Design, recruitment, and microbiological considerations in human challenge studies

Thomas C Darton*, Christoph J Blohmke*, Vasee S Moorthy, Daniel M Altmann, Frederick G Hayden, Elizabeth A Clutterbuck, Myron M Levine, Adrian V S Hill, Andrew J Pollard

Since the 18th century a wealth of knowledge regarding infectious disease pathogenesis, prevention, and treatment has been accumulated from findings of infection challenges in human beings. Partly because of improvements to ethical and regulatory guidance, human challenge studies—invoking the deliberate exposure of participants to infectious substances—have had a resurgence in popularity in the past few years, in particular for the assessment of vaccines. To provide an overview of the potential use of challenge models, we present historical reports and contemporary views from experts in this type of research. A range of challenge models and practical approaches to generate important data exist and are used to expedite vaccine and therapeutic development and to support public health modelling and interventions. Although human challenge studies provide a unique opportunity to address complex research questions, participant and investigator safety is paramount. To increase the collaborative effort and future success of this area of research, we recommend the development of consensus frameworks and sharing of best practices between investigators. Furthermore, standardisation of challenge procedures and regulatory guidance will help with the feasibility for using challenge models in clinical testing of new disease intervention strategies.

Introduction

Human challenge studies, with deliberate exposure of participants to infectious substances, have been an important research approach for nearly 300 years. Although broadly similar in process, the scientific rationale for this type of research is diverse, ranging from conclusively showing Robert Koch’s postulates regarding cause of disease to testing the efficacy of novel treatments or vaccines in a highly-controlled setting. Since inception, challenge studies have been ethically and logistically demanding, especially because they directly flout the Hippocratic principle of Primum non nocere (first do no harm). The perceived acceptability of such research and thus the availability of volunteers, a study site, and a suitably infectious challenge strain, all contribute towards the complex ethical and logistical deliberations necessary before such work can be undertaken. This complexity is increased by subtle nuances in what investigators consider the actual versus perceived risk that these studies pose to participants, investigators, and funders. Additionally, the often murky historical context of deliberate infection studies should not be overlooked. Many early studies clearly and deliberately breached both contemporary ethical and moral standards for individual or an organisation’s own gain.3,4

In the late 20th and early 21st century enhanced research accountability, robust ethical and regulatory scrutiny, and, in some cases, the provision of consensus frameworks of how human challenge studies should be done, has led to their resurgence in popularity.4,5 Terms used to describe the challenge process nowadays include experimental, artificial, or induced, in addition to controlled human infection or deliberate exposure. In this Review, we summarise some of the scientific, technical, and logistical factors pertinent to the design of human challenge studies. Ethical and legal justifications to do such research are beyond the scope of our report, but have been reviewed elsewhere.2,7,8

Why use challenge studies?

Since the 18th century, deliberate infection of individuals with known or putative pathogenic substances, or exposure of those individuals to others presumed to have an infection, has been used to further scientific, clinical, and public health enquiries. The first true challenge with an infectious agent was used to assess the safety of variolation for prevention of smallpox.4 Later, in 1789, Edward Jenner10 emulated this work to show the effective use of vaccination against smallpox infection. At present, challenge studies continue to be valuable in the assessment of protective immunity by allowing investigators to directly measure the efficacy of vaccine candidates against a specific infection (figure; appendix). Furthermore, assessment of natural infection-derived protection has been studied in several pathogen models, including Salmonella enterica serovar Typhi, enterotoxigenic Escherichia coli (ETEC), Giardia lamblia, and a revived model of leishmanisation (Leishmania major).11-14 Usefully, measures of vaccine efficacy in human challenge studies relate closely to those obtained in subsequent clinical trials in endemic areas for many, but not all, infections. In the controlled human malaria infection (CHMI) model4 the Plasmodium falciparum vaccine candidate RTS,S was reported to be 30–50% (sterile protection) effective against this disease,4,5,15 a level of protection subsequently reported in phase 2 and 3 field-trials.16-18

Key to the resurgence of challenge studies for assessment of vaccines is the opportunity for investigators to up-select or down-select potential vaccine candidates at an early stage, avoiding expensive phases of field-testing for development of ineffective prototypes.19 Challenge studies also play an important part in translation of data

See Online for appendix

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and results from one setting, population, or infectious strain to another (bridging studies). This role was exemplified by the demonstration that the oral cholera vaccine Centre for Vaccine Development (CVD) 103-HgR protected against the 01 El Tor Inaba biotype of *Vibrio cholerae* in challenge study participants.\(^{20}\) In combination with previous results of this vaccine against classic biotype infection,\(^{21}\) these data were accepted by regulatory agencies in several regions towards the licensure of CVD 103-HgR for travellers.\(^{22,23}\) Similarly, proof-of-concept studies to assess the efficacy of candidate treatments can be efficiently undertaken in volunteers who are infected with a specific pathogen, such as for development of both classes of available anti-influenza drugs and for various investigational therapeutics in the CHMI and *Shigella flexneri* models.\(^{24-27}\)

Challenge studies can provide invaluable laboratory data, suggesting possible correlates of protection or immunological pathways that are amenable to augmenting vaccine efficacy. For example, findings from a study\(^{28}\) using CHMI have refuted the role of a strong cellular immune response to viral-vectored vaccination in affecting parasite growth rates, suggesting that future vaccines targeting blood-stage infection should be aimed at generating high antibody titres.\(^{28}\)

Human challenge studies offer the most direct approach to show Koch’s postulates of disease causation by a putative pathogen. These studies include well known self-challenge experiments,\(^{29}\) including those by John Hunter\(^ {30} \) in 1767, and by Barry Marshall,\(^ {31,32} \) who in 1985 showed the virulence of Helicobacter pylori in causing acute gastritis. Historically, important work undertaken at the Medical Research Council’s Common Cold Unit (Salisbury, UK) identified rhinovirus as the main cause of the common cold.\(^ {33} \) In addition to discovery of methods for rhinovirus culture in vitro, challenge studies allowed identification of the prophylactic benefit of intranasal interferon in prevention of colds caused by the virus,\(^ {34-36} \) a finding later supported by household-based field trials.\(^ {37,38} \) More recent questions about disease causation include the search for putative virulence factors and mechanisms underlying host susceptibility (appendix). A *Neisseria gonorrhoeae* challenge model directly assessed the potential virulence of IgA1 protease by challenging the immune system of male volunteers (via the urethra) with a wild-type or IgA1 protease-deficient strain.\(^ {39} \) In a different model, monitoring of antigen variation by *G. lambia* during challenge has provided some indication as to how this pathogen might evade the host immune response.\(^ {40} \) Extensive investigations have also used a skin-inoculation model of *Haemophilus ducreyi* infection to assess the effects of pili, cytotoxic distending toxin, or haemolysin deficiency on the rate of pustule formation.\(^ {41,42} \)

A major use of human challenge studies has been to investigate those host-restricted pathogens for which no suitable animal model exists. In several cases, animal

<table>
<thead>
<tr>
<th>Pathogenesis or immune response</th>
<th>Therapeutic intervention</th>
<th>Vaccine study</th>
<th>Vaccine screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection derived immunity</td>
<td></td>
<td>Vaccine Centre for Vaccine Development (CVD) 103-HgR</td>
<td></td>
</tr>
<tr>
<td>Transmission</td>
<td></td>
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<tr>
<td>Dose escalation*</td>
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<tr>
<td>Therapeutic intervention‡</td>
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<tr>
<td>Vaccine study†</td>
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<tr>
<td>Vaccine screening§</td>
<td></td>
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</tbody>
</table>

**Figure:** Use and development stage of some existing active challenge models

Colours represent route of administration for challenge agents: purple is respiratory, blue is cutaneous, green is blood, red is urogenital, and orange is enteric. Several models are at different stages of development and use. Additionally, most models investigate infection pathogenesis mechanisms and host immune responses including serological, cellular, and molecular responses. ETEC = enterotoxigenic *Escherichia coli.* "Dose escalation studies are studies that aim to identify the challenge doses needed to reach a specific attack rate. "Vaccine studies are direct assessment of vaccine efficacy by administration of challenge at some interval after vaccination. "Therapeutic interventions use challenge as a direct therapeutic approach or aim to test the efficacy of a treatment after infection has been caused by challenge. "Vaccine screening uses challenge models to assess potential vaccine candidates before subsequent development stages.

**Table:** Use and development stage of some existing active challenge models

- Rhinovirus
- Respiratory syncytial virus
- Influenza
- *Streptococcus pneumoniae*
- *Necator americanus*
- Dengue
- *Haemophilus ducreyi*
- BCG
- *Plasmodium falciparum*
- *Neisseria gonorrhoeae*
- Norovirus
- *Salmonella enterica serovar Typhi*
- *Shigella flexneri*
- ETEC
- *Vibrio cholerae*
- Helicobacter pylori
environment, most aspects of natural interaction (eg, proximity and frequency) in addition to the natural environment (eg, humidity and temperature) can be controlled, permitting replicable data to be obtained with a precision and level of detail that could not be achieved with different methods or real-life settings.

Frequently, the idiosyncratic nature of infectious disease epidemiology and of emerging epidemics needs urgent data to be gathered for possible vaccine or treatment effectiveness. Alternatively, some infections occur so rarely in nature that study of therapeutic or control strategies is not feasible with naturally occurring cases. This difficulty applies to sporadic seasonal infections, including *Bordetella pertussis*, but is also true for many potential biological warfare pathogens, including *Francisella tularensis*, *Coxiella burnetti*, and *Rickettsia rickettii*. Concerns about the deliberate release of these pathogens places a high priority on research into the prevention and treatment of such infections. In addition to the direct assessment of treatment effects or benefits and the opportunity to glean useful pharmacokinetic or pharmacodynamic data from longitudinal volunteer monitoring, challenge studies might also be used to validate management algorithms. For example, no specific treatments for dengue infection are available; however, use of a human challenge model, a standardised care pathway, and predefined endpoints proved effective in prevention of severe infection.

Contemporary challenge studies are investigating the full extent of the hygiene hypothesis in the potential of therapeutic immunomodulatory effects of helminth infection on various autoimmune and allergic diseases—eg, the WIRMS study. Exciting results have been shown for hookworm (*Necator americanus*) treatment patients with asthma or coeliac disease, and *Trichuris suis* therapy for Crohn’s disease.

**Study design and setting**

Important considerations in the design of human challenge studies include the setting in which the study is to be undertaken and the methods used for screening and volunteer selection.

Human challenge studies are undertaken by academic and commercial organisations in various settings, the choice of which is governed mainly by participant safety, pathogen transmissibility and virulence, and factors related to infection control. Confinement of participants to hospital wards or research units is clearly necessary if potentially virulent pathogens could be transmitted to the environment or the public. Alternatively, common pathogens likely to be encountered under natural conditions—eg, rhinoviruses—can be studied outside isolation facilities. Conversely, participants themselves might need to be isolated to prevent acquisition of naturally encountered infection, which could affect the scientific interpretation of data obtained. Legal requirements in some countries might enforce isolation of participants with specific infections until treatment has started (eg, typhoid or cholera challenge in the USA), or could mandate reporting to relevant public health authorities, despite occurrence in an artificial setting (such as for gonorrhoea). Logistic ramifications of such legislation might include the long-term isolation of participants with infections—eg, typhoid, which might have an incubation period of up to 32 days because of the lengthy incubation period—or declaration to health-insurance providers.

Despite the high cost and, sometimes, difficulty in identification of suitable volunteers, many advantages are associated with an inpatient design. These advantages include the ability to gather detailed observational data, accurate clinical sampling, to initiate adjunctive therapy and treatment as soon as it is needed (eg, oral or intravenous fluid replacement), and to minimise the chance of inadvertent transmission of the challenge pathogen from participants to contacts.

Many early challenge studies used prisoners, residents of custodial institutions, or military personnel. Although use of these populations is now generally deemed unacceptable for ethical reasons, it has led to a keen interest in assessment of participants’ experiences of taking part in such studies.

With appropriate precautions being taken, contemporary challenge studies are often done with ambulant outpatient volunteers. These precautions might include notification of close household members about a volunteer’s participation in a study, having a 24 h on-call medical support team, and frequent outpatient review visits. For example, in present challenge studies for *N gonorrhoeae*, participants have to return to the study site every night to minimise the risk of transmission during high-risk hours.

Because the limits have been developed in which modern human challenge studies can be safely done, attempts are now being made to increase relevance of results from these studies to the target participant or at-risk population. These studies include undertaking challenge in participants with known comorbid disease processes—eg, rhinovirus challenge of individuals with asthma or chronic obstructive pulmonary disease.

For these types of studies additional levels of medical vigilance are needed to address the possible deterioration of a participant’s clinical condition.

Scientific rationale shows the need for such studies of disease susceptibility, vaccine efficacy, and treatment interventions in appropriate endemic settings. For example, models of human challenges with *Shigella sonnet* or *V cholerae* have been established in Thailand by successful collaborations between Thai and US investigators. Although these studies showed feasibility, investigators emphasised the difficulties in screening for participants who are immunologically naive for the pathogen of interest in endemic areas. This
difficulty in screening might lead to the need for higher challenge doses to replicate the attack rates or illness severities noted in volunteers in North America, where the pathogen is not endemic. These studies also emphasise the importance for translations of illness definitions or endpoints between studies, and the benefit of having frozen challenge strains that can be shared between sites and investigators.

Volunteer screening and selection

Careful volunteer selection is important both for participants’ safety and integrity of the scientific question being addressed. Thus, individuals who enrol for challenge studies undergo a rigorous, often several staged, screening process before being allowed to participate (table 1).

In many studies, participants are screened and excluded if pre-existing antibody titres to the organism being investigated are found. For example, in influenza challenge studies, pre-existing immunity not measured by specific antibody screening and present in all adult volunteers as a result of previous influenza infection could greatly affect responses to infectious challenge. By contrast, overly careful selection of healthy volunteers for challenge studies could affect the applicability of the data generated—eg, selection of healthy adult male volunteers without serological evidence of exposure to a particular infection might poorly represent the host responses to wild-type infection seen in the general population.

Furthermore, financial payments are often made to volunteers in these types of time-intensive and labour-intensive studies. Although fraught with ethical and moral issues, the amount paid should be commensurate with the study demands without being coercive. So far, no generally agreed standards specific to experimental, human challenge studies are recognised for the consent or amount of compensation paid, and decisions are the purview of ethical review.

Safety considerations

Participants, staff, public, and environmental safety are clearly the main concerns for deliberate infection studies with pathogens Core to the findings of the Nuremberg trials, which investigated unethical medical practices used during World War 2, and the subsequent Declaration of Helsinki was that individuals must consent to participation in research. To ensure that participants fully understand the implications of participation in such studies can be complex, especially in challenge studies in which there might be several stages of participation. Methods to ensure that informed consent has been given can include separation of the consent procedures for vaccination and challenge (eg, norovirus virus-like particles vaccine and challenge), or to repeat the consent procedure before the challenge (eg, Oxford typhoid and malaria models). Importantly, assessment of the participant’s understanding of consent can be gauged by use of questionnaires or written examinations (eg, CVD including E coli and cholera challenge models) and by obtaining approval from the participant’s primary care practitioner for enrolment in the study (eg, Oxford malaria and typhoid models).

After fully informed consent has been provided, assessment of participant safety continues with vigorous medical screening procedures, which often include obtaining the participant’s medical or vaccination records or psychological assessment in addition to the specific requirements for the infection model (table 1). Participant selection and assessment criteria, although broadly similar between studies, are

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<table>
<thead>
<tr>
<th>Screening considerations</th>
<th>Screening method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptosporidium spp</td>
<td>Protective effect of pre-existing antibodies to Cryptosporidium parum</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>Protective effect of pre-existing antibody to infection and risk of Guillain-Barré syndrome or reactive arthritis</td>
</tr>
<tr>
<td>Salmonella enterica</td>
<td>Chronic carriage—found in about 3.5% of healthy individuals in endemic settings</td>
</tr>
<tr>
<td>Typhi</td>
<td>Individuals who are secretor-negative (non-functional FUT2 gene) are resistant to Norwalk virus infection</td>
</tr>
<tr>
<td>Norovirus</td>
<td>Strain specific neutralising antibodies</td>
</tr>
<tr>
<td>Influenza</td>
<td>Neutralising and haemagglutination-inhibition antibodies</td>
</tr>
<tr>
<td>BCG</td>
<td>Screening for latent tuberculosis</td>
</tr>
<tr>
<td>Dengue</td>
<td>For vaccine trial—neutralising antibody response needed to be detectable after vaccination to be eligible for challenge; screening control participants for flavivirus seronegativity</td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td>Blood group O conveys susceptibility to increased frequency and severity of diarrhoea or disease</td>
</tr>
<tr>
<td>Shigella spp</td>
<td>HLA B27 carriers have a raised risk of inflammatory reactive arthritis after infection or exposure to virus</td>
</tr>
</tbody>
</table>

ELISPOT=enzyme-linked immunospot.

Table 1: Screening considerations for specific challenge types
clearly dynamic and affected by new data and events that happen in challenge models. For example, investigation of an episode of myocarditis in one individual after influenza B challenge resulted in heightened screening for pre-existing cardiac conditions in all influenza challenge studies. The participant made a full recovery; similar events have been reported in the CHMI model, also strengthening the screening protocols used. Staff safety during the challenge study and in handling of potentially infectious samples is also important and risks can be reduced by suitable risk assessment, staff vaccination (when relevant), and infection control services.

Various microbiological factors are equally important to ensure participant safety. These factors include choice of challenge strain (balance between the need for virulence, ensuring a clinically applicable model, with manageable symptom profile and rapid response to treatment), route of delivery, treatment of infection or symptoms, and confirmation of infection clearance or resolution. Examples of pathogen-specific factors include the selection and use of modified strains, such as CagA strains of H pylori (in view of the association between CagA and gastric cancer), or the prevention of campylobacter recrudescence after participants receive antibiotics. Occasionally this close attention to minutiae results in unique observations, such as the in-vivo documentation of development of antibiotic resistance. Use of antibiotic-resistant bacterial strains for the challenge is beyond most ethical boundaries and is regarded as unsafe; however, this is a further example of how removed challenge models might be from most real-life settings.

**Challenge strain**

Crucial to setting up of a human challenge study is the choice and availability of a suitable challenge strain. The repertoire of available challenge strains is quite narrow because of the costs associated with good manufacturing practice production, type of pathogen, and available culture methods (eg, norovirus for which no culture method is available to produce a suitable challenge strain) [table 2], and potential regulatory hurdles. Furthermore, the necessity for extensive characterisation of strains—including genetic sequencing, antibiotic susceptibility testing, identification of attack rates, and investigators’ extensive pre-clinical experience using challenge strains—substantially restricts the expansion of potential strain repositories. Collaborative efforts will hopefully address these issues and increase the size of the repository of challenge strains (panel). Which strain is chosen might have substantial implications for the study findings, because variations in virulence, transmissibility, and genetic stability could greatly alter the infection profile and thus the risk to participants (table 2).

In vaccine studies, pathogen strain choice could be guided by the suggested mechanism of vaccine protection (eg, antibody or cell-mediated immune response) and whether homologous or heterologous protection is to be shown in participants. One successful strategy used in many vaccine studies as the first step towards vaccine development is to use an attenuated strain for vaccination and then the same non-attenuated strain for challenge—eg, for tularaemia live vaccine strain or the live-attenuated intranasal influenza vaccine in infants. Although they do not meet safety profile requirements of

**Table 2: Examples of challenge strains and the specific considerations for their use in human challenge studies**

<table>
<thead>
<tr>
<th>Strain Name</th>
<th>Characteristics and Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enterotoxigenic Escherichia coli</strong></td>
<td>Reliably produces more severe diarrhoeal responses compared with other assessed E coli strains; most virulent challenge strain and increases production of loose stools and higher volume of diarrhoea, but well characterised and therefore is the so-called benchmark strain.</td>
</tr>
<tr>
<td><strong>Helicobacter pylori</strong></td>
<td>CagA positivity is associated with the development of stomach cancer, thus initial challenges use CagA strains; however, most common strains are CagA all strains used are susceptible to antibiotics.</td>
</tr>
<tr>
<td><strong>Cryptosporidium parvum</strong></td>
<td>C. hominis is more frequently associated with asymptomatic infection than is C parvum; purified oocysts from infected calf faeces, administered in gelatin capsules.</td>
</tr>
<tr>
<td><strong>Norovirus strains</strong></td>
<td>Challenge virus derived from stool filtrate—donor followed up for &gt;10 years (syphilis, HCV, HBV, HIV, HTLV); fresh isolate needed due to absence of culture method.</td>
</tr>
<tr>
<td><strong>Giardia lamblia</strong></td>
<td>G lambia GS/M strain was regarded as infective; strain Isr was used as control because no participant developed signs consistent with giardiasis.</td>
</tr>
<tr>
<td><strong>Vibrio cholerae</strong></td>
<td>Prefrozen strains that have a standardised level of infection.</td>
</tr>
<tr>
<td><strong>Salmonella enterica serovar Typhi</strong></td>
<td>Fresh isolate with documented virulence through transmission to family members, reliable expression of virulence factors, frozen Quailes strain manufactured according to GMP, antibiotic sensitive.</td>
</tr>
<tr>
<td><strong>Shigella flexneri 2a strain</strong></td>
<td>Shiga toxin negative, genetically stable, antibiotic insensitive.</td>
</tr>
<tr>
<td><strong>Mycobacterium bovis</strong></td>
<td>BCG challenges are being developed to assess attenuated tuberculosis challenge model.</td>
</tr>
</tbody>
</table>

a vaccine, use of reactogenic strains could be considered to induce sufficient symptoms that accord with the required infection profile, such as the dengue 1 vaccine strain 4SAZS.115,119

Strain availability might vary greatly because of complexities in strain stability. For example, norovirus is difficult to culture and needs a fresh isolate before being used in challenge studies.127 Additionally, standardisation of challenge models across geographically distant sites is often needed so that research findings can be replicated and verified, to compare competing vaccine or treatment candidates, and to broaden the opportunities for participant recruitment. For standardisation to be effective and to achieve consistent challenge profiles, the availability of frozen strains has greatly helped various models, such as cholera, typhoid, and malaria challenge.51,77,83,128

Strain availability is also determined by the various jurisdictional requirements of the country or region in which the study is to be undertaken. Challenge agents in the USA are classed as pharmacologically active agents and therefore investigational new drug approval from the US Food and Drug Administration is required for their release.129 By contrast, challenge agents used in the UK are not classed as pharmaceutical products under the European Clinical Trials Directive (ECTD 2001/20/EC)130 and therefore regulatory approval (from the UK Medicines and Healthcare Products Regulatory Agency) is not required before their use.6,128 For the deliberate attenuation or genetic change of strains additional approvals are necessary for release of genetically modified organisms in most regions, in addition to specific permissions for contained use or deliberate release.

Because of the different approval processes in the USA and northern Europe and between the different countries of the European Union, international multicentre studies are logistically complex and expensive.42 In several places public–private partnerships are starting to address this difficulty by producing strain libraries for use by both academic and industry-sponsored studies, and by larger-scale production of standardised, well characterised, pre-frozen strains for off-the-shelf use.82,91,108

**Dose and route of infection**

How to actually plan and do the challenge once inocula are available is frequently a source of further deliberation, because this aspect is associated with many safety, scientific, and practical considerations. Replication of natural exposure often produces the most scientifically relevant data, but this method might not be physically or logistically practical or judged to be safe. Replication of natural infection needs knowledge of the exposure dose, which, if known, is often lower in the cohort of volunteers who are immunologically naive than in those previously exposed to the pathogen.76,106,120 The natural attack rate of most infections is quite low and often associated with mild or subclinical infections detected by serological responses only. As a result, to study a sufficient number of individuals exposed to and infected, especially if clinical manifestations are used as a study endpoint, larger numbers of study participants would need to be exposed. Experimental challenges, therefore, often use large unnatural exposure doses to induce a high attack rate and to use available resources more efficiently. With most infections a high dose leads to a short incubation period.121 Although increased attack rates often allow for small study cohorts, they can overwhelm vaccine-derived or infection-derived protection, cause more severe infection symptoms, or reduce the physiological relevance of investigational findings,72 motivating many investigators to lower the challenge dose used in enteric challenge studies. Methods to lower the challenge dose include the use of sodium bicarbonate solution to reduce the effects of gastric acidity on bacteria viability in the *Shigella* spp or typhoid challenge models.72,75,89

Reproducing the route of natural exposure can also affect the disease caused and the corresponding host’s response that is being studied (table 3). This drawback is a substantial limitation to challenge studies of respiratory viruses because, generally, aerosol challenge to the lower respiratory tract leads to severe illness with an increased risk of complications.194,195 As a result, nasal instillation is frequently used to deliver respiratory viruses, which leads to the need for nasal dosing of topically delivered treatments, and, in the case of influenza, an illness profile that substantially differs from natural disease. For nasopharyngeal bacterial challenges the endpoints measured include colonisation by the instilled organism.84,120 An exception to this approach through the

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Panel: Major advances and future opportunities in the use of human challenge studies

### Major advances

- Assessment of vaccines in several challenge models to select those with the best possible efficacy to take into field trials
- Understanding of pathogenesis of some infections and testing of new treatments
- Development of ethical and regulatory frameworks for use of human challenge models to improve public health through research with the relevant model system for human health

### Future opportunities

- To develop new human challenge models and capacity in existing models to accelerate development of new vaccines and treatments
- To apply new technologies, such as RNAseq, to investigate the natural and vaccine-induced resistance to infection
- To adapt challenge models for the use of genetically modified organisms to assess putative virulence factors or antigens for development of vaccines
- To develop challenge strain libraries or repositories and to promote increased sharing of protocols and development of model frameworks between study investigators
- To develop challenge models in relevant populations, such as in endemic geographical areas, who might have substantially different and more relevant responses to infection—eg, because of genetic background, previous exposure, or different microbiome
- Maximise possible use of collaborative work between regulatory agencies and to develop a multinational ethical framework for challenge studies to adhere to

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 vectored versus direct subcutaneous or intravenous infection with malaria, dengue, and filaria have also been investigated in some depth in efforts to standardise challenge delivery in addition to understanding disease mechanisms.

### Infection endpoints or criteria

Choice of endpoints in challenge studies is crucial to minimise the risk to participants (the number exposed to infection and severity of infection caused) and to ensure that clinically or scientifically useful outcomes are assessed. Depend on the type of study being undertaken, the endpoint could be diagnosis of infection

### Table 3: Considerations specific to the type and route of infection used in challenge trials

<table>
<thead>
<tr>
<th>Consideration</th>
<th>Approach taken</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory viruses (including influenza, and respiratory syncytial virus)</td>
<td>Risk of more severe infection to lower respiratory tract with aerosolised infection; effects of behaviour or habits on person-to-person transmission</td>
</tr>
<tr>
<td>Nasopharyngeal (including Streptococcus pneumoniae)</td>
<td>Risk of invasive disease including pneumonia or meningitis</td>
</tr>
<tr>
<td>Enteric (eg, Salmonella enterica serovar Typhi, Vibrio cholerae, ETEC, shigellosis, or Helicobacter pylori)</td>
<td>Gastric acid milieu acting as a broad and effective non-specific barrier to infection</td>
</tr>
<tr>
<td>Mosquito bite vs blood stage challenge</td>
<td>Transdermal infection or administration of infected erythrocytes</td>
</tr>
<tr>
<td>Hookworm infection</td>
<td>Transdermal infection</td>
</tr>
<tr>
<td>Gonococcal infection</td>
<td>None</td>
</tr>
</tbody>
</table>

ETEC=enterotoxigenic Escherichia coli.

### Table 4: Examples of model endpoints used in human challenge studies

<table>
<thead>
<tr>
<th>Clinical endpoint</th>
<th>Microbiological endpoint</th>
<th>Other endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholera</td>
<td>Diarrhoea (purge)</td>
<td>No relevant endpoints</td>
</tr>
<tr>
<td>Typhoid-Maryland</td>
<td>Temperature</td>
<td>No relevant endpoints</td>
</tr>
<tr>
<td>Typhoid-Oxford</td>
<td>Temperature (≥38°C for ≥12 h)</td>
<td>No relevant endpoints</td>
</tr>
<tr>
<td>ETEC</td>
<td>Diarrhoea using stool grading and symptoms as severity score</td>
<td>Quantitative stool culture (early shedding predictive of subsequent disease)</td>
</tr>
<tr>
<td>Norovirus</td>
<td>Gastroenteritis (infection or disease): &gt;200 g of watery faeces, one vomiting episode, &gt; three loose or watery stools</td>
<td>Infection: faecal shedding (stool PCR)</td>
</tr>
<tr>
<td>Helicobacter pylori</td>
<td>No relevant endpoints</td>
<td>Culture</td>
</tr>
<tr>
<td>Influenza</td>
<td>Temperature and symptom scores</td>
<td>Histology, rapid urease test, urease breath test</td>
</tr>
<tr>
<td>Malaria</td>
<td>No relevant endpoints</td>
<td>No relevant endpoints</td>
</tr>
<tr>
<td>Shigellosis</td>
<td>Diarrhoea and dysentery symptoms and temperature (two oral temperatures ≥37.6°C at least 5 min apart)</td>
<td>Parasitaemia (by qPCR or microscopy)</td>
</tr>
</tbody>
</table>

ETEC=enterotoxigenic Escherichia coli; qPCR=quantitative PCR. Cholera: stool weight used to grade severity of illness (>3 kg is moderate, >5 kg is severe). Signs either abdominal cramps or pain, nausea, bloating, loose stools, temperature (≥37.6°C), myalgia, or headache. Helicobacter pylori: composite endpoint score for severity of infection (score 0=no infection, infection severity graded 1–4); higher the score the worse the infection. Shigellosis: diarrhoea volume used to grade severity of illness; same definition of diarrhoea is used for many of the enteric challenge studies including those undertaken at the Centre for Vaccine Development (MD, USA).
(measured by a diagnostic test), carriage or infection by specific strain types, development of infection signs and symptoms, or assessment of infection severity. Although predefined endpoint criteria are needed, these have a substantial effect on attack rates, which was shown in a retrospective analysis of the Maryland typhoid challenge studies. Dependent on the pathogen selected, quantitative illness measures (eg, fever, stool volumes, nasal mucus weights, middle ear pressure) can provide objective outcome data. Different endpoint criteria to the standard ones used allows for additional detailed assessments of model outcomes—ie, to distinguish between participants who are infected and those infected and diseased to further stratify so-called challenge take (ie, successful infection by the challenge strain), or assess intervention effects on symptomatic and asymptomatic infections. The challenge study endpoint, which is generally the point at which rescue treatment (eg, antibiotics) is initiated, is often composed of several clinical, microbiological, or serological components (table 4).

The use of development of infection as an endpoint in challenge studies provides a unique opportunity to identify and validate novel biomarkers and diagnostic tests. The norovirus challenge study showed that availability of pre-challenge samples allows baseline susceptibility factors to be assessed. Furthermore, subsequent samples obtained after challenge allows the direct investigation and assessment of putative correlates of protection after infection or vaccination (appendix).

**Conclusions**

Substantial advances in the understanding of disease mechanisms and host responses, together with more affordable high-throughput technologies, have created opportunities to obtain informative, predictive, and often novel data from human challenge studies. Although historically moral and ethical challenges remain and should remain under constant inspection and discussion, never has this type of research been more feasible and valuable. If done appropriately, challenge studies have an excellent safety profile that is probably attributable to careful selection of participants, adherence with study procedures, and heightened investigator vigilance to potential problems. More than 20 challenge models are in use and several thousands of volunteers have so far safely participated in challenge studies.

Furthermore, in many cases, while operating in the appropriate ethical committee mandate, institutions and investigators clearly work in some isolation or operate solely in disease-specific or pathogen-specific areas. Coordination and communication between these different areas of study and models using a horizontal approach by sharing protocols and common experiences could greatly benefit the discipline. This sharing in turn might lead to standardisation of reporting procedures, not only to ethics review boards and regulatory agencies, but also to publication of study results.

A general shortage of challenge strains could be due to the insufficient experience or safety profile with a particular, recently isolated strain, or to the specific technical difficulties in strain isolation or production, characterisation, and storage. Specific challenge agents are especially problematic or unavailable, including *H pylori*, respiratory syncytial virus, noroviruses, and a sufficiently broad range of influenza strains. The narrow repertoire and often attenuated nature of challenge strains used potentially restricts the adequate extrapolation of study findings into clinical settings and ignores the potential effects of strain-to-strain variation. Collaboration and sharing of challenge strains between institutions and study sites might not only effectively address this issue, but also might promote a collegial atmosphere supportive of undertaking such expensive preclinical safety and dose-ranging studies. Such collaboration has been very successful in specific public–private partnerships, including the provision of cryopreserved *P falciparum* strains, and frozen *V cholerae*, and *Salmonella Typhi* Quailes strain. The availability of challenge strains, which might cost more than US$200,000 to make when adhering to good manufacturing practice standards, could facilitate more research teams to consider doing this type of research, including those in resource-limited settings. For example, cholera and *Shigella* spp studies have been undertaken in Thailand, and several African centres recently started to do CHMI studies.

Central to the continuing success of human challenge studies is the careful balance between close regulation of safety and ethical processes and the freedom to make important scientific advances. Although some experimental infection studies were not always done ethically, it has encouraged debate between investigators and led
to many of the advancements in the ethical principles that are now held central to clinical trial work. Despite a sometimes uncertain prospect in the past, human challenge studies could continue to substantially affect medicine and global health for another century.

**Contributors**

TCD and CJB did the literature search and drafted the Review. CJB created figure 1. VSM, DMA, FGH, EAC, MML, AVSH, and AJP reviewed the manuscript and provided further input into the quality of this manuscript.

**Declaration of interests**

FGH reports serving as consultant on respiratory virus vaccines (GliaoxSmithKline (GSK)) and respiratory virus diagnostics (Hologic), a member of Data and Safety Monitoring Board (DSMB) for influenza vaccine study (Sanofi-Pasteur) and a member of DSMB for respiratory syncytial virus antiviral trials (Gilead), with honoraria paid to University of Virginia. In 2013–14 both the University of Virginia and FGH personally received compensation from legal firms and insurance companies for his time in reviewing one patent case regarding zanamivir (Biota and GSK) and medicolegal cases involving fatal influenza and oseltamivir (Roche). FGH has been an unpaid consultant to several companies engaged in developing and marketing respiratory virus antivirals, other therapies, and vaccines. AJP reports grants from Wellcome Trust, Jenner Institute, National Institute of Health, WHO, or European Union.

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**References**


