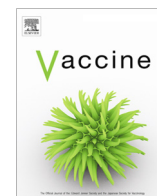


Contents lists available at [ScienceDirect](#)

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Controlled human infection with RSV: The opportunities of experimental challenge

Maximillian S. Habibi^a, Christopher Chiu^{b,*}

^a National Heart and Lung Institute, Imperial College London, London W2 1PG, UK

^b Section of Infectious Diseases & Immunity, Imperial College London, London W12 0NN, UK

ARTICLE INFO

Article history:
Available online xxx

Keywords:
RSV
Clinical trial
Antibody
T cell

ABSTRACT

Despite the recent explosion in RSV vaccine development, there remain substantial hurdles to overcome before licensing of effective vaccines will allow widespread use, particularly in high-risk populations. Incomplete understanding of mechanisms and correlates of protection against RSV mean that, for the time being, successful RSV vaccines must directly demonstrate efficacy, which necessitates large and costly clinical trials in naturally infected patients. To mitigate the risks inherent in progressing to these late-stage trials, experimental human RSV infection studies have recently been re-established, representing the interface between pre-clinical models and observational studies of patients. Not only can they be used for early proof-of-concept clinical trials to test vaccine efficacy, but human challenge studies also offer the potential to better understand protective immunity against RSV infection to improve vaccine design and delivery. In the past, controlled human infection studies with RSV have been instrumental in elucidating the influence of factors such as route of infection and type of inoculum on the course of disease. Recently, efficacy trials of novel RSV antiviral drugs have also been successfully undertaken. Now, with advances in technology, detailed investigations of human mucosal immunity in the RSV-infected airway are possible. These have indicated defects in RSV-induced humoral and CD8+ T cell immunity that may contribute to the recurrent symptomatic infection that occurs throughout life and should be circumvented by optimal vaccines. Here, we discuss the insights derived from RSV human challenge models; the major impediments to their more widespread uptake; and their potential benefit in accelerating vaccine development, including future directions to further enhance the relevance of these models to at-risk patient populations.

Crown Copyright © 2016 Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Respiratory syncytial virus (RSV) is the leading cause of infant respiratory tract infection in the world [1] and a major cause of morbidity and mortality in elderly adults [2]. Recent research has also added to a growing literature supporting a causal role for infant RSV infection in the development of wheeze and asthma in later life [3]. Despite this huge disease burden, potential for major impact on global health and decades of research, neither vaccines nor antivirals are yet available against human RSV. However, recent advances in understanding of its global epidemiology and improved antigenic targets have led to an explosion in activity related to the development of RSV vaccines and therapeutics [4].

Nevertheless, while empirical approaches to development may well lead to effective vaccines, the unique immunobiology of RSV infection means that many hurdles to success remain, with arguably the biggest barrier to progress being the continued gaps in our understanding of pathogenesis and protective immunity in RSV-induced disease.

Whilst controlled RSV infection of laboratory animals provides mechanistic insights, animal models do not fully recapitulate many aspects of human disease. Extrapolating from pre-clinical models is therefore difficult and they remain imprecise guides to understanding both mechanisms of protection and pathogenesis, as well as the likely clinical efficacy of candidate vaccines and therapeutics [5]. Most animals are at most semi-permissive to human RSV and require large, supra-physiological inocula to become infected. On infection, few animals suffer the same clinical syndromes as seen in humans and none clearly suffer from the recurrent symptoms on re-infection that is a special feature of human RSV. Furthermore, although primary infections in animal models have parallels to

* Corresponding author at: Section of Infectious Diseases & Immunity, 8th Floor Commonwealth Building, Imperial College London, Hammersmith Campus, Du Cane Road, London W12 0NN, UK.

E-mail address: c.chiu@imperial.ac.uk (C. Chiu).

<http://dx.doi.org/10.1016/j.vaccine.2016.08.086>

0264-410X/Crown Copyright © 2016 Published by Elsevier Ltd.

This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

infantile bronchiolitis and have been important in dissecting the mechanisms underlying vaccine-enhanced disease, so far none have been shown to adequately replicate RSV disease in elderly adults, who become increasingly susceptible to severe disease with age despite multiple prior infections that induce partial protective immunity [6]. In contrast, studies of hospitalised children and high-risk adults allow observations of natural disease in high-risk patients of greatest interest, but due to the several day incubation period of the virus coupled with frequent delays in diagnosis, it is only possible to assess patients as they approach or are recovering from peak illness [7,8]. While therapeutic trials in hospitalised children and adults are possible, variability of infecting viruses, co-infections, co-morbidities, medical interventions and atypical disease presentations limit interpretation. Robust endpoints for such trials have yet to be clearly defined, so demonstration of efficacy will be complicated and extremely large clinical trials are likely to be required for sufficient statistical power in the face of these confounding factors and low ascertainment rates of certain patient populations hospitalised with RSV. Development of potential vaccines and therapeutics in the face of these limitations has meant long delays with significant associated risks and costs.

Experimental human RSV infection studies therefore offer a vital complementary approach, allowing controlled study with a defined viral inoculum, intensive longitudinal sampling and the opportunity to perform detailed investigations of pre-existing and pre-symptomatic immune responses as well as induced illness [9,10]. Human challenge studies have a long history with the inoculation of James Phipps by Edward Jenner in 1796 regarded as one of the earliest documented deliberate human infection experiments. Whilst controversial today, such experiments eventually led to vaccination and the eradication of smallpox nearly two centuries later, arguably one of the greatest triumphs of infectious disease science. Controlled human infection studies can also provide unique insights into pathogenesis and immunity, further facilitating drug and rational vaccine development. Using relatively small cohorts of volunteers with high attack rates after inoculation, such studies therefore provide a durable yet relatively rapid and cost-effective proof-of-concept for evaluation of potential vaccine candidates and therapies.

2. Logistical and ethical issues in deliberately infecting with RSV

RSV disease is seldom severe in healthy adults and older children, but there remains a theoretical possibility of unexpectedly severe illness in any experimental infection setting [11]. Furthermore, RSV is transmitted via respiratory droplets and fomites and

as such, experimentally-infected individuals pose a potential risk to others including research staff and the wider public [12]. These potential risks therefore pose practical and ethical dilemmas that must be addressed.

One advantage of controlled human infection studies is the ability to select relatively homogeneous groups of volunteers according to pre-defined criteria. In the majority of studies, these are healthy young adults, who are the least likely to suffer severe disease and have substantial physiological reserve so that recovery is swift and complete. However, challenge studies do not need to be conducted exclusively in healthy young adults; for example, deliberate rhinovirus infections of asthmatics and older adults with chronic obstructive pulmonary disease (who may or may not be smokers) are ongoing and findings have been highly relevant to understanding infective exacerbations in these patient groups [13,14]. As long as the central principle is met that the infection is either entirely self-limiting or completely treatable with no long-term sequelae in those studied, it is possible in principle to study defined individuals with some risk-factors. This criterion is certainly fulfilled by RSV challenge models, in which hundreds of individuals have been infected historically (Table 1) and no severe unexpected adverse events have been reported.

The potential for onward transmission means that research staff can and should take appropriate precautions with their own health and use proper personal protective equipment to minimise the possibility of becoming infected (or, conversely, transmit another infection to a research participant). Whether the wider public face a significant additional risk from inoculated research participants when compared to the natural risk of respiratory viral infections in everyday life is debatable. Nevertheless, thorough screening of potential research participants' social backgrounds to ensure they do not have any regular contact during the study period with individuals deemed at high risk of RSV-related complications should be mandatory. In contrast to experimental challenge with human rhinovirus [13] and bacterial pathogens such as *Salmonella typhi* [15] (which have all been carried out as out-patient studies), all contemporary experimental human RSV challenge studies have been carried out using in-patient quarantine to further isolate infected individuals from the community at large for the period of highest potential infectivity. Whether this is absolutely necessary remains unclear. Whilst there may be other advantages from the point of view of experimental control and convenience of obtaining samples, there is no UK or EU regulation that currently mandates the use of specific quarantine facilities [10]. The decision regarding whether to isolate study participants in individual negative-pressure side-rooms, or to co-house in hospital wards or other

Table 1
Historical experimental human RSV challenge studies describing numbers of subjects and their characteristics, particular RSV strain used and main observations and conclusions.

Characteristics and number of subjects	RSV strain used	Main observations	Year and reference
33 males, no history of cardiac, respiratory or allergic disease	RSV (Long) – wild-type strain isolated in 1957	High-dose (5 log TCID50) found to be effective. Titre of nasal wash antibody inversely correlated with susceptibility to infection	Mills et al. [19]
32 young adults, free of atopic disease	RSV (A2, wild-type strain isolated from Australia 1961)	5.2 log TCID50 found to be effective dose. Route of inoculation compared; eye and nose but not mouth found to be viable	Hall et al. [22]
105 healthy adults aged 18–50 years	RSS-2 (wild-type strain) (n = 20) and 4 different ts-mutants (n = 22 per group)	RSS-2 was virulent and induced serological response in 90% and clinical cold in 45%; ts-mutant strains induced much less illness and two strains produced serological response in 68%	McKay et al. [20] and Watt et al. [21]
102 healthy adults aged 18–55 years	RSS-2 (5 subjects challenged with saline)	Study designed to assess prophylactic and therapeutic effect of intranasal interferon α ; benefit seen for prophylaxis but not as therapy	Higgins et al. [23]
15 young healthy adults with recent laboratory confirmed natural RSV infection	RSV A2	Study designed to determine duration of immunity to reinfection; immunity associated with serum neutralising antibody to F and G proteins, but protection short-lived	Hall et al. [24]
36 healthy adults aged 18–45 years	RSV A2 (8 subjects challenged with placebo)	Dose of 4.7 log TCID50 and 3.7 log TCID50 used; no difference in symptoms or infection rates with different doses; infection rate inversely correlated with serum neutralisation titre; virus appeared attenuated (mild symptoms only in all subjects)	Lee et al. [25]

living arrangements therefore remain with the investigators in consultation with their local ethics committee. Since the costs of these facilities may be prohibitively high, flexibility in terms of quarantine has a profound influence on whether academic groups can perform these types of studies. If the field is to expand to address deeper scientific questions about biology and pathogenesis, either quarantine capacity will need to be expanded or alternate methods be used, e.g. ambulatory studies with self-isolation. The latter is attractive from a cost and logistics perspective, and self-isolation is already used by public health authorities to control less severe contagious threats (which RSV would not exceed), although this would require proper scrutiny to ensure due diligence.

Deliberately infecting human beings, especially with the aim of inducing illness, has ethical implications. Such experiments would not normally provide any meaningful benefit to the participants themselves, so potential harms cannot be justified or offset and must therefore be minimised. Whilst the development of potentially life-saving interventions against RSV is relevant to all, the interests of science or society do not override the interests of individual participants [16]. Informed consent is crucial to the proper conduct of infection challenge studies and it is imperative that potential participants are fully aware of the risks and possible harms to which they may be exposed. However, informed consent alone is not sufficient justification for research: there must also be a limit to the harms that potential participants can be exposed to, no matter what the perceived societal need. Safety of participants is of primary concern at all times and screening of potential volunteers should be designed to exclude any potential participant deemed at risk of severe illness.

With respect to RSV, healthy young adults (and even healthy individuals in the older age group) would at most be expected to experience a common cold illness. Such illnesses are a part of normal life and as such, the risk and potential harms of acquiring one experimental cold is not usually considered significant from an ethical point of view. The research procedures themselves should generally be minimally invasive but more invasive sampling, e.g. bronchoscopy or biopsy may be justified scientifically provided the frequency of procedures is kept at a minimum. Recently, these have been permitted on ethical review with assurances that they are carried out in clinical units with track records of safety and evidence of sufficient staffing and training. These have allowed direct access to respiratory mucosa, enabling sampling of local immune cells and epithelia, which is understood to be critical for further understanding of how to limit respiratory viral disease in man [17].

3. Historical RSV challenge studies

In 1961, Kravetz et al. first successfully infected twenty adult volunteers with an RSV strain isolated in 4 years before from a patient with bronchopneumonia [18]. Since that time there have been numerous challenge studies of human RSV involving over 300 subjects using both wild-type and attenuated strains without reported serious adverse events (Table 1) [19–25]. Insights into the influence of route of infection, incubation periods, durations of protective immunity and viral shedding were gained from these studies, but until recently limitations in technology have meant smaller contributions to understanding of immuno-pathogenesis or facilitating the development of effective vaccines. Previous human RSV challenge studies used viruses (e.g. Long and A2) isolated by serial laboratory passage in multiple cell lines or live-attenuated strains. Successful infection required the use of very high doses and were often minimally symptomatic, suggesting that these viral strains had been extensively laboratory-adapted and even so-called wild-type strains were likely attenuated [25,26].

Furthermore, these viruses had first been isolated in the 1960s, so differ somewhat from currently circulating RSV strains, thus affecting the applicability of those findings to modern infection. Also, it has been difficult to ensure consistency between stocks in different laboratories. Together, these issues have posed a problem when extrapolating these historical data to understanding of natural infection, and raise some uncertainties over the generalisability of some findings.

4. The contemporary human RSV challenge model

More recently, a wild-type, low-passage strain of RSV A has been produced according to Good Manufacturing Practice (GMP) guidelines specifically for human challenge. The progenitor virus, RSV Memphis 37 (M37), was isolated from a child with bronchiolitis in 2006 [27]. RSV M37 was grown to high titre in Vero cell culture and individual lots tested extensively for the presence of adventitious agents. While GMP challenge pathogens are generally considered the standard for human experimental infections, in the UK and EU there are no specific regulations that stipulate a requirement for GMP manufacture. Instead, if sufficient testing for adventitious agents can be demonstrated, GMP manufacturing may not be required. Nevertheless, worldwide this is not generally true and in any case, the costs incurred in producing a GMP virus are in large part related to the testing, so are not greatly reduced by pursuing the non-GMP path [28].

The first published study using this virus in humans was by Zaas et al. in 2009 [29]. The objective was to identify a peripheral blood mRNA signature to diagnose acute respiratory viral infection. Nine out of twenty subjects challenged became infected and, while the baseline characteristics of the subjects and course of the illness were not described in detail, it was noted that symptoms peaked at a median 141.5 h post-inoculation. Transcriptomic analysis of whole blood identified a transcriptional signature associated with respiratory viral infection that distinguished symptomatically infected from uninfected individuals as well as those with bacterial infection with at least 93% accuracy. The following year DeVincento et al. [30] reported on a dose-ranging study involving thirty-five healthy adults challenged intranasally with increasing doses of RSV M37 from 3.0 to 5.4 log₁₀ PFU mL⁻¹. Unlike the earlier study, subjects were screened so that only individuals with serum RSV neutralising antibody titres in the lower third of those tested were enrolled. The key findings were an infection rate of 77% with no statistical differences in infection rate for different inoculating doses; a dose of 4 log₁₀ PFU mL⁻¹ has since been commonly used as the standard inoculum. The incubation period was 3.1 days and virus was shed for a mean of 7.4 days. Viral load and symptoms peaked around day 6 post-inoculation and a significant correlation was observed between viral load and respiratory tract symptom scores. These findings have been consistent in subsequent studies including those that we performed several years later with the same virus, indicating the stability of the stock and the clinical syndrome it causes (Fig. 1). Additionally, nasal lavage concentrations of tumour necrosis factor (TNF), interleukin (IL) 6, IL8, and macrophage inflammatory protein (MIP) 1α were noted as biomarkers of clinical disease and a significant positive correlation was observed between cumulative nasal IL6 levels and cumulative viral load. The authors concluded that viral load was the main driver of clinical disease and the study therefore proposed a model by which to demonstrate that antiviral activity by a drug or reduction in virus titre by vaccine-induced immunity could reduce RSV-associated illness.

RSV M37 is currently one of only two GMP strains available for human challenge. It has been shown to consistently cause mild and moderate disease with a relatively high attack rate even if volun-

teers are not selected on the basis of low serum antibody titres. The other, a GMP virus made by reverse genetics using the sequence of the laboratory RSV A2 strain, has recently been developed and the first study using it is currently underway (ClinicalTrials.gov identifier NCT02484417). However, as time progresses, these strains may become outdated as new RSV strains (such as currently circulating RSV A ON1 and RSV B BA strains that contain 60–72 nucleotide duplications in the attachment G protein) emerge and come to predominate globally [31,32].

5. Challenge models enable proof-of-concept for RSV antiviral development

The RSV M37 model as established by DeVincenzo has since been employed in a number of clinical studies as early proof-of-concept for directly acting antiviral drugs. This model was used to study 141 healthy volunteers who were randomised to receive the novel oral RSV fusion inhibitor GS5806 or placebo either on the first day of detectable viral shedding in nasal lavage or day 5 post-inoculation (whichever was earlier) [33]. Participants treated with GS-5806 had significantly decreased lower mean cumulative viral load by area-under-the-curve (AUC), lower mean AUC change from baseline symptom score and lower total mucus weight than those treated with placebo. No serious adverse events were reported. Recently, a second novel compound, ALS-008176, an oral

prodrug of a cytidine nucleoside analogue that inhibits RSV polymerase, was evaluated in 62 healthy adults with low serum RSV neutralising antibody titres using experimental challenge with RSV M37 [34]. ALS-008176 appeared possibly even more potent than GS-5806, achieving a more rapid reduction in viral load and had a higher theoretical genetic barrier to resistance; however, the authors conceded that the sample sizes were too small to conclude any definite difference. Whilst both these studies were only intended as a proofs-of-concept, GS-5806 and ALS-008176 represent a significant advance in RSV antiviral research and demonstrate the power of human experimental challenge in accelerating development of novel treatments for RSV infection.

A second lot of RSV M37 was acquired by Alnylam Pharmaceuticals to test the efficacy of a small interfering RNA (siRNA) directed against the mRNA of RSV N protein [35]. Although a trend was seen in the ALN-RSV01-treated group toward a lower viral load, the study was not powered to detect a statistically significant difference. As one of the only examples of RNA interference (RNAi) as a human therapeutic, again the use of experimental human challenge proved crucial as proof-of-concept.

Together these studies highlight the strengths of experimental challenge as a platform for early tests of efficacy for RSV therapeutics. The primary outcome of reduced viral load offers a clear and definitive target measure to enable go/no-go decisions using relatively small numbers of study subjects. It remains to be seen

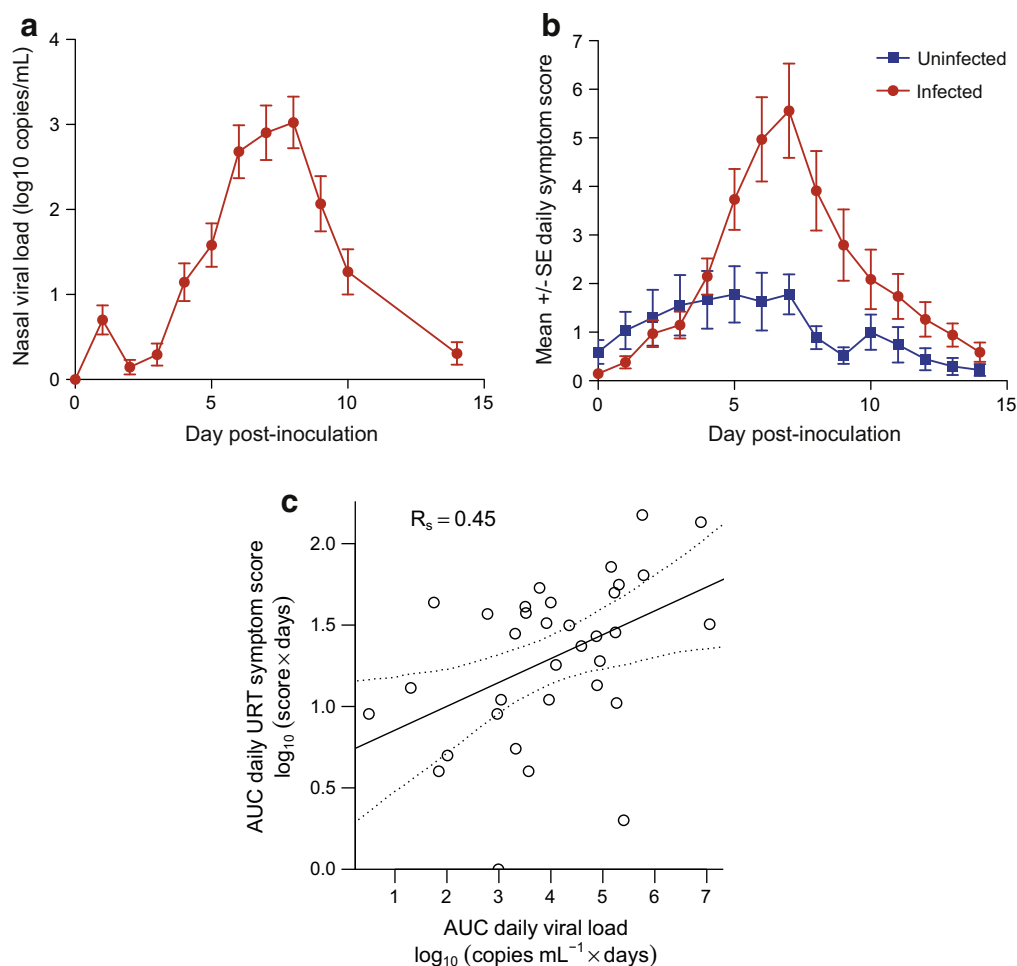


Fig. 1. Viral load and symptoms following experimental infection of 61 volunteers with RSV Memphis 37. Sixty-one healthy young adults were inoculated with $4 \log_{10}$ -PFU mL⁻¹ of RSV Memphis 37. (a) Viral load was measured daily by qPCR of nasal lavage. (b) Daily symptom scores were collected by self-reported symptom diaries. Subjects deemed infected following inoculation are shown in red, those deemed uninfected are shown in blue. (c) Spearman's rank-order correlation between cumulative viral load and symptoms is shown for infected individuals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

whether lowering of viral load to this extent is associated with clinical benefit in severely ill individuals, and indeed whether this degree of viral load reduction can even be achieved in the hospitalised individuals where treatment may be started late. The testing of antivirals is also arguably a more straightforward problem than that of vaccines. Understanding of the mode of action of the antiviral coupled with the ability to deliver it at an optimum time and dosage in relation to infection all help to optimise the model. In contrast, the development of vaccines against RSV involves substantially more complexity, with the nature of the vaccine; the type, level, timing and anatomical site of the immunity induced; and the interaction of host immunity (both pre-existing and vaccine-induced) with infecting virus all playing a role and where absolute protection or significant reductions in viral load may not be the only definitions of success.

6. Experimental challenge provides insights into pathogenesis

Despite the delays and setbacks that have affected the RSV vaccine field, the potentially enormous positive impact of effective RSV vaccines that can protect infants and/or frail elderly adults has continued to drive development, with over 50 vaccine candidates currently recognised [36]. In most other infectious diseases, successful vaccines have been developed empirically, without the *a priori* need to understand the true mechanism of protection but requiring defined measurable correlates of protection to predict efficacy [37]. However, predicting risk of infection based on immune correlates has been difficult to achieve in RSV, which induces only modest and transient immunity meaning that everyone remains susceptible throughout life despite relatively minor viral antigenic variation. This was demonstrated in historical RSV challenge studies, where re-infection by an identical strain through serial re-challenge of a cohort of infected volunteers every 2 months led to approximately 20% of subjects becoming re-infected on each occasion [24]. The partial immunity induced by RSV and resultant lack of individuals who are consistently protected against infection or disease have therefore made robust correlates of protection difficult to clearly determine in population-based studies.

Almost all successful vaccines rely on the generation of high titres of functional pathogen-specific antibodies as correlates of protection [38]. In RSV, serum neutralising antibody titres have been shown to negatively correlate with risk of medical attendance with RSV disease but no upper threshold of protection has been identifiable, with serum neutralising antibodies only broadly dividing individuals into those with minimal antibody (and therefore highly susceptible) and those with adult-like levels [39]. Previous observational studies indicated that local RSV-specific antibodies might be better correlates of protection but suggested that protective antibody levels were not well maintained [40,41]. To address these questions, we used the experimental human infection system to study the relationship between pre-existing local and systemic antibodies with infection [42]. No selection of subjects was made on the basis of pre-existing antibody levels and, among these highly immunologically experienced individuals, approximately 56% become infected following experimental challenge. Thus, while almost all had relatively high levels of serum neutralising antibody, some were still susceptible to infection while others were apparently immune. Serum neutralising antibodies by plaque-reduction neutralisation assay only loosely correlated with protection from PCR-confirmed infection. Instead, we found local mucosal RSV-specific IgA to be significantly more robustly predictive (Fig. 2). Measurement of RSV-specific IgA in respiratory samples have proved technically problematic in the field, with wide variability depending on the consistency of sam-

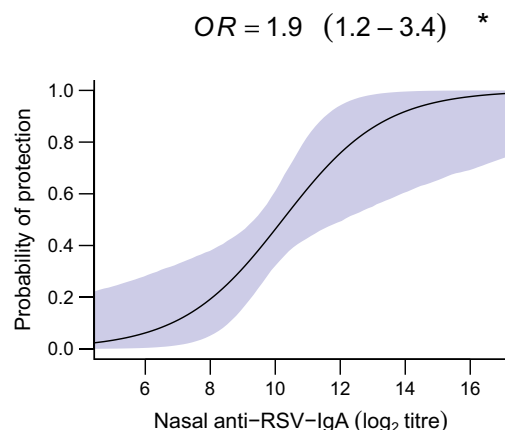


Fig. 2. RSV-specific nasal IgA titres correlate with protection from PCR-confirmed infection. Sixty-one healthy young adults were inoculated with RSV Memphis 37. RSV-specific nasal IgA end-point titres were measured in nasal washes by ELISA and normalised using relative urea concentrations in nasal lavage and serum. Logistic regression was achieved by a generalised linear model using a binomial (logit) link function in R. Odds ratio and 95% confidence intervals (blue) are shown. Figure adapted from Habibi et al. [42]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pling, but these were minimised by the controlled nature of the model and ability to normalise using comparative urea concentrations in nasal lavage and serum. Nevertheless, these data showed that RSV infection was poorly immunogenic and only 21% of subjects studied were predicted to have $\geq 80\%$ protection on the basis of nasal IgA levels induced even 4 weeks following infection. Furthermore, both serum and nasal antibody levels waned to pre-infection levels within 6 months. Analysis of peripheral blood memory B cells (MBCs) revealed an RSV-specific defect in the induction of anti-RSV IgA-secreting MBCs, which for the first time provided a possible explanation of why the natural immune response to RSV does not induce lasting protection from reinfection. These findings suggest that induction of mucosal antibodies may be more effective in preventing RSV infection compared with serum IgG, which might have a preferential role in preventing lower respiratory disease. However, these vaccines must also improve upon naturally-induced anti-RSV immunity by inducing higher antibody titres and circumventing any specific defect in the RSV-specific memory response.

Antibodies can protect against infection but once infection has occurred, they have little role in reducing disease severity [49,50]. Many licensed vaccines, such as those against influenza, remain suboptimal in inducing persistently high levels of antibody, particularly in age groups such as the elderly and young children. The likelihood of infection occurring in spite of having been vaccinated against RSV will therefore be substantial. T cell-inducing vaccines have a number of theoretical advantages, including the amelioration of disease if infection does occur; the potential to protect against multiple strains of the same virus in the face of some antigenic change; and helping B cells produce high affinity antibodies [43]. However, in primary RSV infection, T cells arise late and are not thought to contribute significantly to protection [7]. Furthermore, in mouse models they have been shown to cause immunopathology [44]. While most clinical studies have relied on peripheral blood to analyse T cell responses, animal models have highlighted the unique immunobiology of T cells in the lung. Until recently, it has not been possible to systematically study these in the context of acute RSV infection to clarify their importance and role.

We recently investigated the role of virus-specific T cells in RSV infection in the experimental human infection model [17]. Using MHC-peptide tetramers, we labelled and tracked RSV-specific

CD8+ T cells in blood and airways of experimentally infected volunteers, sampling the lower airway by bronchoscopy. We showed that RSV-specific CD8+ T cells are significantly more abundant in the airway compared with peripheral blood and almost all display the characteristic phenotypic markers (CD69 and CD103) of resident memory T (Trm) cells. Trm cells are a recently described memory T cell subset that are generated at sites of pathogen entry and remain at those sites poised to respond rapidly on re-infection [45]. They have been shown not only to clear infection but also coordinate the early recall response by recognition of antigen and cytokine production in an innate-like manner, altering the inflammatory environment to further recruit other memory T cells [46]. Thus, they are highly specialised to confer early protection, have been shown to provide heterosubtypic immunity against multiple strains of influenza and are strong candidates for the enhancement of vaccine-mediated immunity [47].

In the experimental human infection model, the frequency of RSV-specific CD8+ T cells in the airway (but not peripheral blood) prior to infection correlated with reduction in symptom severity and viral load on subsequent infection [17]. They were generated at extremely high frequencies during acute infection and continued to accumulate well into convalescence. However, they were relatively poor producers of cytotoxic molecules such as granzyme B and perforin, and together with an observed deficiency in production of type 1 cytokines by RSV-specific CD8+ T cells in blood compared to influenza, these data again suggest that RSV-specific adaptive immune responses are relatively impaired. Several RSV vaccine candidates have been developed that can be delivered intranasally with the aim of inducing both antibodies and T cells in the respiratory tract [48,49]. However, the development of Trm cells has not been investigated using any of these. Furthermore, little is yet known about the specificities and functional capacity of CD4+ T cells in human RSV. These remain important research avenues that must be explored to further understand optimal vaccination.

7. Limitations of studies of healthy young adults

While experimental human infections benefit from being conducted in the natural host of RSV in a controlled fashion, there remain a number of limitations. Practical and ethical hurdles have been discussed earlier but a fundamental limitation exists in studying healthy adults who only suffer mild-to-moderate disease, while severe complications of RSV mostly occur in high-risk people. Adult humans represent an immunologically mature and highly RSV-experienced population so the induced disease and observed host responses will inevitably differ from those in bronchiolitis of infants [50]. Extrapolating into that population should therefore be made with caution. Nevertheless, the heterogeneity of clinical outcome following experimental infection of healthy adults does facilitate the inference of protective immune mechanisms that may be universally applicable. Furthermore, whilst we must accept that deliberately infecting an infant or high-risk adult could never be justified ethically or scientifically, experimental rhinovirus colds have been safely induced in adults with mild COPD and moderate asthma [13,14]. It may therefore not be unreasonable to enrol healthy elderly persons in experimental studies with RSV with careful screening and safety considerations addressed.

Adults pre-screened for especially low serum neutralising antibodies do have a greater probability of developing a common-cold illness, making them an ideal group in which to demonstrate efficacy of antivirals, in which the increased attack rate allows reduction in sample size. However, it might be argued that these people are an unusual population group and particularly susceptible to RSV among adults, either due to lack of recent natural exposure

or some intrinsic vulnerability. Extrapolation of study endpoints in these groups to other, more immunologically-experienced individuals, such as the elderly, may underestimate the contribution of the host response. Therefore, it may necessary to accept the increased cost associated with larger sample sizes to power a vaccine challenge study sufficiently to detect statistically significant efficacy in more immunologically representative cohorts.

Successful human infection studies depend on the availability of well-characterised and standardised viral inocula. Although there is no current legal requirement for viral inocula to be prepared in accordance with Good Manufacturing Practice (GMP) in the EU it remains highly desirable to ensure comparability across studies and the safety of participants. Unfortunately, the creation of new and alternate GMP viruses will be time-consuming and costly, with a capital outlay that may be beyond most academic groups. This issue is not limited to RSV and is a major hurdle to the expansion of human infection challenge research in general [10]. Further cooperation between funders and consortia of experimental human infection researchers are likely to be required to enhance the sustainability of all these models in the face of these requirements by sharing of knowledge and infrastructure.

8. Conclusion

Experimental human infection with RSV has already provided proof-of-concept for therapeutics and furthered our understanding of the pathogenesis of the disease. Thus far, no group has published a study evaluating a potential RSV vaccine using this model but, in spite of its limitations, it is likely to be a powerful system in which to do so, given the high attack rate of at least 50% of unselected adults. Experimental human infection with RSV therefore offers a unique and vital opportunity to test antivirals, vaccines, and novel therapeutics, streamlining successful development and exploring mechanisms of pathogenesis of this enigmatic virus in the natural host. Industry and academic groups need to join forces to overcome the considerable tasks of the creation and maintenance of challenge agents as well as the logistics of quarantine in order to maintain the utility of these models, which will be essential in maintaining the momentum currently seen in RSV vaccine research.

Conflicts of interest

MSH declares no conflict of interest. CC holds a Wellcome Trust Translation Award in collaboration with Mucosis BV.

Acknowledgements

This work was funded in part by a Medical Research Council Clinician Scientist fellowship held by CC (G0902266) and the Wellcome Trust (087805/Z/08/Z).

References

- [1] Nair H, Nokes DJ, Gessner BD, Dherani M, Madhi SA, Singleton RJ, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. *Lancet* 2010;375:1545–55. [http://dx.doi.org/10.1016/S0140-6736\(10\)60206-1](http://dx.doi.org/10.1016/S0140-6736(10)60206-1).
- [2] Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. Respiratory syncytial virus infection in elderly and high-risk adults. *N Engl J Med* 2005;352:1749–59. <http://dx.doi.org/10.1056/NEJMoa043951>.
- [3] Blanken MO, Rovers MM, Molenaar JM, Winkler-Seinstra PL, Meijer A, Kimpen JLL, et al. Respiratory syncytial virus and recurrent wheeze in healthy preterm infants. *N Engl J Med* 2013;368:1791–9. <http://dx.doi.org/10.1056/NEJMoa1211917>.
- [4] McLellan JS, Chen M, Joyce MG, Sastry M, Stewart-Jones GBE, Yang Y, et al. Structure-based design of a fusion glycoprotein vaccine for respiratory syncytial virus. *Science* 2013;342:592–8. <http://dx.doi.org/10.1126/science.1243283>.

- [5] Bem RA, Domachowske JB, Rosenberg HF. Animal models of human respiratory syncytial virus disease. *Am J Physiol Lung Cell Mol Physiol* 2011;301:L148–56. <http://dx.doi.org/10.1152/ajplung.00065.2011>.
- [6] Tregoning JS, Wang BL, McDonald JU, Yamaguchi Y, Harker JA, Goritzka M, et al. Neonatal antibody responses are attenuated by interferon- γ produced by NK and T cells during RSV infection. *Proc Natl Acad Sci* 2013. <http://dx.doi.org/10.1073/pnas.1214247110>.
- [7] Heidema J, Lukens MV, van Maren WW, van Dijk ME, Otten HG, van Vught AJ, et al. CD8+ T cell responses in bronchoalveolar lavage fluid and peripheral blood mononuclear cells of infants with severe primary respiratory syncytial virus infections. *J Immunol* 2007;179:8410–7.
- [8] Eun-Hyung Lee F, Falsey AR, Halliley JL, Sanz I, Walsh EE. Circulating antibody-secreting cells during acute respiratory syncytial virus infection in adults. *J Infect Dis* 2010;202:1659–66. <http://dx.doi.org/10.1086/65715>.
- [9] DeVincenzo JP, Wilkinson T, Vaishnav A, Cehelsky J, Meyers R, Nochor S, et al. Viral load drives disease in humans experimentally infected with respiratory syncytial virus. *Am J Respir Crit Care Med* 2010;182:1305–14. <http://dx.doi.org/10.1164/rccm.201002-0221OC>.
- [10] Darton TC, Blohmke CJ, Moorthy VS, Altmann DM, Hayden FG, Clutterbuck EA, et al. Design, recruitment, and microbiological considerations in human challenge studies. *Lancet Infect Dis* 2015;15:840–51. [http://dx.doi.org/10.1016/S1473-3099\(15\)00068-7](http://dx.doi.org/10.1016/S1473-3099(15)00068-7).
- [11] Openshaw PJ, Chiu C. Protective and dysregulated T cell immunity in RSV infection. *Curr Opin Virol* 2013;3:468–74. <http://dx.doi.org/10.1016/j.coviro.2013.05.005>.
- [12] Graham BS. Protecting the family to protect the child: vaccination strategy guided by RSV transmission dynamics. *J Infect Dis* 2014. <http://dx.doi.org/10.1093/infdis/jiu07>.
- [13] Zhu J, Message SD, Qiu Y, Mallia P, Kebadze T, Contoli M, et al. Airway inflammation and illness severity in response to experimental rhinovirus infection in asthma. *Chest* 2014;145:1219–29. <http://dx.doi.org/10.1378/chest.13-1567>.
- [14] Mallia P, Message S, Kebadze T, Parker H, Kon O, Johnston S. An experimental model of rhinovirus induced chronic obstructive pulmonary disease exacerbations: a pilot study. *Respir Res* 2006;7:116.
- [15] Blohmke CJ, Darton TC, Jones C, Suarez NM, Waddington CS, Angus B, et al. Interferon-driven alterations of the host's amino acid metabolism in the pathogenesis of typhoid fever. *J Exp Med* 2016;213:1061–77. <http://dx.doi.org/10.1084/jem.20151025>.
- [16] Hope T, McMillan J. Challenge studies of human volunteers: ethical issues. *J Med Ethics* 2004;30:110–6.
- [17] Jozwik A, Habibi MS, Paras A, Zhu J, Guvenel A, Dhariwal J, et al. RSV-specific airway resident memory CD8+ T cells and differential disease severity after experimental human infection. *Nat Commun* 2015;6:10224. <http://dx.doi.org/10.1038/ncomms10224>.
- [18] Kravetz HM, Knight V, Chanock RM, Morris JA, Johnson KM, Rifkind D, et al. Respiratory syncytial virus. III. Production of illness and clinical observations in adult volunteers. *JAMA. J Am Med Assoc* 1961;176:657–63.
- [19] Mills J, Van Kirk JE, Wright PF, Chanock RM. Experimental respiratory syncytial virus infection of adults. Possible mechanisms of resistance to infection and illness. *J Immunol Baltim Md* 1971;107:123–30. 1950.
- [20] McKay E, Higgins P, Tyrrell D, Pringle C. Immunogenicity and pathogenicity of temperature-sensitive modified respiratory syncytial virus in adult volunteers. *J Med Virol* 1988;25:411–21.
- [21] Watt PJ, Robinson BS, Pringle CR, Tyrrell DA. Determinants of susceptibility to challenge and the antibody response of adult volunteers given experimental respiratory syncytial virus vaccines. *Vaccine* 1990;8:231–6.
- [22] Hall CB, Douglas RG, Schnabel KC, Geiman JM. Infectivity of respiratory syncytial virus by various routes of inoculation. *Infect Immun* 1981;33:779–83.
- [23] Higgins PG, Barrow GI, Tyrrell DAJ, Isaacs D, Gauci CL. The efficacy of intranasal interferon- α 2a in respiratory syncytial virus infection in volunteers. *Antiviral Res* 1990;14:3–10. [http://dx.doi.org/10.1016/0166-3542\(90\)90061-B](http://dx.doi.org/10.1016/0166-3542(90)90061-B).
- [24] Hall C, Walsh E, Long C, Schnabel K. Immunity to and frequency of reinfection with respiratory syncytial virus. *J Infect Dis* 1991;163:693–8.
- [25] Lee FE-H, Walsh EE, Falsey AR, Betts RF, Treanor JJ. Experimental infection of humans with A2 respiratory syncytial virus. *Antiviral Res* 2004;63:191–6. <http://dx.doi.org/10.1016/j.antiviral.2004.04.00>.
- [26] Cherrie AH. Human immune responses to respiratory syncytial virus PhD Thesis. London: Dept Medicine, St Mary's Hospital Medical School, Imperial College; 1992.
- [27] Kim Y-I, DeVincenzo JP, Jones BG, Rudraraju R, Harrison L, Meyers R, et al. Respiratory syncytial virus human experimental infection model: provenance, production, and sequence of low-passaged Memphis-37 Challenge Virus. *PLoS ONE* 2014;9:e113100. <http://dx.doi.org/10.1371/journal.pone.0113100>.
- [28] U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER). Guidance for Industry Influenza: Developing Drugs for Treatment and/or Prophylaxis. 2011. <<http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm091219.pdf>> [accessed 31.07.16].
- [29] Zaas AK, Chen M, Varkey J, Veldman T, Hero AO, Lucas J, et al. Gene expression signatures diagnose influenza and other symptomatic respiratory viral infection in humans. *Cell Host Microbe* 2009;6:207–17. <http://dx.doi.org/10.1016/j.chom.2009.07.006>.
- [30] DeVincenzo JP, Wilkinson T, Vaishnav A, Cehelsky J, Meyers R, Nochor S, et al. Viral load drives disease in humans experimentally infected with respiratory syncytial virus. *Am J Respir Crit Care Med* 2010;182:1305–14. <http://dx.doi.org/10.1164/rccm.201002-0221OC>.
- [31] Eshaghi A, Duvvuri VR, Lai R, Nadarajah JT, Li A, Patel SN, et al. Genetic variability of human respiratory syncytial virus A strains circulating in Ontario: a novel genotype with a 72 nucleotide G gene duplication. *PLoS ONE* 2012;7:e32807. <http://dx.doi.org/10.1371/journal.pone.0032807>.
- [32] Hotard AL, Laikhter E, Brooks K, Hartert TV, Moore ML. Functional analysis of the 60-nucleotide duplication in the respiratory syncytial virus buenos aires strain attachment glycoprotein. *J Virol* 2015;89:8258–66. <http://dx.doi.org/10.1128/JVI.01045-15>.
- [33] DeVincenzo JP, Whitley RJ, Mackman RL, Scaglioni-Weinlich C, Harrison L, Farrell E, et al. Oral GS-5806 activity in a respiratory syncytial virus challenge study. *N Engl J Med* 2014;371:711–22. <http://dx.doi.org/10.1056/NEJMoa1401184>.
- [34] DeVincenzo JP, McClure MW, Symons JA, Fathi H, Westland C, Chanda S, et al. Activity of oral ALS-008176 in a respiratory syncytial virus challenge study. *N Engl J Med* 2015;373:2048–58. <http://dx.doi.org/10.1056/NEJMoa1413275>.
- [35] DeVincenzo J, Cehelsky JE, Alvarez R, Elbashir S, Harborth J, Toudjarska I, et al. Evaluation of the safety, tolerability and pharmacokinetics of ALN-RSV01, a novel RNAi antiviral therapeutic directed against respiratory syncytial virus (RSV). *Antiviral Res* 2008;77:225–31. <http://dx.doi.org/10.1016/j.antiviral.2007.11.009>.
- [36] Guvenel AK, Chiu C, Openshaw PJ. Current concepts and progress in RSV vaccine development. *Expert Rev Vaccines* 2014;13:333–44. <http://dx.doi.org/10.1586/14760584.2014.878653>.
- [37] Pulendran B, Ahmed R. Immunological mechanisms of vaccination. *Nat Immunol* 2011;12:509–17.
- [38] Plotkin SA. Complex correlates of protection after vaccination. *Clin Infect Dis* 2013;56:1458–65. <http://dx.doi.org/10.1093/cid/cit048>.
- [39] Piedra PA, Jewell AM, Cron SG, Atmar RL, Paul Glezen W. Correlates of immunity to respiratory syncytial virus (RSV) associated-hospitalization: establishment of minimum protective threshold levels of serum neutralizing antibodies. *Vaccine* 2003;21:3479–82. [http://dx.doi.org/10.1016/S0264-410X\(03\)00355-4](http://dx.doi.org/10.1016/S0264-410X(03)00355-4).
- [40] Walsh EE, Falsey AR. A simple and reproducible method for collecting nasal secretions in frail elderly adults, for measurement of virus-specific IgA. *J Infect Dis* 1999;179:1268–73.
- [41] Falsey A, Singh H, Walsh E. Serum antibody decay in adults following natural respiratory syncytial virus infection. *J Med Virol* 2006;78:1493–7.
- [42] Habibi MS, Jozwik A, Makris S, Dunning J, Paras A, DeVincenzo JP, 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;191:1040–9. <http://dx.doi.org/10.1164/rccm.201412-2256OC>.
- [43] Altenburg AF, Rimmelzwaan GF, de Vries RD. Virus-specific T cells as correlate of (cross-)protective immunity against influenza. *Vaccine* 2015;33:500–6. <http://dx.doi.org/10.1016/j.vaccine.2014.11.054>.
- [44] Cannon MJ, Openshaw PJ, Askonas BA. Cytotoxic T cells clear virus but augment lung pathology in mice infected with respiratory syncytial virus. *J Exp Med* 1988;168:1163–8. <http://dx.doi.org/10.1084/jem.168.3.1163>.
- [45] Park CO, Kupper TS. The emerging role of resident memory T cells in protective immunity and inflammatory disease. *Nat Med* 2015;21:688–97. <http://dx.doi.org/10.1038/nm.3883>.
- [46] Schenkel JM, Fraser KA, Vezys V, Masopust D. Sensing and alarm function of resident memory CD8+ T cells. *Nat Immunol* 2013;14:509–13. <http://dx.doi.org/10.1038/ni.2568>.
- [47] Wu T, Hu Y, Lee Y-T, Bouchard KR, Benechet A, Khanna K, et al. Lung-resident memory CD8 T cells (TRM) are indispensable for optimal cross-protection against pulmonary virus infection. *J Leukoc Biol* 2014;95:215–24. <http://dx.doi.org/10.1189/jlb.0313180>.
- [48] Rigger A, Widjaja I, Versantvoort H, Coenjaerts FEJ, van Roosmalen M, Leenhouts K, et al. A protective and safe intranasal RSV vaccine based on a recombinant prefusion-like form of the F protein bound to bacterium-like particles. *PLoS ONE* 2013;8:e71072. <http://dx.doi.org/10.1371/journal.pone.0071072>.
- [49] Green CA, Scarselli E, Sande CJ, Thompson AJ, de Lara CM, Taylor KS, et al. Chimpanzee adenovirus- and MVA-vectored respiratory syncytial virus vaccine is safe and immunogenic in adults. *Sci Transl Med* 2015;7. <http://dx.doi.org/10.1126/scitranslmed.aac574>. 300ra126–300ra126.
- [50] Lambert L, Sagfors AM, Openshaw PJM, Culley FJ. Immunity to RSV in early-life. *Immunother Vaccines* 2014;5:466. <http://dx.doi.org/10.3389/fimmu.2014.00466>.