



The TB vaccine H56 + IC31 dose-response curve is peaked not saturating: Data generation for new mathematical modelling methods to inform vaccine dose decisions



Sophie J. Rhodes^{a,*,1}, Andrea Zelmer^{b,1}, Gwenan M. Knight^{a,c}, Satria Arief Prabowo^b, Lisa Stockdale^b, Thomas G. Evans^d, Thomas Lindenstrøm^e, Richard G. White^{a,2}, Helen Fletcher^{b,2}

^a TB Modelling Group, CMMID, TB Centre, London School of Hygiene and Tropical Medicine, UK

^b Immunology and Infection Department, London School of Hygiene and Tropical Medicine, UK

^c National Institute for Health Research Health Protection Research Unit in Healthcare Associated Infection and Antimicrobial Resistance, Imperial College London, UK

^d Tomovax, Portland, OR, United States

^e Statens Serum Institut, Copenhagen, Denmark

ARTICLE INFO

Article history:

Received 12 July 2016

Received in revised form 4 October 2016

Accepted 22 October 2016

Available online 2 November 2016

ABSTRACT

Introduction: In vaccine development, dose-response curves are commonly assumed to be saturating. Evidence from tuberculosis (TB) vaccine, H56 + IC31 shows this may be incorrect. Mathematical modelling techniques may be useful in efficiently identifying the most immunogenic dose, but model calibration requires longitudinal data across multiple doses and time points.

Aims: We aimed to (i) generate longitudinal response data in mice for a wide range of H56 + IC31 doses for use in future mathematical modelling and (ii) test whether a 'saturating' or 'peaked' dose-response curve, better fit the empirical data.

Methods: We measured IFN- γ secretion using an ELISPOT assay in the splenocytes of mice who had received doses of 0, 0.1, 0.5, 1, 5 or 15 μ g H56 + IC31. Mice were vaccinated twice (at day 0 and 15) and responses measured for each dose at 8 time points over a 56-day period following first vaccination. Summary measures Area Under the Curve (AUC), peak and day 56 responses were compared between dose groups. Corrected Akaike Information Criteria was used to test which dose-response curve best fitted empirical data, at different time ranges.

Results: (i) All summary measures for dose groups 0.1 and 0.5 μ g were higher than the control group ($p < 0.05$). The AUC was higher for 0.1 than 15 μ g dose. (ii) There was strong evidence that the dose-response curve was peaked for all time ranges, and the best dose is likely to be lower than previous empirical experiments have evaluated.

Conclusion: These results suggest that the highest, safe dose may not always optimal in terms of immunogenicity, as the dose-response curve may not saturate. Detailed longitudinal dose range data for TB vaccine H56 + IC31 reveals response dynamics in mice that should now be used to identify optimal doses for humans using clinical data, using new data collection and mathematical modelling.

© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Vaccines are one of the most important and cost-effective interventions in public health [1]. However, development from vaccine discovery to licensure is costly; in the region of US\$0.8 billion [2]. Mistakes in vaccine development may cause not only a waste of resources (both financial and experimental) but also ultimately,

delay licensure of an effective vaccine. A key decision in development is vaccine dose amount (hereafter dose), which, if chosen optimally would achieve maximum vaccine efficacy, with minimal side effects.

It is common practise in pre-clinical and clinical trials that vaccine dose is increased incrementally until a maximum safe dose that promotes an effective response (usually an antibody response) is achieved; it is assumed that this response will then saturate [3]. This saturating relationship between dose and host response has been the standard assumption in vaccine development and many vaccines have proceeded through to the late stages of development with this method as a basis for dose choice [4,5].

* Corresponding author.

E-mail address: sophie.rhodes@lshtm.ac.uk (S.J. Rhodes).

¹ Joint first authors.

² Joint senior authors.

However, in tuberculosis (TB) vaccine development, early pre-clinical studies in mice with the IC31 adjuvanted fusion protein TB10.4 /Ag85B (H4) revealed that low antigen doses were both more immunogenic and provided increased protection relative to high doses [6]. In accordance, a clinical study showed that responses after vaccination with the same H4 + IC31 vaccine were not different between the 5 and 15 µg doses, decreased at 50 µg and were minimal for the 150 µg dose [7]. In a latently infected target population, vaccination with an analogous vaccine H56 (an ESAT-6/Ag85B/Rv2620 fusion protein vaccine) also adjuvanted with IC31 (H56 + IC31) showed that out of two doses tested in Quantiferon-positive (QTF+) individuals, the lowest dose (15 µg H56 + IC31) was more effective at inducing polyfunctional CD4+ T cell responses than the higher dose (50 µg H56 + IC31) [8]. Of note, the higher dose (50 µg) of the related first-generation Hybrid vaccine H1 + IC31 was taken forward to a First-in-man study, which may have led to suboptimal vaccine evaluation [9]. Although immunogenicity not only depends on antigen dose, but indeed also on the type and nature of the adjuvant employed, the incorrect assumption that a higher dose is preferred per se for protein vaccines (or other platforms) has further been brought into question by vaccines using other types of adjuvants [10].

Translational quantitative analysis methods to inform dose decision-making already exist in the drug development world. Pharmacokinetic/Pharmacodynamic (PK/PD) modelling uses mechanistic mathematical methods to describe how dose influences drug dynamics over time [11,12]. Translational modelling to predict human PK/PD parameters based on animal data is a key stage in model-based drug dose decision-making [12–14], and is often required by regulators during development. Although pharmacokinetic data is often not available for vaccines, pharmacokinetics is dependent on dose and regimen, and thus analogies to dose finding for vaccines are relevant. No translational quantitative methods are applied in vaccine development, as the chosen vaccine dose to be tested in a clinical environment is usually based on qualitative assessment of the pre-clinical data [15], which has the potential to ignore or underutilise dose-response information.

To address this gap, we are proposing the new field of *Immunosimulation/Immunodynamic (IS/ID) modelling*, analogous to that of PK/PD modelling, to make more informed human vaccine dosing decisions based on animal response data. In this field, models will be created to describe the underlying mechanisms that determine the immune response dynamics (immunodynamics) following vaccination, e.g. the influence of the innate and regulatory systems for T cell expansion and contraction (immunostimulation). These models will then be calibrated to dose ranging data from animals and model parameters “mapped” to known human response data. Subsequently, dose-response curve in humans can be predicted, providing information on the most effective range of doses to be first evaluated in clinical trials. In this larger body of work, we will apply these methods on the aforementioned TB vaccine, H56 + IC31 by measuring IFN-γ after vaccination over time.

As in model-based drug development, extensive longitudinal data are required. Published data on a wide dynamic range of doses and time points do not exist for H56 + IC31, where dose-ranging studies have only ever been conducted on minimal pre-specified time points [6]. As such, we conducted and report here an experiment in which we vaccinated mice with a wide range of doses of H56 + IC31 and measured responses extensively over time. These data outlined in this paper will be used in future IS/ID modelling to further our knowledge in mice, non-human primates and humans. In this paper, we aim to (i) generate longitudinal response data in mice for a wide range of H56 + IC31 doses for use in future mathematical modelling and (ii) test whether a ‘saturating’ or ‘peaked’ dose-response curve, better fit the empirical data.

2. Materials and methods

2.1. Ethics statement

All animal work was carried out in accordance with the Animals (Scientific Procedures) Act 1986 under a license granted by the UK Home Office (PPL 70/8043), and approved by the LSHTM Animal Welfare and Ethics Review Body.

2.2. Animals

Female CB6F1 mice were acquired from Charles River UK at 6–8 weeks of age. Animals were housed in specific pathogen-free individually vented cages, were fed ad libitum, and were allowed to acclimatize for at least 5 days before the start of any experimental procedure.

2.3. Vaccination

The experimental vaccine H56 (comprising *Mycobacterium tuberculosis* antigens Ag85B-ESAT-6-Rv2660c [16], provided by Statens Serum Institute (SSI), Copenhagen, Denmark) was formulated in IC31® adjuvant (provided by SSI on behalf of Valneva Technologies) and 10 mM Tris-HCl buffer (pH 7.4) as described in [17] to obtain a final volume of 200 µl/mouse. The adjuvant IC31® consists of a mixture of the cationic peptide KLK (NH₂-KLKL5KLK-COOH) and the oligodeoxynucleotide ODN1a (oligo-(dIdC)13). Adjuvant doses were 100 nmol peptide and 4 nmol oligonucleotide for every vaccine (H56) dose. Antigen doses of 0.1, 0.5, 1, 5 or 15 µg of H56 + 100/4 nmol IC31 (hereafter, H56 + IC31) were administered per animal at day 0 and 15, the same dose was used at both vaccination times within a group. Control animals received no vaccination. The vaccine was administered subcutaneously into the left or right leg flap.

2.4. IFN-γ ELISPOT

IFN-γ secreting CD4+ T cells were measured using the ELISPOT assay. Single cell suspensions of mouse splenocytes were prepared by mechanical disruption of spleens through a 100 µm cell strainer on the day of sacrifice. After lysis of red blood cells, single cell suspensions were made up in antibiotic-free media [RPMI-1640 (Sigma-Aldrich, Dorset, UK) + 10% heat-inactivated FBS (Labtech International Ltd, Uckfield, UK) + 2 mM L-Glutamine (Fisher Scientific, Loughborough, UK)]. 96-well microtiter ELISPOT plates (MAIPS4510, Millipore, Watford, UK) were coated with 10 µg/ml rat anti-mouse IFN-γ (clone AN18, Mabtech, Nacka Strand, Sweden). Free binding sites were blocked with RPMI 1640 supplemented as described above. 2.5×10^5 of total splenocytes were added and incubated in duplicate with H56 (10 µg/ml), supplemented RPMI as a negative control, or Phorbol myristate acetate (PMA) (50 µg/ml, Sigma-Aldrich) and Phytohemagglutinin (PHA) (10 µg/ml, Sigma-Aldrich) as a positive control. After 24 or 48 h of incubation at 37 °C in 5% CO₂, IFN-γ was detected with 1 µg/ml biotin labelled rat anti-mouse antibody (clone R4-6A2, Mabtech) and 1 µg/ml alkaline phosphatase-conjugated streptavidin (Mabtech). The enzyme reaction was developed with BCIP/NBT substrate (5-Bromo-4-chloro-3-indolyl phosphate/Nitro blue tetrazolium) (MP Biochemicals, UK) and stopped by washing the plates with tap water when individual spots could be visually detected (up to 5 min). ELISPOT plates were analysed using an automatic plate reader. IFN-γ-specific cells are expressed as number of spot-forming units (SFU) per million spleen cells after non-specific background was subtracted using negative control wells.

2.5. Experimental schedule

ELISPOTs were carried out at 2, 7, 9, 14, 16, 21, 28, and 56 days after the first vaccination for all doses. Five mice were used per time point per dose group (equating to 40 mice in a dose group from initiation to conclusion of the experiment). This schedule was designed to reflect the H56 + IC31 phase I clinical trial schedule [8] and previous experimental schedules in mice using the H-series vaccines by SSI in CB6F1 mice [6,16,18,19].

2.6. Statistical methods

2.6.1. Summary of IFN- γ response data after two vaccinations with TB vaccine H56+IC31 for future mathematical modelling

The Wilcoxon test was used to test for differences in IFN- γ responses generated as a result of the two ELISPOT incubation times (on data pooled across dose groups and time points). The following summary measures were used to quantify responses over time: Area Under the Curve (AUC), day 56 response, peak response between first and second vaccination and peak response post-second vaccination (peaks may occur at different times as they were defined as the highest median response measured in the respective time period). As IFN- γ responses over time within a dose group were not dependent (each taken from an individual mouse spleen), AUC was calculated using 200 samples of the possible combinations of the five mice per dose group over time. Full details of this method are outlined in [Supplementary material and Fig. S1](#). The non-parametric Dunn test was used to compare the summary measures between the dose groups and a Bonferroni correction applied to account for comparisons across multiple groups. A p -value < 0.05 was considered significant.

2.6.2. Determine the shape of dose-response curve when examined at varying sample times and the best dose predicted by fitted curves

To assess the shape of the dose-response curve (IFN- γ SFU per million splenocytes versus dose), a saturating or peaked curve was fitted to all IFN- γ responses against the (\log_{10} transformed) doses using nonlinear regression, in the software Prism (v 7 for Mac, GraphPad Software, California USA, www.graphpad.com). Briefly, the aim of nonlinear regression is to find the parameters that minimise the sum of the squared residuals from all data points to the curve (see [Supplementary material for description](#)). We choose a sigmoidal equation as the saturating curve and the gamma probability density function (pdf) as the peaked curve (see [Supplementary for equations](#)). The choice of the gamma pdf was due to the hypothesis that the dose response, once peaked, will not increase again and will never decrease to a zero response. To establish which of the shapes best described the dose-response curves the goodness-of-fit measure, the corrected Akaike information criteria (AICc) was compared, where a lower AICc indicates a better fit. A difference in AICc value between curve fits of greater than seven was considered strong evidence of a better fit and a difference in AICc of greater than ten was considered absolute evidence of a better fit [20] ([Table S1](#)). To assess how the dose-response curve changed with time, the response data was pooled into three time ranges: between the first and second vaccination (day 2, 7, 9, 14 aggregated), post-second vaccination (day 21, 28 and 56 aggregated) and day 56 responses. The best dose was defined as the dose that produced the maximum IFN- γ response as predicted by the fitted curves.

3. Results

3.1. Summary of IFN- γ response data after two vaccinations with TB vaccine H56+IC31 for future mathematical modelling

Splenocyte-derived IFN- γ responses did not differ for the 24 versus the 48 h ELISPOT incubation times when responses were pooled over all dose groups and time points (p -value = 0.67, [Fig. S2](#)). Therefore, an incubation time of 24 h was used in the following analyses.

The IFN- γ responses over time for each dose group are shown in [Fig. 1](#) (significance of the changes in dynamics over time are in [Table S2](#)). Out of the samples taken to calculate the AUC, the common significance trend showed that dose groups 0.1, 0.5 and 1 μ g had significantly higher AUC than the control group, and the dose group 0.1 μ g had significantly higher AUC than dose group 15 μ g ([Fig. 2, Table S3](#)). Peak responses between first and second vaccination were significantly higher in the dose groups 0.1, 1, 5 μ g than in the control group and for post-second vaccination, dose groups 0.1, 0.5 and 5 μ g were significantly higher than the control group ([Figs. 1 and 2, Table 1](#)). Similarly, day 56 responses were significantly higher for the dose groups 0.1, 0.5 and 1 μ g than the control group and the dose group 0.5 μ g was higher than 15 μ g. However, this did not reach statistical significance ([Figs. 1 and 2, Table 1](#)). For all summary measures, no other comparisons between dose groups were statistically significantly different ([Fig. 2, Table 1](#)).

3.2. Determine the shape of dose-response curve when examined at varying sample times and the best dose predicted by fitted curves

To establish the shape of the dose-response curve (IFN- γ SFU per million splenocytes versus dose), we fitted either a saturating or peaked function for three time ranges. There was strong evidence that the peaked curve was a better fit to the response data between first and second vaccination ([Fig. 3A, Table S4](#)) with a AICc difference of 7.5 favouring the peaked (gamma) curve. The AICc difference was 18.8 and 10.9 for the post-second vaccination ([Fig. 3B, Table S5](#)) and day 56 ([Fig. 3C, Table S6](#)) response data, suggesting absolute support for the peaked curve for both these time ranges. The best dose predicted by the peaked fitted curve for the time ranges; between first and second vaccination, post-second vaccination and day 56 were 0.026, 0.11 and 0.25 μ g, respectively (transformed from \log_{10} scale, [Fig. 3](#)). Due to the right-skewed nature of the responses the best saturation curve had an almost immediate increase followed by immediate plateau for all time ranges ([Fig. 3](#)). As such it was not possible to obtain a best predicted dose using the saturation model as, in this case, all doses generated the same response.

4. Discussion

Our future aim is to apply the new field of Immunostimulation/immunodynamic (IS/ID) modelling to translate vaccine dose-response information between animals and humans and thus quantitatively inform vaccine dose decision-making. To begin initial examination of such methods, we conducted a longitudinal dose-ranging experiment of the novel TB vaccine H56 + IC31 in mice, and a mathematical analysis of the dose-response curve over time.

We successfully generated an intensive time course of IFN- γ response data to vaccination where AUC and peak analysis showed a trend toward higher responses over time in the lower doses than in the higher doses. By using mathematical curve fitting, we showed that the IFN- γ dose-response follows a peaked shape instead of the commonly assumed saturation shape for all time

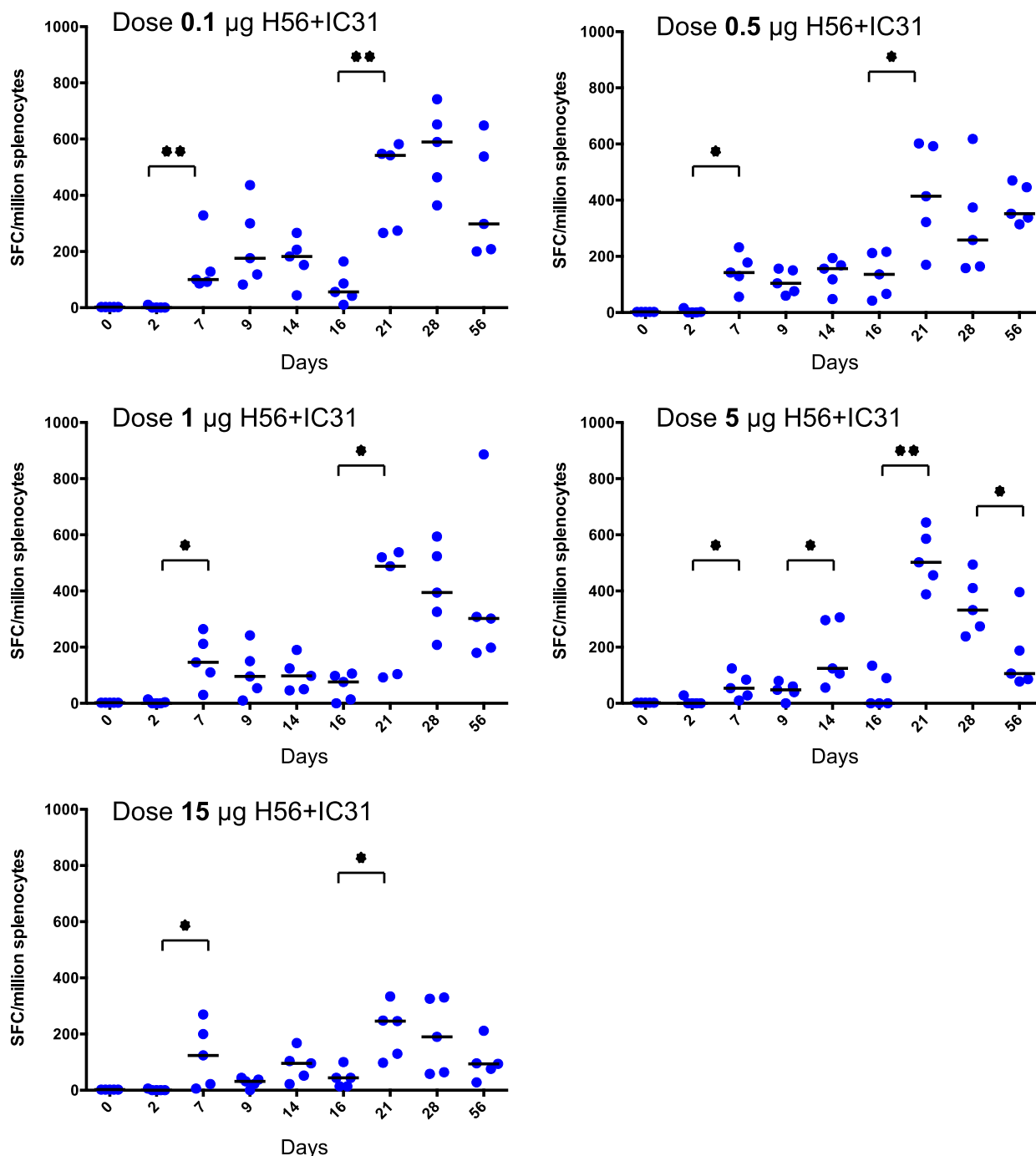


Fig. 1. Median IFN- γ responses (horizontal black bars) and responses of individual mice per time point (blue points) for each dose. As the control group did not receive H56 + IC31, the median of all responses from the control group (which did not significantly change throughout the experiment) was used to represent all mice at baseline. The Wilcoxon test was used to compare consecutive time points, where *equates to p-value < 0.05 and **p-value < 0.01 (Table S2). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ranges. This was most apparent post-second vaccination, and trended toward that curve shape after the first dose. Using the peaked fitted curve, we were able to determine which dose may provide the maximal predicted IFN- γ response in mice. Our results indicate this was at a low range; between 0.02 and 0.25 μ g H56 + IC31. It must be noted that, there is uncertainty associated with our predictions for best dose which is apparent in Tables S4–S6, where the standard error was high for some gamma distribution function parameters, particularly for the time ranges between first and second vaccination and post-second vaccination. This is poten-

tially due to a lack of response information between dose 0 and 0.1 μ g, which would provide information on the increase of the peaked curve. Despite this, as we show a definitive decline in the dose-response at the higher dose range (approximately after dose 1 μ g H56 + IC31), our predicted best dose range show compelling evidence that lower doses than previously explored in mice using very similar vaccines [6,16,18,21,22], would be preferential. Importantly, as previous evidence suggest the human dose-response may be of a similar shape for the H-series of vaccines from SSI [7,8] (although higher in magnitude), this implies that previously tested

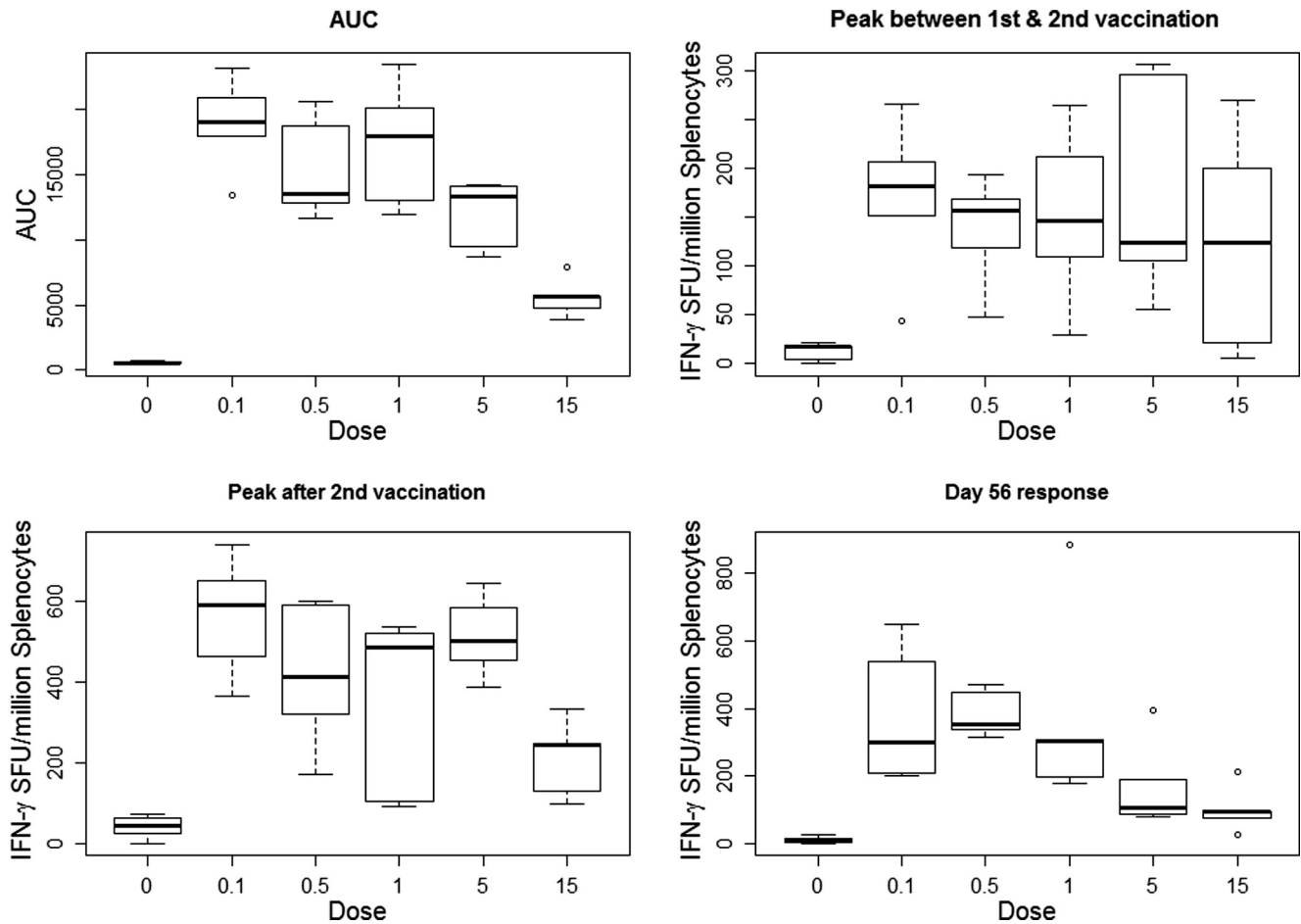


Fig. 2. AUC calculated from number of IFN- γ secreting CD4+ T cells after two vaccinations with H56 + IC31 from individual mice spleens, number of IFN- γ secreting CD4+ T cells between first and second vaccination, after second vaccination and day 56 by dose (μ g H56 + 100 nmol IC31). Dose 0 equates to the control group. The plot for AUC is representative of the 200 samples taken to establish statistical differences in AUC by dose (see methods). X axis not to scale.

Table 1

Median (IQR) values for the summary measures day 56 response, peak between first and second vaccination and post-second vaccination IFN- γ responses by dose. Significance in the summary measures was tested between dose groups using the non-parametric Dunn test with a Bonferroni correction. Those comparisons between dose groups with a p-value < 0.05 or 0.05 < p-value < 0.1 (starred) are displayed. Abbreviations: IQR = interquartile range, vacc = vaccination.

Dose group (μ g H56 + IC31)	Median day 56 response (IQR)	Significantly different from dose	Median Peak between 1st and 2nd vacc (IQR)	Significantly different from dose	Median Peak post 2nd vacc (IQR)	Significantly different from dose
Control	10 (2–14)	0.1, 0.5, 1	18 (4–18)	0.1, 1, 5	44 (26–64)	0.1, 0.5, 5
0.1	298 (208–538)	Control	182 (152–206)	Control	590 (464–652)	Control
0.5	352 (338–446)	Control, 15*	156 (118–168)	–	414 (322–592)	Control
1	302 (198–308)	Control	146 (110–212)	Control	488 (104–520)	–
5	106 (86–188)	–	124 (106–296)	Control	502 (456–586)	Control
15	94 (76–96)	0.5*	124 (22–200)	–	246 (130–248)	–

clinical dose ranges may also have been too high to capture the optimal response in terms of immunogenicity.

We use the frequency of IFN- γ secreting CD4+ T cells measured using the ELISPOT assay as our chosen immune response readout to reflect the current convention in TB vaccine development for dose selection. IFN- γ is a cytokine shown to be associated with control of infection or decreased risk of TB disease [23], however these findings have been a topic of controversy in TB vaccine development [24]. In previous work by Aagaard et al. in the vaccine H4 + IC31, results showed that mice vaccinated with a lower dose (0.5 μ g H4) experienced significantly higher IFN- γ responses than higher doses (5 and 15 μ g H4) and, significantly stronger protection against *Mtb.* infection (measured by *Mtb.* colony forming units) than the higher doses, in two independent challenge exper-

iments [6]. These results support our choice of IFN- γ as a readout. We also compliment Aagaard et al.'s study by measuring the IFN- γ response at *multiple* time points, thus assessing trends in the dose-response curve over time.

It is possible that varying dose may alter the function or type of CD4 T cell secreting IFN- γ , which the ELISPOT assay will not detect. Further lab assays such as flow cytometry should be used in the future work to determine the function or type of cell produced as a result of varying dose, which may then be used in clinical trials for human dose selection.

Dose concentration feasibility and animal cost and overall numbers limited the size of our study. By testing in larger groups of mice at potentially fewer time points, we may have gained greater certainty in response differences and fitted dose-response curves.

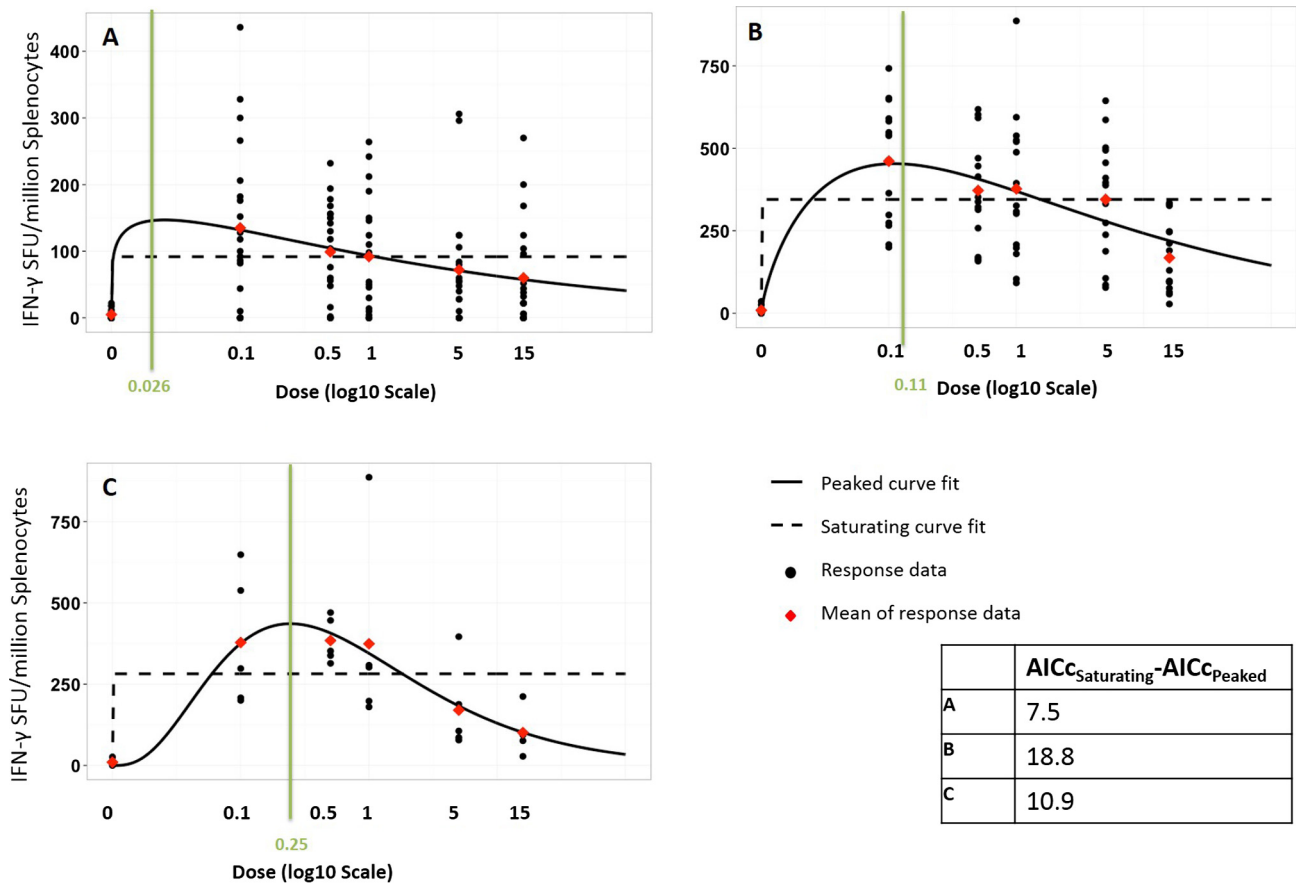


Fig. 3. Results of the dose-response curve fitting analysis for the time ranges (A) between first and second vaccination (aggregated responses from days 2, 7, 9 and 14), (B) post-second vaccination (days 21, 28 and 56) and C. Day 56 (last time point). Black points correspond to the number of IFN- γ secreting CD4+ T cells from one mouse spleen in response to vaccination for the relevant time range, red diamonds show the mean of the responses, the black solid lines are the peaked (gamma) fit, the black dashed line show the saturating (sigmoidal) curve fit and the vertical green line indicated the best dose as predicted by the peaked curve fit. The table shows the differences in AICc for A,B and C between the saturating and peaked curve fits. The x-axis is $\log_{10}(\text{dose: } \mu\text{g H56 + IC31}) + 2$ to transform the dose to a log scale and to ensure positivity, but x-axis labels show the non-logged value for clarity (to avoid infinite values, control (dose 0) is not logged).

However, we chose the extensive time course to determine detailed dynamics of the vaccine response and dose-response curves over time, since this has been performed infrequently in the past. We believe a higher maximum dose may have better defined the decline in the dose-response curve. As the size of the study was limited, we concentrated efforts on the lower doses, where previous exploration is lacking; however, we found that the probable best dose is still lower than the minimum dose used here.

We have identified the following areas for further research. The mathematical curve fitting we conducted has provided the basis for further optimised studies in animals, i.e. we now know that the dose range that captures the probable best dose should be 0–0.3 $\mu\text{g H56 + IC31}$ (based on our predicted best dose values) not the initial 0–15 $\mu\text{g H56 + IC31}$ (a reduction of 98% in the range). Expanding the dose range in the lower end between 0 and 0.1 μg will further increase our best dose predictions, as the peaked curve parameters will be estimated with greater certainty. This warrants further animal studies to investigate in greater detail the host response to low dose vaccination.

Additionally, we used AUC to quantify the magnitude of the immune response for the duration of the experiment and compared AUC between dose groups. However, we do not interpret this measure as the cumulative immune response over time, as would be appropriate in drug AUC measures, but an indication of overall higher immune response magnitude. In vaccine development, dose

decisions are made based on the dose response curve at one time point (usually a long-term time point) and as such it would be advantageous to know how dose effects the dynamics of the immune response that may lead to higher average response in the long-term. As such, our future aim is to apply the new field of Immunostimulation/immunodynamic (IS/ID) modelling, where by a mechanistic mathematical model incorporating the fundamental T cell subsets involved in a Th1 immune response will be calibrated to the ELISPOT or another given measure over time. The IS/ID model will allow us to quantify how the dynamics of the immune response differ between dose groups (and across the entire mouse population) by assessing the change in model parameters by dose. To achieve this, we will use the robust statistical framework, Nonlinear Mixed Effects Modelling to calibrate the model to the data and characterise the parameters and associated parameter variation. IS/ID modelling will also be used to translate vaccine dose-response information between animals and humans. To achieve this we will utilise known allometric scaling factors for H56 + IC31 between mouse and human we aim to “map” IS/ID model parameters from one dose of the outlined experiment to existing human H56 + IC31 dose data (one dose 50 $\mu\text{g H56 + 500 nmol IC31}$). The effect of heterogeneity in target human populations (due to HIV status, existing latent infection, etc.) on IS/ID modelling parameters will be taken into account. Following this mapping, we can then use the remaining doses to predict the dose response curve in humans. This predicted human dose response

curve will be used as a guide to select further doses to test in clinical trials.

Additionally, as the antigen dose-response may also vary with the adjuvant dose and type of adjuvant, in order to fully characterise and optimise complete vaccine (H56 + IC31) dose it would be necessary to perform a checkerboard interaction pattern, the design of which could be informed by IS/ID modelling through optimal design analysis [25].

5. Conclusion

Our results suggest that the highest, safe dose is not always optimal in terms of host response as the dose-response curve is not saturating, which may also be true for vaccines against diseases other than TB. Mathematical modelling can be used on the detailed longitudinal dose range data for TB vaccine H56 + IC31 to simulate responses to optimise further experiments in mice and help to identify optimal doses for humans.

Funding

Funding for the experiment was provided by the Bill and Melinda Gates Foundation. SR is supported by a LSHTM studentship funded by Aeras. The views expressed are those of the author(s) and not necessarily those of the SSI, the NHS, the NIHR, the Department of Health or Public Health England. RGW is funded the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement that is also part of the EDCTP2 programme supported by the European Union (MR/J005088/1), the Bill and Melinda Gates Foundation (TB Modelling and Analysis Consortium: OPP1084276) and UNITAID (4214-LSHTM-Sept15; PO #8477-0-600).

Conflicts of interest

There are no conflicts of interest.

Acknowledgements

We would like to thank our collaborators at Staten Serum Institute Peter Andersen and Else Marie Agger (now at Novo Nordisk) for advising the mouse experiment schedule and Steven Kern at the Bill and Melinda Gates Foundation for facilitating experiment funding.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.vaccine.2016.10.060>.

References

- [1] Han S. Clinical vaccine development. *Clin Exp Vaccine Res* 2015;4:46–53.
- [2] Dickson M, Gagnon JP. The cost of new drug discovery and development. *Discovery Med* 2004;4:172–9.
- [3] Quinn CP, Sabourin CL, Niemuth NA, Li H, Semenova VA, Rudge TL, et al. A three-dose intramuscular injection schedule of anthrax vaccine adsorbed generates sustained humoral and cellular immune responses to protective antigen and provides long-term protection against inhalation anthrax in rhesus macaques. *Clin Vaccine Immunol*: CVI 2012;19:1730–45.
- [4] Little SF, Webster WM, Norris SL, Andrews GP. Evaluation of an anti-rPA IgG ELISA for measuring the antibody response in mice. *Biol: J Int Assoc Biol Standardization* 2004;32:62–9.
- [5] Semenova VA, Schiffer J, Steward-Clark E, Soroka S, Schmidt DS, Brawner MM, et al. Validation and long term performance characteristics of a quantitative enzyme linked immunosorbent assay (ELISA) for human anti-PA IgG. *J Immunol Methods* 2012;376:97–107.
- [6] Aagaard C, Hoang TT, Izzo A, Billeskov R, Troudt J, Arnett K, et al. Protection and polyfunctional T cells induced by Ag85B-TB10.4/IC31 against Mycobacterium tuberculosis is highly dependent on the antigen dose. *PLoS ONE* 2009;4:e5930.
- [7] Geldenhuys H, Mearns H, Miles DJ, Tameris M, Hokey D, Shi Z, et al. The tuberculosis vaccine H4:IC31 is safe and induces a persistent polyfunctional CD4 T cell response in South African adults: a randomized controlled trial. *Vaccine* 2015;33:3592–9.
- [8] Luabeya AK, Kagina BM, Tameris MD, Geldenhuys H, Hoff ST, Shi Z, et al. First-in-human trial of the post-exposure tuberculosis vaccine H56:IC31 in Mycobacterium tuberculosis infected and non-infected healthy adults. *Vaccine* 2015;33:4130–40.
- [9] van Dissel JT, Arend SM, Prins C, Bang P, Tingskov PN, Lingnau K, et al. Ag85B-ESAT-6 adjuvanted with IC31 promotes strong and long-lived Mycobacterium tuberculosis specific T cell responses in naive human volunteers. *Vaccine* 2010;28:3571–81.
- [10] Evans TG, McElrath MJ, Matthews T, Montefiori D, Weinhold K, Wolff M, et al. QS-21 promotes an adjuvant effect allowing for reduced antigen dose during HIV-1 envelope subunit immunization in humans. *Vaccine* 2001;19:2080–91.
- [11] Wright DF, Winter HR, Duffull SB. Understanding the time course of pharmacological effect: a PKPD approach. *Br J Clin Pharmacol* 2011;71:815–23.
- [12] Mould DR, Upton RN. Basic concepts in population modeling, simulation, and model-based drug development. *CPT: Pharmacometrics Syst Pharmacol* 2012;1:e6.
- [13] Knibbe CA, Zuideveld KP, Aarts LP, Kuks PF, Danhof M. Allometric relationships between the pharmacokinetics of propofol in rats, children and adults. *Br J Clin Pharmacol* 2005;59:705–11.
- [14] Dubois VF, de Witte WE, Visser SA, Danhof M, Della Pasqua O, Cardiovascular Safety Project T, et al. Assessment of interspecies differences in drug-induced QTc interval prolongation in cynomolgus monkeys, dogs and humans. *Pharm Res* 2016;33:40–51.
- [15] Plotkin SA, Orenstein WA, Offit PA. Vaccines. 6th ed. Saunders; 2013.
- [16] Aagaard C, Hoang T, Dietrich J, Cardona PJ, Izzo A, Dolganov G, et al. A multistage tuberculosis vaccine that confers efficient protection before and after exposure. *Nat Med* 2011;17:189–94.
- [17] Lalvani A, Pathan AA, McShane H, Wilkinson RJ, Latif M, Conlon CP, et al. Rapid detection of Mycobacterium tuberculosis infection by enumeration of antigen-specific T cells. *Am J Respir Crit Care Med* 2001;163:824–8.
- [18] Hoang T, Aagaard C, Dietrich J, Cassidy JP, Dolganov G, Schoolnik GK, et al. ESAT-6 (EsxA) and TB10.4 (EsxH) based vaccines for pre- and post-exposure tuberculosis vaccination. *PLoS ONE* 2013;8:e80579.
- [19] Christensen D, Lindstrom T, van de Wijdeven G, Andersen P, Agger EM. Syringe free vaccination with CAF01 Adjuvanted Ag85B-ESAT-6 in bioinocules provides strong and prolonged protection against tuberculosis. *PLoS ONE* 2010;5:e15043.
- [20] Burnham KP, Anderson DR. Multimodel inference: understanding AIC and BIC in model selection. *Sociol. Methods Res.* 2004;33:261–304.
- [21] Ciabattini A, Prota G, Christensen D, Andersen P, Pozzi G, Medaglini D. Characterization of the antigen-specific CD4(+) T Cell response induced by prime-boost strategies with CAF01 and CpG adjuvants administered by the intranasal and subcutaneous routes. *Front Immunol* 2015;6:430.
- [22] Elvang T, Christensen JP, Billeskov R, Thi Kim Thanh Hoang T, Holst P, Thomsen AR, et al. CD4 and CD8 T cell responses to the M. tuberculosis Ag85B-TB10.4 promoted by adjuvanted subunit, adenovector or heterologous prime boost vaccination. *PLoS ONE* 2009;4:e5139.
- [23] Fletcher HA, Snowden MA, Landry B, Rida W, Satti I, Harris SA, et al. T-cell activation is an immune correlate of risk in BCG vaccinated infants. *Nature Commun* 2016;7:11290.
- [24] Kagina BM, Abel B, Scriba TJ, Hughes EJ, Keyser A, Soares A, et al. Specific T cell frequency and cytokine expression profile do not correlate with protection against tuberculosis after bacillus Calmette-Guerin vaccination of newborns. *Am J Respir Crit Care Med* 2010;182:1073–9.
- [25] Chien JY, Friedrich S, Heathman MA, de Alwis DP, Sinha V. Pharmacokinetics/pharmacodynamics and the stages of drug development: role of modeling and simulation. *AAPS J* 2005;7:E544–59.