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Potential for use of retinoic acid as an oral vaccine adjuvant

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Despite the heavy burden of diarrhoeal disease across much of the tropical world, only two diarrhoea-causing pathogens, cholera and rotavirus, are the target of commercially available vaccines. Oral vaccines are generally less immunogenic than the best parenteral vaccines, but the reasons for this are still debated. Over the past decade, several lines of evidence from work in experimental animals have suggested that all-*trans* retinoic acid (ATRA), a form of vitamin A which is highly transcriptionally active, can alter the homing receptor expression of T lymphocytes. Increased expression of α4β7 integrin and the chemokine receptor CCR9 following exposure to ATRA can be used to redirect T cells to the gut. Early work in human volunteers suggests that oral ATRA administration 1 h prior to dosing with oral typhoid vaccine can augment secretion of specific IgA against vaccine-derived lipopolysaccharide into gut secretions. In this review, we set out the rationale for using ATRA in this way and assess its likely applicability to vaccination programmes for protection of children in low-income countries from the considerable mortality caused by diarrhoeal disease. Comparison of recent work in experimental animals, non-human primates and men suggests that a more detailed understanding of ATRA dosage and kinetics will be important to taking forward translational work into human vaccinology.

1. Introduction

Diarrhoeal disease remains a major cause of morbidity and mortality in children in low-income and tropical countries [1,2]. Despite decades of intensive research, only about half of all diarrhoea cases can be attributed to any given pathogen [3]. While the ultimate solution to this problem undoubtedly lies in improved living conditions, better water quality and quantity, safer food and better sanitation, there is no evidence that these determinants of diarrhoeal disease burden are improving across the world, and inequality may actually be getting worse [4]. While humankind wrestles with these large issues, in the meantime we need to work towards ways of preventing disease, and vaccination is a very attractive option.

2. There is a lack of vaccines against diarrhoeal disease

Oral vaccines represent a major challenge for vaccine development [5]. There are at least three reasons for this. First, while a few major pathogens dominate morbidity and mortality in any age group [3], there are many pathogens which contribute small percentages to the overall burden, and developing a range of vaccines which will prevent a majority of diarrhoea cases is a daunting task. Second, the luminal environment in the gastrointestinal tract is hostile to peptides and complex carbohydrates, degrading most antigenic epitopes delivered in soluble form. Third, mucosal tolerance protects against unwanted immune responses to digested antigens [6]. Notwithstanding these obstacles, a number of oral vaccines developed have been successful, all using particulate antigen (live attenuated pathogens or whole cell inactivated). Commercially available oral vaccines include oral polio vaccine (OPV), which has been largely responsible for the eradication of polio virus serotype 2 and huge progress towards eradication of all polio virus

Table 1. Commercially available oral vaccines.

vaccine type	disease	vaccine constituents	route	protection	commercial name
live attenuated	typhoid	*S. typhi* (Ty21a)	oral	67% over 3 years	Vivotif
live attenuated	cholera	CVD103-HgR	oral	80–90%	Orochol
live attenuated	RV	attenuated virus	oral	85–100%	Rotarix
live attenuated	RV	human-bovine re-assortant viruses	oral	74%	RotaTeq
inactivated	cholera	heat-killed *V. cholerae* + CTB	oral	80–90%	Dukoral

infections [7], Vivotif a typhoid vaccine, Dukoral for cholera and the rotavirus (RV) vaccines Rotarix and RotaTeq (table 1).

3. Oral vaccines have low efficacy in Asia, Africa and Latin America

Although oral vaccines are available, they have shown high efficacy in industrialized countries but much lower efficacy in developing countries [8]. This is confirmed for RV, cholera and poliovirus. The live cholera vaccine CVD 103-HgR elicited a significant (fourfold or greater) rise in serum vibriocidal antibody in North American adults, but the same vaccine demonstrated diminished immunogenicity in Indonesia, Thailand, Peru and Ecuador [8]. Oral RV vaccine was found to be 78% effective against severe RV diarrhoea in Finland [9], but was only 35% effective in Malawi [10]. Although the reported efficacy of RV in Malawi and other poor settings [11,12] was low, it was found that the population level benefits of the vaccination were likely to be greater in these poor settings with highest incidence. Mahdi *et al*. [10] showed that because of the high incidence of severe disease, a vaccine efficacy of 61.2% resulted in a substantial vaccine-attributable overall reduction in severe gastroenteritis of 5.0 cases per 100 infant-years. They also compared the severe gastroenteritis episode cases from Malawi and South Africa and found that although vaccine efficacy was higher in South Africa, there were more episodes (6.7 episodes prevented) of severe RV gastroenteritis per 100 infant-years prevented by vaccination in Malawi than in South Africa (4.2 episodes prevented). These data showed that even though the efficacy of RV is low, it is still worth giving in developing countries. OPV also is much less efficacious in developing countries [13,14], and in recent campaigns in northern India up to 20 doses have been administered per child.

The reasons for the impaired efficacy of oral vaccines in low- and middle-income countries are not clear. Several possible factors could contribute to this phenomenon. Possibilities include interference from the high titres of antibody in maternal breast milk, nutritional factors such as vitamin A deficiency (VAD) and environmental enteropathy [15]. At least for polio virus type 1, it is highly likely that interference by concurrent infections such as non-polio enteroviruses contribute substantially to impaired vaccine efficacy, and efficacy is also lower in the presence of diarrhoea [14]. Counter-intuitively, *Helicobacter pylori* infection, which is common in those populations where oral vaccines are less efficacious, seems unlikely to explain reduced vaccine immunogenicity as there is some evidence that it actually increases it [16].

Part of the solution may be adjuvants. Adjuvants generally boost vaccine responses by creating an innate immune-mediated cytokine milieu in which antigen presentation leads to an immune response which is quantitatively and qualitatively enhanced. Intriguing data published a decade ago suggest that an alternative pathway of adjuvanticity, through a derivative of vitamin A, may be worth exploring. Before dealing with this in greater detail, we will summarize the literature on vitamin A and vaccine responsiveness.

4. Vitamin A supplementation

Vitamin A is the term given to a collection of different but related molecules [17]. These include retinol, retinyl esters, retinoic acid (RA) and β-carotene, most of which are interconvertible and can replace each other in the treatment of the VAD state. VAD is clinically recognizable as night blindness, progressing to keratomalacia, and this is the only absolute indication for vitamin A treatment using high doses. There have been many studies which show an association between increased infectious disease and evidence of compromised vitamin A status, but these are confounded by the fact that serum retinol concentration, and probably bioavailability to tissues, are impaired during an acute phase response [18]. The most reliable data therefore come from intervention studies. In Ghana, supplementation with retinol palmitate capsules (200 000 IU) every four months was associated with a 34% (95%CI 8–53%) reduction in deaths due to diarrhoeal disease in children under the age of 7.5 years and a reduction of 19% (95%CI 2–32%) in all-cause mortality [19]. It was on the basis of this and other studies that vitamin A supplementation programmes, using intermittent treatment with mega-doses of retinol were widely adopted in the 1990s. Since then large trials have been conducted on the impact of vitamin A on child health. A meta-analysis conducted in 2011 [20] included 43 trials from low- and middle-income countries representing over 215 000 children. In summary, they found a 24% reduction in all-cause mortality, a 28% reduction in deaths due to diarrhoea, a 15% reduction in incidence of diarrhoea and a 50% reduction in measles incidence [20]. Since then, the world's largest ever clinical trial, DEVTA, published results from a study involving over 1 million Indian children found no evidence of benefit (rate ratio 0.96, 95%CI 0.89–1.03; $p = 0.22$) [21]. Whether there is a significant difference between India and Africa, or whether the impact of vitamin A has waned over time remains to be determined. As the meta-analysis has provided considerable evidence that vitamin A has a beneficial effect on morbidity and mortality (most of which is assumed to be infectious in aetiology), it would appear worthwhile examining the hypothesis that vitamin A has positive effects on immune function [22,23]. Before going on to discuss the immunological effects of retinoids, it is necessary at this point to explore the different retinoids and how they are related.

5. Source and handling of all-*trans* retinoic acid *in vivo*

Vitamin A is present in the diet either as retinyl esters (with fatty acids, usually in the all-*trans* isomeric configuration) or as plant precursors of which the greatest share is β-carotene which comprises two retinol molecules. Interconversion of these forms of vitamin A is under enzymatic control [24] and occurs in liver and intestine. Retinyl esters are hydrolysed in the intestinal lumen or in the enterocyte, and retinol is then taken up against its concentration gradient by complexing with cellular retinol-binding proteins (cRBP)-I and -II in the enterocyte [17]. Uptake is increased in the presence of fat [25]. cRBP-II is upregulated by dietary fat [17]. cRBP-I also functions to promote retinol esterification, and cRBP-I null mice exhibit increased synthesis of RA because of diversion of retinol to RA. Carotenoids are hydrolysed in the enterocyte to retinol, retinal or apocarotenoids. There is also evidence that all-*trans* retinoic acid (ATRA) can be produced directly from β-carotene by excentric cleavage [17]. Retinal is reduced to retinol. Retinol is then re-esterified and exported as chylomicrons which are absorbed in the liver, and retinyl esters are stored in stellate cells. ATRA is transported from the liver to peripheral tissues complexed to retinol-binding protein (RBP), in holo-RBP, and transthyretin [24]. Holo-RBP is recognized by specific receptors and retinol taken up across the plasma membrane. The remaining particle, apo-RBP, is degraded in the kidney. Altered retinoid metabolism may be caused by alcohol intake/abuse [26], as alcohol dehydrogenase is the same enzyme which oxidizes retinol, and baseline vitamin A status as many of the absorption and transport proteins for vitamin A are induced or regulated by RA itself [27–29].

(a) Molecular effects of RAs

The transcriptional effects of retinol at a molecular level appear to be mediated principally by RAs, which are powerful transcriptional regulators playing a major role in embryo development. There are three major isoforms of RA (9-*cis*-RA, 13-*cis*-RA and ATRA), apart from 11-*cis*-RA which is only required as the substrate for the synthesis of rhodopsin in the retina. There are two classes of RA receptors including retinoic acid receptors (RARs) and retinoid X receptors (RXRs). The receptors are part of the steroid/thyroid/retinoid nuclear receptor family [30]. The receptors exist in three different isotypes (α, β and γ) which are expressed in specific tissues [31]. ATRA only binds RAR, but 9-*cis*-RA can bind either RAR or RXR. RAR and RXR receptors form either homodimers (RXR–RXR) or heterodimers (RAR–RXR) [30] and can also form heterodimers with other nuclear receptors such as human constitutive androstenedione receptor or pregnane X receptor. RAR–RXR heterodimers, in the absence of ligand, act as transcriptional repressors by binding a repressor complex which includes NCo-R or SMRT and a protein which confers histone deacetylase activity. Upon ligand binding, proteins in this complex are exchanged for activators such as SRC proteins and histone acetylases, and RA-responsive genes are switched on. This can only happen if RAR/RXR are bound to retinoic acid response elements (RAREs) in the promoter regions of retinoid-responsive genes [30]. RAREs consist of a direct repeat of a core hexameric sequence 5′-(A/G)G(G/T)TCA-3′ or a more relaxed 5′-(A/G)G(G/T)(G/T)G/C)A-3′ motif separated by one, two or five base pairs [32].

(b) Immune effects of vitamin A

This subject has recently been reviewed and it is clear that available data do not permit a consensus understanding of the effects of retinoids on human immunology [22,23,33]. In experimental animals, the situation is fairly clear-cut. VAD has been much studied. VAD compromises antibody responses in rats to T cell-dependent antigens such as tetanus toxoid, but responses to other antigens, such as lipopolysaccharide, are undiminished. In these models, it appears that antibody responses are dependent on retinoids, but conditionally dependent on the nature of the antigen [23]. Rats immunized during VAD can generate normal IgG and IgM responses following rescue with retinol or ATRA, indicating that memory cell formation is not the defect [34]. RA is known to enhance T cell activation by mitogens, and augments antibody production by B cells in the presence of a TLR3 agonist [35]. RA also contributes towards class switching in B cells, maturation of B cells and the formation of germinal centres, so it clearly plays a significant role in development of humoural immunity in these models [33].

In children, however, the situation is much less clear. There is some evidence of altered T cell subsets in VAD. VAD children in Indonesia had lower CD4/CD8 ratios, lower proportions of CD4 naive T cells and higher proportions of CD8, CD45RO T cells than non-VAD children, and these abnormalities were all reversed after treatment with 60 mg retinol [36]. A different research group found that VAD was associated with reduced interferon-γ production in response to stimulation [37]. But these are fairly isolated unequivocal findings in a difficult field. Some excellent reviews [22,23,33,38] suggest that the impact of vitamin A status, or vitamin A supplementation, is modest at best. A systematic analysis [38] concludes that there is no direct evidence of an effect of vitamin A supplementation on BCG responses, but that in a subgroup analysis, there may be a small sex- and age-dependent effect. They found very few discernible effects of vitamin A status or supplementation on responses to measles, OPV, diphtheria, pertussis, rabies, tetanus, cholera, influenza, hepatitis B, pneumococcus or *Haemophilus influenza* B vaccines [38].

Similar findings (table 2) have also been reported in a number of human and animal studies which focused on effects of vitamin A supplementation on mucosal vaccine responses.

Although it is true that effects may be different in VAD compared with vaccinated individuals, only a few studies have investigated the systemic and mucosal B and T cell responses to vaccines in both experimental and non-experimental VAD conditions. Even so, these studies focused on the effect of vitamin A and not specifically RA. However, Kaufman in 2011 [46] investigated the impact of VAD on mucosal-homing marker upregulation on vaccine-elicited $CD8^+$ T lymphocytes from mice. Following immunization, α4β7 integrin upregulation on the proliferating $CD8^+$ T lymphocytes was markedly reduced in mice receiving the VAD diet but was completely restored after administration of RA to these mice.

6. Therapeutic uses of retinoic acids

9-*cis*-RA (known as alitretinoin) is used orally for the treatment of eczema at between 10 and 30 mg d^{-1}. 13-*cis*-RA (isotretinoin) is used orally for the treatment of severe acne in a dose of 25–50 mg d^{-1}. ATRA (tretinoin) is used in the treatment of promyelocytic leukaemia (PML), but at much higher doses (45 mg m^{-2} daily for 90 days). In PML, the RARα gene is

Table 2. Studies of the effect of vitamin A supplementation on mucosal vaccine response.

author	population	supplement	mucosal vaccine	main findings
Rahman *et al.* [39]	infants	50 000 IU vitamin A	OPV	vitamin A supplementation had no effect on seroconversion
Bahl *et al.* [40]	mothers infants	60 mg retinol equivalent (RE) vitamin A 7.5 mg RE	OPV type 1,2,3	vitamin A supplementation did not interfere with antibody response to any of the three polioviruses and enhanced the response to polio virus type 1
Lisulo *et al.* [41]	adult Zambian men	all-*trans* RA	oral typhoid vaccine (Ty21a)	specific IgA in whole gut lavage fluid against LPS and protein extract was increased in vaccine recipients who were given ATRA compared with those who were not given ATRA
Semba *et al.* [42]	infants at six, 10 and 14 weeks	15 mg or 7.5 mg RE vitamin A	1.15 ml trivalent OPV	oral vitamin A does not affect antibody response to polio vaccine
Surman *et al.* [43,44]	VAD mice	600 IU retinyl palmitate or 300 μg retinol	250–500 PFU Sendai virus	responses in VAD were significantly reduced but virus-specific IgA responses were improved in mice that received vitamin A
Surman *et al.* [43,44]	VAD mice	300 μg/mouse RA 600 IU/mouse retinyl palmitate	30 μl FluMist	IgA responses in VAD mice treated with RA were improved. There was also a statistically significant increase in the number of IgA-producing AFC in the diffuse nasal-associated lymphoid tissue
Chattha *et al.* [45]	VAD and VAS pigs	50 000 and 100 000 IU vitamin A	attenuated human rotavirus G1P[8] vaccine virulent HRV G1P	VAD pigs had higher diarrhoea and Th1 responses. They showed lower serum IgA HRV Ab titres, lower intestinal IgA antibody secreting cells and compromised T-reg response

rearranged and fused to the PML gene so that retinoid-responsive genes become silenced by epigenetic mechanisms. This leads to maturational arrest in the myeloid cell lineage with accumulation of immature promyelocytes, and this can be overcome by pharmacological doses leading to restoration of normal differentiation.

It is important to note that much anxiety surrounds the issue of the possible teratogenicity of retinoids. Retinoids have multiple, critical roles in embryogenesis and development, and there is evidence from experimental animals that they are teratogenic [47]. Most worryingly, 13-*cis*-RA, prescribed for acne, was found to induce severe teratogenicity in babies whose mothers took 1–1.5 mg kg d^{-1} during pregnancy. The congenital problems encountered included retardation, cerebellar and brainstem abnormalities, spontaneous abortion, premature delivery and death [47]. Clearly, RAs are not safe in women of childbearing age and cannot be given except under extreme medical circumstances.

7. Studies of all-*trans* retinoic acid as an adjuvant in experimental mice

(a) Dendritic cells secrete all-*trans* retinoic acid during antigen presentation

Dendritic cells (DCs) have been shown to induce imprinting of tissue tropism of effector T cells, and this has been shown to involve ATRA. During vitamin A metabolism, the irreversible conversion of retinal to RA is catalysed by retinal dehydrogenases (RALDH). Iwata *et al.* [48], in a key paper for this field showed that the mRNA of three different isoenzymes of RALDH (*RALDH1*, *RALDH2* and *RALDH3*) was expressed by DCs from Peyer's patches and mesenteric lymph nodes. The RALDH allows the intestinal DCs to convert retinal to RA which in turn induces T cell expression of the gut-homing receptors α4β7 and CCR9 on lymphocytes during antigen presentation [48]. It has subsequently been shown that ATRA can imprint the CDs themselves, defining a set of bone marrow-derived DCs which subsequently preferentially express CD103 and home to the intestine [49]. Recently, it has been shown that the ability of DCs to synthesize ATRA may be at least partly dependent on vitamin D_3 [50].

(b) Effects on T cells and their trafficking

ATRA [51] and 9-*cis*-RA [52] have been shown to inhibit activation-induced cell death in thymocytes and T cells, but the importance of the demonstration that DCs can synthesize ATRA is in its ability to alter T cell trafficking [53]. Selective migration of the effector T cells to the gut requires expression of α4β7-integrin and chemokine receptor CCR9. Naive T cells circulating in the bloodstream express receptor CCR7 and L-selectin. The markers help the T cells migrate to the Peyer's patches. Here, they are presented with antigen complexed to DCs causing them to become activated. This leads

Table 3. Studies of the effect of ATRA on expression of gut-homing markers.

author	study design population	intervention	main findings
Iwata *et al.* [48]	naive $CD4^+$ T cells from mouse model	various doses of all-*trans* RA, retinaldehyde, retinol (nM)	0.1 nM all-*trans* RA gave significant upregulation of $\alpha4\beta7$. RA-induced mRNA expression of CCR9
Hammerschmidt *et al.* [54]	transgenic mice	subcutaneous application of RA	$CD4^+$ T cells upregulated $\alpha4\beta7$-integrin and CCR9 after subcutaneous antigen plus RA application
Tan *et al.* [55]	six- to eight-week-old female mice	induction of exogenous ATRA during systemic vaccination	all-*trans* RA doses more than or equal to 10 nM increased levels of CCR9, $\alpha4\beta7$and CD103 in mouse T cells
Bernardo *et al.* [56]	colonic biopsies from ulcerative colitis patients, monocytes from healthy volunteers	culturing of cells in complete medium with different doses (10^{-6} M, 10^{-7} M, 10^{-8} M) of RA and LPS (0.1 μg ml^{-1})	RA induced an immature, gut-homing phenotype on MoDC although expression was in a dose-dependent manner. RA-conditioned MoDC had decreased T cell stimulatory capacity and increased gut-homing imprinting capacity on stimulated T cells
Lisulo *et al.* [41]	randomized control trial, Zambian adult men	ATRA given alongside oral typhoid vaccine	specific IgA in whole gut lavage fluid against LPS and protein extract was increased in vaccine recipients who were given ATRA compared with those who were not given ATRA
Saurer *et al.* [57]	pathogen-free pigs were used as blood donors	PBMCs stimulated with 100 ng ml^{-1} SEB in presence or absence of exogenous RA, the RARα antagonist or coculture supernatants	RA-treated monocyte-derived DC led to an increased mucosal-homing receptor expression. Effect seen with both naive and Ag-experienced lymphocytes
Evans & Reeves [58]	*Rhesus macaques* and chimpanzee cells, PBMCs from human donors	exogenous ATRA treatment of cells in a dose- and time-dependent manner	upregulation of $\alpha4\beta7$and CCR9 were both concentration and time-dependent. ATRA-induced expression of $\alpha4\beta7$ was conserved among three primate species

to the loss of CCR7 and L-selectin molecules and the gain of $\alpha4\beta7$-integrin and CCR9 chemokine receptor. The adhesion molecule $\alpha4\beta7$-integrin expressed by antigen-stimulated T cells helps them to bind to the endothelial cells lining the blood vessels in mucosal tissues via the mucosal addressin cell adhesion molecule-1. This binding triggers the signal for migration of effector T cells into the lamina propria.

Several studies (table 3) have now shown that RA is a key mediator in T cell homing to the gut. Iwata *et al.* [48] demonstrated that stimulated $CD4^+$ T cells cultured *in vitro* with ATRA enhanced the expression of gut-homing receptors $\alpha4\beta7$-integrin. They further demonstrated that RA treatment induced a strong chemotactic activity in $CD4^+$ T cells towards the CCR9 ligand TECK (CCL25).

Two studies in 2011 demonstrated the ability of ATRA to act as an adjuvant for vaccination against intestinal or mucosal infection. Hammerschmidt *et al.* [54] showed that ATRA, when given subcutaneously alongside a subcutaneous antigen, can upregulate $\alpha4\beta7$ expression on lymphocytes and increase T cell trafficking to the gut. This approach was able to confer enhanced protection against cholera toxin-mediated diarrhoea and invasive salmonellosis [54]. Tan *et al.* [55] showed that ATRA upregulated expression of CCR9 and $\alpha4\beta7$ on $CD8^+$ T cells and that this was able to protect against challenge with a recombinant-modified vaccinia virus Ankara expressing LCMVgp.

(c) Effects on B cell trafficking and class switching

Like their counter part, T lymphocytes, naive B cells are primed in the Peyer's patches and mesenteric lymph nodes. They are first stimulated via B cell receptors to IgM-producing B cells and then undergo class switching to IgA production which is controlled by the cytokine TGF-β. Effector B cells, just like effector T cells, need gut-homing molecules in order to be redirected to the gut. Mora *et al.* [59] showed that gut-associated DCs were able to induce T cell-independent expression of IgA and gut-homing receptors on B cells [59]. They also found that the addition of RA to activated murine spleen B cells induced high levels of $\alpha4\beta7$ and maintained a robust CCR9 expression on B cells [59], consistent with earlier findings about the effects of ATRA on $CD4^+$ T cells (see §7b). They went

on to show that B cells cultured with peripheral lymph node DCs and RA plus IL-5 and or IL-6 substantially enhanced IgA production. This effect was also seen when B cells were cultured with Peyer's patches in the presence of IL-5, IL-6 and RA. Recently, ATRA has been shown to potentiate the effects of CD1d activation in driving the differentiation of B cells towards antibody production [60].

8. Studies of all-*trans* retinoic acid as an adjuvant in pigs, non-human primates and humans

The IgA that is produced by effector B cells has to be transported across the epithelium to reach its target antigen in the gut lumen. This is achieved by a transmembrane glycoprotein called polymeric immunoglobulin receptor (pIgR) [61]. The molecule transports immunoglobulins by transcytosis to the luminal epithelium. Secretory IgA is a hybrid molecule consisting of one or more joining chains and an epithelial portion called bound secretory component which is linked to one of the IgA subunits [61]. The pIgR has an affinity for the J-chain of the immunoglobulin. Studies in human cell lines showed that ATRA upregulates pIgR in enterocytes [62].

In peripheral blood mononuclear cells (PBMCs) from *Rhesus macaques*, ATRA upregulated α4β7 expression on unstimulated DCs, but CCR9 was not upregulated, indicating for the first time that there may be species differences in these effects [58]. Importantly, the effect was maximal at much higher ATRA concentrations than were used in the mouse studies (100 nmol l^{-1}), a concentration which would likely be toxic in humans. The effect was also seen on human and chimpanzee PBMCs, but CCR9 was not analysed [58].

In PBMCs isolated from pathogen-free pigs, the effect of ATRA was again confirmed. ATRA was able to confer on DCs the ability to upregulate α4β7 and CCR9 expression on co-cultured lymphocytes, but again the concentration of ATRA required was high (up to 1000 nmol l^{-1}) [57]. In human monocyte-derived DCs (MoDCs) treated with ATRA *ex vivo*, the ability to upregulate α4β7 was conferred by conditioning with 10–100 nmol l^{-1} [56].

To our knowledge, following a search of PubMed and ISRCTN databases, there has only been one study of the use of ATRA in humans [41]. Initial pharmacokinetic studies confirmed that an oral dose of 10 mg of ATRA produces a rapid rise in serum ATRA concentration from which it can be inferred that ATRA is bioavailable to intestinal cells both directly during absorption and then by delivery from the circulation. Daily doses of ATRA 10 mg for 8 days, beginning 1 h before vaccination, generated an increased amount of IgA directed against vaccine-derived lipopolysaccharide and protein in gut lavage fluid. Further work is ongoing to determine if this effect can be generalized to other vaccines and if it depends on baseline vitamin A status (ISRCTN89702061).

9. Conclusion

The weight of evidence that ATRA plays a key role in shaping the mucosal immune response is now too great to ignore. In a range of experimental animals and in non-human primates, and *ex vivo* in humans, ATRA has important effects on gut-homing behaviour of lymphocytes. Early data suggest that this can translate into effects on gut IgA secretion against oral vaccine antigens, but corroborative work is needed. However, it is important to note that there is significant uncertainty surrounding the dose of ATRA required in humans to achieve the immunological effects which are needed for successful use as an adjuvant for mucosal immunology. We suggest that further work on dose and timing will be required for successful translation of these basic science findings to protection of children from intestinal infectious disease.

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