Gmail **Christine, of the Massey family <cmssyc@gmail.com>**

FOIA request to CDC re: scientific proof of "RSV", or purification

Christine Massey <cmssyc@gmail.com> Sat, Nov 12, 2022 at 9:41 AM
To: "FOIA Requests (CDC)" <FOIARequests@cdc.gov>

November 12, 2022

To:
Roger Andoh
Freedom of Information Officer
1600 Clifton Rd NE MS T-01
Atlanta, Georgia 30333
Email: FOIARequests@cdc.gov
Phone: 770-488-6277
Fax: 770-488-6200

Dear Roger,

I require access to general records, as per the *Freedom of Information Act.*

Description of Requested Records:

1. All studies/reports in the possession, custody or control of the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) that scientifically prove the existence of the alleged "RSV" (respiratory syncytial virus).

Note:
Scientific proof is NOT

- Opinions
- Speculation
- Review papers
- Descriptive papers

Scientific proof requires

- Use of the scientific method
- Repeatable and falsifiable hypotheses that have been tested using valid, controlled experiments where only 1 variable differs between the experimental and control groups
- In this case, the 1 manipulated variable would be the presence/absence of purified particles suspected of being a "virus"
- Consistent results from valid, controlled experiments (i.e. identical "genomes", consistent in vivo effects)

Records that do not describe the testing of falsifiable, repeatable hypotheses regarding the existence of this alleged "virus" (meaning the existence of the alleged particle and its alleged causation of disease) are disqualified from my request.

2. **If the CDC has no studies responsive to #1 above**, please indicate such explicitly, and provide all studies and/or reports in the possession, custody or control of the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) describing the **purification** of this alleged virus, directly **from bodily fluid/tissue/excrement or from a cell culture, with purification confirmed via EM imaging** (the images must be available as well).

Please note that I am not requesting studies/reports where researchers failed to **purify** the suspected "virus" and instead:

- cultured an unpurified sample or other unpurified substance, and/or
- performed an amplification test (i.e. a PCR test), and/or
- produced an in silico "genome", and/or

- produced electron microscopy images of unpurified things.

For further clarity, please note I am already aware that according to virus theory a "virus" requires host cells in order to replicate, and I am **not** requesting records describing the **replication** of a "virus" without host cells.

Further, I am **not** requesting records that describe a suspected "virus" floating in a vacuum; I am simply requesting records that describe its **purification** (**separation** from everything else in the patient sample, as per standard laboratory practices for the purification of other small things).

General Note:
Please also note that my request is **not limited** to records that were authored by the CDC or ATSDR or that pertain to work done at/by the CDC or ATSDR. Rather, my request includes any record matching the above description authored by anyone, anywhere, ever.

Publicly Available Records
If any records match the above description of requested records and are currently available to the public elsewhere, please assist me by providing enough information about each record so that I may identify and access each one with certainty (i.e. title, author(s), date, journal, where the public may access it). Please provide URLs where possible.

Format:
Pdf documents sent to me via email; I do not wish for anything to be shipped to me.

Contact Information:
Christine Massey
Ontario, Canada
Email: cmssyc@gmail.com

Thank you in advance and best wishes,
Christine Massey

Gmail **Christine Massey <cmssyc@gmail.com>**

Your CDC FOIA Request #23-00263-FOIA

MNHarper@cdc.gov <MNHarper@cdc.gov> Wed, Nov 16, 2022 at 12:32 PM
To: cmssyc@gmail.com

November 16, 2022

Request Number: 23-00263-FOIA

Dear Ms. Massey:

This is regarding your Freedom of Information Act (FOIA) request of November 12, 2022, for 1. All studies/reports in the possession, custody or control of the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) that scientifically prove the existence of the alleged "RSV" (respiratory syncytial virus)..

Please see the attached letter.

Sincerely,
CDC/ATSDR FOIA Office
770-488-6399

2 attachments

23-00263 Acknowledgement (Complex) 30 Days (027).pdf
130K

FOIA request to CDC re_ scientific proof of _RSV__ or purification.msg
103K

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control
and Prevention (CDC)
Atlanta GA 30333

November 16, 2022

Christine Massey
[redacted]
Via email: cmssyc@gmail.com

Dear Ms. Massey:

The Centers for Disease Control and Prevention and Agency for Toxic Substances and Disease Registry (CDC/ATSDR) received your attached Freedom of Information Act (FOIA) request dated November 12, 2022. Your request assigned number is 23-00263-FOIA, and it has been placed in our complex processing queue.

In unusual circumstances, an agency can extend the twenty-working-day limit to respond to a FOIA request.

We will require more than thirty working days to respond to your request because we reasonably expect that two or more CDC centers, institutes, and offices (C/I/Os) may have responsive records.

To process your request promptly, please consider narrowing the scope of your request to limit the number of responsive records. If you have any questions or wish to discuss reformulation or an alternative time frame for the processing of your request, you may contact the analyst handling your request Mark Harper at 770-488-8154 or our FOIA Public Liaison, Roger Andoh at 770-488-6277. Additionally, you may contact the Office of Government Services (OGIS) to inquire about the FOIA mediation services they offer. The contact information for OGIS is as follows: Office of Government Information Services; National Archives and Records Administration; 8601 Adelphi Road-OGIS; College Park, Maryland 20740-6001; e-mail at ogis@nara.gov; telephone at 202-741-5770; toll free at 1-877-684-6448; or facsimile at 202-741-5769.

Because you are considered an "Other requester" you are entitled to two hours of free search time, and up to 100 pages of duplication (or the cost equivalent of other media) without charge, and you will not be charged for review time. We may charge for search time beyond the first two hours and for duplication beyond the first 100 pages. (10 cents/page).

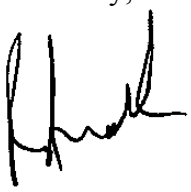
If you don't provide us with a date range for your request, the cut-off date for your request will be the date the search for responsive records starts.

You may check on the status of your case on our FOIA webpage https://foia.cdc.gov/app/Home.aspx and entering your assigned request number. If you have any questions regarding your request, please contact me at 770-488-8154 or via email at wzj6@cdc.gov.

We reasonably anticipate that you should receive documents by February 6,2023. Please know that this date roughly estimates how long it will take the Agency to close requests ahead of your request in the queue and complete work on your request.

The actual date of completion might be before or after this estimated date.

Sincerely,



Roger Andoh
CDC/ATSDR FOIA Officer
Office of the Chief Operating Officer
(770) 488-6399
Fax: (404) 235-1852

23-00007-FOIA

Gmail

Christine, of the Massey family <cmssyc@gmail.com>

Your CDC FOIA Request #23-00263-FOIA

MNHarper@cdc.gov <MNHarper@cdc.gov> Wed, Jan 11, 2023 at 7:52 AM
To: cmssyc@gmail.com

January 11, 2023

Request Number: 23-00263-FOIA

Dear Ms. Massey:

This is regarding your Freedom of Information Act (FOIA) request of November 12, 2022, for 1. All studies/reports in the possession, custody or control of the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) that scientifically prove the existence of the alleged "RSV" (respiratory syncytial virus).

Please see the attached letter.

Sincerely,
CDC/ATSDR FOIA Office
770-488-6399

2 attachments

Final Response 23-00263 Jan 11 20223.pdf
194K

82_2022_257.pdf
572K

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Centers for Disease Control
and Prevention (CDC)
Atlanta GA 30333

January 11, 2023

Ms. Christine Massey
[redacted]
Via email: cmssyc@gmail.com

Dear Ms. Massey:

This letter is in response to your Centers for Disease Control and Prevention and Agency for Toxic Substances and Disease Registry (CDC/ATSDR) Freedom of Information Act (FOIA) request of November 12, 2022, for:

> *Dear Roger,*
>
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> - *Use of the scientific method*
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> - *In this case, the 1 manipulated variable would be the presence/absence of purified particles suspected of being a "virus"*
> - *Consistent results from valid, controlled experiments (i.e. identical "genomes", consistent in vivo effects)*
>
> *Records that do not describe the testing of falsifiable, repeatable hypotheses regarding the existence of this alleged "virus" (meaning the existence of the alleged particle and its alleged causation of disease) are disqualified from my request.*

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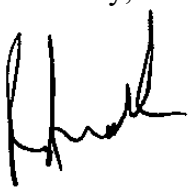
***Format**:*
Pdf documents sent to me via email; I do not wish for anything to be shipped to me.

Beyond the attached publication provided by the NCIRD subject matter expert which may or may not meet you exclusionary criteria, a search of our records failed to reveal any documents pertaining to your request.

You may contact our FOIA Public Liaison at 770-488-6246 for any further assistance and to discuss any aspect of your request. Additionally, you may contact the Office of Government Information Services (OGIS) at the National Archives and Records Administration to inquire about the FOIA mediation services they offer. The contact information for OGIS is as follows: Office of Government Information Services, National Archives and Records Administration, 8601 Adelphi Road-OGIS, College Park, Maryland 20740-6001, e-mail at ogis@nara.gov; telephone at 202-741-5770; toll free at 1-877-684-6448; or facsimile at 202-741-5769.

If you are not satisfied with the response to this request, you may administratively appeal to the Deputy Agency Chief FOIA Officer, Office of the Assistant Secretary for Public Affairs, U.S. Department of Health and Human Services, via the online portal at https://requests.publiclink.hhs.gov/App/Index.aspx. Please mark both your appeal letter and envelope "FOIA Appeal." Your appeal must be electronically transmitted by April 11, 2023.

Sincerely,



Roger Andoh
CDC/ATSDR FOIA Officer
Office of the Chief Operating Officer
(770) 488-6399
Fax: (404) 235-1852

#23-00263-FOIA

Controlled Human Infection Challenge Studies with RSV

Pete Dayananda, Christopher Chiu, and Peter Openshaw

Contents

Abstract Despite considerable momentum in the development of RSV vaccines and therapeutics, there remain substantial barriers to the development and licensing of effective agents, particularly in high-risk populations. The unique immunobiology of RSV and lack of clear protective immunological correlates has held back RSV vaccine development, which, therefore, depends on large and costly clinical trials to demonstrate efficacy. Studies involving the deliberate infection of human volunteers offer an intermediate step between pre-clinical and large-scale studies of natural infection. Human challenge has been used to demonstrate the potential efficacy of vaccines and antivirals while improving our understanding of the protective immunity against RSV infection. Early RSV human infection challenge studies determined the role of routes of administration and size of inoculum on the disease. However,

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National Heart and Lung Institute, Imperial College London, London, UK
e-mail: p.openshaw@imperial.ac.uk

Current Topics in Microbiology and Immunology
https://doi.org/10.1007/82_2022_257

inherent limitations, the use of highly attenuated/laboratory-adapted RSV strains and the continued evolutionary adaptation of RSV limits extrapolation of results to present-day vaccine testing. With advances in technology, it is now possible to perform more detailed investigations of human mucosal immunity against RSV in experimentally infected adults and, more recently, older adults to optimise the design of vaccines and novel therapies. These studies identified defects in RSV-induced humoral and CD8+ T cell immunity that may partly explain susceptibility to recurrent RSV infection. We discuss the insights from human infection challenge models, ethical and logistical considerations, potential benefits, and role in streamlining and accelerating novel antivirals and vaccines against RSV. Finally, we consider how human challenges might be extended to include relevant at-risk populations.

1 Introduction

Human respiratory syncytial virus (RSV) is an enveloped single-stranded RNA virus that primarily infects ciliated bronchial epithelial cells. RSV infection is dependent on two glycoproteins on the surface of the virion—the attachment protein (RSV G) and fusion protein (RSV F). The fusion protein exists in two conformations. The pre-fusion (pre-F) conformation is metastable and is primarily found on infectious virions. Conformational change from the pre-F to the more stable post-fusion (post-F) mediates fusion of the viral envelope with the cell membrane (Russell et al. 2017). Antibodies directed against pre-F are more potent in neutralising viruses (Crank et al. 2019) and recent vaccine development has generally been directed to the generation of antibodies against pre-F epitopes (Ruckwardt et al. 2021; Williams et al. 2020; Sadoff et al. 2021).

RSV was first isolated from a colony of chimpanzees with coryza in 1956 and subsequently recognised as a cause of viral bronchiolitis in human infants (Hall 2001). RSV is a ubiquitous pathogen affecting almost all children by 2 years of age (Bont et al. 2016). In 2015, there were an estimated 33.1 million episodes of RSV associated lower respiratory tract infections (LRTI) in children under 5 years old, including 3.2 million hospitalisation episodes and 118,000 deaths. Approximately 45% of hospitalisations (and deaths) occur in infants under 6 months of age (Shi et al. 2017).

Despite decades of intensive research, RSV continues to be one of the commonest causes of LRTIs in childhood, accounting for 22% of all acute episodes worldwide (Barr et al. 2019). In addition, RSV associated LRTIs are associated with recurrent chest infections, wheezing (Verwey and Nunes 2020; Zar et al. 2020) and the development of chronic respiratory disease in adulthood, particularly if hospitalisation is required (Bui et al. 2018). Recurrent wheezing significantly impacts the child's education and development as well as being a significant public and global health problem. Direct costs of asthma management in the UK is estimated to be at least £1.1 billion (Mukherjee et al. 2016). A better understanding of the unique immunobiology of RSV disease and reduction of subsequent recurrent wheeze would have the potential to improve the quality of life of children as well as having considerable economic benefits.

In comparison, RSV in adults has received relatively little attention. Although RSV regularly reinfects throughout life, RSV infections are much less serious in young adults compared with infants; infection is generally limited to the upper respiratory tract with full recovery post-infection (Falsey and Walsh 2000). Hence, controlled human infection challenge studies have been conducted almost exclusively in healthy young adults (Habibi and Chiu 2017). However, RSV is a major cause of morbidity and mortality in hospitalised older adults, perhaps even comparable to that caused by influenza (Shi et al. 2020; Ackerson et al. 2018; Zhang et al. 2020). Globally, Shi et al. estimated that there were approximately 1.5 million episodes of RSV associated acute respiratory infections in older adults (age $\geq$ 65 years old), leading to 336 000 hospital admissions and 14,000 in-hospital deaths (Shi et al. 2020). A subsequent prospective cohort study found that the annual incidence in healthy community-dwelling older adults is between 1.6 and 7% (Korsten et al. 2020). Although older adults are generally more susceptible to RSV disease, studies of natural infection with RSV in older adults suggest that most patients hospitalised with RSV associated acute respiratory tract infection had underlying comorbidities that would increase the risk of complications following any respiratory tract infection (Falsey and Walsh 2005; Colosia et al. 2017).

Despite the high disease burden, the potential for large-scale benefits for global health and finance, and decades of research, no effective antiviral or vaccine is yet available against human RSV. However, advances in our understanding of RSV immunobiology in recent years have led to renewed interest and activity relating to the development of RSV vaccines and therapeutics (Mazur et al. 2018). However, gaps in our understanding of the unique features of human RSV disease, including the propensity for reinfection secondary to incomplete immunity following natural infection, and difficulty in identifying and validating correlates of protection, still represent barriers to success.

Animal studies

While controlled infection of animals provides mechanistic insights, these do not fully replicate all aspects of human disease. Importantly, significant differences exist in the ways that RSV infects and causes disease in different species. Although human RSV is genetically related to RSV isolated from animals, there is no animal reservoir for the human virus (Taylor 2017). Direct extrapolation of results from animal studies remains difficult and offers limited guidance to understanding correlates of protection, pathogenesis and treatment (Bem et al. 2011). The imperfections of animal models mean that different models have to be deployed depending on which aspect of infection, immunology and pathogenesis is under investigation (Altamirano-Lagos et al. 2019). Mice, cotton rats and non-human primates are commonly used to study different aspects of human RSV disease but are only semi-permissive for human RSV infection and display clinical features that may or may not recapitulate human disease. Primary infection of several animal models such as mice, neonatal lambs (Sitthicharoenchai et al. 2020) and cotton rats show parallels with infantile bronchiolitis, but pathological changes in the lungs are not identical to those seen in human infection. The closest animal model is non-human primates, but ethical considerations limit their use (Taylor 2017).

Age-related changes in immune responses to RSV can also be seen in animal models. Infected aged BALB/c mice generate weak RSV-specific CD8+ T cell responses following infection, which is associated with a delay in viral clearance compared with young mice (Fulton et al. 2013). Similarly, RSV infection leads to excessive activation of type I interferon pathways in aged BALB/c mice but impaired viral clearance from the lungs (Pennings et al. 2018). These models offer potential explanations for the increased susceptibility to RSV in older adults. Regrettably, however, they do not adequately replicate RSV disease in older adults, who with advancing age become increasingly susceptible despite an increasing number of prior infections that induce partial immunity (Branche and Falsey 2015). A comparison of advantages, disadvantages and potential applications of different animal models and human infection challenge studies is shown in Fig. 1.

Observational and intervention studies in humans

Observational studies of natural RSV infection in humans provide important insights into disease pathogenesis and outcomes in the natural host. One of the hallmarks of RSV infection is its propensity for reinfection throughout life, even with antigenically similar strains, suggesting that immune protection is short-lived and/or incomplete. Birth cohort studies monitoring children over several RSV epidemic seasons have shown that children can be naturally infected with the same strain of virus within the same epidemic (Agoti et al. 2012), although following infection, there is a temporary (approximately 6 months) reduction in the rate of reinfection (Ohuma et al. 2012) and viral shedding (Okiro et al. 2010). Similarly, in young and older adults, reinfection with the same RSV strain has been

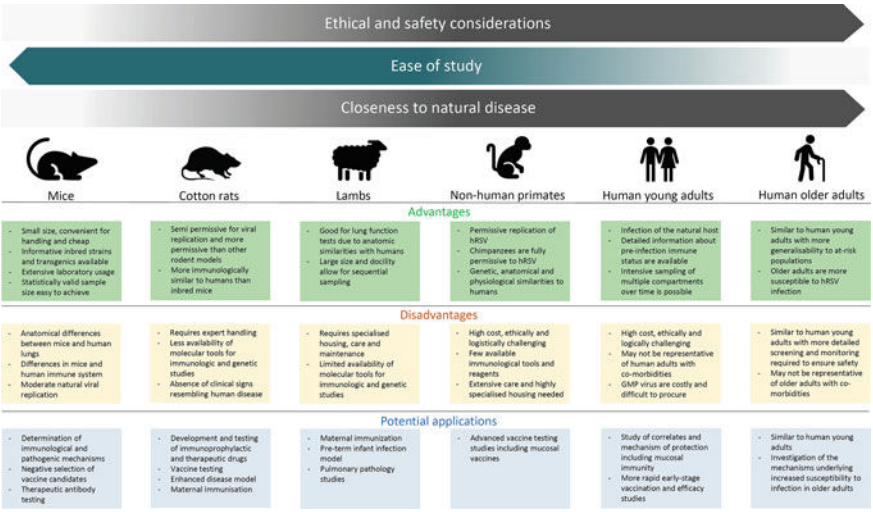

Fig. 1 Advantages, disadvantages and potential applications for different animal models and human infection challenge studies. The choice of the model will depend on the hypothesis under investigation, local expertise and the inherent advantages and disadvantages of each model (Habibi and Chiu 2017; Taylor 2017; Altamirano-Lagos et al. 2019)

demonstrated naturally (Falsey and Walsh 2000, 2005) and experimentally (Hall et al. 1991). A more recent prospective surveillance study identified robust antibody responses in adults acutely infected with RSV, but adults who were infected at some point prior to enrolment had a sharp decrease in RSV neutralising antibody titres within 60 days (Blunck et al. 2021). Taken together, these findings suggest the induction of partially protective immunity across all age groups following RSV infection (Lambert et al. 2014; Ascough et al. 2018).

Though informative, these studies are limited by the frequency and type of samples collected and potential delays in diagnosis, which means that patients may only be seen at the peak or beyond the peak of their infection (Habibi and Chiu 2017). While therapeutic trials in hospitalised children and adults are possible, there are multiple confounding factors such as variability in infecting viruses, coinfections, comorbidities, medical interventions, atypical and late presentations that limit the interpretation of findings. In addition, very large numbers of participants are required to achieve sufficient statistical power in view of the multiple confounding factors and low RSV ascertainment rates in certain populations. Taken together, this has led to long delays and relative paucity in new potential RSV vaccines and therapeutics with significant associated risks and costs.

Experimental human RSV infection challenge offers a cost-effective complimentary approach, in which investigators know with certainly the time of exposure and can intensively monitor responses following infection with a pre-defined viral inoculum. This approach also allows intensive longitudinal sampling, and the opportunity to perform detailed investigations of pre-existing, pre-symptomatic and immune responses following an induced illness.

Intentional infection of humans with pathogens with the aim of medical discovery goes back to 1796, when Sir Edward Jenner performed a human infection challenge with smallpox to demonstrate the resistance to disease conferred by cowpox (Jamrozik et al. 2021). This experiment paved way for future vaccination against smallpox and eventual eradication (Strassburg 1982). Controlled human infection studies provide unique insights into pathogenesis, immunity and facilitate drug and vaccine development in certain ways that animal models cannot. Investigators can also select participants with low pre-existing immunity to the challenge pathogen to increase infection rates, and thus statistically significant results can be achieved with smaller numbers of selected volunteers. Such studies can maximise participants' safety while providing a relatively rapid, robust and cost-effective proof-of-concept platform for evaluating potential vaccine and therapeutic candidates.

2 Logistical and Ethical Considerations in Deliberate Infection with RSV

Controlled human infection challenge studies are ethically complex as they directly violate the Hippocratic Oath *Primum non nocere* ('first, do no harm') (Shirley and McArthur 2011). Deliberate infection of healthy volunteers with the aim of

inducing illness may seem inherently unethical and, unfortunately, there are numerous examples of early studies which clearly breached contemporary ethical and moral standards (Hope and McMillan 2004; Paul and Brookes 2015). According to current ethical principles, well-designed and carefully conducted studies may be justified where there is a compelling reason and clear benefit, especially if infections are mild, self-limiting or easily and fully treatable in the selected volunteers (Franklin and Grady 2001). Due to the complex ethical and logistical challenges, a variety of factors including the perceived acceptability of research, availability of volunteers, suitability of infection challenge strain and research site. The team must be considered with great care before such work is undertaken. Additionally, there may be differences in the investigators' perceived risk and the actual risk posed by the study to the participants, investigators and funders (Darton et al. 2015). Controlled human infection challenge studies are often characterised as non-therapeutic research in that they do not normally provide any direct benefit to the participants (Jamrozik and Selgelid 2020). Therefore, potential harm cannot be justified or offset and must be minimised.

Study design and setting

One of the important considerations in the design of human infection challenge studies is the study setting and the selection of volunteers governed by the need to ensure participant safety, limit transmission of pathogen to the wider community and infection control. While there is no UK or EU regulation that mandates the use of specific quarantine facilities (Darton et al. 2015), confinement of participants to hospital wards or designated research units may be necessary to limit transmission of potentially virulent pathogens to the environment or members of the public. Alternatively, experimental challenge studies involving ubiquitous human pathogens such as human rhinovirus have been safely and successfully carried out as outpatient studies (Mallia et al. 2006).

Adopting an inpatient design for the study is costly, not always readily available and may deter prospective participants, but offers many advantages over the outpatient design. These include the ability to closely monitor and collect observational data, accurate clinical sampling, timely initiation of supportive or rescue treatment as required, limiting inadvertent transmission of the challenge pathogen to contacts and limiting the acquisition of other infection(s) which may affect the scientific interpretation of data.

All contemporary RSV human infection challenge studies involve a period of residential quarantine to limit potential transmission to the community when participants are at peak viral loads, and therefore, highest potential infectivity. However, whether residential quarantine is strictly required is debatable and this decision remains with the investigators in consultation with the local ethics committee and Patient and Public Involvement groups. With a pathogen that circulates freely in nature, it can be argued that studies should be performed on subjects living normally at home, with advantages in terms of cost and real-world relevance.

Volunteer screening and selection

Careful screening and selection of prospective participants is critical for ensuring participants' safety while maintaining the integrity of the scientific question being addressed. Investigators can apply pre-defined selection criteria to ensure that the participants are relatively homogenous. Most studies enrol healthy young adults who are least likely to suffer from severe disease and have the most physiological reserve for a quick and complete recovery post-infection. In some studies, such as influenza, participants may also be screened for pre-existing antibodies to the infection challenge strain as this may greatly affect study results and outcomes. This practice is more varied in RSV challenge studies where some investigators preferentially include participants with low levels of pre-existing serum RSV neutralising antibody levels which improves infection rate (Bagga et al. 2013; DeVincenzo et al. 2019). However, a difficult balance exists between careful selection of healthy participants to ensure safety and maintain the applicability of results to the general population. For example, as many infection challenge studies are restricted to healthy young adult volunteers because of safety considerations, there is a limit on the applicability of results to older adults and patients with underlying comorbidities who are at greater risk of severe disease.

Safety considerations

Ensuring the safety of participants, research staff and the wider public is paramount for human infection challenge studies. Experimentally infected participants pose a potential risk for onward transmission. As such, research staff involved with the study must take all reasonable precautions such as maintaining good hand hygiene practices and using appropriate personal protective equipment. This minimises the chances of staff becoming infected or conversely transmitting pathogen(s) to the research participants. In addition, the risk posed by inoculated participants to the wider public when compared with the natural risk of ubiquitous respiratory viral infection remains unclear. Nevertheless, a thorough screening of the participants' social history to ensure that they do not have regular close contact with individuals deemed to be at high risk of severe infection or complications such as older adults, pregnant women or the immunocompromised should be mandatory.

Consent

Informed consent is crucial to the proper conduct of human infection challenge studies and it is imperative that all prospective participants are fully aware of the implications, potential risks and possible harm that may arise from taking part. Additionally, there must also be a limit to the harm that potential participants can be exposed to, irrespective of perceived societal needs or scientific merits. After receiving informed consent, a detailed assessment of participant safety continues with rigorous screening procedures and tests, including obtaining the participant's medical records to ensure that any potential participant deemed to be at risk of severe illness is excluded.

With respect to RSV, healthy adults would at most be expected to experience a mild common cold-like illness following RSV infection, but there remains a theoretical possibility of this becoming a more severe infection in the experimental infection setting (Yoon et al. 2020). While older adults are generally more susceptible to infection due to immunosenescence, 'inflammaging', comorbidities and age-related physiological changes, the majority of older adults also at most experience mild disease not requiring medical attention (Korsten et al. 2020). Only high-risk patients (i.e. with congestive heart failure or chronic pulmonary disease) ever develop a disease severe enough to require hospital treatment following infection with RSV (Falsey and Walsh 2005; Walsh et al. 2004; Loubet et al. 2017). Owing to the ubiquitous nature of respiratory viruses, common cold illness is considered a part of normal life, and therefore, the risk and potential harm of acquiring an experimentally induced cold is generally not considered significant from an ethical perspective. Hence, controlled infection studies involving healthy adults and older adults could be considered.

Generally, research procedures are minimally invasive, but more invasive sampling such as bronchoscopy may be justified scientifically if the frequency of the procedure is kept to a minimum and are carried out in clinical units with records of safety, adequate staffing and training. These procedures allow direct sampling of the respiratory mucosa and lead to a better understanding of local immune cells and epithelial function, which is critical for characterising local immune responses against viral infections in humans (Jozwik et al. 2015; Habibi et al. 2020).

Extending the study to older adults

With the projected continued increase in the ageing population (Kingston et al. 2018), there is a growing unmet medical and public health need to develop vaccines and therapeutics for the management of RSV disease. To date, most RSV human infection challenge studies have enrolled healthy young adult volunteers (Table 1) and all have been safely completed without unexpected adverse outcomes. However, the applicability of findings to older adults who are at greater risk of severe disease due to age-related differences in immune responses and physiology are limited (Ackerson et al. 2018). With increasing expertise and evidence on the safety of modern human infection challenge studies, investigators are looking to extend these studies to increase the relevance and applicability of results to target participants or at-risk populations (National Library of Medicine [NLM], NCT03919591; Dayananda et al. 2020). As long as the central principle remains that the pathogen is detectable and is either self-limiting or completely treatable with no residual long-term sequelae for the participants (Gordon et al. 2017), it is possible, in principle, to extend the study to participants with some risk factors.

For example, human rhinovirus infection challenge in adult asthmatics and older adults with chronic obstructive pulmonary disorders (who may or may not have been smokers) has been successfully and safely conducted (Mallia et al. 2006; Zhu et al. 2014). Findings from these ongoing studies further contribute towards a better

Table 1 Historical human infection challenge studies using RSV describing the characteristics and number of subjects, RSV strain used, main findings and conclusion

Participant characteristics and number	RSV strain used	Findings and conclusion	Year and reference
33 males, no history of respiratory, cardiovascular or allergic disease	RSV Long—wild type strain isolated in 1957	High-dose inoculum (5 log TCID50) was found to be effective at inducing RSV infection. Neutralising antibody titres in the nasal wash inversely correlated with susceptibility to infection	Mills et al. (1971)
21 males, no history of cardiopulmonary disease or allergies pre-selected based on lowest levels of neutralising antibodies to RSV in nasal secretions	RSV A2—wild type strain (isolated from Australia 1961) (n = 8) RSV A2—temperature sensitive strain (ts − 1; unable to form plaques at 37–39 °C) (n = 13)	Ts-1 strain caused less extensive infection compared with wild type virus with a corresponding reduction in serological response. Previous challenge with Ts-1 strain induced protection against reinfection with wild type strain 45 days after the first challenge	Wright et al. (1971)
32 healthy adult volunteers, no history of atopy	RSV A2—wild type strain	5.2 log TCID50 was found to be the most effective at inducing RSV infection with comparable infection rates (3 out of 4 participants) when inoculated via the nose or eye. Inoculation via the mouth is not a viable route	Hall et al. (1981)
105 healthy adults aged 18–55	RSS-2 (wild-type strain) (n = 19), 4 different temperature sensitive (ts) mutants (n = 20–22 per group)	RSS-2 strain is effective at inducing RSV infection and induced serological response in 90% and clinical cold in 45%; ts mutants are less virulent and two strains produced serological responses in 68% of participants	Mckay et al. (1988), Watt et al. (1990)

(continued)

Table 1 (continued)

Participant characteristics and number	RSV strain used	Findings and conclusion	Year and reference
102 healthy volunteers aged 18–55	RSS-2 (5 subjects challenged with saline)	Intra-nasal interferon α was effective as prophylaxis against RSV challenge but no benefit seen when administered post infection	Higgins et al. (1990)
15 young healthy adults with laboratory confirmed natural RSV infection	RSV A2	Immunity to RSV is associated with serum neutralising antibodies against F and G proteins but is short lived following infection	Hall et al. (1991)
394 adult volunteers with no acute or chronic illness and not on regular medications aged 18–54	RSV (n = 40) Other participants received other respiratory viruses or saline (n = 26)	Psychological stress is associated with an increased risk of respiratory tract infection in a dose response manner	Cohen et al. (1991)
22 adult volunteers aged 21–50	RSV A2 − ts mutant (ts1C) [unable to produce plaques in MRC-5 cells at 37 °C]	Ts1C mutant RSV A2 strain induced infection in 15/22 (68%) of volunteers. Ts1C strain is more attenuated than a previous ts mutant (Ts1B) and may be more suitable as a live vaccine	Pringle et al. (1993)
116 adult volunteers aged 18–53	RRS-2 (n = 11) Other participants received other respiratory viruses (n = 105)	Compared with other respiratory viruses (rhinovirus types 2, 9 and 14, coronavirus type 229E) participants infected with RSV developed symptoms slower (after 5 days post inoculation)	Tyrrell et al. (1993)
36 healthy adults aged 18–45	RSV A2 (8 participants challenged with placebo)	No differences in symptoms or infection rates observed between 2 different doses (4.7 log TCID50 vs 3.7 log TCID50). Infection rate was inversely correlated with serum neutralising titre	Lee et al. (2004)

understanding of infective exacerbations in these more vulnerable patient groups (Hewitt et al. 2015; Singanayagam et al. 2019). Recently, we have extended our RSV challenge model to 12 older adults (median age 67.5; range 61–73) and showed that the procedure is safe and well tolerated with a good attack rate (75%) and symptom distribution (Dayananda et al. 2020). For these studies, additional monitoring and medical vigilance were in place to readily respond to potential adverse events or deterioration.

3 Historical Human RSV Infection Challenge Studies

In 1961, twenty adult participants were inoculated with an RSV strain isolated from a patient with bronchopneumonia (Kravetz et al. 1961). Since then, there have been multiple human infection challenge studies using different strains of RSV without reports of serious adverse events (Table 1). Early human infection challenge studies unveiled information on the route of transmission, incubation times, duration of protective immunity and viral shedding. However, limitations in diagnostic technology restricted contributions from these studies towards better understanding of RSV immunopathology or contribution towards RSV therapeutics and vaccines.

In earlier RSV challenge studies, participants were inoculated with viruses (i.e. Long and A2) isolated by serial laboratory passage in multiple cell lines or live attenuated strains. As a result, successful infection required high doses and infected participants were minimally symptomatic, suggesting that these strains had been laboratory-adapted or attenuated (Lee et al. 2004). In addition, these viruses were first isolated in the 1950s and 1960s, and likely differ from the currently circulating strains, further limiting applicability to modern infection (Pandya et al. 2019). Furthermore, as each laboratory generated and maintained its own stock of challenge viruses, it was difficult to ensure consistency and comparability. Taken together, these issues raise uncertainties when extrapolating these historical data to further our understanding of modern-day natural infection with RSV.

Common laboratory strains of RSV

Since RSV was first isolated in 1956, most research on RSV has focused on the use of a limited number of maintained historical isolates. While these studies have played a critical role in understanding the general virology of RSV infection, differences in cytopathology, immunogenicity and pathogenicity exist between laboratory strains and clinical isolates (Table 2) (Pandya et al. 2019). More recently, an objective protocol has been proposed for RSV genotyping, suitable for adoption as an international standard to support the global expansion in RSV surveillance (Goya et al. 2020).

Table 2 Comparative summary of RSV clinical isolates and laboratory strains (Pandya et al. 2019)

Virus strain	Type and designation	Characteristics and use
Long	RSV A (laboratory strain)	RSV Long strain was first isolated in 1956 and passaged 11–13 times in HEp-2 cells. It is primarily used in immunogenicity and neutralisation studies RSV Long strain is the first prototypic RSV A model
A2	RSV A (laboratory strain)	RSV A2 was first isolated in Melbourne in 1961 and has since been established as the prototypic RSV A strain and used in the development of live attenuated vaccine candidates
Line 19 and A2-line19F	RSV A (laboratory strain)	RSV Line 19 was first isolated in 1967 and initially expanded in WI-38 cells. This is primarily used in pathogenesis and immunology studies as it causes pathological changes in mice that more closely resemble human disease
CH-18537	RSV B (laboratory strain)	RSV CH-18537 was first isolated in 1962 and initially expanded in Wistar 26 cells. This has been established as the prototypical RSV B strain
M37	RSV A (laboratory strain)	RSV M37 was first isolated in Memphis in 2001 from a child with bronchiolitis; it was plaque purified and expanded in Vero cells for use in human infection challenge studies
Clinical isolates	RSV A and RSV B	Clinical isolates vary in genetic diversity and differ from conventional laboratory strains The predominant subtypes circulating today (ON1 and BA genotypes) exhibit characteristic nucleotide duplications (72 and 60-nucleotides, respectively) in G (Trento et al. 2003; Cui et al. 2020)

4 Contemporary Human RSV Infection Challenge Studies

In recent years, a wild type, low-passage strain of RSV A has been produced in accordance with Good Manufacturing Process (GMP) guidelines specifically for human challenge studies. The virus, RSV Memphis 37 (RSV M37), was first isolated by nasal aspirate from a 4-month-old child presenting with bronchiolitis in 2001. The initial isolate was plaque purified, screened extensively for adventitious agents and expanded as a GMP lot in FDA-approved Vero cells for use in human challenge studies (Pandya et al. 2019). While GMP virus is considered the standard for human infection challenge studies, it is not mandated by the UK or EU regulations.

Zaas et al. conducted the first published study using this virus in humans in 2009. Following the viral challenge, 9 of 20 (45%) of the participants developed symptoms and had confirmed viral shedding. Participants reported symptoms that

peaked at 141.5 h post-inoculation. Using this model, the authors found a unique peripheral blood transcriptional signature identifying respiratory viral infection from bacterial infection with a 93% accuracy (Zaas et al. 2009). DeVincenzo et al. challenged a further 35 healthy adult volunteers with RSV M37 with incremental dosing from 3.0 to 5.4 $\log_{10}$ PFU per ml with an overall infection rate of 77.1% and no significant differences between the infection rates. Contrary to the previous study, all participants were screened for pre-existing RSV antibody neutralising titres and only the lower third of those tested were enrolled. The viral load and symptom scores peaked around 6 days post-inoculation and a significant correlation was seen between the respiratory tract symptom scores and viral load (DeVincenzo et al. 2010). In addition, the authors observed a significant correlation between the cumulative concentration of nasal wash interleukin (IL)-6 and cumulative viral load and identified nasal wash concentration of IL-6, IL-8, tumour necrosis factor (TNF)-α and macrophage inflammatory protein (MIP)-1α as potential markers of disease severity. They concluded that the viral load appeared to be the main driver of RSV disease in humans and showed that this was a reproducible and safe model in which investigators could demonstrate the efficacy of potential vaccines or therapeutics for RSV disease (DeVincenzo et al. 2010).

5 Utility of RSV Human Infection Challenge

The utility of the RSV human challenge model (Fig. 2) pioneered by DeVincenzo (DeVincenzo et al. 2010) has been demonstrated in many subsequent studies investigating antiviral therapy and vaccines as early-proof-of-concept studies and insights into RSV pathogenesis. In general, healthy prospective adult volunteers (age 18–45 inclusive) are screened and in some cases pre-selected for sero-suitability for the challenge strain of RSV (generally defined as having low serum RSV neutralising antibody levels prior to screening, although no absolute cut-off values have been defined). Nasal wash samples from experimentally challenged volunteers are tested twice daily for the presence of RSV and (for intervention studies) can be started on the pre-specified treatment or placebo on the first day of a positive RSV nasal wash or 5 days post-inoculation whichever comes first. Participants are also asked to complete a symptom diary and the total weight of mucus produced during the study are sometimes measured daily (DeVincenzo et al. 2014).

Fusion inhibitors

Using this model, DeVincenzo examined the role of GS-5806 (presatovir), a novel fusion inhibitor, in 140 healthy volunteers. Participants receiving GS-5806 had significant reductions in viral load by the area under the curve (AUC), total weight of mucus produced and total symptom scores without serious adverse events, supporting further study of GS-5806 in at-risk participant groups (DeVincenzo et al.

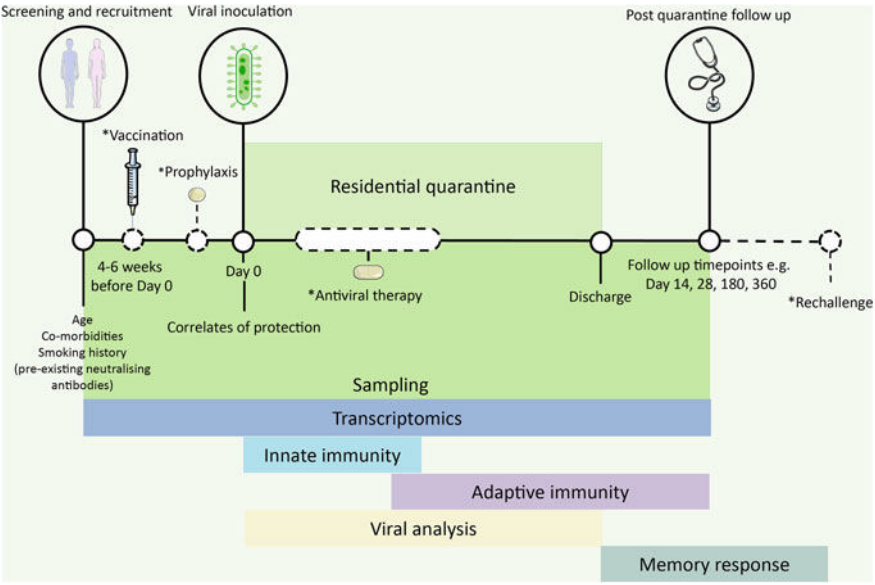

Fig. 2 Outline of a Human Infection Challenge Model. Solid lines represent the steps shared between all studies and dotted lines represent potential additions or modifications to the model. The study starts with screening and recruitment of healthy volunteers using pre-specified selection criteria. Depending on the type of study and hypothesis under investigation, the volunteers may also be vaccinated or receive prophylaxis prior to exposure to a GMP virus, after which the volunteers will be asked to remain in residential quarantine until discharge. During quarantine, volunteers may also be given antiviral therapy. Following discharge, the volunteers are followed up at different pre-specified time points and may be re-invited for rechallenge with a heterologous or homologous GMP viral strain. Potential outputs from the study, outlined below, may include transcriptomic and differential gene expression analyses, innate and adaptive responses following viral exposure, viral analyses and memory responses

2014). Presatovir did not significantly reduce the viral load or improve clinical outcomes in hospitalised adults with RSV treated relatively late in the course of the disease (Hanfelt-Goade et al. 2018). A further study, however, did identify a trend towards antiviral effect and clinical benefit in haematopoietic stem cell transplant (HCT) recipients with patients with lymphopenia, suggesting a potential niche role for presatovir (Gottlieb et al. 2018).

More recently, Stevens and colleagues evaluated JNJ-53718678 (rilematovir), in 69 experimentally challenged healthy adults. The authors found that participants receiving JNJ-53718678 had reduced RSV viral load and duration of viral shedding which correlated with lower symptom scores and mucus production. A subsequent Phase 1b study of JNJ-53718678 in hospitalised infants ($> 1 - \leq 24$ months) infected with RSV also demonstrated that JNJ-53718678 is well tolerated and a greater reduction of RSV viral load was observed compared with the placebo group. The findings support further clinical development of JNJ-53718678 as a

potential treatment for RSV infection (Martinón-Torres et al. 2020). Rilematovir is currently undergoing a Phase 3 randomised double-blind, placebo-controlled study in infants, children and neonates hospitalised with RSV related acute respiratory tract infection (DAISY) (estimated primary completion date May 2024) (National Library of Medicine [NLM], NCT04583280) and a randomised placebo-controlled study in post-haematopoietic stem cell transplant adult and adolescent infected with RSV related upper respiratory tract infection (FREESIA) (estimated primary completion date August 2022) (National Library of Medicine [NLM], NCT04056611).

Similarly, DeVincenzo studied the effects of RV521 (sisunatovir), in 66 healthy adults challenged with RSV. Reassuringly, treatment with RV521 resulted in a significant reduction in the RSV viral load measured using the area under the curve (AUC) and severity measured using symptom scores and daily nasal mucus weight compared with placebo. Sisutonavir is currently being investigated further as a Phase 2 randomised double-blind, placebo-controlled study in post-haematopoietic stem cell transplantation presenting with RSV related upper respiratory tract infection (REVIRAL2) with an estimated primary completion date in June 2022 (National Library of Medicine [NLM], NCT04267822).

Nucleoside viral replication inhibitor

In 2015, DeVincenzo examined the role of ALS-008176 (lumicitabine), a prodrug of a cytidine nucleoside analogue which inhibits RSV polymerase in 62 experimentally challenged healthy adults also with low pre-existing levels of RSV neutralisation antibody titres. Consistent with the different mechanism of action, ALS-008176 achieved a more rapid reduction in the viral load and symptom scores than GS-5806 with a theoretically higher barrier to resistance (DeVincenzo et al. 2015). The authors also developed a model of RSV kinetics and pharmacokinetics using data from the human infection challenge study to guide further dosage selection in adult and paediatric patients (Patel et al. 2018). Lumicitabine has unfortunately been suspended from further development (Waghmare and Englund 2021).

Non-fusion replication inhibitor

Coakley and colleagues analysed the effects of EDP-938, a novel non-fusion inhibitor of RSV (Rhodin et al. 2021) in 115 experimentally challenged healthy adult subjects using the human infection challenge model (Fig. 2). EDP-938 significantly reduced RSV viral load, symptom scores and mucus weights and the study supports further clinical evaluation of EDP-938 (Coakley et al. 2019). EDP-938 is currently undergoing a Phase 2b randomised controlled trial in HCT recipients with acute RSV infection and symptoms of upper respiratory tract infections (URTI) with an estimated primary completion date in December 2022 (National Library of Medicine [NLM], NCT04633187).

PC786, a nebulised non-nucleoside RSV polymerase inhibitor was also investigated using the human infection challenge model in 56 healthy adult volunteers. Nebulised PC786 demonstrated significant antiviral effect and a trend towards reduction of symptom score and mucus weight. Despite the suboptimal testing conditions (PC786 is not optimised for nasal delivery or evaluation using a nasal viral challenge model), the findings support further investigation of PC786 in patients naturally infected with RSV (DeVincenzo et al. 2020).

Vaccination

A better understanding of the mode of action, optimal timing and dose of antivirals have helped optimise the RSV human infection challenge model for proof-of-concept studies. However, adapting the model to demonstrate the efficacy of vaccines adds extra layers of complexity, with the type of vaccine; level, timing and anatomical site of the immunity induced; and the interplay between host immunity (pre-existing and induced) and the infecting virus all playing a role in determining the efficacy of the vaccine. By design, the human infection challenge model has the advantage of knowing with certainty the time of exposure and inoculum dose as well as allowing for intensive monitoring of responses and longitudinal sampling. It is, therefore, possible to investigate participants following vaccination to better understand the mechanisms of action and immune correlates of protection.

Early experiences with formalin-inactivated alum adjuvanted RSV vaccines (FI-RSV) in the 1960s had been disastrous and highlighted the complexity of RSV immunobiology which can be both protective and harmful (Openshaw et al. 2017). Children who received FI-RSV preferentially developed non-neutralising antibodies and cell mediated responses that enhanced disease severity during subsequent natural RSV infection (Openshaw et al. 2017; Murphy et al. 1986). This supports the due consideration warranted not only to the quantity of antibodies generated and cell mediated responses, but also their functionality.

As a result of the early clinical trials, there are currently no formalin-inactivated RSV vaccines in development (PATH 2021). Fifty-three healthy adult volunteers with low pre-existing levels of RSV neutralising antibodies took part in the first RSV human infection challenge to investigate an intramuscular RSV vaccine candidate, Ad.26.RSV.preF. The volunteers were challenged with RSV M37 28 days after receiving Ad.26.RSV.preF or placebo. The primary end point was significant reduction of viral load AUC determined by RT-PCR. Following the infection challenge, 14 of 27 (51.9%) volunteers' viral load remained below the lower limit of quantification compared with 6 out of 26 (23.1%) volunteers in the placebo group. The median viral load was also significantly lower in Ad.26.RSV.preF group (0.0 vs 236.0; $p = 0.012$), and therefore, the primary end point was met. There was also a significant reduction in cumulative symptom scores [Ad26.RSV.preF (35.0) vs placebo (167)], mucus weight and an increase in the fold-change in neutralising antibodies titres in the Ad.26.RSV.preF group (5.8 vs 0.9) following infection challenge (Sadoff et al. 2021). Following the successful proof-of-concept study, Ad.26.RSV.preF was combined with a pre-fusion (pre-F) protein induction

and further evaluated in a Phase 2b field trial (CYPRESS study) in adults. The study team reported efficacy of 80% (CI 52.2–92.9%) against confirmed RSV associated lower respiratory tract disease and 70% (CI 42.7–85.1%) against any symptomatic RSV associated acute respiratory infection. In older adults aged 65 and older, the candidate vaccine generated robust humoral and cellular immune response, including neutralising antibodies 14 days following vaccination (Johnson Johnson 2021). This vaccine candidate is currently undergoing a Phase 3 study (EVERGREEN study) to specifically investigate the safety, efficacy and immunogenicity against RSV related lower respiratory tract disease in older adults aged 60 and older (National Library of Medicine [NLM], NCT04908683).

Similarly, a human infection challenge study investigating another intramuscular RSV prefusion F subunit vaccine (RSVpreF) candidate (National Library of Medicine [NLM], NCT04785612) concluded in August 2021 and supported the further evaluation of RSVpreF in older adults over 60 years old with an estimated completion date in June 2024 (National Library of Medicine [NLM], NCT05035212). Following on, a mucosal vaccine candidate MV-012-968 completed its Phase 2 study using a human infection challenge model in September 2021 (National Library of Medicine [NLM], NCT04690335).

Uncompleted studies

Not all studies were successful in demonstrating efficacy in the human challenge model. Human infection challenge studies examining the and prophylactic effects of MEDI-557 (motavizumab-YTE) a monoclonal antibody (National Library of Medicine [NLM], NCT01475305) and the antiviral effects of BTA-C585 (enzaplatovir) an oral RSV fusion protein inhibitor (National Library of Medicine [NLM], NCT02718937) were both registered on the clinical trials database in 2011 and 2016, respectively. The study examining the effects of MEDI-557 was terminated after recruiting 7 out of 90 subjects prohibiting any meaningful interpretation of results. To the best of our knowledge, no further information on BTA-C585 is available in the public domain.

Taken together, these 'fail-fast' studies highlight recent advances in RSV antiviral and vaccine research and the utility of the experimental human challenge model in accelerating their development as well as identifying and discontinuing candidates that are more at risk of failing in late-stage clinical trials. The primary outcome of reduction in viral load offers a clear indication to guide whether a potential treatment or vaccine warrants further investigation and investment using a relatively small number of study subjects. However, key differences between the human infection challenge model and natural infection such as the severity of infection, immune status, comorbidities and time of intervention means that direct extrapolation of findings to clinical practice should be undertaken with caution and, instead, human challenge may be better positioned to inform further field studies.

6 Experimental Infection Challenge Provides Insight into Pathogenesis

The development of effective vaccines for RSV has been hit with delays and setbacks due to various factors, including concerns of enhanced respiratory disease following vaccination and the absence of an absolute correlate of protection against clinically relevant RSV infections. Yet, the large-scale potential impact of an effective vaccine that can protect infants and/or frail older adults continues to drive development, with over 30 vaccine and monoclonal antibody candidates in different stages of development (PATH 2021). For much of history, vaccines against infectious diseases have been successfully developed empirically without the involvement of immunologists or the need to define the true correlates of protection, but rather defined measurable surrogates of protection to predict efficacy (Pollard and Bijker 2021). This approach has been proven to be unsuccessful in hard-to-target pathogens such as RSV where immune memory is transient and individuals remain susceptible throughout life despite relatively little genetic diversity (Graham 2017). The partial immunity induced following RSV infection and resultant difficulty in identifying individuals who are consistently protected against infection or disease have muddied identification of the true correlates of protection in population-based studies.

Almost all current vaccines rely on the generation of high titres of pathogen-specific antibodies in serum or mucosa that correlate with blockade of infection or bacteraemia/viraemia (Plotkin 2010). Specifically for RSV, serum neutralising antibodies correlate negatively with the risk of RSV associated hospitalisation across different age groups (Piedra et al. 2003). This is largely in agreement with other observational studies suggesting that higher levels of RSV-specific antibodies correlate with protection against symptomatic natural infection (Falsey and Walsh 1998; Luchsinger et al. 2012), although these levels are not well maintained following infection (Falsey et al. 2006). To further investigate the relationship between local and systemic immunity with infection, we enrolled 61 healthy adult volunteers without pre-selection for low RSV-specific serum neutralising antibodies for RSV infection challenge (Habibi et al. 2015). Almost all participants had relatively high levels of serum neutralising antibodies at baseline, yet thirty-four (56%) participants became infected following the infection challenge. We found that serum neutralising antibodies by plaque reduction neutralisation assay only loosely correlated with protection from PCR confirmed infection. Further, transcriptomic profiling of nasal tissue in participants with symptomatic infection pre-inoculation revealed a neutrophilic inflammatory response associated with a suppression of early IL-17 response in the pre-symptomatic period. On the other hand, RSV-specific nasal IgA level was found to be more strongly correlated with protection from infection. Transcriptomic profiling of nasal tissues from participants who resisted infection showed a transient upregulation of mucosal markers of innate immune activation following inoculation but no subsequent viral

replication (Habibi et al. 2020). RSV infection appeared to be poorly immunogenic as serum and nasal antibodies waned to pre-infection levels within 6 months of inoculation. Further analysis of peripheral blood memory B cells (MBCs) revealed a defect in the induction of anti-RSV IgA-secreting MBCs (Habibi et al. 2015). This may in part explain the lack of complete protection against RSV following natural infection but suggests that induction of sustained mucosal antibody response may be more effective in preventing RSV infection, compared with serum IgG which may have a preferential role in preventing lower respiratory tract involvement.

Antibodies play a role in protection against infection, but once the infection is established, antibodies have a limited role in reducing disease severity (Alansari et al. 2019). In addition, currently licensed vaccines such as those against influenza are suboptimal at inducing persistently high levels of protective immunity in older adults and young children (Andrew et al. 2019). Hence, the likelihood of RSV infection even after vaccination remains reasonably substantial. Vaccines that are also able to induce cellular immunity, therefore, have theoretical advantages that include ameliorating disease, protection against different variants or strains of the same virus in the event of antigenic changes, and interaction with B cells to enhance the production of long-lived high-affinity antibodies (Panagioti et al. 2018). However, T cells arise late following RSV infection and were not previously thought to contribute to protection against infection but rather viral clearance (Schmidt and Varga 2018).

The majority of clinical studies to date have utilised peripheral blood for T cell analysis, and much of our understanding of the unique T cell immunobiology in the lungs are from animal studies (Kinnear et al. 2018; Openshaw and Chiu 2013). Our group investigated the role of virus-specific T cells in blood and airway following RSV infection in 49 healthy adults following RSV infection challenge (Jozwik et al. 2015). We found that RSV-specific CD8+ T cells were significantly more abundant in the airway compared to peripheral blood, and displayed the T resident memory (Trm) cell markers CD69 and CD103. The frequency of RSV-specific CD8+ T cells in neither blood nor the airway had any impact on the likelihood of PCR confirmed RSV infection. Instead, higher frequencies of CD8+ T cells in the airway correlated with a lower cumulative symptom score and viral load in infected participants. In contrast, we have shown no correlation between CD4+ T cells in the airway with disease severity or viral clearance following RSV infection (Guvenel et al. 2020). Further studies with mouse models have shown that CD8+ Trm cells protect against RSV infection in absence of circulating effector CD8+ T cells or T cells from secondary lymphoid organs (Luangrath et al. 2021). Taken together, these studies suggest that in addition to neutralising antibody titres and circulating peripheral T cells, Trm cells also represent an important new target for vaccine design (Zens et al. 2016).

7 Limitations

While human infection challenge studies benefit from being conducted in the natural host in a controlled fashion, there are several important limitations to this model. In addition to the practical and ethical considerations discussed previously, a fundamental limitation exists in studying healthy adults with few to no known underlying health conditions and who experience only mild-to-moderate upper airway illness following inoculation. Severe RSV disease disproportionately affects the extremes of age and findings from healthy adults may not be directly extrapolatable to high-risk populations. Adult humans are anatomically and immunologically different from infants, so observed host responses following infection challenge are likely to be different from infantile bronchiolitis (Florin et al. 2017). Nevertheless, the heterogenicity of clinical outcome data following infection challenge does facilitate inference of protective correlates that may be universally applicable.

Furthermore, while the experimental infection of infants or high-risk adults cannot be ethically or scientifically justified, experimental infection with rhinovirus led the way for the human challenge in older adults and those with comorbidities (Mallia et al. 2006; Zhu et al. 2014). Therefore, it is not unreasonable to extend the RSV infection challenge model to include healthy older adults following careful screening and other safety considerations.

Some studies examining the antiviral activity of novel compounds against RSV pre-select healthy volunteers with especially low serum neutralising antibody levels. This increases the probability of developing common cold illness following infection challenge, thereby allowing for smaller sample size and lower costs at the proof-of-concept stage. However, it is arguable that these individuals represent an unusual population that is particularly susceptible to RSV either due to a lack of recent exposure or an intrinsic vulnerability. In addition, human infection challenge studies are very tightly controlled, and participants are initiated on antiviral therapy as early as 12 h post-symptom onset (DeVincenzo et al. 2014). This is significantly earlier than in individuals who generally presented to the hospital at a later stage, with potentially more established and greater disease severity. As the benefits of early administration of effective antivirals are well described (Li et al. 2010), the same clinical efficacy demonstrated in a human infection challenge model receiving early interventions may not necessarily be replicated in patient populations (Gottlieb et al. 2018). Equally, while early treatment with presatovir significantly reduces RSV viral load and clinical disease severity compared with placebo (DeVincenzo et al. 2014), there are conflicting reports on the association between RSV viral loads and clinical disease severity (Jozwik et al. 2015; DeVincenzo et al. 2010; Piedra et al. 2017; Garcia-Mauriño et al. 2019). By extension, it is yet unclear how the reduction in RSV viral load and disease severity may impact subsequent inflammatory and RSV-specific immune responses.

Moreover, as participants generally only experience upper airway symptoms/illness, the efficacy of nebulised antivirals that target the lower airway may be

underestimated in this setting and may benefit from utilising a more representative cohort (DeVincenzo et al. 2020). Further development of successful human infection studies will depend on the availability of a well characterised and standardised viral inoculum. Although there are no consistent regulations necessitating the use of viral inoculum manufactured in accordance with Good Manufacturing Practice (GMP), this remains highly desirable to ensure comparability of results between different cohorts of participants and safety. Most contemporary human infection challenge studies [based on DeVincenzo's study (DeVincenzo et al. 2010)] have RSV M37 manufactured in accordance with GMP. Recently, a more recent RSV-A strain (rRSV A/Maryland/001/11) has been manufactured and is currently under investigation for use as a human RSV challenge agent (National Library of Medicine [NLM], NCT03624790). The creation of alternative GMP viruses is time-consuming and prohibitively costly to most academic groups. However, there is currently no RSV-B strain manufactured under GMP for use in human challenge studies and, since extrapolating findings from these studies to RSV-B should be undertaken with caution, there remains a strong rationale for a wider range of challenge viruses to be made (Midulla et al. 2019; Ciarlitto et al. 2019).

Over the last 20 years, two new genotypes of RSV (ON1 and BA) have emerged and taken precedence showing continual evolution by RSV. Although our understanding of the biology of RSV continues to be driven by extensive study of relatively limited prototypic laboratory strains (Table 2), it is not clear whether these strains accurately depict the infectivity, replication or cytopathology compared with presently circulating strains. Therefore, better understanding of the extent of variation in genetics and phenotypes among RSV strains and exploring key differences in immune responses and correlates between laboratory and clinical strains will be required to ensure that results generated remain relevant and applicable (Pandya et al. 2019).

8 Conclusion

Experimental infection with RSV has improved our understanding of RSV pathogenesis in humans and provides a platform for novel vaccine and antiviral proof-of-concept studies. Human infection challenges can be an effective tool for streamlining the evaluation of novel prophylactic or therapeutic modalities. With increasing experience, evidence on the safety of human infection challenge studies, public awareness and acceptability, attempts have been made to extend these studies to increase the relevance of results to target participants or at-risk populations, including adults with underlying respiratory illness and older adults. A better understanding of correlates of protection with age and comorbid status will help maintain the current momentum and further facilitate the development of novel treatments for RSV disease.

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