Retinoic acid elicits a coordinated expression of gut homing markers on T lymphocytes of Zambian men receiving oral Vivotif, but not Rotarix, Dukoral or OPVERO vaccines

Mpala Mwanza-Lisulo,⇑ Mumba S. Chomba, Mubanga Chama, Ellen C. Besa, Evelyn Funjika, Kanekwa Zynambo, Rose Banda, Mercy Imikendi, Sandie Sianongo, Robert E.W. Hancock, Amy Lee, Roma Chilingi, Andy J. Stagg, Boniface Namangala, Paul M. Kelly

⇑ Corresponding author at: TROPGAN Office, Department of Internal Medicine, University of Zambia School of Medicine, University Teaching Hospital, Nationalist Road, Lusaka, Zambia.

E-mail address: mpalamwanza123@gmail.com (M. Mwanza-Lisulo).

Abbreviations: ATRA, all-trans retinoic acid; RA, retinoic acid; CCR9, chemokine receptor 9; WGLF, whole gut lavage fluid.

1. Introduction

There is strong evidence of diminished immunogenicity and efficacy of oral vaccines in developing country populations [1]. For example, oral rotavirus vaccine affords excellent (80–90%) protection against severe rotavirus diarrhea in industrialized and middle income countries but has been less efficacious when tested in infants in the developing world [2,3]. The trivalent oral polio vaccine (OPVERO) has also been found to be less effective in children in the developing world [4] with estimated efficacies of around 21% in India compared to 50% in the United States [5]. In view of this, there is an urgent need to identify ways to improve immune responses of these oral vaccines in the settings where they are most needed.

Vitamin A supplementation significantly reduces mortality from diarrheal infections as evidenced by results of several studies [6–9]. The mechanism of protection however is unclear [6,9]. Vitamin A is oxidized to four isoforms of retinoic acid (RA); these include all-trans RA (ATRA), 9-cis RA, 13-cis RA, and 11-cis RA. The RAs have important immunological functions especially on intestinal defense and mucosal immune responses.

Selective migration of the effector T cells to the gut requires the expression of α4β7-integrin and CCR9. Integrin α4β7 is the homing receptor for the small and large intestine, whereas CCR9 is the small intestine-specific homing receptor [10]. Several studies have shown that RA is a key mediator of T cell homing to the gut [11–13]. Iwata and colleagues [11] demonstrated that CD4+ T cells activated in vitro in the presence of ATRA...
had enhanced expression of the gut-homing receptors α4β7-integrin and CCR9. Hammerschmidt and colleagues [12] demonstrated that RA-assisted subcutaneous immunization resulted in pronounced localization of antigen-specific plasma cells in the small intestine and a robust immunoglobulin A (IgA) response in serum and intestinal washes. In recent preliminary work [14], we found that ATRA could enhance gut IgA responses to Vivottif typhoid vaccine in intestinal washes. Collectively these observations suggest that RA-assisted immunization generates intestinal immunity and confers the benefits of mucosal protection.

Retinoic acid has been known to affect expression of the polymeric immunoglobulin receptor (pIgR) [15] which transports polymeric immunoglobulin A (pIgA) into external secretions as secretary IgA (S-IgA). This suggests that vitamin A might be required for the proper regulation of IgA transport in response to mucosal infection, which would be a desirable characteristic of an orally-active adjuvant [16,17].

The present study investigated whether the ATRA adjuvanticity seen with Vivottif vaccine in African men could be generalized to other oral vaccines. We asked if ATRA given alongside oral vaccines could improve the immune response by enhancing expression of α4β7-integrin and CCR9 gut homing molecules, and if so, under what circumstances.

2. Methods

2.1. Recruitment

We recruited 94 adult male volunteers aged between 18 and 60 years from a high-density township in Lusaka, Zambia, as previously described [14]. A 3-stage consent process was used [20,21]. Nutritional assessment and HIV testing was done. Details in Supplementary material methods.

2.2. Ethical Approval

Approval for the study was obtained from the Biomedical Research Ethics Committee of the University of Zambia (references 012-06-12 and 013-01-14).

2.3. Vaccine administration and sample collection

Recruited volunteers (n = 94) were randomized to receive one of four oral vaccines: Vivottif (Crucell, Switzerland) [22,23], Dukoral (SBL Vaccines), Rotarix (GSK), and OPVERO (Sanofi Pasteur). Each vaccine was given with or without ATRA on day 1. ATRA was administered as 10 mg daily for 8 days and 1 h before vaccination. Vivottif was given on days 1, 3 and 5 while Dukoral, Rotarix and OPVERO were administered as single doses. Whole gut lavage fluid was collected as previously described [14,25] on days 0 and 14 (see supplementary material methods). Blood samples were collected from 8 male individuals given both ATRA and Vivottif on day 0 (baseline) and day 8. Each sample comprised 2.5 ml of blood directly drawn into a PAXgene blood RNA tube (Qiagen), inverted 10 times, then kept at room temperature for 2 h before being stored at −80°C until RNA extraction (see Supplementary material methods for more details).

2.4. Detection of Vivottif and Dukoral-specific IgA and IgG in serum and gut lavages

Vivottif and Dukoral LPS antigen preparations were made using a modified phenol extraction technique as previously described [14,22]. Analysis of vaccine specific antibodies in serum and gut lavages was performed as described previously [14].

2.5. Detection of Rotarix-specific IgA and IgG in serum and gut lavages

Analysis of IgA and IgG antibody responses was done using ELISA (see supplementary material).

2.6. Detection of OPVERO-specific IgA and IgG in serum

Analysis of OPVERO-specific antibodies in serum was performed by neutralization.

2.7. Analysis of effects of ATRA on polymeric Ig receptor (plgR)

Biopsies (<20 mg of tissue) were collected from the jejunum of 13 participants and analyzed for the effect of ATRA on expression of plgR (for details refer to Supplementary material methods).

2.8. Analysis of baseline serum retinol concentrations

Retinol in serum was assayed by high performance liquid chromatography (HPLC) modified version [26].

2.9. Transcriptome analysis of effects of ATRA during vaccination

Blood samples were collected from 8 male individuals given both ATRA and Vivottif on day 0 (baseline) and day 8. Antibody titers in whole gut fluid against Vivottif LPS antigen were significantly increased (Fig. 1A) in the group that received Vivottif vaccine as well as ATRA, consistent with our previous findings [14]. There was no significant change in mucosal responses to Rotarix antigens or Dukoral (whether LPS or CTB) antigens in gut lavage samples (Fig. 1B-D). Antibody titers in serum showed no significant change in any of the vaccine groups (Supplementary Fig. 2). This increase in specific IgA was not a consequence of increased plgR expression in intestinal biopsies (Supplementary Fig. 3).

2.10. Statistical analyses

All graphical and statistical tests were performed using GraphPad Prism 6.0 (La Jolla, CA) and STATA 12 (Stata Corp, College Station, TX, USA). Statistical comparisons were done using nonparametric tests (Mann-Whitney, Kruskal-Wallis and Spearman’s rank correlation coefficient). Gut lavage responses are expressed as change in log titer values; P values <0.05 were assumed significant.

3. Results

In total, 94 participants were randomized to receive one of 4 vaccines and participant demographics are described in Table 1. The HIV prevalence in this study cohort was 21% with average age being 29 years and nutritional status comparable in each group. We report combined data for HIV infected and non-infected, as we found HIV infection had no effect on the intestinal IgA response to Vivottif (Supplementary Fig. 1).

3.1. ATRA increased gut IgA directed at Vivottif LPS, but not other vaccine antigens

Antibody titers to Vivottif, Dukoral and Rotarix were measured as changes in log titers in response to vaccination with or without daily administration of 10 mg ATRA for 8 days. Antibody titers in whole gut fluid against Vivottif LPS antigen were significantly increased (Fig. 1A) in the group that received Vivottif vaccine as well as ATRA, consistent with our previous findings [14]. There was no significant change in mucosal responses to Rotarix antigens or Dukoral (whether LPS or CTB) antigens in gut lavage samples (Fig. 1B-D). Antibody titers in serum showed no significant change in any of the vaccine groups (Supplementary Fig. 2). This increase in specific IgA was not a consequence of increased plgR expression in intestinal biopsies (Supplementary Fig. 3).

3.2. Effect of ATRA was vaccine and time dependent

We then analyzed whether expression of gut homing markers was vaccine dependent. We found that there was an increase in
Table 1
Baseline characteristics of study participants.

| Vaccine | Number | Age (years) | BMI (kg/m²) | HIV Positive (n) |
|---------|--------|-------------|-------------|-----------------|
| None    | 13     | 34 (22–42)  | 20 (19–23)  | 5               |
| Dukoral | 6      | 28 (22–43)  | 19 (18–24)  | 4               |
| Opvero  | 4      | 39 (27–53)  | 19 (18–19)  | 3               |
| Rotarix | 7      | 21 (19–25)  | 20 (19–21)  | 1               |
| Vivotif | 33     | 38 (29–40)  | 22 (19–23)  | 1               |
| Vivotif | 13     | 21 (19–25)  | 20 (19–21)  | 1               |
| Dukoral | 6      | 28 (22–43)  | 19 (18–24)  | 3               |
| Dukoral | 6      | 39 (37–40)  | 19 (18–19)  | 3               |
| Dukoral | 6      | 20 (19–42)  | 19 (18–19)  | 3               |
| Rotarix | 7      | 29 (25–33)  | 19 (17–25)  | 3               |
| Opvero  | 5      | 31 (28–40)  | 20 (18–24)  | 3               |
| Dukoral | 13     | 28 (25–33)  | 19 (18–24)  | 3               |
| Vivotif | 13     | 29 (19–42)  | 19 (18–24)  | 3               |
| Dukoral | 6      | 19 (17–25)  | 19 (18–24)  | 3               |
| Dukoral | 6      | 20 (18–24)  | 19 (18–24)  | 3               |

The number of participants randomised to Vivotif includes those in the time course experiments, vaccine comparison experiments and pIgR experiments. Volunteers were all healthy adult men randomised to receive one or none of the vaccines with or without 10 mg ATRA given daily for 8 days. For vaccine comparisons 8 volunteers were recruited, pIgR studies had 13 volunteers while the time course studies had 12 volunteers all given Vivotif + ATRA. These are shown as pooled Vivotif vaccine groups. Continuous variables are shown as median and interquartile range (IQR). ATRA: all-trans retinoic acid, BMI: body mass index.

Fig. 1. Change in specific IgA in intestinal lavage fluid in volunteers given one of three vaccines. 'Change' is the difference in log titre values of IgA pre- and 14 days post vaccination. Antigen-specific IgA responses to Vivotif and Dukoral LPS, Dukoral CTB and Rotarix antigen were measured in whole gut lavage fluid (WGLF). (A) Specific IgA in WGLF against Vivotif LPS was increased (*P = 0.01) in Vivotif recipients who received ATRA. No change was seen in responses to the other vaccine antigens: (B) Rotarix (P = 0.19), (C) Dukoral LPS (P = 0.83) or (D) Dukoral Cholera Toxin B subunit (CTB) (P = 0.84). Data were analyzed using Mann-Whitney test. Responses are shown measured as change in log titers.
α4β7+CD4+ (Fig. 2A) and α4β7+CD8+ T cells (Fig. 2B) in the Vivotif and Rotarix groups when compared to those that received only ATRA. We only observed slightly enhanced CCR9+ CD4+ T cells (Fig. 2C) but not CCR9+CD8+ T cells (Fig. 2D) in those given both Vivotif and ATRA. When analyzing only activated (HLA-DR+) CD3+ T cells, it was the CD8+ cells which showed the greatest change in α4β7 and CCR9 expression (Supplementary Fig. 4). Conversely, a slight decrease in α4β7+ DR+CD4+ T cells was observed at day 3 ($P = 0.02$) and day 8 ($P = 0.01$) in participants that received both Vivotif and ATRA (Supplementary Fig. 5). These data indicate that the effect of ATRA is seen only in the presence of antigen and on day 14 post vaccination.

3.3. ATRA caused a coordinated response of α4β7 integrin and CCR9 only if given simultaneously with Vivotif vaccination

Having established that the co-administration of Vivotif and ATRA resulted in increased α4β7+CD4+ and CCR9+CD4+ T cells at day 14, we explored the correlation of these two gut-homing markers. Spearman's rank test revealed a strong correlation ($\rho = 0.83; P < 0.0001$) of these gut homing markers on CD4+ T cells (Fig. 3A), but only when ATRA was given alongside Vivotif. We designated those individuals with a coordinated increase in these two gut homing markers as positive responders and those with a coordinated decrease as negative responders. A coordinated increase in both gut homing markers was observed in 57% (12/21) of volunteers that received Vivotif and ATRA simultaneously. This effect was not seen with Vivotif alone (Fig. 3B), ATRA alone (Fig. 3C) or Rotarix given with ATRA (Fig. 3D). Our data therefore suggest that the coordinated gut homing response to ATRA is restricted to the situation when co-administered with Vivotif.

3.4. Baseline serum retinol was lower in individuals that showed coordinated responses to α4β7 integrin and CCR9

Baseline serum retinol concentrations (Table 2) in the positive responders were significantly lower (median of 1.41 μmol/L, interquartile range 1.06–2.48 μmol/L) than the retinol markers.
concentration in the negative responders (median 2.68 \text{ mmol/L}, interquartile range 1.68–3.29; \( P = 0.03 \)). In the positive responder group, 40% (4/10) of the subjects had serum retinol concentrations of \(<1.05 \text{ mmol/L} \), consistent with vitamin A deficiency, compared to 0% (0/7) of negative responders.

3.5. The change in \( \alpha 4 \beta 7 \) on \( \text{DR}^+ \text{CD4}^+ \) T cells correlated strongly with the intestinal \( \text{IgA} \) response to Vivotif

We then looked for a correlation between the change in gut homing marker expression in blood and the intestinal \( \text{IgA} \) response to Vivotif (i.e. gut lavage \( \text{IgA} \) to Vivotif LPS). The change in \( \alpha 4 \beta 7 \) marker expression on CD4\(^+\) T cells was strongly correlated with the change in specific \( \text{IgA} \) response to Vivotif LPS in the intestine in the positive responders only (Fig. 4A). In negative responders (Fig. 4B), or those given Vivotif alone (Fig. 4C), there was no correlation.

3.6. Transcriptome analysis

To elucidate the transcriptional changes that occurred during co-administration of ATRA and the oral vaccine Vivotif, we performed RNA-Seq on blood samples. Transcriptomic changes in immune-related pathways, particularly including interferon \( \alpha /\beta \) signaling pathway, membrane-ECM interactions and immune hubs were observed (Supplementary Table 1). Key immune hubs identified include fibronectin 1 (FN1), AXL Tyrosine-protein kinase receptor, complement component 1 (C1QB), complement component 4 binding protein (C4BPA) and HLA-DRB1 (Supplementary Fig. 6).

4. Discussion

The global burden of diarrheal disease and the reduced efficacy of oral vaccines in populations from developing nations have highlighted the need for new strategies for vaccination against...
diarrheal diseases, for example by using oral adjuvants. This study has revealed four important effects of ATRA on immune responses to oral vaccination: First, we have confirmed earlier findings [14] that ATRA can increase gut mucosal IgA directed at an oral live attenuated typhoid vaccine, and that this increase is not attributable to the increased pIgR expression as had been suggested previously [15]. Secondly, our data suggests that ATRA can increase the number of circulating CD4+ T cells expressing the gut homing markers $\alpha_4\beta_7$ and CCR9 in a coordinated and vaccine-specific fashion in a subset of individuals. Thirdly, integrin $\alpha_4\beta_7$ increase was strongly correlated with the intestinal IgA responses in positive responders, i.e. those individuals with a coordinated increase in the gut homing markers. Lastly, we found that ATRA treatment may cause changes in immune related pathways, including interferon $\alpha/\beta$ signaling pathway (adjusted p-value = 0.0015), membrane-ECM interactions, and immune hubs including fibronectin 1, AXL kinase, complement pathway components and major histocompatibility complex components such as HLA-DRB1. Since these elements were impacted by the end of the 8 day ATRA treatment, we propose that they are responsible, in part, for the increased responses to Vivotif vaccine.

We have demonstrated previously [14] that, in whole gut fluid, ATRA is able to enhance specific IgA against vaccine antigens suggesting that ATRA could play an adjuvant role when given alongside Vivotif. To determine whether the adjuvanticity of ATRA could be translated to other oral vaccines, we evaluated its effect on three other vaccines; Rotarix, Dukoral and OPVERO vaccines, all orally administered one hour after administration (or not) of 10 mg ATRA given daily for 8 days. Our data revealed that ATRA adjuvanticity in this study only occurred with Vivotif and not the other vaccines, suggesting that the ATRA effect might be vaccine and/or antigen specific and not a generalizable adjuvant property. There may be several contributors to the specificity of ATRA in promoting adjuvanticity with Vivotif and not the other vaccines. The most important factor is likely to be the type of immune responses being elicited by the vaccines. Vivotif induces a vigorous T-cell response that favours the production of interferon gamma (IFN-\(\gamma\)), tumour necrosis factor-\(\alpha\) (TNF-\(\alpha\)), interleukin -1 (IL-1) and IL-6, indicative of a Th1-type response probably through Class II antigen presentation [31,32]. Rotavirus has been shown to drive the secretion of TGF-\(\beta\) resulting in inhibition of Th1 responses by dendritic cells [33]. In one study [34], Rotarix vaccination showed

**Fig. 4.** Correlation of change in gut IgA to change in $\alpha_4\beta_7^{+}DR^{+}CD4^{+}$ cells in the positive and negative responders. (A) The positive responders showed a strong correlation ($p = 0.82; P = 0.02$) of gut IgA to gut marker expression. (B) This was not observed in the negative responders ($p = 0.31, P = 0.2$) or those given (C) Vivotif alone ($p = 0.37; P = 0.2$). Correlations were analyzed using the Spearman rank correlation test.
no discernible effect on IFN-γ and TNF-α response in a considerable proportion [76% (16/21)] of vaccinees. Another study [35] showed that oral polio vaccine was associated with down regulation of cytokine (IFN-γ and TNF-α) production when co-administered with BCG. The authors speculated that polio virus might have specific immune modulatory molecules that down-regulate immune responses to antigens to which immune responses are being mounted simultaneously. Finally, the inactivated bacterial vaccine Dukoral tends to elicit diminished or skewed CD4+ T cell responses towards development of a Th2 T cell phenotype because of the presence of CTB [36]. Of the vaccines studied, Vivotif was the only vaccine that is likely to elicit a Th1-type immune response through Class II antigen presentation. Intriguingly, ATRA led to an increase in several MHC class II components including a major component enabling antigen recognition by T cells, HLA DRB1. We hypothesize here that it is the nature of the context of antigen presentation, in this case for a vaccine that generates an IFN-γ dominant response, which determines whether ATRA has an effect [37]. Also, in a pro-inflammatory context involving cytokines, such as IL-15, ATRA probably acts through dendritic cells to decrease conversion of naive T cells into T regs and to enhance Th1 cell polarization. However, this hypothesis will have to be tested empirically by inclusion of another vaccine group that elicits a Th1 type response, and that the experimental protocol would need to include direct demonstration of Th1 and Tregs in comparison with Th2 and Th17 responses.

To our knowledge, this is only the second study to evaluate ATRA effects on immune function in humans in vivo, since most other studies [11,13,38–43] have used in vitro systems or animal models. Our findings showed an increase in CD4 T cells expressing α4β7 and CCR9 following oral administration of 10 mg ATRA but only when given with Vivotif but not Rotarix or OPVERO vaccines. These results are consistent with other studies in animal models [11,12,39,40].

There is also evidence that ATRA has effects on other cell types that also possibly migrate to the gut. Pantazi and colleagues [13] demonstrated in a murine model, that retinoic acid signaling in B cells is not essential for their homing to Peyer’s Patches but is necessary in generating antigen-specific IgA responses in the gut. ATRA has also been found to enhance the migratory properties of dendritic cells, which is crucial for their antigen presenting function during infection. We must emphasize that while it would have been of interest to extend the scope of our work to include B cells, NK cells and DCs, in a human study one is always constrained by the experimental procedures which we can conduct, acceptable volumes of blood to be drawn, and the invasiveness of endoscopic procedures. Our attention in this study was drawn to T cells because of the mouse work by other groups [11,41,42] and our own previous study [14] suggesting that ATRA can augment responses to Vivotif vaccine.

For safety reasons, one limitation of this study is that it was carried out only in men due to the teratogenicity of ATRA [18,19]; and with no clear safe dose, ATRA could not be used in women who might become pregnant during the study. As other studies [44,45] confirm differences between males and females in response to vaccination and we suspect that responses in women would have been different. A further limitation is that we did not analyse the gut-resident cells to see the effect of ATRA on the gut immune responses, as we did not have ethical approval to perform gut lavage and endoscopic biopsies on the same participants.

When the baseline serum retinol concentration was analyzed, we found that 4/10 (40%) of the positive responders had a clear or borderline vitamin A deficiency but none of the negative responders had such deficiency. The difference observed might signify that the effect of ATRA is seen predominantly in people with borderline vitamin A deficiency. Also, the data could suggest that the vitamin A deficiency seen in the positive responders may have resulted in the dendritic cells being altered toward being more inflammatory producing high levels of IL-13 and TNF-α [46] thus creating a favorable environment in which ATRA has an effect. The response to ATRA and Vivotif was also not explained by HIV infection. A high proportion of this variability therefore remains unexplained, as evident from our RNA-Seq studies, so the possibility of an immunogenetic predisposition needs to be considered in future work.

It must be pointed out here that ATRA is also obtained from vitamin A in the diet, and the authors did not control or attempt to control for diet in the cohort because of the recall bias that doing a food frequency questionnaire has. It is also apparent from pharmacological data [14] that oral administration of 10 mg ATRA over a period of 8 days generates a sharp rise in serum concentrations of ATRA required to produce the desired immune effects that were the focus of the current study. Also of note is that ATRA levels were not monitored longitudinally but only evaluated at two time points; baseline and day 14 post vaccination.

Acknowledgements

We thank Dr. David Allen, Public Health England, Colindale, UK for assistance with analysis of OPVERO specific antibodies in serum. We thank the Tropical Diseases Research Centre, Ndola Central Hospital, Ndola, Zambia (TDRC) for help with analyzing the retinol concentrations in serum samples. We are also grateful to Dr. Yusuf Ahmed, Dr. Patrick Musonda and Dr. Andrew Prendergast for work on the Data and Safety Monitoring Board.

Funding

This work was supported by the Bill and Melinda Gates Foundation through a Grand Challenges Explorations grant (OPP1043696).

Disclosure

The authors declare no conflicts of interest.

Author contributions

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

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2018.04.083.

References

[1] Levine MM. Immunogenicity and efficacy of oral vaccines in developing countries: lessons from a live cholera vaccine. BMC Bio 2010;9:129.
[2] 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: randomized double-blind controlled study. Lancet 2007;370:1757–63.
[3] Madhi SA, Cunliffe NA, Steele D, et al. Effect of human Rotavirus vaccine on severe diarrhea in African infants. N Engl J Med 2010;362:289–98.
[4] Grassly NC, Jafari H, Bahl S, Durran S, Wenger J, Sutter RW, et al. Asymptomatic wild-type poliovirus infection in India among children with previous oral poliovirus vaccination. J Infect Dis 2010;201:1315–43.
[5] Grassly NC, Fraser C, Wenger J, Deshpande JM, Sutter RW, Heymann DL, et al. New strategies for the elimination of polio from India. Science 2006;314:1150–3.
Ghana Vitamin A Supplementation Trials (VAST) Study Team. Vitamin A supplementation in Northern Ghana: effects on clinic attendances, hospital admissions and child mortality. Lancet 1994;344:228–31.

Long K, Rosado JL, DuPont HL, Hertzmark E, Santos J. Supplementation with vitamin A reduces watery diarrhea and respiratory infections in Mexican children. Br J Nutr 2007;97:337–43.

Hall JA, Cannons JL, Dos Santos LM, et al. Essential role for retinoid acid in the promotion of CD4+ T cell effector responses via retinoid receptor alpha. Immunology 2011;34:435–77.

Kim HM, Taparovsky EJ, Kim CH. Retinoid acid differentially regulates the migration of immature lymphoid cell subsets to the gut. Immunity 2015;43:107–19.

Iwata M, Hirakayama A, Eshima Y, Kagechika H, Kato C, Song S. Retinoid acid imprints gut-homing specificity on T cells. Immunity 2004;21:527–38.

Hammerschmidt S, Friedrichsen M, Boepter J, Lyszkiewicz M, Kremmer E, Pabst O, et al. Retinoid acid induces homing of protective T and B cells to the gut after subcutaneous immunization of mice. J Clin Invest 2011;121:3051–61.

Pantazi E, Marks E, Stolarczyk E, Lycke N, Noelle R, Elgueta R. Retinoic acid signaling in B-cells is essential for oral immunization and microfold cell composition. J Immunol 2015;195(4):1368–71.

Lisulo MM, Kapulu MC, Banda R, Sinkala E, Kayamba V, Sianongo S, et al. Adjuvant potential of low dose all-trans retinoid acid during oral typhoid vaccination in Zambian men. Clin Exp Immunol 2014;175:468–75.

Takenouchi ON, Asano M, Chihaya H, Chung-Hsuing Wu, Ishikasa K, Moro I. Retinoid acid enhances the gene expression of human polymeric immunoglobulin receptor (pIGR) by TNF-α. Clin Exp Immunol 2004;135:448–54.

Reed GS, Ori MT, Fox CB. Key role of adjuvants in modern vaccines. Nat Med 2013;19:1597–608.

Pulendran B, Ahmed R. Immunological mechanisms of vaccination. Nat Immunol 2011;12:509–17.

Lee L, Leung CY, Tang WW, et al. A paradoxical teratogenic mechanism for vitamin A metabolites induce gut-homing FoxP3+ regulatory T cells. J Immunol 2007;179:3724–33.

Kuchta A, Rahman T, Sennott ET, et al. Vibrio cholerae O1 infection induces proinflammatory CD4+ T cell responses in blood and intestinal mucosa of infected humans. Clin Vaccine Immunol 2011;18:1371–7.

Brown CC, Estherhazy D, Sarde A, et al. Retinoid acid is essential for Th1 cell lineage stability and prevents transition to a Th17 cell program. Immunity 2015;42:499–511.

Kang S, Lim H, Andrisani O, Broxmeyer H, Kim C. Vitamin A metabolites induce gut-homing FoxP3+ regulatory T cells. J Immunol 2007;179:3724–33.

Luderitz O, Risse HJ, Schulte-Holthausen H, Strominger JL, Sutherland IW, Westphal O. Biochemical studies of the smooth-rough mutation in Salmonella minnesota. J Bacteriol 1965;89:343–54.

Whitworth JA, McSharry DC, et al. Live oral typhoid vaccine provides with Bacille Calmette-Guerin vaccine at birth: a randomized trial. Vaccine 2014;32:5949–56.

Kugener S, Herold MC. Oral polio vaccine provides the immune response to BCG vaccination. A natural experiment. PLoS ONE 2010;5(5):e10328. https://doi.org/10.1371/journal.pone.0010328.

Jensen KJ, Karkov HS, Lund N, et al. The immunological effects of oral polio vaccine provided with BCG vaccine at birth: a randomized trial. Vaccine 2014;32:5949–56.

Kugener S, Herold MC. Oral polio vaccine provides the immune response to BCG vaccination. A natural experiment. PLoS ONE 2010;5(5):e10328. https://doi.org/10.1371/journal.pone.0010328.

Farag A, et al. Retinoic acid induces homing of protective T and B cells to the gut after subcutaneous immunization of mice. J Clin Invest 2011;121:3051–61.

Stolarczyk E, Lycke N, Noelle R, Elgueta R. Retinoic acid signaling in B-cells is essential for oral immunization and microfold cell composition. J Immunol 2015;195(4):1368–71.

Lisulo MM, Kapulu MC, Banda R, Sinkala E, Kayamba V, Sianongo S, et al. Adjuvant potential of low dose all-trans retinoid acid during oral typhoid vaccination in Zambian men. Clin Exp Immunol 2014;175:468–75.

Takenouchi ON, Asano M, Chihaya H, Chung-Hsuing Wu, Ishikasa K, Moro I. Retinoid acid enhances the gene expression of human polymeric immunoglobulin receptor (pIGR) by TNF-α. Clin Exp Immunol 2004;135:448–54.

Reed GS, Ori MT, Fox CB. Key role of adjuvants in modern vaccines. Nat Med 2013;19:1597–608.

Pulendran B, Ahmed R. Immunological mechanisms of vaccination. Nat Immunol 2011;12:509–17.

Lee L, Leung CY, Tang WW, et al. A paradoxical teratogenic mechanism for vitamin A metabolites induce gut-homing FoxP3+ regulatory T cells. J Immunol 2007;179:3724–33.

Kuchta A, Rahman T, Sennott ET, et al. Vibrio cholerae O1 infection induces proinflammatory CD4+ T cell responses in blood and intestinal mucosa of infected humans. Clin Vaccine Immunol 2011;18:1371–7.

Brown CC, Estherhazy D, Sarde A, et al. Retinoid acid is essential for Th1 cell lineage stability and prevents transition to a Th17 cell program. Immunity 2015;42:499–511.

Kang S, Lim H, Andrisani O, Broxmeyer H, Kim C. Vitamin A metabolites induce gut-homing FoxP3+ regulatory T cells. J Immunol 2007;179:3724–33.

Eksteen B, Mora JR, Haughton EL, et al. Gut homing receptors on CD8 T cells are retinoid acid dependent and not maintained by liver dendritic or stellate cells. Gastroenterology 2009;137:320–9.

Tang X, Gudas LJ. Retinoids, retinoic acid receptors and cancer. Annu Rev Pathol Mech Dis 2011;6:345–64.

Kaufman DR, De Calisto J, Simmons NL, Cruz AN, Villablanca EJ, Mora JR, et al. Vitamin A deficiency impairs vaccine-elicited gastrointestinal immunity. J Immunol 2011;187:1877–83.

Mora JR, Bono MR, Manjunath N, et al. Selective imprinting of gut-homing T cells by peyer’s patch dendritic cells. Nature 2003;424:88–93.

Auby P, Jensen H, Gomes J, Fernandes M, Lisse IM, et al. The introduction of diphtheria-tetanus-pertussis vaccine and child mortality in rural Guinea-Bissau: an observational study. Int J Epidemiol 2004;33:374–80.

Klein S, Mariott I, Fish E. Sex-based differences in immune function and responses to vaccination. Trans R Soc Trop Med Hyg 2015;109:9–15.

Yokota-Nakatsuka A, Takeuchi H, Okhaya Y, et al. Retinoid acid prevents mesenteric lymph node dendritic cells from producing IL-13 producing inflammatory Th2 cells. Mucosal Immunol 2014;7(4):786–801. https://doi.org/10.1038/mi.2013.96.

Epub 2013 Nov 13.

Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL, TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol 2013;14(4):R36.

Li H, Handsaker B, Wysoker A, et al. The Sequence alignment/map (SAM) format and SAMtools. Bioinformatics 2009;25:2078–9.

Anders S, Pyl PT, Huber W, HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics 2015;31(2):103–10.

Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014;15:550.

Further reading

Lucas MES, Dean JL, von Seidlein L, et al. Effectiveness of mass oral cholera vaccination in Beira, Mozambique. N Engl J Med 2005;352:757–67.