

Adjuvant potential of low dose all-*trans* retinoic acid during oral typhoid vaccination in Zambian men

M. M. Lisulo,* M. C. Kapulu,*
R. Banda,* E. Sinkala,* V. Kayamba,*
S. Sianongo* and P. Kelly*†

*Tropical Gastroenterology & Nutrition Group,
Department of Medicine, University of Zambia
School of Medicine, Lusaka, Zambia, and †Blizard
Institute, Barts & The London School of Medicine,
Queen Mary University of London, London, UK

Accepted for publication 7 November 2013
Correspondence: P. Kelly, Blizard Institute, Barts
and The London School of Medicine, Queen
Mary University of London, Turner Street,
London E1 2AD, UK.
E-mail: m.p.kelly@qmul.ac.uk

Summary

There is an urgent need to identify ways of enhancing the mucosal immune response to oral vaccines. Rotavirus vaccine protection is much lower in Africa and Asia than in industrialized countries, and no oral vaccine has efficacy approaching the best systemic vaccines. All-*trans* retinoic acid (ATRA) up-regulates expression of $\alpha 4\beta 7$ integrin and CCR9 on lymphocytes in laboratory animals, increasing their gut tropism. The aim of this study was to establish the feasibility of using ATRA as an oral adjuvant for oral typhoid vaccination. In order to establish that standard doses of oral ATRA can achieve serum concentrations greater than 10 nmol/l, we measured ATRA, 9-*cis* and 13-*cis* retinoic acid in serum of 14 male volunteers before and 3 h after 10 mg ATRA. We then evaluated the effect of 10 mg ATRA given 1 h before, and for 7 days following, oral typhoid vaccine in eight men, and in 24 men given various control interventions. We measured immunoglobulin (Ig)A directed against lipopolysaccharide (LPS) and protein preparations of vaccine antigens in whole gut lavage fluid (WGLF) and both IgA and IgG in serum, 1 day prior to vaccination and on day 14. Median [interquartile range (IQR)] C_{max} was 26.2 (11.7–39.5) nmol/l, with no evidence of cumulation over 8 days. No adverse events were observed. Specific IgA responses to LPS ($P = 0.02$) and protein ($P = 0.04$) were enhanced in WGLF, but no effect was seen on IgA or IgG in serum. ATRA was well absorbed, well tolerated and may be a promising candidate oral adjuvant.

Keywords: IgA, oral vaccines, retinoic acid, retinoid pharmacokinetics, typhoid vaccine

Introduction

The global burden of intestinal infectious disease represents a major obstacle to the realization of health goals for many low- and middle-income countries [1,2]. Diarrhoea remains a major cause of death in children in the tropics, and this is compounded by malnutrition. While many infectious diseases are controlled by immunization, this has not proved possible for diarrhoeal diseases, and only now is immunization against rotavirus becoming a reality for many children who would benefit from it [3]. Immunization against diarrhoea-causing pathogens remains a challenge not only because of the large (and expanding) number of potential pathogens [4], but also because oral vaccines are intrinsically less effective than the best systemic

vaccines, such as yellow fever or hepatitis B vaccines. Even those vaccines that are most effective, such as rotavirus vaccine, are substantially less effective in tropical than in temperate regions [3]; for example, while the pentavalent oral rotavirus vaccine is 98% effective against severe rotavirus diarrhoea in the United States and Finland and other relatively affluent countries [5], it is only 48% effective in Bangladesh and Vietnam [6]. Similarly, the single strain rotavirus vaccine was 90% effective against severe diarrhoea in Europe [7], but was only 49% effective in Malawi [8]. An adjuvant that could enhance the effect of oral vaccines could have a dramatic impact on programmes for controlling diarrhoeal disease, with major benefits for child health in developing countries. Several strategies for adjuvanticity are currently being explored [9], including AB5

enterotoxins and their derivatives, ligands at innate immune receptors, mucoadhesives, particulates, saponins, oil emulsions and cytokines.

Retinoic acid is the oxidation product of retinol and comprises four isoforms: all-*trans*, 9-*cis*, 11-*cis* and 13-*cis* retinoic acids. All-*trans* retinoic acid (ATRA) binds to the heterodimeric retinoic acid receptor (RAR/RXR), whereas 9-*cis* RA binds to the RXR homodimer [10]; the ligand-receptor unit then binds other co-regulatory molecules, and these complexes then bind to specific retinoic acid response elements (RAREs) in the regulatory regions of specific genes [11,12]. Recent work has elucidated a complex role for ATRA in immune regulation. First, ATRA up-regulates expression of the gut-homing phenotype markers $\alpha 4\beta 7$ integrin and CCR9 through the RAR/RXR heterodimer [13]. Secondly, ATRA up-regulates expression of the polymeric immunoglobulin (Ig)A receptor, which exports secretory IgA into the gut lumen [14]. Thirdly, ATRA is implicated in T cell subset allocation following antigen presentation [15]. Fourthly, ATRA is implicated in lineage commitment of forkhead box protein 3 (FoxP3⁺) regulatory T cells [15,16], and with the tissue tropism of ROR γ ⁺ Th17 cells [17]. Fifthly, ATRA acts in a positive feedback loop to encourage DCs to promote gut homing [18]. Sixthly, vitamin A is required for development of oral tolerance [19]. For the purpose of this work, the first two of these are of considerable interest as these are highly desirable characteristics of an orally active adjuvant. Recent work in mice has demonstrated that ATRA can induce mucosal immune responses to antigens injected by the subcutaneous [20] or intramuscular [21] routes.

In clinical oncology, ATRA (tretinoin, marketed as Vesanoid; Roche, Welwyn Garden City, UK) has been used for 20 years to induce myelocytic differentiation in patients with promyelocytic leukaemia [22]. In this condition, but at high doses (45 mg/m²/day for up to 3 months), ATRA has a considerable number of adverse effects [23]. The concentration of ATRA required to produce the immune effects described above [13] was approximately 10 nmol/l (which is equivalent to 3 ng/ml), several orders of magnitude lower than the C_{max} generated by the very high doses used in oncological practice [24]. One other adverse effect of ATRA, teratogenicity [25], is of greater relevance as it occurs at concentrations much closer to those found in healthy post-prandial adults [26]. The purpose of this study was to determine the dose required to produce concentrations into the sort of range used *in vitro* [13], then to determine if ATRA has adjuvant properties when given alongside oral vaccines to adult male volunteers in a population where this adjuvant potential is most needed.

Materials and methods

Fifty adult men were recruited from a cohort of adults in Misisi compound, a high-density township in Lusaka,

Zambia, in which we have carried out several studies on intestinal infection and immunity [27,28]. In consecutive studies, volunteers participated in pharmacokinetic assessments, then in an assessment of the impact of ATRA on responses to oral typhoid vaccine. Volunteers were interviewed and examined including nutritional assessment [body mass index (BMI) and body composition determined using a Tanita BC-418 and grip strength using a Takeda dynamometer], screened for helminths by examination of a single stool sample using the Kato-Katz technique, and offered HIV testing. Participation was deferred if participants gave any history of diarrhoea within 1 month, taking antibiotics within 2 weeks or vaccination within 6 months.

Ethical approval

Approval for this study was obtained from the Biomedical Research Ethics Committee of the University of Zambia (reference 020-06-10) and the Pharmaceutical Regulatory Authority of the Ministry of Health of the Government of the Republic of Zambia (DMS/7/9/22/TROPGAN003).

Measurement of retinoic acid isoforms in blood

To confirm the dose of tretinoin required to achieve C_{max} concentrations of 10 nmol/l or more, eight men were given ATRA (Vesanoid; Roche) 10 mg daily for 8 days. On days 1 and 8, volunteers attended while fasting and a sample of blood was collected at 08:00 h and 3 h post-dose, as 3 h is a good estimate of T_{max} for retinoic acids [24,29–31]. The pre- and post-dose samples on days 1 and 8 were designated t1, t2, t3 and t4, respectively; t1 and t3 were thus measures of C₀ and t2 and t4 were measures of C_{max}. A further six men were given 60 mg retinyl palmitate (Accucaps Industries Ltd, Windsor, ON, Canada; supplied by the World Health Organization) as a single dose on day 1 and only one sample was collected on day 8, giving time-points t1, t2 and t3; t1 was thus a measure of C₀, t2 was C_{max} and t3 was a late post-dose measure.

All blood samples were collected into clot activator Vacutainer tubes (Becton Dickinson, San Jose, CA, USA) and kept in the dark, refrigerated for 20 min, before being centrifuged at 461 g and serum stored at –80°C. Aliquots of serum were shipped to Craft Laboratories (Wilson, NC, USA) for analysis by high-performance liquid chromatography (HPLC).

Vaccine administration

Volunteers ($n = 27$), screened for the absence of helminths, were randomized to receive one of two vaccines, Vivotif or Dukoral, with or without ATRA given as 10 mg daily for 8 days. Volunteers were not given any vaccine, which they had received previously. A further five volunteers were evaluated, but no vaccine or ATRA was given. These vaccine and

Table 1. Demographic and nutritional characteristics of study participants.

	Vaccine study					P
	Group 1	Group 2	Group 3	Group 4	Group 5	
<i>n</i>	8	6	7	6	5	
Vaccine	Vivotif	Vivotif	Dukoral	Dukoral	None	
ATRA	+	–	+	–	–	
Age (years)	35 (28–45)	38 (29–40)	31 (28–40)	39 (37–40)	21 (19–22)	0.07
HIV-positive (<i>n</i>)	2	2	3	4	0	
BMI (kg/m ²)	21 (18–24)	22 (19–23)	20 (18–24)	19 (18–19)	19 (18–20)	0.47
Fat (%)	10 (6–19)	15 (10–20)	14 (9–25)	13 (10–13)	9 (6–12)	0.29
Grip strength (kg)	40 (29–43)	35 (32–39)	39 (35–42)	36 (33–40)	40 (35–41)	0.87

All participants were men. All participants in the Pharmacokinetics study subsequently participated in the vaccine study, so these are shown broken down by study group. Continuous variables are shown as median and interquartile range (IQR). Fat is expressed as percentage body weight, measured by impedance. ATRA: all-*trans* retinoic acid; BMI: body mass index.

control groups are summarized in Table 1. As this was a Phase I study, no attempt was made at concealment of treatment allocation and no placebo was used, but allocation was concealed from laboratory staff. Two vaccines were used, one to evaluate responses and one as a control to exclude non-specific responses. Vivotif (Ty21a; Berna Biotech, Bern, Switzerland) is the only licensed oral typhoid vaccine; it also confers immunity against *Salmonella paratyphi*. Vivotif induces anti-lipopolysaccharide (LPS) systemic IgG and IgA, antibody-secreting cells in peripheral blood and cell-mediated immunity (CMI). CMI to Vivotif is associated with interferon (IFN)- γ secretion characteristic of a Th1 response. Immunity conferred by Ty21a lasts up to 7 years, but uniquely requires three to four doses given only 1–2 days apart. Safety has been established in more than 200 million vaccines [32]. The control vaccine, Dukoral (Berna Biotech), is administered as a liquid preparation on a single occasion. This vaccine has been shown to be safe and protective in several trials conducted in settings where cholera is endemic. Of relevance to the setting of this study, Dukoral administered in a mass immunization campaign in Mozambique was associated with 78% protection against cholera [33].

Dukoral was given as a single dose; Vivotif was given on days 1, 3 and 5 (or days 1, 4 and 6 if a weekend made this more convenient, so that all doses of vaccine could be administered under supervision). ATRA was administered 1 h before the first dose of vaccine; this was supervised on day 1 and unsupervised on days 2–8.

Measurement of mucosal IgA responses using whole gut lavage

Immunological assessment was carried out on days 0 and 14. Following an overnight fast, volunteers underwent whole gut lavage as described previously [34], and a 10-ml blood sample was collected. Briefly, each volunteer drank a sufficient quantity (usually 2–4 litres) of a gut lavage solution (Klean-Prep; Norgine, Uxbridge, UK) to yield a clear

fluid per rectum. Once the rectal output had almost cleared of visible particulates a 50 ml aliquot was collected and protease inhibitors added: ethylenediamine tetraacetic acid (EDTA) (Sigma, Poole, UK) was added to a final concentration of 10 mmol/l, and one tablet of Sigmafast protease inhibitor cocktail (Sigma) was dissolved in the fluid. This contains 4-(2-aminoethyl) benzenesulphonylfluoride hydrochloride, arginine, pepstatin A, bestatin, pancreatic trypsin inhibitor, acetyl-Leu-Leu-arginal, N-(*trans*-epoxysuccinyl)-L-leucine 4-guanidinobutylamide, phosphoramidon and 1S-octyl- β -D-thioglucopyranoside. The tube was centrifuged in a refrigerated centrifuge at 4°C at 2626 g for 20 min and aliquots of supernatant stored at –80°C.

Analysis of IgA responses to oral vaccination

Three different vaccine antigen preparations were made: a suspension of whole bacteria (2×10^7 in each well), a protein extract (30 ng in each well) and a preparation of LPS made using a modified phenol extraction technique [35] (1 μ g in each well). Briefly, vaccine bacteria were heated in distilled water at 67°C, mixed with an equal volume of preheated 90% aqueous phenol (pH 8.0) and incubated for 15 min, then centrifuged at 16 000 g for 5 min. Plates were coated with antigen in phosphate-buffered saline (PBS) overnight at 4°C, and casein in PBS (Perbio Science, Cramlington, UK) was used as blocking buffer for 1 h before adding 100 μ l of serum (dilution 1:100) or 100 μ l whole gut lavage fluid (WGLF; undiluted) and incubating for 2 h at room temperature (22°C). Washing was carried out six times using PBS with 0.05% Tween20. Secondary antibody, goat anti-human IgA or anti-human IgG conjugated to alkaline phosphatase (Cambridge Bioscience, Cambridge, UK) was incubated for 1 h at room temperature and washed six times. Following 20 min incubation with p-nitrophenyl phosphate (Sigma) the plate was read at 405 nm in an EL800 plate reader (BioTek, Potton, UK). All work was carried out with pyrogen-free pipette

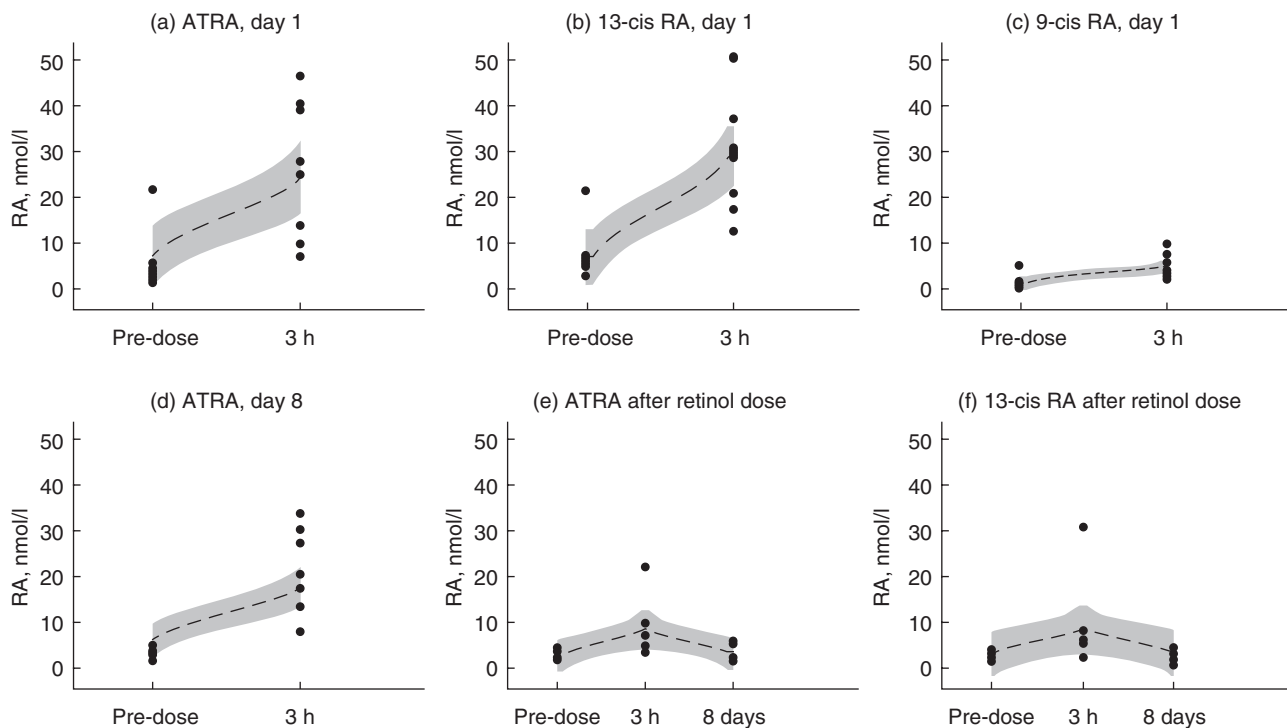


Fig. 1. Pharmacokinetics. (a–c) Serum concentrations of (a) all-*trans* retinoic acid (ATRA), (b) 13-*cis* retinoic acid (RA) and (c) 9-*cis* RA just before or 3 h after an oral dose 10 mg ATRA on day 1. Although pure ATRA was given, immediate isomerization occurs and the peak concentration of 13-*cis* RA exceeds that of ATRA. (d) Serum concentration of ATRA before and after 10 mg ATRA on the eighth day; there is no evidence of accumulation, but C_{max} appears to be reduced compared with day 1, probably reflecting induction of degradative enzymes. (e,f) Serum concentrations of (e) ATRA and (f) 13-*cis* RA before and 3 h after a single dose of 60 mg retinyl palmitate, and then on day 8 at the same time as the baseline sample was taken for the volunteers shown in (d). Dotted lines show the polynomial smoothed curve, and the shaded band the 95% confidence limits.

tips (Starlab, Milton Keynes, UK). Secretory IgA in WGLF was assayed by ELISA (Immunodiagnostik AG, Bensheim, Germany).

Data analysis

Pharmacokinetic data are expressed as median and interquartile range (IQR); values at individual time-points were compared in different groups using the Kruskal–Wallis test. Graphic representation was performed using polynomial (Epanechnikov) smoothing of the time-points at 0 and 3 h in order to demonstrate individual data points and summary data with 95% confidence intervals. Serum and WGLF responses to vaccine antigens are expressed as optical density (OD) values, but total IgA is expressed in ng/ml; values are expressed as median and IQR and the Kruskal–Wallis test was used.

Results

Of 50 men recruited, 18 had intestinal helminth infections and were excluded, leaving 32 men aged between 18 and 50 years (mean age 31) who participated in the study. Of these, 11 men (34%) were HIV-seropositive, which is consistent

with previous studies in this community [28]. Mean BMI was 20.4 kg/m², mean percentage body fat was 13.2% and mean grip strength was 36.8 kg; no significant difference in these parameters was apparent using the Kruskal–Wallis or *t*-tests in volunteers assigned to different study groups (Table 1).

Pharmacokinetics

Predose concentrations of ATRA, 9-*cis* and 13-*cis* RA were similar in men allocated to retinyl palmitate or to tretinoin, but post-dose ATRA and 13-*cis* RA were much higher following administration of tretinoin (Fig. 1). Post-dose concentrations exceeded 10 nmol/l on all but two occasions. These were two HIV-seropositive men whose C_{max} on day 1 failed to rise above 10 nmol/l, one rising to 9.6 and the other to 6.7 nmol/l; in the second case the C_{max} on day 8 was also low at 7.8 nmol/l. C_{max} values in HIV-seropositive men (median 13.8 nmol/l, IQR 6.7–24.8) appeared to be lower than in HIV-seronegative men (median 38.9, IQR 27.6–40), but the difference was not significant ($P = 0.10$). There was a suggestion that C_{max} on day 8 was lower than on day 1, which would indicate induction of glucuronidation and oxidation enzymes, but this was not significant in this small

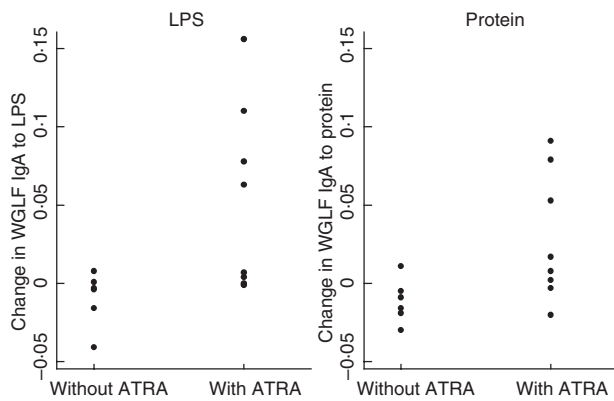


Fig. 2. Summary of changes in specific immunoglobulin (Ig)A in Vivotif recipients. Responses are shown measured as change in optical density (OD) values at 405 nm, in response to vaccination with or without 10 mg *all-trans* retinoic acid (ATRA) daily for 8 days ($P = 0.02$ for lipopolysaccharide (LPS), $P = 0.04$ for protein extract).

group. No evidence of accumulation of any of the RA isoforms over the 8 days was observed, as trough concentrations remained low on day 8. No adverse events were reported by these men during the 8 days of treatment.

Effect of tretinoin on responses to oral vaccines

Specific IgA in whole gut lavage fluid (WGLF) against LPS and protein extract was increased in vaccine recipients who were given ATRA 10 mg daily for 8 days compared with those who were not given ATRA, or those who were given Dukoral with or without ATRA (Fig. 2). There was no difference in changes in total WGLF IgA concentrations between ATRA recipients and controls ($P = 0.18$; Fig. 3). There were three individuals whose total IgA in WGLF increased, but two of these showed no rise in specific IgA in WGLF, and of the four participants with a clear rise in specific IgA in WGLF, only one had a rise in total IgA. No effect was seen on serum IgA or IgG responses to either of the vaccine antigens evaluated (Fig. 5).

Correlations between serum and WGLF responses

We explored correlations between gut and serum IgA responses to LPS and protein extracts and in relation to total IgA in WGLF (Fig. 4). Correlations between responses to LPS and protein were strong in both WGLF and serum (Fig. 4a,b). There was a suggestion of a threshold effect of total IgA in WGLF such that only WGLF samples with total IgA concentrations of 200 ng/ml showed detectable specific IgA in WGLF (Fig. 4c,d). Correlations between specific IgA responses in WGLF and serum were less strong than within compartments (Fig. 4e,f).

We also explored the extent to which these results might be influenced by HIV status. Of the participants who were given Vivotif, two of eight who were also given ATRA were

HIV-seropositive, and two of six who were not given ATRA were HIV-seropositive. The increases in specific IgA in WGLF from days 0 to 14 were -0.02 and 0.091 in the two HIV-seropositive participants compared with median (IQR) increases of 0.0125 (0.002 , 0.053) in the six HIV-seronegative participants. While these numbers are small, we found no evidence of an inability to generate ATRA-augmented vaccine responses in HIV infection. While the strong correlations between specific IgA responses to LPS and protein within compartments (serum or WGLF) were unaffected by HIV status, the correlations between specific IgA in WGLF and total IgA in WGLF was restricted to HIV-uninfected (seronegative) participants (for example, $\rho = 0.72$ and $P < 0.0001$ for responses to protein in HIV-negative compared with $\rho = 0.21$ and $P = 0.61$ in HIV-seropositive participants).

Discussion

In view of the global burden of diarrhoeal disease and the reduced efficacy of oral vaccines in tropical populations, the need for effective oral adjuvants is urgent [36], and new strategies are needed for vaccination against diarrhoeal diseases. We have explored the possibility that *all-trans* retinoic acid could be utilized as an oral adjuvant in a population with a high burden of HIV. We have demonstrated that ATRA can be given safely in a simple commercially available oral capsule and that rapid rises in serum concentration are predictable. The great majority of adults in this population demonstrated a C_{max} higher than the concentration (10 nmol/l), which was associated with changes in lymphocyte homing markers *in vitro* [13]. We went on to examine gut and serum responses to Vivotif antigens (crude extracts of LPS and protein from vaccine bacteria) in vaccinees given Vivotif and in controls given an irrelevant vaccine, Dukoral. Our results suggest that ATRA could play an adjuvant role when given alongside Vivotif, an oral typhoid

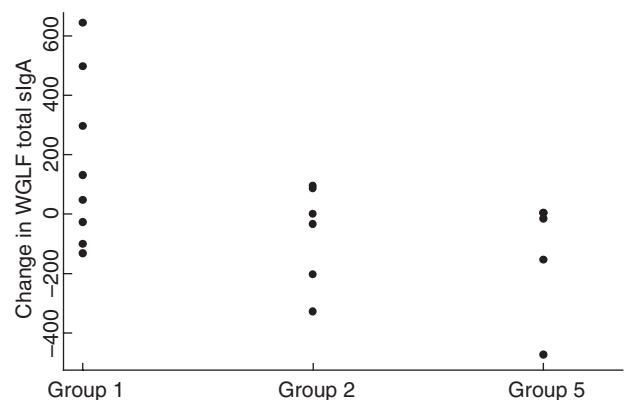
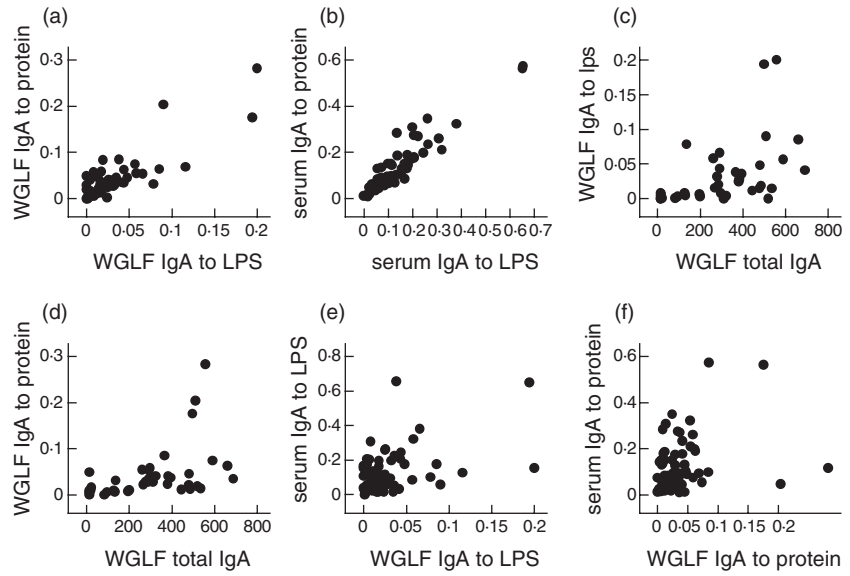


Fig. 3. Secretory immunoglobulin (Ig)A concentrations in whole gut lavage fluid. Total IgA (ng/ml) in men in different groups was not statistically significant ($P = 0.07$).

Fig. 4. Correlations between whole gut lavage fluid (WGLF) and serum specific and total immunoglobulin (Ig)A responses. (a) Specific IgA responses to lipopolysaccharide (LPS) and protein extract in WGLF were closely correlated ($\rho = 0.71$; $P < 0.0001$). (b) Specific IgA responses to LPS and protein extract in serum were very closely correlated ($\rho = 0.91$; $P < 0.0001$). (c) Specific IgA to LPS in WGLF against total IgA in WGLF were correlated ($\rho = 0.58$; $P = 0.0001$). (d) Specific IgA to protein extract in WGLF against total IgA in WGLF were correlated ($\rho = 0.60$; $P = 0.0001$). (e) WGLF and serum-specific IgA to LPS were correlated moderately ($\rho = 0.34$; $P = 0.006$). (f) WGLF and serum-specific IgA to LPS were correlated moderately ($\rho = 0.28$; $P = 0.03$). Specific IgA concentrations given as optical density (OD) values at 1:200 dilution, and total IgA in WGLF as ng/ml.



vaccine. Our results need to be confirmed in other populations and with other vaccines, but suggest that immunological effects of ATRA demonstrable *in vitro* and *in vivo* [13,20] could potentially be translated into an important strategy for enhancing responses to easily administered vaccines.

The dose of tretinoin used in this study (10 mg/day, about 6–7 mg/m²) is approximately one-eighth of that used in oncological practice and generated serum C_{max} concentrations of 6.7–42 nmol/l. We extrapolated from *in-vitro* data that a concentration of 10 nmol/l would be required to be sure of an effect on homing receptor expression in lym-

phocytes, although the concentration in intestinal epithelial cells would, of course, be higher. These values of C_{max}, while satisfactory for this purpose, are lower than might be expected based on data from adults [24,29] or children [30]. To set this in context, 45 mg/m² in adults generated C_{max} concentrations of 508 nmol/l in adults [29]. Even at much lower doses (25 mg/m²) in children, C_{max} varied between 48.5 and 513 nmol/l [30]. We surmise that the low C_{max} we observed in some participants (and high values in none) reflects the impact of environmental enteropathy [28,37], and in those individuals with HIV infection C_{max} was lower still. A great deal of work has been conducted on

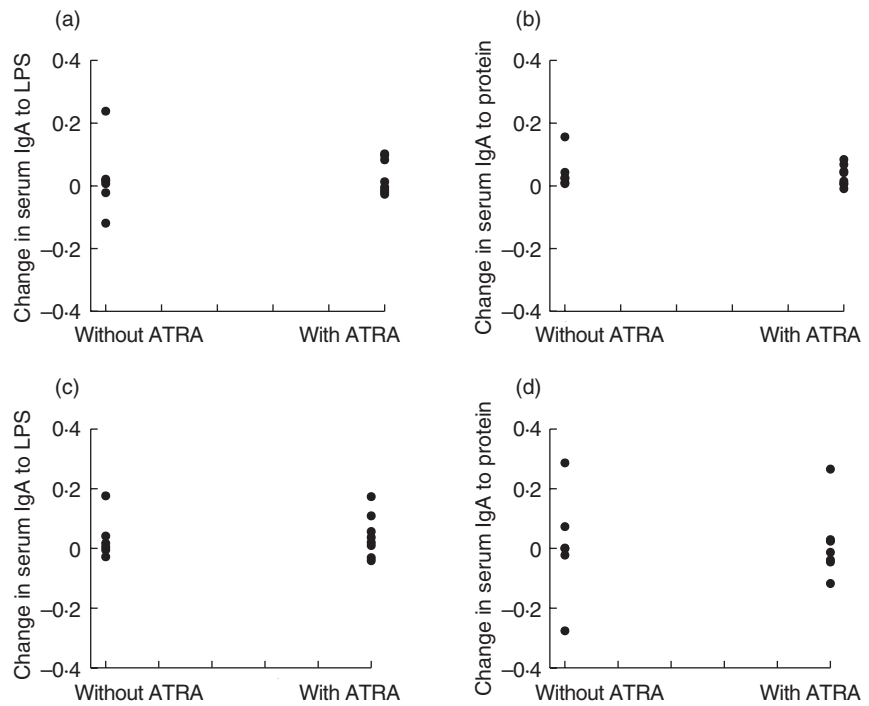


Fig. 5. Changes in serum immunoglobulin (Ig)A and IgG in Vivotif recipients. No difference in serum responses to the vaccine antigens was seen in the vaccinees given 10 mg all-*trans* retinoic acid (ATRA) daily for 8 days alongside vaccination.

vitamin A (retinol and carotenoid) absorption, but many questions remain unanswered [38,39], and mechanisms of absorption of retinoic acids and their derivatives remain largely unexplored. Nevertheless, these values provide a starting-point for future work directed at immunological manipulation rather than oncological treatment.

The effect of vitamin A supplementation (VAS) on vaccine efficacy has been the focus of a modest number of studies that have generated disparate results [40]. There is also some evidence that the effects of VAS on responses to vaccination (specific and non-specific) may be sex-dependent, with a greater benefit in boys than in girls [41], and this may apply particularly to diphtheria–tetanus–pertussis (DTP) vaccination [41]. It is apparent from our pharmacokinetic data that oral administration of retinoic acid generates a much sharper rise in serum concentration of ATRA than does oral dosing with retinyl palmitate, and thus the immunological effects may be different, or at least clearer.

The adjuvanticity of ATRA we observed in this study applied only to the mucosal compartment; no effect was observed on serum IgA or IgG responses. Further work will be required to ascertain whether ATRA may increase IgA transport into the gut, through up-regulation or enhanced function of the polymeric immunoglobulin receptor (pIgR). However those individuals with the clearest increases in specific IgA to LPS and protein extracts in WGLF were not those in whom we found evidence of increased total IgA, which would be expected if the principal effect were on pIgR. The correlations between specific IgA to LPS and both serum-specific IgA and total WGLF IgA suggest to us that gut antibody responses to this vaccine are dependent upon both enhanced induction of the immune response and increased antibody secretion, but this remains to be established.

In order to avoid having to reject volunteers who are HIV-seropositive, which could lead to stigmatization in this close-knit community, we included men who are HIV-infected alongside men without HIV infection. This opportunity for comparison yielded data that suggest that absorption of ATRA is reduced in HIV-infected men compared with uninfected men. Despite this apparent impairment, there was no evidence of impairment of immune induction in the gut, and it would appear that HIV-infected men were as able to recognize the vaccine alloantigens as uninfected men. The quantity and quality of vaccine responses in HIV infection is impaired for some (for example with hepatitis B vaccine [42]), but not all, vaccines (human papilloma virus vaccine is effective [43]), but we found no evidence in this early study that mucosal immune responses are impaired due to HIV.

While we believe that these results are promising, we must point out that retinoic acids are embryotoxic [25], with no clearly safe dose, and ATRA could not be used for vaccination programmes for women of childbearing age.

ATRA appears to be safe in men at the doses we used in this study, and teratogenicity is not a concern in children at the ages at which vaccines are given. Nevertheless, the programmatic implications of this safety issue would need to be considered carefully. If the potential adjuvanticity of ATRA can be confirmed with a range of other vaccines, it might help to overcome the impaired vaccine efficacy in populations with the greatest need of vaccination against diarrhoea pathogens.

Acknowledgements

We are grateful to John Mbewe, Coillard Kaunga, Rose Soko, Themba Banda, Stayner Banda and Vera Yambayamba for work on data collection, and to the Craft Laboratories for analysis of retinoic acid isoforms. Funding was granted by the Bill & Melinda Gates Foundation through a Grand Challenges Explorations grant (OPP1018115).

Disclosures

None.

Author contributions

P. K. originated the study and is guarantor. M. M. L., M. C. K. and P. K. designed the study and the outcome measures. R. B., E. S., V. K. and P. K. supervised clinical data collection and S. S. designed and carried out the parasitological evaluations. P. K. wrote the first draft of the manuscript. All authors contributed to and approved the final manuscript, including the authorship list.

References

- 1 Petri WA, Miller M, Binder HJ *et al.* Enteric infections, diarrhea, and their impact on function and development. *J Clin Invest* 2008; **118**:1277–90.
- 2 Checkley W, Epstein LD, Gilman RH, Cabrera L, Black RE. Effects of acute diarrhea on linear growth in Peruvian children. *Am J Epidemiol* 2003; **157**:166–75.
- 3 Grimwood K, Lambert SB, Milne RJ. Rotavirus infections and vaccines. *Pediatr Drugs* 2010; **12**:235–56.
- 4 Pasetti MF, Simon JK, Szein MB, Levine MM. Immunology of gut mucosal vaccines. *Immunol Rev* 2011; **239**:125–48.
- 5 Vesikari T, Matson DO, Dennehy P *et al.* Safety and efficacy of a pentavalent human–bovine (WC3) reassortant rotavirus vaccine. *New Engl J Med* 2006; **354**:23–33.
- 6 Zaman K, Dang DA, Victor JC *et al.* Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in Asia: a randomised, double-blind, placebo-controlled trial. *Lancet* 2010; **376**:615–23.
- 7 Vesikari T, Karvonen A, Prymula R *et al.* Efficacy of human rotavirus vaccine against rotavirus gastroenteritis in the first two years of life in European infants: randomised double-blind controlled study. *Lancet* 2007; **370**:1757–63.

- 8 Mahdi SA, Cunliffe NA, Steele D *et al.* Effect of human rotavirus vaccine on severe diarrhea in African infants. *New Engl J Med* 2010; **362**:289–98.
- 9 Lycke N. Recent progress in mucosal vaccine development: potential and limitations. *Nat Rev Immunol* 2012; **12**:592–605.
- 10 Germain P, Chambon P, Eichele G *et al.* International Union of Pharmacology. LX. Retinoic acid receptors. *Pharmacol Rev* 2006; **58**:712–25.
- 11 Tang X-H, Gudas LJ. Retinoids, retinoic acid receptors, and cancer. *Annu Rev Pathol-Mech* 2011; **6**:345–64.
- 12 Bastien J, Rochette-Egly C. Nuclear retinoid receptors and the transcription of retinoid-target genes. *Gene* 2004; **328**:1–16.
- 13 Iwata M, Hirakiyama A, Eshima Y *et al.* Retinoic acid imprints gut-homing specificity on T cells. *Immunity* 2004; **21**:527–38.
- 14 Takenouchi-Ohkubo N, Asano M, Chihaya H *et al.* Retinoic acid enhances the gene expression of human polymeric immunoglobulin receptor (pIgR) by TNF-alpha. *Clin Exp Immunol* 2004; **135**:448–54.
- 15 Coombes JL, Coombes JL, Siddiqui KR *et al.* A functionally specialised population of mucosal CD103+ DCs induces Foxp3 regulatory T cells via a TGFβ and retinoic acid-dependent mechanism. *J Exp Med* 2007; **204**:1757–64.
- 16 Golovina TN, Mikheeva T, Brusko TM *et al.* Retinoic acid and rifamycins differentially affect and synergistically promote the *ex vivo* expansion of natural human T regulatory cells. *PLOS ONE* 2011; **6**:e15868.
- 17 Wang C, Kang SG, Hogen Esch H, Love PE, Kim CH. Retinoic acid determines the precise tissue tropism of inflammatory Th17 cells in the intestine. *J Immunol* 2010; **184**:5519–26.
- 18 Villablanca E, Wang S, DeCalisto J *et al.* MyD88 and retinoic acid signaling pathways interact to modulate gastrointestinal activities of dendritic cells. *Gastroenterology* 2011; **141**:176–85. epub before print.
- 19 Cassani B, Villablanca EJ, Quintana FJ *et al.* Gut tropic T cells that express integrin α4β7 and CCR9 are required for induction of oral tolerance in mice. *Gastroenterology* 2011; **141**:2109–18.
- 20 Hammerschmidt SI, Friedrischsen M, Boelter J *et al.* Retinoic acid induces homing of protective T and B cells to the gut after subcutaneous immunization in mice. *J Clin Invest* 2011; **121**:3051–61.
- 21 Tan X, Sande JL, Pufnock JS, Blattman JN, Greenberg PD. Retinoic acid as a vaccine adjuvant enhances CD8+ T cell response and mucosal protection from viral challenge. *J Virol* 2011; **85**:8316–27.
- 22 Sanz MA, Lo-Coco F. Modern approaches to treating acute promyelocytic leukaemia. *J Clin Oncol* 2011; **29**:495–503.
- 23 Rogers JE, Yang D. Differentiation syndrome in patients with acute promyelocytic leukemia. *J Oncol Pharm Pract* 2011; **18**:109–14. epub ahead of print.
- 24 Takitani K, Koh M, Inoue A, Kawakami C, Kuno T, Tamai H. Pharmacokinetics of all-*trans* retinoic acid in adults and children with acute promyelocytic leukemia. *Am J Hematol* 2006; **81**:720–1.
- 25 Adams J. The neurobehavioral teratology of retinoids: a 50-year history. *Birth Defects Res (Part A)* 2010; **88**:895–905.
- 26 Hartmann S, Brors O, Bock J *et al.* Exposure to retinyl esters, retinol, and retinoic acids in non-pregnant women following increasing single and repeated doses of vitamin A. *Ann Nutr Metab* 2005; **49**:155–64.
- 27 Kapulu MC, Simuyandi M, Sianongo S *et al.* Differential changes in expression of intestinal antimicrobial peptide genes during *Ascaris lumbricoides* infection in Zambian adults do not respond to helminth eradication. *J Infect Dis* 2011; **203**:1464–73.
- 28 Kelly P, Menzies I, Crane R *et al.* Responses of small intestinal architecture and function over time to environmental factors in a tropical population. *Am J Trop Med Hyg* 2004; **70**:412–9.
- 29 Ozpolat B, Lopez-Berestein G, Adamson P, Fu CJ, Williams AH. Pharmacokinetics of intravenously administered liposomal all-*trans*-retinoic acid (ATRA) and orally administered ATRA in healthy volunteers. *J Pharm Pharm Sci* 2003; **6**:292–301.
- 30 Lanvers C, Reinhardt D, Dubbers A *et al.* Pharmacology of all-*trans* retinoic acid in children with acute promyelocytic leukemia. *Med Pediatr Oncol* 2003; **40**:293–301.
- 31 McNamara PJ, Jewell RC, Jensen BK, Brindley CJ. Food increases the bioavailability of acitretin. *J Clin Pharmacol* 1988; **28**:1051–5.
- 32 Dietrich G, Griot-Wenk M, Metcalfe IC, Lang AB, Viret J-F. Experience with registered mucosal vaccines. *Vaccine* 2003; **21**:678–83.
- 33 Lucas MES, Deen JL, von Seidlein L, Wang X-Y, Ampuero J *et al.* Effectiveness of mass oral cholera vaccination in Beira, Mozambique. *New Engl J Med* 2005; **352**:757–67.
- 34 Daley AC, Randall R, Darsley M *et al.* Genetically modified enterotoxigenic *Escherichia coli* vaccines induce mucosal immune responses without inflammation. *Gut* 2007; **56**:1550–6.
- 35 Luderitz O, Risse HJ, Schulte-Holthausen H *et al.* Biochemical studies of the smooth-rough mutation in *Salmonella minnesota*. *J Bacteriol* 1965; **89**:343–54.
- 36 Pulendram B, Ahmed R. Translating innate immunity into immunological memory: implications for vaccine development. *Cell* 2006; **124**:849–63.
- 37 Prendergast A, Kelly P. Enteropathies in the developing world; neglected effects on global health. *Am J Trop Med Hyg* 2012; **86**:756–63.
- 38 Harrison EH. Mechanisms involved in the intestinal absorption of dietary vitamin A and provitamin carotenoids. *Biochim Biophys Acta* 2012; **1821**:70–7.
- 39 D'Ambrosio DN, Clugston RD, Blaner WS. Vitamin A metabolism: an update. *Nutrients* 2011; **3**:61–103.
- 40 Savy M, Edmond K, Fine PE *et al.* Landscape analysis of interactions between nutrition and vaccine responses in children. *J Nutr* 2009; **139**:2154S–218S.
- 41 Benn CS. Combining vitamin A and vaccines: convenience or conflict? *Dan Med J* 2012; **59**:B4378.
- 42 Kim HN, Harrington RD, Crane HM, Dhanireddy S, Dellit TH, Spach DH. Hepatitis B vaccination in HIV-infected adults: current evidence, recommendations and practical considerations. *Int J STD AIDS* 2009; **20**:595–600.
- 43 Wilkin T, Lee JY, Lensing SY *et al.* Safety and immunogenicity of the quadrivalent human papillomavirus vaccine in HIV-1-infected men. *J Infect Dis* 2010; **15**:1246–53.