with a
reduction in symptoms in younger adults and remain active for several weeks after resolution of the clinical disease [38, 40]. It remains unknown how this specific population changes with age and whether this could be related to rates of severe disease in the elderly or could be a suitable target for intra-nasal or aerosol vaccination. A specific ethical obstacle for using an experimental RSV challenge in the elderly is the clear risk of causing severe lower respiratory tract disease. Promising results from new anti-viral drugs for RSV, such as ALS-008176 which was tested in an RSV challenge model with younger adults, means that an effective anti-viral drug for RSV could be available for the termination of an RSV challenge virus in the elderly, making study in this population more acceptable [50]. This could herald a safe means to explore age-related immune factors in greater detail and the ability to test novel vaccine candidates quickly and relatively cheaply for potential protective efficacy. The variable and low seasonal attack rate in the elderly means a very large study population (and associated cost) is needed for phase 3 vaccine trials aiming to demonstrate protection and apply for licensure.

In summary, there is high annual burden of hospitalisation and death from RSV in the elderly, and a lack of universal and cost-effective preventative measures which makes RSV a major priority for vaccine development. The disease burden is set to increase with an increasing aging population. Our understanding of immunity to RSV is incomplete, and the hope for a future RSV vaccine for elderly adults would be to redress age-related losses in humoral and cellular RSV immunity to restore protection from disease progression, and to reduce hospitalisation and death. Recent
advances in the structural biology of the RSV F protein, the introduction of new vaccine designs for the elderly, and a better understanding on where to target vaccine immunogenicity mean we can hopeful for a successful vaccine in the future.

REFERENCES

1. Hart, R.J., An outbreak of respiratory syncytial virus infection in an old people's home. J Infect, 1984. 8(3): p. 259-61.
2. Sorvillo, F.J., et al., An outbreak of respiratory syncytial virus pneumonia in a nursing home for the elderly. J Infect, 1984. 9(3): p. 252-6.
3. Papenburg, J., et al., Molecular Evolution of Respiratory Syncytial Virus Fusion Gene, Canada, 2006–2010. Emerging Infectious Diseases, 2012. 18(1): p. 120-124.
4. Hall, C.B., et al., Respiratory syncytial virus infections within families. N Engl J Med, 1976. 294(8): p. 414-9.
5. Deshpande, S.A. and V. Northern, The clinical and health economic burden of respiratory syncytial virus disease among children under 2 years of age in a defined geographical area. Arch Dis Child, 2003. 88(12): p. 1065-9.
6. Lozano, R., et al., Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet, 2012. 380(9859): p. 2095-128.
7. Hall, C.B., C.E. Long, and K.C. Schnabel, Respiratory syncytial virus infections in previously healthy working adults. Clin Infect Dis, 2001. 33(6): p. 792-6.
8. O'Shea, M.K., et al., Symptomatic respiratory syncytial virus infection in previously healthy young adults living in a crowded military environment. Clin Infect Dis, 2005. 41(3): p. 311-7.
9. Whimbey, E., et al., Community respiratory virus infections among hospitalized adult bone marrow
transplant recipients. Clin Infect Dis, 1996. **22**(5): p. 778-82.

10. Ebbert, J.O. and A.H. Limper, *Respiratory syncytial virus pneumonitis in immunocompromised adults: clinical features and outcome*. Respiration, 2005. **72**(3): p. 263-9.

11. McCarthy, A.J., et al., *The outcome of 26 patients with respiratory syncytial virus infection following allogeneic stem cell transplantation*. Bone Marrow Transplant, 1999. **24**(12): p. 1315-22.

12. Ljungman, P., et al., *Respiratory virus infections after stem cell transplantation: a prospective study from the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation*. Bone Marrow Transplant, 2001. **28**(5): p. 479-84.

13. Renaud, C., et al., *Mortality rates of human metapneumovirus and respiratory syncytial virus lower respiratory tract infections in hematopoietic cell transplantation recipients*. Biol Blood Marrow Transplant, 2013. **19**(8): p. 1220-6.

14. Fransen, H., et al., *Infections with viruses in patients hospitalized with acute respiratory illness, Stockholm 1963-1967*. Scand J Infect Dis, 1969. **1**(2): p. 127-36.

15. Falsey, A.R., et al., *Acute respiratory tract infection in daycare centers for older persons*. J Am Geriatr Soc, 1995. **43**(1): p. 30-6.

16. Falsey, A.R. and E.E. Walsh, *Respiratory syncytial virus infection in elderly adults*. Drugs Aging, 2005. **22**(7): p. 577-87.

17. Sundaram, M.E., et al., *Medically attended respiratory syncytial virus infections in adults aged >/= 50 years: clinical characteristics and outcomes*. Clin Infect Dis, 2014. **58**(3): p. 342-9.

18. Branche, A.R. and A.R. Falsey, *Respiratory syncytial virus infection in older adults: an under-recognized problem*. Drugs Aging, 2015. **32**(4): p. 261-9.

19. Falsey, A.R., et al., *Respiratory syncytial virus infection in elderly and high-risk adults*. N Engl J Med, 2005. **352**(17): p. 1749-59.

20. Falsey, A.R., et al., *Respiratory syncytial virus and influenza A infections in the hospitalized elderly*. J Infect Dis, 1995. **172**(2): p. 389-94.
21. Ellis, S.E., et al., *Influenza- and respiratory syncytial virus-associated morbidity and mortality in the nursing home population.* J Am Geriatr Soc, 2003. 51(6): p. 761-7.

22. Lee, N., et al., *High morbidity and mortality in adults hospitalized for respiratory syncytial virus infections.* Clin Infect Dis, 2013. 57(8): p. 1069-77.

23. Fleming, D.M., et al., *Modelling estimates of the burden of Respiratory Syncytial virus infection in adults and the elderly in the United Kingdom.* BMC Infect Dis, 2015. 15: p. 443.

24. Thompson, W.W., et al., *Mortality associated with influenza and respiratory syncytial virus in the United States.* JAMA, 2003. 289(2): p. 179-86.

25. van Asten, L., et al., *Mortality attributable to 9 common infections: significant effect of influenza A, respiratory syncytial virus, influenza B, norovirus, and parainfluenza in elderly persons.* J Infect Dis, 2012. 206(5): p. 628-39.

26. Widmer, K., et al., *Rates of hospitalizations for respiratory syncytial virus, human metapneumovirus, and influenza virus in older adults.* J Infect Dis, 2012. 206(1): p. 56-62.

27. Zhou, H., et al., *Hospitalizations associated with influenza and respiratory syncytial virus in the United States, 1993-2008.* Clin Infect Dis, 2012. 54(10): p. 1427-36.

28. Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. The IMpact-RSV Study Group. *Pediatrics,* 1998. 102(3 Pt 1): p. 531-7.

29. Green, C.A., et al., *Humoral and cellular immunity to RSV in infants, children and adults.* Vaccine, 2018. 36(41): p. 6183-6190.

30. Watt, P.J., et al., *Determinants of susceptibility to challenge and the antibody response of adult volunteers given experimental respiratory syncytial virus vaccines.* Vaccine, 1990. 8(3): p. 231-6.

31. Hall, C.B., et al., *Immunity to and frequency of reinfection with respiratory syncytial virus.* J Infect Dis, 1991. 163(4): p. 693-8.
32. Luchsinger, V., et al., Role of neutralizing antibodies in adults with community-acquired pneumonia by respiratory syncytial virus. Clin Infect Dis, 2012. 54(7): p. 905-12.

33. Falsey, A.R., et al., Comparison of respiratory syncytial virus humoral immunity and response to infection in young and elderly adults. J Med Virol, 1999. 59(2): p. 221-6.

34. Falsey, A.R. and E.E. Walsh, Relationship of serum antibody to risk of respiratory syncytial virus infection in elderly adults. J Infect Dis, 1998. 177(2): p. 463-6.

35. Walsh, E.E. and A.R. Falsey, Humoral and mucosal immunity in protection from natural respiratory syncytial virus infection in adults. J Infect Dis, 2004. 190(2): p. 373-8.

36. Cherukuri, A., et al., Adults 65 years old and older have reduced numbers of functional memory T cells to respiratory syncytial virus fusion protein. Clin Vaccine Immunol, 2013. 20(2): p. 239-47.

37. Kim, Y.J., et al., Respiratory syncytial virus in hematopoietic cell transplant recipients: factors determining progression to lower respiratory tract disease. J Infect Dis, 2014. 209(8): p. 1195-204.

38. Habibi, M.S., et al., Impaired Antibody-mediated Protection and Defective IgA B Cell Memory in Experimental Infection of Adults with Respiratory Syncytial Virus. Am J Respir Crit Care Med, 2015.

39. Green, C.A., et al., Chimpanzee adenovirus- and MVA-vectored respiratory syncytial virus vaccine is safe and immunogenic in adults. Sci Transl Med, 2015. 7(300): p. 300ra126.

40. Jozwik, A., et al., RSV-specific airway resident memory CD8+ T cells and differential disease severity after experimental human infection. Nat Commun, 2015. 6: p. 10224.

41. de Bree, G.J., et al., Respiratory syncytial virus-specific CD8+ memory T cell responses in elderly persons. J Infect Dis, 2005. 191(10): p. 1710-8.

42. Cusi, M.G., et al., Age related changes in T cell mediated immune response and effector memory to Respiratory Syncytial Virus (RSV) in healthy subjects. Immun Ageing, 2010. 7: p. 14.
43. Bagga, B., et al., Effect of Preexisting Serum and Mucosal Antibody on Experimental Respiratory Syncytial Virus (RSV) Challenge and Infection of Adults. J Infect Dis, 2015. 212(11): p. 1719-25.

44. Mills, J.t., et al., Experimental respiratory syncytial virus infection of adults. Possible mechanisms of resistance to infection and illness. J Immunol, 1971. 107(1): p. 123-30.

45. Mazur, N.I., et al., The respiratory syncytial virus vaccine landscape: lessons from the graveyard and promising candidates. The Lancet Infectious Diseases, 2018. 18(10): p. e295-e311.

46. Falsey, A.R., et al., Detection of respiratory syncytial virus in adults with chronic obstructive pulmonary disease. Am J Respir Crit Care Med, 2006. 173(6): p. 639-43.

47. McLellan, J.S., et al., Structure-based design of a fusion glycoprotein vaccine for respiratory syncytial virus. Science, 2013. 342(6158): p. 592-8.

48. Magro, M., et al., Neutralizing antibodies against the preactive form of respiratory syncytial virus fusion protein offer unique possibilities for clinical intervention. Proc Natl Acad Sci U S A, 2012. 109(8): p. 3089-94.

49. Correia, B.E., et al., Proof of principle for epitope-focused vaccine design. Nature, 2014. 507(7491): p. 201-6.

50. DeVincenzo, J.P., et al., Activity of Oral ALS-008176 in a Respiratory Syncytial Virus Challenge Study. N Engl J Med, 2015. 373(21): p. 2048-58.