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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	ELISA data collected using MultiSkan Go Instrument with SkanIt Software version 4.1 (Thermo Fisher Scientific). Multiplex data collected using MagPix instrument with xPonent software version 4.2 (Luminex Corporation). Bacterial counts were acquired with Protocol3 instruments (Synbiosis).
Data analysis	R v3.8. The R code used to generate all analyses in this study has been deposited in the Zenodo repository under accession code 13327362 [https://zenodo.org/records/13347362]. Raw ELISA data was analysed with Prism versions 8-9 (Graphpad), multiplex data was analysed with Belysa software (Merck) and OPK data was analysed with Opsititer software version 3 (UAB Research Foundation).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The ELISA and multiplex bead-based assay data generated in this study and the human challenge study clinical outcome data have been deposited in the Zenodo repository under accession code 13327362 [https://zenodo.org/records/13347362]

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Participant sex was determined based on self report. Participants were recruited to ensure a balance of both sexes (13M, 12F). As there was no difference in the prevalence of pharyngitis between males and females (starting dose, 18/20 with pharyngitis – 9/10 males and 9/10 females; lower dose, 1/5 with pharyngitis - 0/3 males and 1/2 females), we have opted not to further compare sex differences in our cohort of 25 individuals, as at this scale differences would likely reflect inter-individual variability rather than a sex-bias in responses.
Reporting on race, ethnicity, or other socially relevant groupings	The CHIVAS-M75 participants included 17/25 who identified as white. The remainder were from various Asian ethnic groups. As for sex and gender, there was a general effort to recruit a balanced sample but without purposeful stratification and too few total participants to enable meaningful analyses by race or ethnicity.
Population characteristics	25 healthy adults, 12 females and 13 males, mean age 27.6 years, 19 with pharyngitis and 6 without.
Recruitment	Recruitment of participants was originally described in "Osowicki, J. et al. A controlled human infection model of Streptococcus pyogenes pharyngitis (CHIVAS-M75): an observational, dose-finding study. The Lancet Microbe (2021)". This paper is cited within the current manuscript.
Ethics oversight	Alfred Hospital Human Research Ethics Committee (500/17).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The study includes results from 25 healthy adults in the CHIVAS-M75 dose-finding human challenge trial. Samples are from 4 time points: pre-infection (0h), approximately 7 days after discharge from the inpatient challenge admission, 1 month and 3 months after discharge. Further detail of the sample size for the study from which these samples were derived can be found in "Osowicki, J. et al. Controlled human infection for vaccination against Streptococcus pyogenes (CHIVAS): Establishig a group A Streptococcus pharyngitis human infection study. Vaccine (2019)".
Data exclusions	No data were excluded. In a few instances, we focused our on individuals for which we had paired data at multiple time-points, which is described clearly in the figure legends and main text.
Replication	Antibody responses were measured in duplicate, and either reported as the mean of repeated measures or averaged prior to extrapolation from the standard curve. ELISA results were discarded and the assay repeated if the R2 of the reference curve was ≤0.993 or the standard deviation between replicates was high (>10%).The multiplex assay has been extensively qualified as described in "Whitcombe, A. et al. An eight-plex immunoassay for Group A streptococcus serology and vaccine development. Journal of Immunological Methods (2022)". For the OPK results inclusion criteria were: non-specific killing <30%, number of CFU between 100-200, killing in positive control and no killing in negative control. All assays were run in technical replicates and across duplicate assays.
Randomization	N/A. This study was based on comparing participants who did develop pharyngitis after challenge (n=19) with those who did not (n=6) in an initial non-randomised dose-finding human challenge trial to establish a new model of S. pyogenes pharyngitis.

Blinding	N/A. CHIVAS-M75 was an unblinded trial. The investigators on this study were not blinded to disease outcomes.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Horseradish peroxidase-labelled goat anti-human IgG (Southern Biotech Goat anti-human HRP-IgG cat#2040-05 lot#B3919-XD29C diluted 1:5000) or IgA (Southern Biotech Goat anti-human HRP-IgA cat#2050-05 lot#G0919-QC50D, diluted 1:2000) secondary antibodies (Southern Biotech) were added at 50 µL/well for ELISA assays, and phycoerythrin labelled anti-human IgG (Jackson ImmunoResearch Labs cat#709-116-098 lot#144968) used for Multiplex bead assays
Validation	The primary antibodies used throughout this study were human antibodies measured in serum and saliva. Secondary antibodies employed were: IgG antigen-specific ELISA = SouthernBiotech Goat anti-human IgG HRP, cross adsorbed with human IgM and IgA; may react with IgG from other species, quality tested for ELISA (https://www.southernbiotech.com/goat-anti-human-igg-hrp-2040-05); IgA antigen-specific ELISA = SouthernBiotech Goat anti-human IgA HRP, cross adsorbed with human IgG and IgM; may react with IgG from other species, quality tested for ELISA (https://www.southernbiotech.com/goat-anti-human-iga-hrp-2050-05); total IgA ELISA kit = abcam Human IgA ELISA kit, This kit recognizes both native and purified Human IgA protein in serum, plasma, milk, urine, saliva, and cell culture media samples only. Human IgG, human IgM, and human IgE were prepared at 20 ng/mL and 10 ng/mL in Sample Diluent NS and assayed for cross reactivity. No significant cross reactivity was observed for human IgG, human IgM, or human IgE at either concentration with a mean OD deviation from background of 0.006. Other species reactivity was determined by measuring 1:200,000 (dilution) serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution. Reactivity < 3% was determined for the following species: Mouse, Rat, Hamster, Guinea Pig, Rabbit, Dog, Goat, Pig, Cow (https://www.abcam.com/en-us/products/elisa-kits/human-iga-elisa-kit-ab196263#tab=datasheet). Multiplex bead assays = R-Phycoerythrin AffiniPure™ F(ab')$_2$ Fragment Donkey Anti-Human IgG, Fcγ fragment specific, No antibody was detected against human IgM or IgA, or against non-immunoglobulin serum proteins. The antibody has been tested by ELISA and/or solid-phase adsorbed to ensure minimal cross-reaction with bovine, horse and mouse serum proteins, but it may cross-react with immunoglobulins from other species (https://www.jacksonimmuno.com/catalog/products/709-116-098).

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Detroit 562 from ATCC (cat# CCL-138) were isolated from the pharynx of a female, white, pharyngeal cancer patient.
Authentication	The cell line was not authenticated.
Mycoplasma contamination	The cell line was not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	ClinicalTrials.gov, NCT03361163.
Study protocol	Included in the supplementary appendix of "Osowicki, J. et al. A controlled human infection model of Streptococcus pyogenes pharyngitis (CHIVAS-M75): an observational, dose-finding study. The Lancet Microbe (2021)". https://doi.org/10.1016/

S2666-5247(20)30240-8

Data collection: Participants challenged between July 10, 2018 (first participant, first dose), and Sept 23, 2019 in Melbourne, Australia.

Outcomes: For functional assays (osponophagocytosis & adhesion): bacterial growth by culture. Antibody binding assays: Luminex 200 - concentration. ELISA - arbitrary ELISA units.

Plants

Seed stocks: NA

Novel plant genotypes: NA

Authentication: NA