Vaccine 37 (2019) 3485–3494

Contents lists available at ScienceDirect

Vaccine

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

Controlled human infection for vaccination against *Streptococcus pyogenes* (CHIVAS): Establishing a group A *Streptococcus* pharyngitis human infection study

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ARTICLE INFO

Article history:
Received 5 February 2019
Received in revised form 14 March 2019
Accepted 26 March 2019
Available online 14 May 2019

Keywords:
Group A *Streptococcus*
Streptococcus pyogenes
Human infection studies
Controlled human infection
Human challenge
Vaccine development

ABSTRACT

Group A *Streptococcus* (GAS) is a highly-adapted and human-restricted pathogen responsible for a high global burden of disease across a diverse clinical spectrum. Vaccine development has been impeded by scientific, regulatory, and commercial obstacles. Human infection studies (HIS) are increasingly contributing to drug, diagnostics, and vaccine development, reducing uncertainty at early stages, especially for pathogens with animal models that incompletely reproduce key elements of human disease. We review the small number of historical GAS HIS and present the study protocol for a dose-ranging inpatient study in healthy adults. The primary objective of the study is to establish a new GAS pharyngitis HIS with an attack rate of at least 60% as a safe and reliable platform for vaccine evaluation and pathogenesis research. According to an adaptive dose-ranging study design, *emm*75 GAS doses manufactured in keeping with principles of Good Manufacturing Practice will be directly applied by swab to the pharynx of carefully screened healthy adult volunteers at low risk of severe complicated GAS disease. Participants will remain as closely monitored inpatients for up to six days, observed for development of the primary outcome of acute symptomatic pharyngitis, as defined by clinical and microbiological criteria. All participants will be treated with antibiotics and followed as outpatients for six months. An intensive sampling schedule will facilitate extensive studies of host and organism dynamics during experimental pharyngitis. Ethics approval has been obtained and the study has been registered at ClinicalTrials.gov (NCT03361163).

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https://doi.org/10.1016/j.vaccine.2019.03.059
0264-410X/

1. Introduction

Group A Streptococcus (GAS, *Streptococcus pyogenes*, 'Strep A') is a formidable and ubiquitous human pathogen with a substantial disease burden spanning mild to severe, acute and chronic, infectious and non-infectious syndromes [1]. While control efforts, including antibiotics, have altered the global profile of GAS disease, it remains a prominent cause of infection-related mortality. GAS disease persists across all settings, every age group, and all socioeconomic strata [2,3]. Conservative estimates are of 615 million incident cases of GAS pharyngitis, 162 million prevalent cases of impetigo, 660,000 incident cases of invasive infection causing 150,000 deaths, 470,000 incident cases of acute post-streptococcal glomerulonephritis (APSGN), and 470,000 incident cases of acute rheumatic fever (ARF) [2,4–7]. Over 27% of an estimated 61 million annual cases of cellulitis are likely due to GAS [8,9]. Rheumatic heart disease (RHD) alone is responsible for more than 33 million prevalent cases and 320,000 deaths annually [3]. RHD and APSGN are important contributors to non-communicable diseases including heart failure, stroke, and chronic kidney disease [10,11]. Pharyngitis and impetigo cause a less conspicuous but major impact on healthcare utilisation, absenteeism, and antibiotic use [9,12,13].

A vaccine against GAS is a global health imperative and stated priority of the World Health Organization [14,15]. No alternative healthcare interventions have a realistic prospect of achieving a sizeable and sustainable reduction in the global burden of GAS diseases. Even with major public investment, primary and secondary prevention strategies targeting ARF and RHD have had only a partial effect [16,17]. Patients affected by invasive GAS often require intensive care support and/or surgery despite prompt appropriate antibiotic therapy, with case-fatality rates of over 25% for the most severe syndromes (e.g. toxic shock, necrotizing fasciitis) [6,7]. In spite of the pressing global need, GAS vaccine development has been impeded by a combination of scientific, regulatory, and commercial barriers [15,18,19]. Of four current candidate vaccines, none has entered efficacy trials [20–23]. Other potentially promising vaccines are at various stages of pre-clinical development, targeting a wide array of antigens. Recent efforts have focussed on promoting critical research to address roadblocks and fast-track vaccine development, building momentum towards Phase 2b/3 trials to demonstrate vaccine safety and efficacy against pharyngitis, and possibly impetigo, as the most relevant and realistic endpoint (s) for vaccine field trials [15,24].

A GAS pharyngitis human infection study (HIS)[1] to support early vaccine proof of concept evaluation is a priority activity in the WHO Vaccine Development Technology Roadmap for GAS vaccines [15,25]. As a highly-adapted and human-only pathogen, in vitro assays, genomics, and animal models do not fully capture or predict the dynamic nature of human infection by GAS [26,27]. A HIS may bridge this translational divide, to down-select candidate vaccines and provide early proof of concept evidence for vaccine protective efficacy to reduce uncertainty and give confidence to industry, funders, and regulators that GAS vaccine development is viable as well as valuable [28]. HIS could also inform development of assays, diagnostic tools, and standardised case definitions to help establish the epidemiologic, microbiologic, and immunologic frameworks necessary for future field trials.

[1] We use 'Human Infection Study' rather than 'Human Challenge' or 'Controlled Human Infection Model', as the preferred term emerging from a Wellcome Trust initiative 'Exploring terminology and naming for Controlled Human Infection Models'. Available at: https://wellcome.ac.uk/sites/default/files/exploring-terminology-and-naming-for-controlled-human-infection-models.pdf (viewed January 2019).

2. Historical perspective

In a 1956 lecture, Charles H. Rammelkamp Jr. reviewed the work of the Streptococcal Disease Laboratory, Warren Air Force Base, Wyoming, and associated groups, and briefly described, "a volunteer inoculated by the direct transfer of infectious material from a patient with a type 19 infection. . .Symptoms of infection developed 44 h after. . ." [29].

Subsequent to this, three human infection studies with 172 total adult participants were conducted in the 1970s (Table 1) [30–33]. These were double-blind, placebo-controlled trials of parenteral and/or mucosal monovalent purified M-protein vaccines for protection against experimental pharyngitis from direct pharyngeal application by swab of M1, M3 or M12 GAS strains. Participants were monitored for signs and symptoms of pharyngitis as inpatients for five days, with regular throat swab cultures, leukocyte counts, and measurement of vital signs including temperature. For participants who developed symptoms and signs of pharyngitis, most occurred 36–72 h after challenge. Among the 84 unvaccinated (control) participants, 57 reported sore throat (68%) and 77 had at least one sign of pharyngitis (92%) including fever, pharyngeal erythema or exudates, or lymphadenopathy. Antibiotic treatment with intramuscular benzathine penicillin G was initiated after five days, or within 18–24 h of symptom onset if more severe features were present. Further penicillin injections were given if throat swab cultures were positive for GAS after 24–48 h and/or two weeks later. All participants responded to antibiotics and no serious adverse events occurred during the admission or in the three to four weeks after treatment. Longer term follow-up was not reported and these studies pre-dated routine clinical echocardiography.

Vaccine efficacy (VE) against pharyngitis caused by a homologous M1 strain was 89% (95% CI 23–98) in the initial study of a parenteral M1 vaccine [30] and 68% (28–86) in the subsequent study of a mucosal M1 vaccine [31]. Efficacy could not be demonstrated in the final underpowered study of parenteral and mucosal M3 and M12 vaccines, with too few participants spread across too many treatment arms [33]. Across the three studies, colonisation (positive throat cultures) was significantly reduced by mucosal but not parenteral vaccination. Any vaccination was associated with statistically significant reductions in every symptom and sign of pharyngitis (Supplementary Table S1). There were inconsistent quantitative (complement fixation, hemagglutination, radioimmune precipitation) and functional (serum bactericidal assay) antibody responses in serum and nasal secretions following vaccination and challenge, and none was predictive of protection against pharyngitis or colonisation. An additional (unpublished) study at the University of Florida found no protective effect against experimental GAS pharyngitis from ingestion of hyperimmune bovine milk containing high immunoglobulin titers to the homologous M-protein (Robert D'Alessandri, personal communication, December 19th, 2017).

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2019.03.059.

These important historical studies show that a GAS pharyngitis HIS in healthy adult volunteers is possible, highly unlikely to cause early severe adverse effects, and may be used to explore microbiological and immunological aspects of GAS disease and assess vaccine protective efficacy. However, a new study protocol must now meet more stringent scientific, ethical, and regulatory standards for HIS specifically, and human research broadly [34–37]. Viewed through a modern lens, perhaps unfairly, reporting of methods and results in these historical studies is broadly inadequate [30–33]. Examples include the limited details provided

Table 1
Group A *Streptococcus* Pharyngitis Human Infection Studies.

Citation	Study design	Subjects and screening	Interventions	Pharyngitis endpoint			
				Vaccine (%)		Placebo (%)	P*
Fox EN et al.	Randomized double-blind placebo-controlled trial of parenteral monovalent M1 protein vaccine for protection against challenge with M1 CDC SS-496 GAS	Healthy male prisoners, 21–35 years, at Florida State Correctional Institution, Raiford. History, physical examination, blood and urine tests, ECG. Other exclusion criteria: history of heart or kidney disease or known allergies; serum M1 bactericidal antibodies; positive penicillin allergy skin test; delayed-type hypersensitivity to intradermal injection of M1 protein	*At 0, 1, 2 months:* 1. SC vaccine [M1 protein + $Al(OH)_3$ + RL], *or* 2. SC placebo [$Al(OH)_3$ + RL]*30–50 days after the third dose:* Challenge by direct oropharyngeal application by swab dipped once in solution of $\sim 10^6$ CFU/ml GAS	1/19 (5) *VE = 89% (95% CI 23–98)*		12/25 (48)	0.002
Polly SM et al.	Randomized double-blind placebo-controlled trial of mucosal monovalent M1 protein vaccine for protection against challenge with M1 CDC SS-496 GAS	Healthy male and female volunteers, 18–25 years old, at University of Florida. History, physical examination, blood and urine tests, ECG. Other exclusion criteria: history of heart or kidney disease, ARF, scarlet fever, or known allergies; serum M1 bactericidal antibodies; positive penicillin allergy skin test	*At 0, 1, 2 months:* 1. Aerosol spray of mucosal vaccine [M1 protein + thimerosal + RL], *or* 2. placebo [RL] into nostrils and onto pharynxChallenge as above	5/21 (24) *VE = 68% (28–86)*		17/23 (74)	0.002
D'Alessandri R et al.	Randomized double-blind placebo-controlled trial of mucosal and parenteral monovalent M3 and M12 vaccines for protection against challenge with homologous M3 and M12 strains (identity and provenance not stated)	Healthy male and female volunteers, 18–25 years old, at University of Florida. History, physical examination, blood and urine tests, ECG. Other exclusion criteria: history of heart or kidney disease, ARF, or penicillin allergy; serum M3 or M12 bactericidal antibodies	*At 0, 1, 2 months:* 1. SC vaccine (M3 or M12 protein Al$(OH)_3$ + 0.5 ml RL) & mucosal placebo, *or* 2. Mucosal vaccine (M3 or M12 protein + thimerosal + RL) & SC placebo [$Al(OH)_3$ + RL], *or* 3. SC placebo & mucosal placeboChallenge as above, with M3 or M12 GAS (homologous)	All vaccines: SC (M3 + M12): Mucosal (M3 + M12): SC M3: Mucosal M3: SC M12: Mucosal M12:	15/48 (31) 9/20 (45) 6/28 (21) 3/7 (43) 3/12 (25) 6/13 (46) 3/16 (19)	15/36 (42)	0.364 1.00 0.111 1.00 0.493 1.00 0.129
			All studies	21/88 (24)		44/84 (52)	0.0001

* Two-tailed P-value by Fisher's exact test, ARF: acute rheumatic fever; CFU: colony-forming units; CI: confidence interval; ECG: electrocardiogram; GAS: Group A *Streptococcus*; RL: Ringer's lactate buffer; SC: subcutaneous; VE: vaccine efficacy.

regarding the challenge strains and outpatient follow-up procedures, and inclusion of a vulnerable group (prisoners) without explicit ethical justification.

3. Study protocol for a 21st century GAS pharyngitis human infection study

The goal of this initial Controlled Human Infection for Vaccination Against Streptococcus (CHIVAS) study is to establish a reliable and safe HIS for future use in testing GAS vaccines and therapeutics. The full study protocol meets standards set out in the Guideline for Good Clinical Practice, the SPIRIT 2013 Statement [38], and will enable compliance with previously proposed reporting standards for HIS [34].

3.1. Study design

This is a dose-ranging inpatient human infection study of the pharyngitis attack rate and the dynamic clinical, bacteriological, and immune responses in healthy adult volunteers following direct pharyngeal application of *emm*75 GAS (thus the study name "CHIVAS-M75"). The study is based at a clinical trials facility operated by a private contract research organization (CRO) and supported by a co-located tertiary academic hospital in Melbourne, Australia.

3.2. Study objectives and outcomes

The primary objective of the study is to develop a safe HIS of GAS pharyngitis, establishing the dose of GAS M75 required to cause a reproducible pharyngitis attack rate of 60% or greater within five days of direct application by swab to the oropharynx. The related primary outcome is the proportion of participants at each dose level who develop GAS pharyngitis, according to a combined clinical and microbiological case definition (Fig. 1).

The secondary objective of the study is to enable a wide-ranging exploratory effort to describe host and organism responses during experimental GAS pharyngitis. Blood, saliva, and throat swab samples will be used to characterize experimental GAS pharyngitis pathogenesis and systemic, mucosal, humoral, and cellular host immune responses, including to potential vaccine antigens. The secondary exploratory outcomes will relate to comparisons between participants who develop pharyngitis and those who do not, including the following: quantitative antibody responses (e.g. ELISA, multiplex bead assays); functional antibody responses (e.g. opsonophagocytic killing); phenotypic and functional changes in leukocyte subsets including memory B cells; cytokines; serial bacterial cultures; quantitative bacterial PCR; and host and organism gene expression.

3.3. Recruitment and eligibility criteria

Recruitment will use an established CRO database of healthy volunteers for clinical studies and may, with ethics approval of all materials, also advertise in print, radio, and social media. Healthy adults aged 18–40 years without risk factors for severe GAS disease will be considered for inclusion. General health will be determined by history, physical examination, non-clinically significant blood and urine test results, electrocardiography, and transthoracic echocardiography. Current or planned pregnancy during the study period and lactation are criteria for exclusion. Females of childbearing potential must agree to using a barrier method of contraception from screening until thirty days after

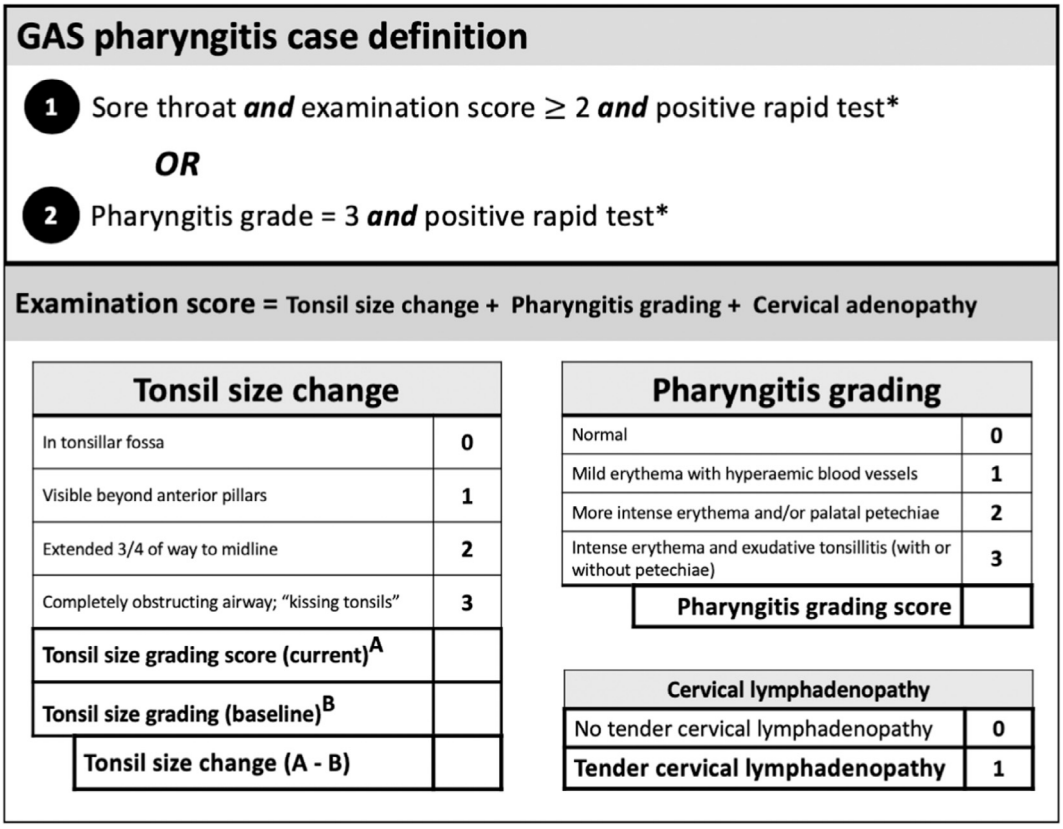


Fig. 1. Group A *Streptococcus* Human Infection Study Pharyngitis Case Definition. *Rapid test: highly sensitive molecular point-of-care test (Alere™ i Strep A) GAS: group A *Streptococcus*.

the final dose of rifampin. Exclusion due to use of certain restricted concomitant medications focuses most on the month preceding challenge until expected complete recovery by the first outpatient visit. In this critical period for the primary outcome and for potential development of infective complications, there are multiple restrictions on use of certain drugs including antibiotics, vaccines, systemic and intranasal corticosteroids, immunomodulators, anti-inflammatory therapy.

Study-specific exclusion criteria are: a personal or family history of severe GAS infection or post-infectious sequelae (ARF, RHD, APSGN); history of tonsillectomy; intolerance of throat swab procedure (exaggerated gag reflex); known hypersensitivity or other contraindication to beta-lactam, rifamycin, macrolide, or lincosamide antibiotics, or soya protein (the bacterial culture medium is soya based); and echocardiographic evidence of subclinical RHD [39]. Participants with evidence of pre-existing immunity to the challenge strain will also be excluded, defined for this study as a high serum IgG to a peptide comprising the first fifty amino acids of the M75 protein (N-terminal hypervariable region) measured by ELISA [40,41].

3.4. Study procedures

Each participant will be challenged once, using a procedure analogous to a clinical throat swab done "in reverse", that is, direct oropharyngeal application using a sterile Dacron swab to transfer GAS from a thawed single-dose vial. Participants will be fasted (food and water) for 90 min before and after inoculation. The challenge will take place in a dedicated space in the trial facility, observing droplet and contact precautions, consistent with hospital and community (e.g. school exclusion) infection control recommendations [42,43]. The vial will be removed from its double-bag, inverted eight times to mix, and the sterile swab dipped in the vial for ten seconds. The participant will tilt their head backwards and widely open their mouth with a tongue depressor used to hold the tongue in place. Taking care not to touch the teeth, buccal mucosa, or tongue, the inoculum will be applied liberally over the tonsillar arches (lateral posterior oropharyngeal walls) then rolled back and forth over the posterior oropharynx. The single-dose vials will be recapped, marked as used, and returned to storage in the pharmacy. Accountability processes will track every dispensed dose from the pharmacy to pharynx and back again, with reconciliation of all doses received, dispensed, and consumed at the end of the study.

Participants will be confined as inpatients for up to 6 days from admission (on the day prior to challenge) until discharge (approximately 24 h after antibiotic treatment), then followed periodically for six months (Fig. 2). In clinical practice, GAS pharyngitis at any age rarely if ever requires inpatient admission. For this initial HIS, inpatient admission for up to five nights from challenge and early outpatient visits will enable close monitoring, including blood cultures, during the period of highest risk for acute infectious complications. Analgesia with paracetamol will be administered on request at standard doses. Non-steroidal anti-inflammatory drugs have been linked to increased risk of severe GAS and respiratory infections [44,45] and will not to be used during the inpatient period. In the unlikely event a participant deteriorates acutely, empiric sepsis treatment will be started in the clinical research unit and close support from the co-located adult tertiary hospital will ensure a rapid clinical response and transfer if required. Once discharged, participants will have access to 24-h telephone advice and early outpatient clinical review as required.

All participants will receive antibiotic treatment aimed at eradicating the challenge strain from the oropharynx (single-dose intramuscular benzathine penicillin G 900 mg plus oral rifampin 300 mg twice a day for eight doses). Antibiotics will be administered for participants diagnosed with pharyngitis by medically-qualified CRO staff, usually immediately afterwards and always within 24 h. Participants who have not developed pharyngitis by the fifth day after challenge will also be treated. Throat swabs at subsequent outpatient visits will be done to detect prolonged carriage of the challenge strain, with further antibiotic treatment as indicated. Echocardiography, blood and urine studies will be repeated during six months outpatient follow-up to rule out interval development of subclinical immune-mediated complications (i.e. RHD, APSGN).

3.5. Dosing of challenge strain

Dosing will begin at an order of magnitude lower ($1–3 \times 10^5$ CFU/ml) than historical GAS pharyngitis HIS. The study is primarily based on a dose-escalation algorithm, with a contingency plan for dose de-escalation should an unexpectedly high attack rate occur at the starting level (Fig. 3). Up to four dose levels are planned for testing, corresponding to a minimum of 20 and maximum of 80 participants. Total numbers of participants will depend on the observed proportion of participations developing pharyngitis at each dose level.

3.6. Outcome measures

The pharyngitis case definition (Fig. 1) borrows elements from GAS pharyngitis clinical prediction rules including the Centor and McIsaac scores (sore throat, exudative tonsillitis, tender cervical lymphadenopathy) [46], as well as a measure of tonsil size shared with a non-human primate GAS pharyngitis model [47], and a microbiological criterion assessed in real time from a throat swab using the Alere™ i Strep A Test (Abbott), a highly sensitive rapid molecular point-of-care test [48].

3.7. Challenge strain

A broadly antibiotic-susceptible *emm*75 GAS strain ('GAS M75') collected from a child with pharyngitis in a previous study [49] was selected for initial use in this HIS. As previously described in a paper detailing the rationale for strain selection, the results of in vitro assays, whole genome sequencing, and a murine model of invasive disease suggest it is fit for purpose, with a favourable safety profile and broad representation of relevant candidate vaccine antigens [50]. Processes for strain manufacture (Fig. 4) followed the principles of Good Manufacturing Practice (GMP) [51], including use of a dedicated laboratory and Class II biosafety cabinet using strict aseptic techniques with dedicated and sterile product contact equipment, high quality materials supplied with Certificates of Analysis, pre-approved batch manufacturing records and pre-approved product specifications. The Victorian state microbiological reference laboratory was contracted to provide independent quality control testing in parallel and in addition to tests conducted in the research laboratory.

To maximize the model's sustainability, scalability, and portability, we manufactured single-dose vials of the challenge strain, requiring thawing only prior to inoculation. An experienced external quality reviewer assisted in planning and subsequent oversight of all manufacturing processes, reviewing batch manufacturing records and test results for the master cell bank and final single-dose vials of challenge material, including genetic and phenotypic stability assessment by extended passaging [50]. Prior to release of the doses to the trial centre, certificates of analysis were approved and signed by the principal investigator (manufacturer), an independent laboratory representative (quality control), and the external quality reviewer.

	Screening	INPATIENT PERIOD D -1 Admission	D +1 Challenge	D +2 Until...	Treatment	Discharge	PERIODIC OUTPATIENT FOLLOW-UP 1 week	1 month	3 months	6 months
STUDY ITEMS										
Throat swabs	X	X		12-hourly	12-hourly	X	X	X	X	X
Saliva	X	X	X	X	X	X	X	X	X	
Serum	X	X	X	X	X	X	X	X	X	X
Plasma		X	X	X	X	X	X	X	X	X
PBMC		X	X	X	X	X	X	X	X	X
CRP		X		X	X	X	X			
Blood RNA		X	X	X	X	X	X	X	X	X
Host DNA		X								
Pharynx photo		X		X	X	X	X	X		
SAFETY ITEMS										
Echo & ECG	X									X
Urinalysis	X							X		X
Safety bloods	X							X		X
Blood culture				X						
Urine drug screen	X	X								
Alcohol breath test	X	X								
Pregnancy test +/- FSH	X	X								

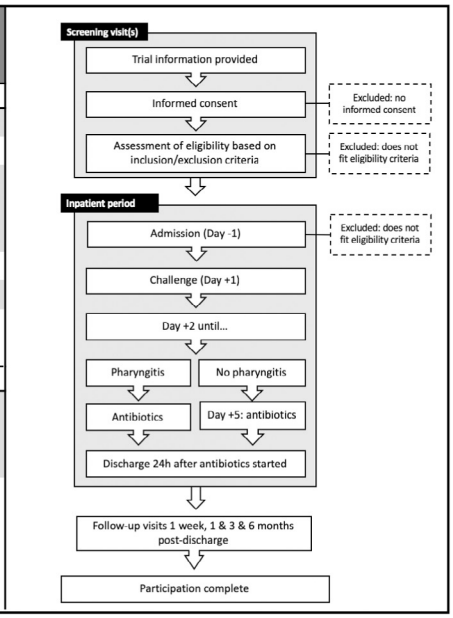
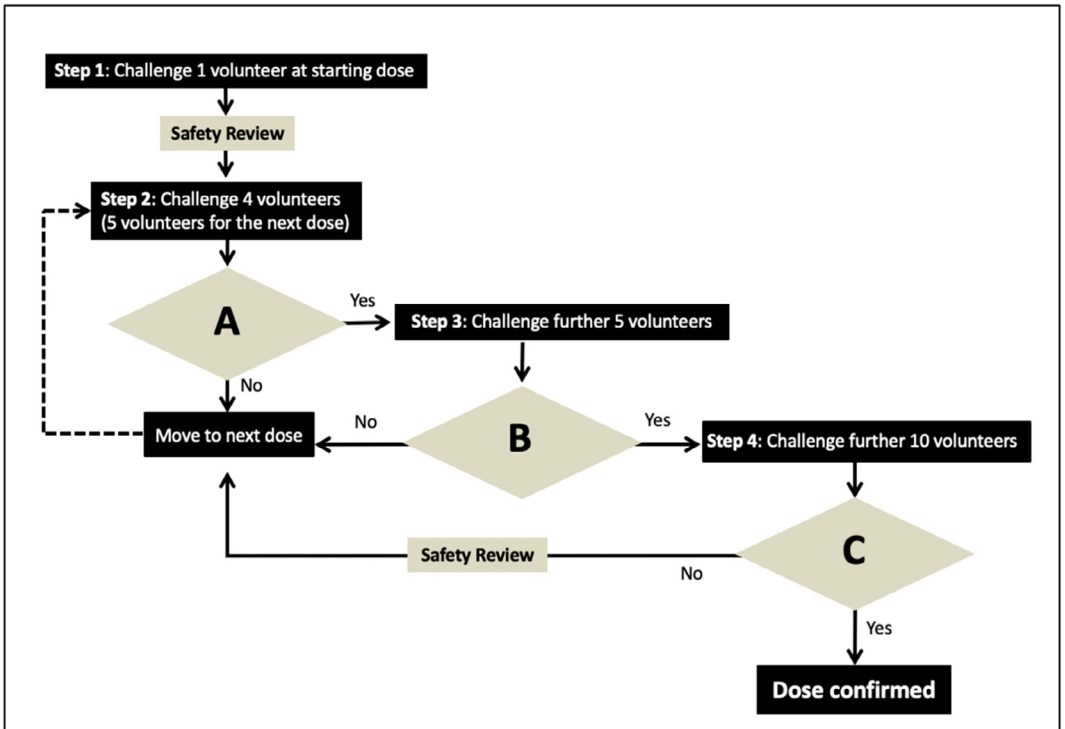


Fig. 2. Group A *Streptococcus* Human Infection Study Schedule. CRP: C-reactive protein; ECG: electrocardiogram; echo: echocardiogram; FSH: follicle stimulating hormone; PBMC: peripheral blood mononuclear cells; Safety bloods: hematology, biochemistry, renal and liver function, coagulation profile.



Dose level		A	B	C
Starting dose	1-3 x 10^5 CFU/mL	≥ 2/5 develop pharyngitis	≥ 7/10*	≥ 15/20*
	1-3 x 10^6 CFU/mL	≥ 2/5	≥ 7/10	≥ 14/20
	1-3 x 10^7 CFU/mL	≥ 2/5	≥ 6/10	≥ 13/20
	1-3 x 10^8 CFU/mL	≥ 2/5	≥ 6/10	≥ 12/20

Fig. 3. Group A *Streptococcus* Human Infection Dose-ranging Study Design. *In the unlikely event of very high attack rates at the starting dose (i.e. ≥9/10 or ≥18/20), consideration will be given to dose de-escalation to 1–3 × 10^4 CFU/ml using the same cohort targets as for the starting dose (≥2/5, ≥7/10, ≥15/20). CFU: colony forming units.

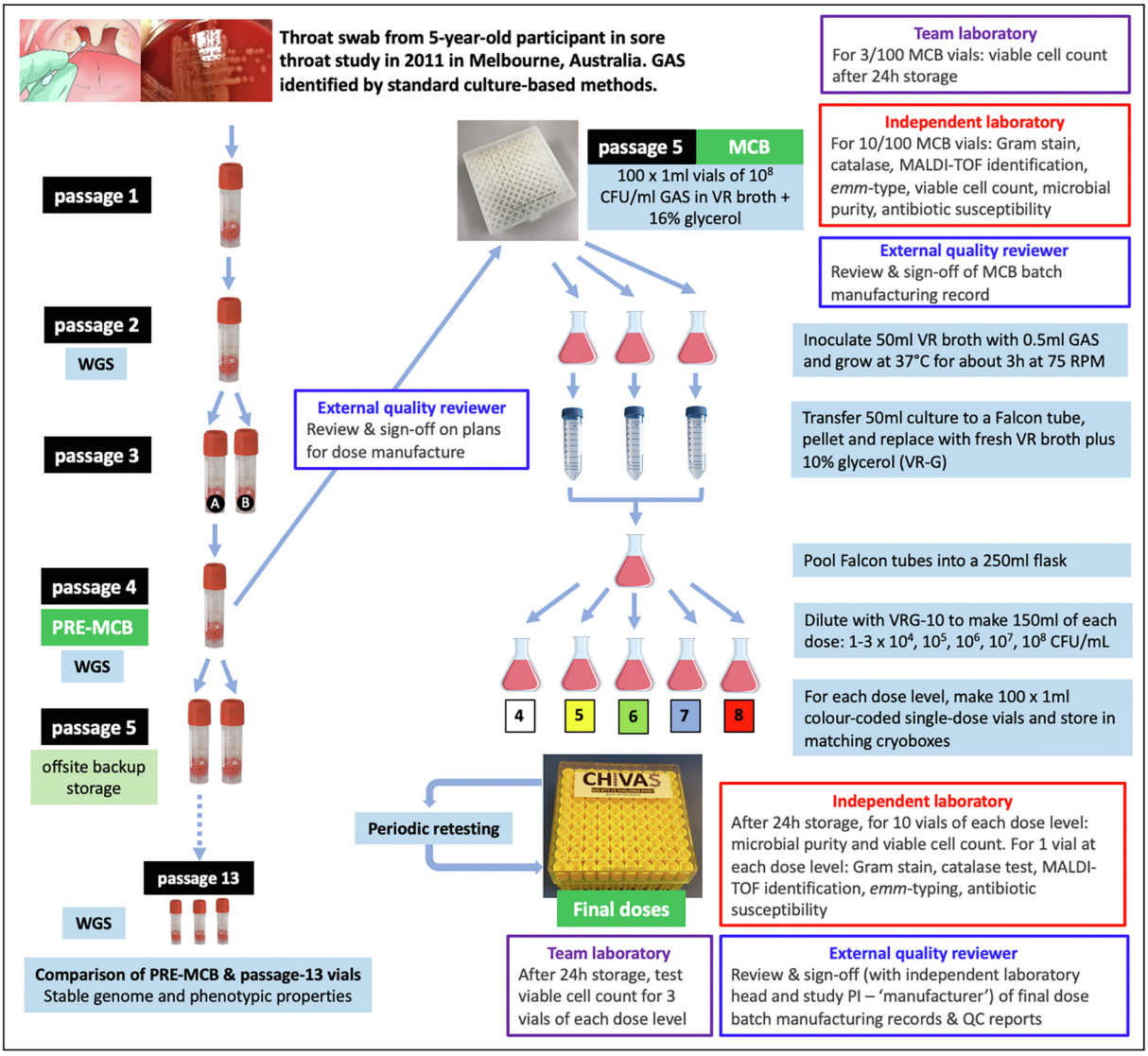


Fig. 4. Group A *Streptococcus* Challenge Strain Manufacturing Process. CFU: colony-forming units; GAS: group A *Streptococcus*; MCB: master cell bank; QC: quality control; VR broth: GMO-free Veggietone Soya Peptone (Oxoid) 2% (w/v) in RPMI-Medium 1640 - No Phenol Red (Gibco); VR-G: VR broth plus 10% (v/v) glycerol; WGS: whole genome sequencing.

3.8. Safety measures

In addition to standard definitions of Serious Adverse Events or Reactions (SAE/SAR), Medically Significant Events specific to this study are: invasive GAS infections including local suppurative complications, bacteremia and focal sterile site infections; post-infectious complications including acute rheumatic fever, new clinical or subclinical evidence of rheumatic heart disease, new choreiform movement disorder, glomerulonephritis; secondary cases of *emm*75 GAS (challenge strain) infections affecting non-participants (i.e. staff, participants in other studies); relapse of acute (symptomatic) pharyngitis caused by the challenge strain; failure of initial antibiotic therapy to achieve clinical cure; and antibiotic adverse reactions.

Day-to-day decisions during the study will be made by a Study Management Team comprising medically-qualified investigator representatives and CRO staff, supported by a larger Study Steering Committee with broad expertise in GAS science, HIS research, vaccine development, and clinical trials.

3.9. Sample size considerations

The study has been explicitly designed with a view to future double-blind randomized controlled vaccine-challenge trials (i.e. participants randomized to receive vaccine or control intervention, then challenged to determine VE against experimental pharyngitis). Whatever the result, the proportion of participants with pharyngitis compared to those who do not develop pharyngitis in this dose-ranging study will only be a point-estimate derived from a small sample. Sample size calculations for future vaccine-challenge studies should consider the confidence interval for the true attack rate not only the point-estimate from one study. The 95% confidence interval if 12/20 (60%) participants develop pharyngitis in the dose-ranging study is 36–81%, requiring a minimum 23 participants per treatment arm in a vaccine-challenge trial if VE is 80% (Supplementary Table S2). This assumes 90% power and alpha of 0.05, with no adjustment for loss to follow-up or multiple testing (2-sided Fisher's exact test). By these assumptions, 18 participants per arm would be required if 14/20

(70%, 95% CI 46–88%) develop pharyngitis in the dose-ranging study. In anticipation of drop-outs, the sample size could be increased by 10–20% and/or participants dropping out prior to the efficacy end point could be replaced.

3.10. Regulation, governance, and ethics

In Australia, infective agents for use in HIS are not considered therapeutic products regulated by the Therapeutic Goods Administration (TGA). Nonetheless, with the aim of setting and surpassing high clinical and manufacturing standards, a TGA Clinical Trials Notification (CTN) has been completed for the fully-characterised non-genetically modified challenge strain and dose manufacture followed Good Manufacturing Practice principles. All dose-escalation decisions (and serious adverse events) will be reviewed by a Safety Review and Dose Escalation Committee chaired by an independent clinician-scientist with extensive clinical research experience including HIS. This study protocol has been reviewed and approved by The Alfred Hospital Ethics Committee (500/17) and is registered at ClinicalTrials.gov (NCT03361163). The sponsor is the Murdoch Children's Research Institute, with indemnity insurance for this study covered by an existing institutional policy.

4. Discussion

This will be the first GAS human infection study since 1975. In the context of continuing global efforts to overcome barriers to product development for prevention and treatment of GAS, and specifically vaccine development, strategic and scientific impetus will flow from a successful HIS [14,15,25]. Once established, the model could deliver the first evidence of human GAS vaccine efficacy in more than forty years. Samples from this HIS will also contribute to human GAS pathogenesis and immunology studies of unprecedented scope and precision, using methods not available at the time of previous GAS pharyngitis HIS.

This study emerges in the setting of growing global recognition of the capacity for HIS ('human challenge', 'controlled human infection') to accelerate product development for control of infectious diseases, and specifically vaccine development [28,36,52]. Apart from the generic qualities of HIS research, there are specific areas of strength in the CHIVAS study protocol. The protocol integrates lessons learned from the experiences of historical GAS pharyngitis HIS and successful modern HIS for other diseases. A simplified dichotomous pharyngitis case definition was preferred over the more complicated scales used in the 1970s HIS [30,31,33]. All 58 participants with 'obvious', 'definite', or 'probable' pharyngitis in the 1970s studies would also clearly have pharyngitis by the new definition. Of the participants who were recorded as not having pharyngitis, 3/97 with tonsillar exudates but otherwise relatively mild symptoms and signs would likely have pharyngitis by the new definition. Use of a rapid molecular test will expedite diagnosis, delivery of antibiotic treatment, and minimize participant discomfort and risk while maintaining diagnostic performance. The chosen swab method for delivering the GAS inoculum to the pharynx ensures it reaches the intended site. However, no two challenges will be identical, depending on how the swab is rolled over the unique contours of each pharynx and how the procedure is tolerated. Alternative methods discussed included intranasal injection as in the non-human primate GAS nasopharyngitis model [47] and HIS for other respiratory pathogens [53–56]. The record of successful and safe induction of experimental pharyngitis in the 1970s was ultimately decisive in choosing the swab method.

The CHIVAS protocol includes multiple precautions reflecting the highest priority given to protecting participants, staff, and other contacts from foreseeable risks. Examples include: the approach to strain selection, manufacture and delivery; universal antibiotic therapy; infection control precautions; and beginning with an inpatient dose-ranging study in healthy adults starting at a lower dose than in the successful 1970s studies. Although GAS is an important cause of severe life-threatening syndromes globally, outside of established outbreaks first-episode ARF is rarely observed following acute pharyngitis in otherwise healthy adults, even in hyper-endemic settings [57]. Prompt antibiotic treatment of GAS pharyngitis prevents subsequent ARF, including in outbreaks [58]. The risk of infective (suppurative) and immune (non-suppurative) complications in healthy adults who might be considered for inclusion in this study is sufficiently low that diagnostic and treatment guidelines for sore throat in Australia, New Zealand and Europe do not recommend routine microbiological sampling and/or antibiotic treatment [59,60,61]. The latent period from an antecedent GAS infection to onset of ARF or APSGN rarely exceeds six weeks, so that in the highly unlikely event that a participant were to develop either syndrome related to their involvement in the study, the six month outpatient follow-up period will be sufficient to capture all cases and allow for prompt referral for clinical care. Although the risk of developing RHD is negligible in this study follow-up echocardiography has been included in the protocol as it will be important in early phase vaccine trials given the history of concerns that a GAS vaccine may promote development of ARF and RHD [62,63].

There are intrinsic limitations in development of a new HIS. The GAS M75 challenge strain was isolated from a child with pharyngitis and selected for its relatively limited and predictable virulence profile [50]. The encouraging historical record of experimental pharyngitis due to M1, M3, and M12 strains may not translate to this strain. The age distribution of GAS pharyngitis is strongly suggestive of the evolution of adaptive immunity through repeated exposure. While the presence of baseline antibodies against the type-specific M protein will be measured to rule out participants with suspected immunity to the challenge strain, immune responses to natural infection are inconsistent and no established human immune correlates of protection exist, so exclusion on the basis of M protein antibodies may be unnecessary [40,64]. Like every HIS, the small sample size means the model is vulnerable to unpredictable individual variation. The sample size calculations are speculative, incorporate assumed answers to the very questions inspiring development of the model, and are based on the primary rather than exploratory secondary outcomes. This apparent weakness may eventually prove to be a strength as unexpected findings generate new hypotheses, testable in future HIS and vaccine field trials.

As with any model, the findings from the GAS pharyngitis HIS will need to be interpreted with caution, especially when attempting to generalize them to other subjects, strains, syndromes, and settings. Opportunities to study HIS samples alongside relevant comparable samples from natural history studies, inclusion of additional strains in the HIS, and possibly a GAS superficial skin infection HIS may be valuable. Ultimately, successful development of a GAS pharyngitis HIS in healthy adults will be an important step towards the truest test of external validity: large-scale field trials of GAS vaccines.

Contributors

JO, ACS, and KIA wrote the protocol and paper. CB, CSW, MP, TS, AG, ACC, AJP, JSM, MFG, MJW, JBD, MRB, JRC, and PRS assisted and advised writing the protocol and paper.

Funding

This work is supported by the Australian National Health and Medical Research Council [GNT1099183].

Competing interests

JBD is the inventor of technologies related to the development of group A streptococcal vaccines. The University of Tennessee Research Foundation has licensed these technologies to Vaxent, LLC, of which JBD is the chief scientific officer and a member. MP and MFG are inventors on patents related to group A streptococcal vaccines. Griffith University, Australia has licensed some of these technologies to Olymvax Pharmaceuticals, China.

Acknowledgements

We thank Fiona Williams, Carolyn Stewart, and Kate Scarff from the Melbourne Children's Trial Centre at the Murdoch Children's Research Institute for valuable protocol development guidance. For their involvement in strain manufacture, we thank external quality reviewer Jim Ackland from Global BioSolutions and Dr. Deborah Williamson, Dr. Kate Worthing and staff at the Microbiological Diagnostic Unit Public Health Laboratory, The Peter Doherty Institute for Infection and Immunity, The University of Melbourne. JO, AJP, JSM, and ACS are members of the Human Infection Challenge Network for Vaccine Development (HIC-Vac), which is funded by the UK Global Challenges Research Fund (GCRF) Networks in Vaccines Research and Development, which was co-funded by the Medical Research Council (MRC) and Biotechnology and Biological Sciences Research Council (BBSRC).

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