Retinol binding protein and vitamin D associations with serum antibody isotypes, serum influenza virus-specific neutralizing activities and airway cytokine profiles

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Summary

Vitamin A supports the induction of immunoglobulin (Ig)A responses at mucosal surfaces in mice, but much less is known about the influence of vitamins on antibody isotype expression in humans. To address this knowledge gap, we examined 46 residual blood samples from adults and children, some of whom were experiencing influenza virus infections of the respiratory tract. Assays were performed for retinol binding protein (RBP, a surrogate for vitamin A), vitamin D (a related vitamin) and antibody isotypes. Results showed that all but two tested samples exhibited RBP and/or vitamin D insufficiencies or deficiencies. Vitamin D correlated with blood IgM and IgG3, while RBP correlated with IgG4 and IgA. RBP also correlated positively with age and with influenza virus-specific antibody neutralization titres. Individuals with low blood RBP levels exhibited the highest frequencies of over-expressed cytokines and growth factors in nasal wash samples, an indication of inflamed mucosal tissues. While cause–effect relationships were not discerned, results support a hypothesis that vitamins directly influence B cell isotype expression in humans, and by so doing may help protect mucosal surfaces from respiratory viral disease.

Keywords: antibodies, B cell, cytokines, host–pathogen interactions, viral

Introduction

The World Health Organization estimates that 250 million preschool children are deficient for vitamin A and that 1 billion people worldwide are deficient for vitamin D. Each of these deficiencies, now known to affect populations in both developed and developing countries, associate with a wide variety of health-care concerns, including vulnerability to respiratory pathogens [1–4].

Murine studies demonstrate that vitamins A and D are critical for healthy immune responses at mucosal surfaces [5]. The vitamins are inter-related as they differentially bind heterodimeric receptors (e.g. RAR-RXR; VDR-RXR) that, in turn, influence the expression of immune response genes. Immunoglobulin (Ig)A, a first line of defence against mucosal pathogens, is particularly dependent upon vitamin A in mice [6–9]. IgA is well suited for protection of the upper respiratory tract mucosa, because it can be shuttled to the lumen by transcytosis through epithelial cells, chaperoned by the poly-Ig receptor. Following transcytosis, secretory component is cleaved so that IgA can be released in soluble form into mucosal secretions or tethered onto the airway lining. The plethora of IgA functions include antigen binding, virus neutralization, antibody-dependent cell-mediated cytotoxicity and modulation of cytokine release by innate immune effectors [10]. In transfer studies, polymeric IgA traffics to the upper respiratory tract airway more effectively than IgG1 [11–13]. Early capture and destruction of pathogens in the upper respiratory tract by antibodies can prevent trafficking to the lung and thereby prevent serious lower respiratory tract disease.

Given that: (i) vitamin deficiencies correlate with IgA reduction in mice, (ii) IgA serves as a first line of defence against mucosal pathogens and (iii) humans with vitamin deficiencies are vulnerable to mucosal infections, we sought to define direct correlations between vitamin levels and antibody isotype patterns in humans. Results show that human blood levels of vitamins A and D correlate with antibody isotypes and isotype ratios.

Table 1. Human blood samples tested for vitamins, antibodies and nasal wash cytokines

Label	Age (years)	Contact/index flu type (day)	RBP (ng/ml)	Vit D (ng/ml)	IgG1[‡]	IgA[‡]	Flu Nab titre	+Nasal Cyt>2X
Influenza Virus Study								
F5028C21	7	FP contact-H1 (7)	5933	1	110	20	20	9
F5030C29	22	Neg contact (0)	9698	16	289	50	<10	3
F5006C05	19	Neg contact (24)	11 952	6	453	154	10	4
F5001*	12	Index-H1 (0)	15 228	24	568	84	<10	**32**
F5004	29	Index-H1 (0)	17 670	1	462	104	<10	**29**
F5012C10	19	Neg contact (0)	18 463	8	433	87	40	**11**
F5020C17	70	FP contact-H1 (0)	18 623	6	409	122	10	**10**
F5011	30	Index-H1 (0)	18 862	6	366	66	<10	**13**
F5021†	12	Index-H1(0)	19 101	14	514	86	<10	**25**
F5001*	12	Index-H1 (25)	19 821	22	674	76	10	**25**
F5013C10	21	CV contact-BA (0)	20 383	10	421	92	40	**10**
F5009C08	30	CV contact-H1 (0)	20 463	6	348	89	10	9
F5015C14	26	Neg contact (0)	21 999	27	223	43	40	5
F5014	8	Index-H1 (0)	22 732	n/a	594	41	<10	4
F5021†	12	Index-H1 (32)	25 286	14	519	79	80	8
F5018C17	25	Neg contact (0)	28 740	13	340	96	80	9
F5002C01	30	Neg contact (0)	30 373	8	842	322	80	4
F5040C39	33	FP contact-H1 (0)	35 770	10	369	137	20	6
F5022C21	32	CV contact–H1 (0)	35 861	1	260	147	40	0
F5019C17	43	Neg contact (0)	50 676	17	170	99	10	3
F5052C51	38	Neg contact (0)	56 431	9	476	175	40	2
Tennessee Blood Services samples								
R242680	27	–	17 071	6	414	91	n.d.	n.d.
R275699	45	–	21 428	20	667	283	n.d.	n.d.
R275708	22	–	21 428	14	355	69	n.d.	n.d.
R242682	38	–	22 370	11	401	52	n.d.	n.d.
R275696	37	–	22 375	16	369	127	n.d.	n.d.
R275693	32	–	22 440	26	505	133	n.d.	n.d.
R242676	28	–	22 605	19	563	131	n.d.	n.d.
R275704	22	–	23 088	24	224	28	n.d.	n.d.
R242688	30	–	24 591	9	525	157	n.d.	n.d.
R242683	39	–	25 238	6	358	55	n.d.	n.d.
R275703	31	–	26 678	19	517	157	n.d.	n.d.
R275709	56	–	27 342	15	610	287	n.d.	n.d.
R242675	27	–	27 632	13	1031	104	n.d.	n.d.
R275700	63	–	28 454	36	607	81	n.d.	n.d.
R275702	44	–	28 488	28	305	113	n.d.	n.d.
R242674	58	–	31 871	16	387	245	n.d.	n.d.
R242677	40	–	32 049	19	921	196	n.d.	n.d.
R275695	45	–	32 132	28	511	186	n.d.	n.d.
R242678	59	–	32 859	14	313	243	n.d.	n.d.
R275698	41	–	33 779	11	270	56	n.d.	n.d.
R275697	39	–	34 035	45	484	148	n.d.	n.d.
R275705	33	–	37 487	18	358	133	n.d.	n.d.
R275694	39	–	42 198	27	329	66	n.d.	n.d.
R242679	56	–	55 550	20	640	197	n.d.	n.d.
R275692	48	–	65 146	14	337	317	n.d.	n.d.

Retinol bonding protein (RBP) and vitamin D levels were tested for 21 plasma samples from the Influenza Virus Study and 25 serum samples from the Tennessee Blood Services (Memphis, TN) Column 3 indicates the type of sample, type of virus infection and day of analysis. 'Index' was the person from a household who first received (on day 0) an influenza virus diagnosis (index case). 'FP contact' was a household contact of the index case who was also diagnosed on day 0 with an influenza virus infection. 'CV contact' was a household contact who was diagnosed with an influenza virus infection at some point after day 0, usually within a few days. 'Neg contact' was a household member who was never diagnosed with an influenza virus infection. The strain of influenza virus infection is noted ('H1' for H1N1 virus, 'BA' for apparent co-infection with influenza B and influenza A viruses) as well as the study day that the sample was collected post-enrolment. The day of collection is indicated relative to the day of index case entry into the study. 'Flu NAb titre' is the serum neutralizing antibody titre against influenza virus. Areas with dark background mark samples with vitamin A sufficiencies. Vitamin D values were scored as '1' if vitamins were below detection in the enzyme-linked immunosorbent assay (ELISA). +The numbers of cytokines, chemokines or growth factors per sample that scored more than twice the lowest detectable value in the series. When numbers were ≥10, they are shown in bold type. *†Two individuals were tested twice, once on day 0 and again approximately 1 month later. ‡Antibody isotype values (ng/ml) were from samples diluted 1 : 16 000, as per the manufacturer's instructions (Milliplex MAP human isotyping magnetic bead panel HGAMMAG-301K); n/a = no available value. For statistical analyses, neutralization values of < 10 were given a score of 5; n.d. = not done or not applicable.

Materials and methods

Sample source

Blood samples were collected from two sources in Memphis, TN (Table 1). First, residual plasma were available from a previously described, Institutional Review Board-approved study of adults and children with acute influenza virus infections (index cases) and their asymptomatic household contacts ($n = 21$) [14]. Informed consent was received from all study participants. This source will be referred to as the 'Influenza Virus Study'. Secondly, adult sera ($n = 25$) were available from the Tennessee Blood Services (TBS) in Memphis, TN. All personal identifiers were removed from samples prior to use.

Vitamin tests

RBP was tested by enzyme-linked immunosorbent assay (ELISA) [R&D Systems (Minneapolis, MN, USA) human RBP4 Quantikine ELISA kit] and used as a surrogate for retinol due to its superior stability profile (retinol is sensitive to light and temperature, factors that may confound analyses of residual blood samples). RBP shuttles retinol through the blood, generally existing at a 1 : 1 molar ratio [15]. Vitamin D was tested in the Pathology Department at St Jude using the Roche Elecsys Vitamin D ELISA (Roche, Indianapolis, IN, USA) that measures 25-hydroxylated metabolites of cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2). Cut-offs for vitamin deficiencies and insufficiencies were based on Institute of Medicine standards and practice guidelines of the US Endocrine Society (vitamin A deficiencies: ≤ 0.7 μM or $\leq 16\,000$ ng/ml RBP; vitamin A insufficiencies: >0.7 to ≤ 1.05 μM or $>16\,000$ to $\leq 22\,000$ ng/ml RBP; vitamin D deficiencies: <20 ng/ml 25(OH)D; vitamin D insufficiencies: ≥ 20 to <30 ng/ml 25(OH)D). When vitamin D levels were below detection, they were given a value of 1.00 for numerical analyses. For one sample, the vitamin D level was not determined.

Cytokines, growth factors and antibody tests

Cytokines and growth factors were tested in nasal washes (NW) and plasma samples from individuals in the Influenza Virus Study using a Milliplex MAP human cytokine/chemokine immunoassay (Millipore, St Charles, MO, USA) in a Luminex®xMAP™ system according to the manufacturer's protocol. The following cytokines/chemokines and growth factors were measured: epidermal growth factor (EGF), eotaxin, fibroblast growth factor (FGF)-2, Fms-like tyrosine kinase 3 (Flt-3) ligand, fractalkine, granulocyte–colony-stimulating factor (G-CSF), granulocyte–macrophage colony-stimulating factor (GM-CSF), growth regulated oncogene (GRO), interferon (IFN)-α2, IFN-γ, interleukin (IL)-10, IL-12(p40), IL-12(p70), IL-13, IL-15, IL-17, IL-1Rα, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6,

IL-7, IL-8, IL-9, IFN-γ-induced protein 10 (IP-10), monocyte chemoattractant protein (MCP)-1, MCP-3, macrophage-derived chemokine (MDC) (CCL22), macrophage inflammatory protein 1 (MIP-1)α, MIP-1β, transforming growth factor (TGF)-α, tumour necrosis factor (TNF)-α, TNF-β, vascular endothelial growth factor (VEGF), sCD40L and sIL-2Rα. Three additional factors were measured using NW: platelet-derived growth factor (PDGF)-AA, PDGF-AB/BB and regulated upon activation normal T cell expressed and secreted (RANTES). If a sample scored below or above the standard curve, it was assigned the curve's lowest or highest detectable value respectively for graphing and statistical evaluations.

Antibody isotypes were tested from blood samples from both the Influenza Virus Study and TBS. The Milliplex® MAP human isotyping magnetic bead panel-isotyping multiplex assay (HGAMMAG-301K) was used. Blood samples were diluted 1 : 16 000 prior to isotype assays as per the manufacturer's instructions; concentrations are reported for the diluted samples. If a sample scored below or above the standard curve, it was respectively assigned the curve's lowest or highest detectable value for graphing and statistical evaluations.

Microneutralization assay

Plasma were tested against the A(H1N1)pdm09 strain in the reverse genetic 6 + 2 backbone (rg-A/Tennessee/1-560/2009), the circulating H1N1 strain. Briefly, plasma were heat-inactivated at 56°C and serially diluted with a starting dilution of 1 : 10. Diluted samples were incubated with 100 tissue culture infectious doses-50 ($TCID_{50}$) of the virus for
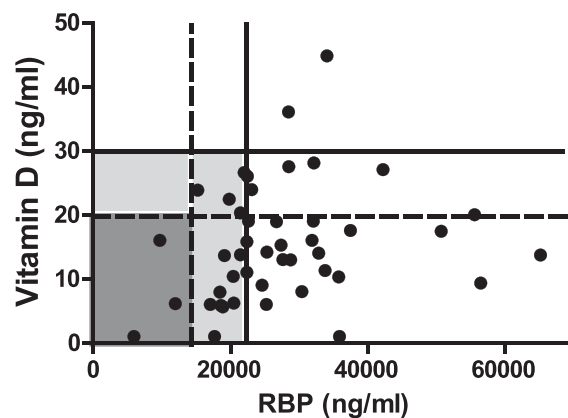

Fig. 1. Retinol binding protein (RBP) comparisons with vitamin D and age. Comparisons were made between RBP (ng/ml) and vitamin D (ng/ml). Levels are shown for 45 samples and each symbol represents a different blood sample. One vitamin D sample was not evaluable among 46 tested samples. Cut-offs for vitamin A (RBP) and D deficiencies (solid lines) and insufficiencies (dotted lines) are shown. Dark grey areas identify individuals with double deficiencies for RBP and vitamin D, and light grey areas identify individuals with insufficiencies for RBP, vitamin D or both.

1 h at 37°C. After incubation, the plasma–virus mixture was transferred to Madin Darby canine kidney (MDCK) cells preseeded in a 96-well plate. After 3 days, 50 µl of culture supernatant were tested for the presence of virus using a haemagglutination assay with 0·5% turkey red blood cells. The antibody titre was defined as the reciprocal of the highest dilution that resulted in inhibition of virus infection. A score of 5 was given for statistical evaluations if neutralizing activity was not detected.

Statistical methods

Spearman's rank correlation was applied to evaluate the correlation of RBP and vitamin D with antibody isotypes, neutralizing antibodies and cytokines/chemokines/growth factors. To assess the impact of age on correlations of interest, Spearman's rank correlation coefficients were estimated by dividing the patient sample into young and old groups

at the median age (31 years for the whole population and 25 years for the Influenza Virus Study). The Wilcoxon–Mann–Whitney test was used to compare cytokine levels between RBP sufficient and insufficient/deficient individuals. Statistical analyses did not adjust for multiple comparisons; a *P*-value of 0·05 or less was considered significant.

Results

Low levels of vitamins A and D in children and adults in Memphis, TN

RBP levels (surrogates for serum retinol [15]) and vitamin D levels for 46 individuals in Memphis, TN were measured and results are shown in Table 1, with entries for samples from an Influenza Virus Study and from the TBS. Entries are listed in order of increasing RBP levels. In Fig. 1,
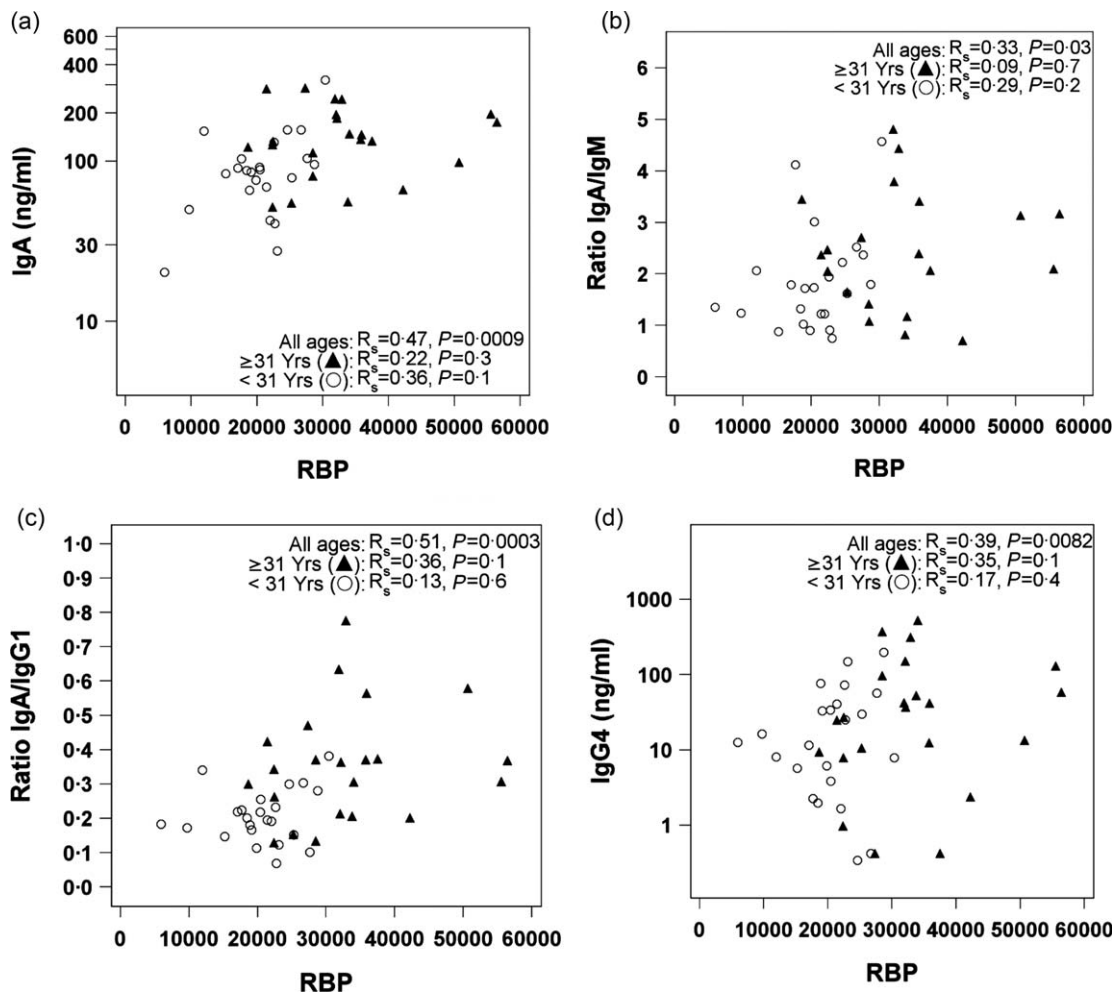

Fig. 2. Retinol binding protein (RBP) comparisons with isotypes and isotype ratios. RBP levels (ng/ml) were plotted against isotypes or isotype ratios in individual panels as follows: (a) immunoglobulin (Ig)A, (b) IgA/IgM, (c) IgA/IgG1 and (d) IgG4. Blood samples were diluted 1 : 16 000 prior to isotype assays. Antibody concentrations are reported for the diluted samples in ng/ml. Statistical evaluations were with Spearman's rank correlation coefficients, estimated using combined data or with data from groups of younger or older individuals, subdivided by the median age (31 years).

measurements for RBP and vitamin D are plotted on an *x*–*y* axis. Vertical and horizontal lines indicate cut-offs for vitamin deficiencies and insufficiencies. Among the 46 samples, one vitamin D level was not acquired. For the remaining samples, all but two exhibited deficient or insufficient levels for RBP, vitamin D or both. We found that RBP and vitamin D levels were correlated with each other ($R_S = 0.31$, $P = 0.04$). We also observed a direct correlation between RBP and age ($R_S = 0.62$, $P < 0.0001$).

High RBP concentrations associate with high levels of IgA and IgG4

Previous studies in vitamin A-deficient (VAD) mice demonstrated a significant deficit in virus-specific IgA antibody secreting cells and IgA antibodies compared to controls [7–9]. To determine if vitamin A in humans (as measured by RBP) associated similarly with IgA production, we tested the 46 blood samples for IgM, IgG1, IgG2,

IgG3, IgG4 and IgA. Numerical values for isotypes and isotype ratios were determined. Values for total IgG1 and IgA are shown for each individual in Table 1. We compared RBP levels with serum antibody isotype distributions and evaluated data using Spearman's rank correlation tests. Samples were examined within the whole population and also after stratification of young and old age groups (defined by the median of 31 years). As shown in Fig. 2, correlations were not significant in the age-separated subsets, but when the population was evaluated as a whole, RBP correlated significantly with IgA (a) ($P = 0.0009$), IgA/IgM (b) ($P = 0.03$), IgA/IgG1 (c) ($P = 0.0003$) and IgG4 (d) ($P = 0.0082$). IgA also correlated with age, as expected based on previous reports [16–18] and based on our finding that RBP and age were correlated. As shown in Fig. 3, vitamin D correlated with IgM, IgG3, IgG3/IgG1 ratios and IgG3/IgA ratios.

To examine functional antibodies, influenza virus-specific neutralization assays were performed on samples
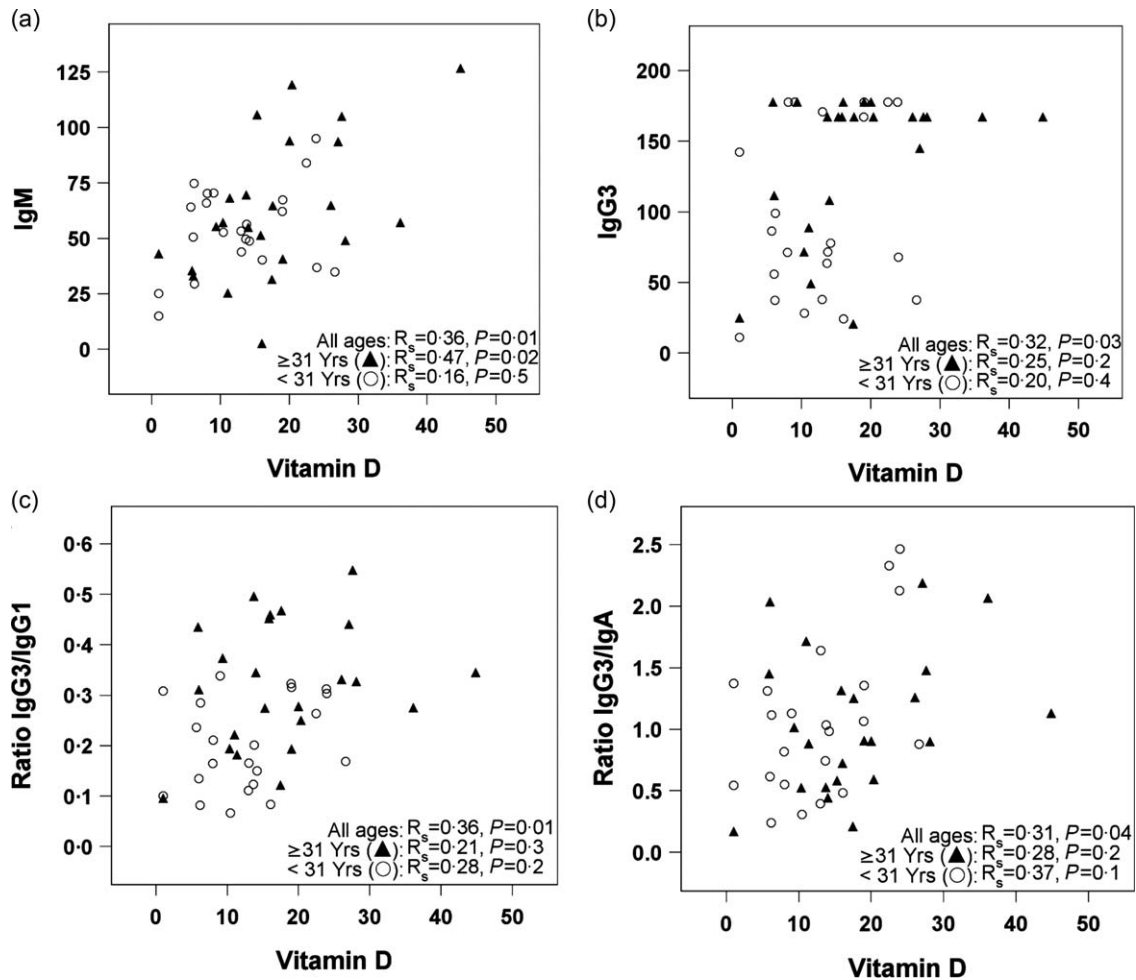

Fig. 3. Vitamin D comparisons with isotypes and isotype ratios. Vitamin D levels (ng/ml) were plotted against isotypes or istoype ratios in individual panels as follows: (a) immunoglobulin (Ig)M, (b) IgG3, (c) IgG3/IgG1 and (d) IgG3/IgA. Blood samples were diluted 1 : 16 000 prior to isotype assays. Antibody concentrations are reported for the diluted samples in ng/ml. Statistical evaluations were with Spearman's rank correlation coefficients, estimated using combined data or with data from groups of younger or older individuals, subdivided by the median age (31 years).

from the influenza virus study. Neutralization correlated significantly with RBP levels (Fig. 4, $R_S = 0.52$, $P = 0.015$), but not with total IgG1 ($R_S = -0.09$, $P = 0.7$) or total IgA ($R_S = 0.34$, $P = 0.13$). Neutralization activities were probably a reflection of acute as well as historical exposures to influenza viruses. As shown in Table 1, not all individuals in this study had evidence of a recent infection, although they may have been exposed to influenza virus by contact with an infected household member.

Sufficient RBP associates with reduced cytokines in nasal passages

Because vitamin A-deficient individuals are vulnerable to respiratory pathogens [7–9], and because respiratory tract disease is often indicated by increased cytokine and growth factor levels in the upper respiratory tract [14,19–21], we asked if RBP levels correlated inversely with cytokine levels in NW samples. To address this question, we examined historical cytokine records for the 21 blood and NW samples from the Influenza Virus Study [14]. Again, samples were grouped by age based on the median age for the 21 participants (median age = 25 years). When cytokine and growth factor levels were evaluated from blood, vitamin D, but not RBP, associated with increases in two cytokine levels, eotaxin ($P = 0.01$) and IL-8 ($P = 0.03$). These correlations were each significant in younger, but not older study participants. When NW cytokines and growth factors were evaluated, negative correlations were observed with RBP, but not vitamin D. Cytokines and growth factors that were correlated negatively with RBP included PDGF-AA (Fig. 5a), granulocyte–colony-stimulating factor (G-CSF) (Fig. 5b), IL-1α (Fig. 5c), IP-10 (Fig. 5d), EGF (Fig. 5e), MCP-1 (Fig. 5f) and IL-8 (not shown). These associations were also significant in older, but not younger individuals. Each sample from the Influenza Virus Study was scored for
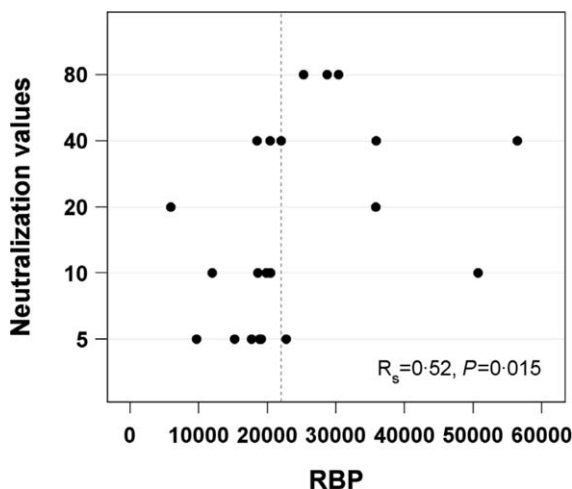
the number of NW cytokines or growth factors exceeding twice the lowest detectable value in the assay. As shown in Table 1, eight of the 13 individuals with insufficient/ deficient levels of RBP exhibited 10 or more elevated cytokines or growth factors in NW samples, whereas such high numbers were not observed in any of the eight individuals with sufficient levels of RBP. Even when individuals with sufficient RBP were experiencing influenza virus infections at the time of sampling (F5014 and F5040C39 in Table 1), cytokine levels were relatively low in NW, perhaps indicative of healthier nasal tissues.

Discussion

Results in this report demonstrate relationships between RBP (a surrogate of vitamin A), vitamin D, antibody isotype patterns, neutralizing antibodies and NW cytokine and growth factor patterns in humans. Specifically, individuals with higher RBP levels exhibited higher levels of IgA, IgA/IgM, IgA/IgG1, IgG4 and virus-specific neutralizing antibodies, and lower levels of NW cytokines and growth factors (indicative of healthy mucosal tissue). RBP also associated with age which, in turn, associated with increased IgA concentrations [16–18,22]. RBP correlated with vitamin D, and vitamin D correlated with IgM, IgG3, IgG3/IgG1 and IgG3/IgA. While cause–effect relationships could not be discerned in this human study, results from small-animal studies support the hypothesis that vitamins directly influence isotype expression [7–9]. Perhaps in humans, vitamins also instruct B cell development and the antibody isotype switch. Vitamin sufficiency may then support effective antibody function, effective virus control and mucosal tissue integrity.

What might be the mechanism for vitamin A and vitamin D association with antibody isotype expression patterns? There are multiple influences of vitamins on the immune system. Vitamins may support dendritic cell development necessary for antigen presentation, T cell activation and homing, B cell activation and division, B cell heavy chain switch rearrangement, maturation of B cells and/or the stabilization of end-differentiated antibody-producing cells. When focusing on the heavy chain class switch rearrangement (CSR) event, we note that functional C_H genes are arranged in the order 5′-Cμ, Cδ, Cγ3, Cγ1, Cα1, Cγ2, Cγ4, Cε, Cα2-3′ along the locus. Because intervening DNA sequences are usually deleted during CSR, isotype switching will generally progress irreversibly in the 5′→3′ direction. B cells that express IgM and IgG3 at early stages of development (as may be prompted by vitamin D) have the potential to continue switching. However, once B cells express IgA or IgG4 (as may be prompted by vitamin A), continued CSR is limited due to deletion of upstream genes Cμ, Cδ, Cγ3 and Cγ1. Preferential expression of IgA and IgG4 relative to others over time may be a simple consequence of successive CSR. However, there are also

Fig. 4. Retinol binding protein (RBP) association with neutralizing antibodies. Neutralizing antibody titres are shown relative to RBP (ng/ml). A score of 5 was used when the response was below detection.
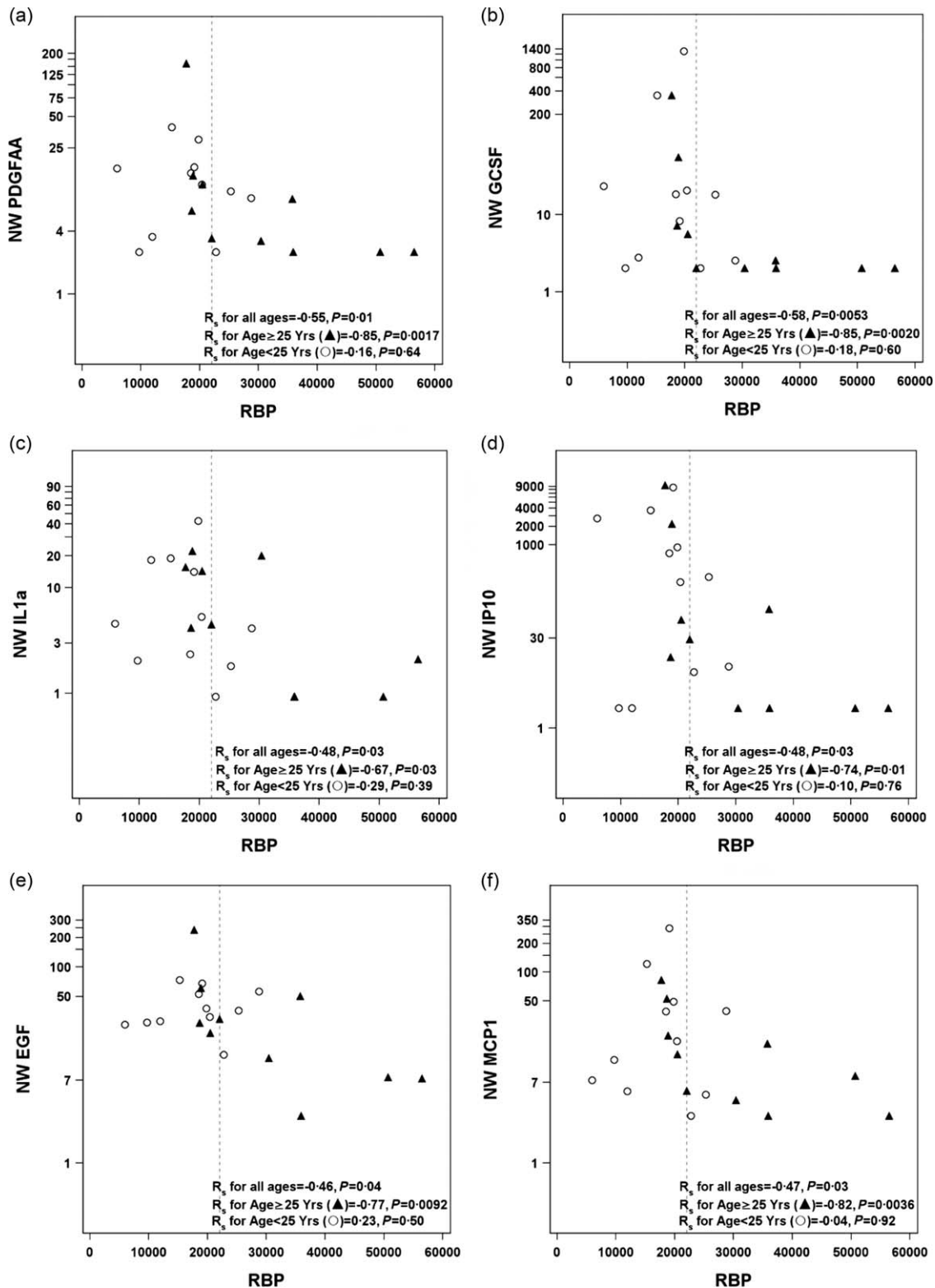
Fig. 5. Retinol binding protein (RBP) association with nasal wash (NW) cytokines. RBP (ng/ml) associations with six NW cytokines/growth factors are shown. These were (a) platelet-derived growth factor (PDGF)-AA, (b) granulocyte-colony stimulating factor (G-CSF), (c) interleukin (IL)-1α, (d) interferon gamma-induced protein 10 (IP-10), (e) epidermal growth factor (EGF) and (f) Monocyte chemoattractant protein-1 (MCP-1). Experiments were performed as per the manufacturer's instructions and then scored as pg/ml. When scores were below or above the standard curve, they were plotted with the assay's lowest or highest detectable values, respectively.

environmental and developmental conditions that differentially influence the rearrangement apparatus [23–26]. Our previous *in-vitro* studies showed that IgA production by stimulated B cells was enhanced by retinol in the presence of epithelial cells that expressed retinal dehydrogenase (RALDH) (necessary for metabolism of retinal to retinoic acid [6]). This IgA expression was associated with up-regulation of IL-6 and GM-CSF within the culture system, and was inhibited when cytokines were blocked. IL-6, like vitamin A, is multi-faceted in that it has a direct influence on IL-6R-bearing B cells, but will also influence B cells indirectly via T cell help and innate cell partners [27]. Clearly, vitamin influences on CSR and stabilization of switched cells are multi-faceted and warrant continued investigation [28].

An additional striking feature of the data described here is the frequency of insufficient vitamins A or D observed in the study populations. Whereas low levels of vitamins in residents of developing countries have been reported routinely [29,30], the extent of these insufficiencies and deficiencies in inner cities of the United States may not be fully appreciated. In our study, there were only two of 45 samples that scored in the 'sufficient' range for vitamin D (>30 ng/ml) [2], and only about half of the RBP samples were in the sufficient range (> 22 000 ng/ml). A few individuals were suffering from influenza virus infections (Table 1), a possible explanation for low levels, but the majority of individuals were not infected. Multiple factors may lend to nutritional deficits. For individuals of low socio-economic status there is ready access to processed foods, but limited access to fresh produce or fortified foods [31]. Skin exposure to UVB light is no longer encouraged [32]. Genetics will also influence vitamin status. RBP values differ among races, and polymorphisms in RBP will alter serum concentrations [33,34]. A close examination of nutritional habits and genetic backgrounds (e.g. RBP single nucleotide polymorphisms, SNPs) is now warranted to provide explanations for the low vitamin levels that we have observed.

In conclusion, our results show that Memphian samples in this study exhibited low levels of RBP and vitamin D. Vitamin D correlated with IgM and IgG3, while RBP correlated with IgA and IgG4. Individuals with the higher RBP levels exhibited higher influenza virus neutralization titres in blood and lower levels of cytokines in NW indicative of healthy mucosal tissues. Given that the surveillance of respiratory tract mucosa is often mediated by IgA, a reduction in this antibody isotype as a consequence of insufficient or deficient vitamin A may explain an individual's increased vulnerability to respiratory viral disease. Results encourage correction of vitamin deficiencies/insufficiencies among individuals in both developed and developing countries, particularly at the time of respiratory virus vaccination [8,9] to improve vaccine efficacy and confer protection against respiratory tract disease.

Disclosure

There were no competing interests associated with this work.

Author contributions

B. G. J., C. M. O. and S. S. W. conducted the experiments. J. L. H., B. G. J., C. M. O., S. S. W., R. W. and P. G. T. designed the experiments. J. L. H., L. T., Y. S., R. W., R. B., S. S. W., C. M. O. and P. G. T. evaluated the data. J. L. H., R. W. and P. G. T. provided financial support. J. L. H., L. T., Y. S., R. W., R. B., S. S. W., C. M. O. and P. G. T. contributed to manuscript preparation.

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