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Vitamin D_3 correlates inversely with systemic dendritic cell numbers and bone erosion in chronic rhinosinusitis with nasal polyps and allergic fungal rhinosinusitis

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Summary

Vitamin D_3 (VD_3) is a steroid hormone that regulates bone health and numerous aspects of immune function and may play a role in respiratory health. We hypothesized that T helper type 2 (Th2) disorders, chronic rhinosinusitis with nasal polyps (CRSwNP) and allergic fungal rhinosinusitis (AFRS) would have VD_3 deficiencies, resulting in increased mature dendritic cells (DCs) and bone erosion. We conducted a retrospective study examining VD_3 levels in patients with AFRS ($n = 14$), CRSwNP ($n = 9$), chronic rhinosinusitis without nasal polyps (CRSsNP) ($n = 20$) and cerebrospinal fluid leak repair (non-diseased controls) ($n = 14$) at time of surgery. Circulating immune cell levels were determined by immunostaining and flow cytometric analysis. Plasma VD_3 and immune regulatory factors (granulocyte–macrophage colony-stimulating factor and prostaglandin E_2) were measured by enzyme-linked immunosorbent assay. It was observed that CRSwNP and AFRS demonstrated increased circulating DCs, while chronic rhinosinusitis without nasal polyps displayed increased circulating macrophages. CRSwNP and AFRS were to found to have insufficient levels of VD_3 which correlated inversely with circulating numbers of mature DCs, DC regulatory factors and bone erosion. CRSsNP displayed no change in circulating DC numbers or VD_3 status compared to control, but did display increased numbers of circulating macrophages that was independent of VD_3 status. Lastly, VD_3 deficiency was associated with more severe bone erosion. Taken together, these results suggest support a role for VD_3 as a key player in the immunopathology of CRSwNP and AFRS.

Keywords: allergic fungal rhinosinusitis, bone erosion, chronic rhinosinusitis, dendritic cells, vitamin D

Introduction

While the exact cause of the persistent symptomatic inflammation associated with chronic rhinosinusitis (CRS) is unknown, it is thought to be the result of numerous interactions between environmental factors and the host immune system. CRS can be subdivided into two categories: CRS without nasal polyps (CRSsNP), which displays elevated levels of T helper type 1 (Th1) and Th2 cytokines, and CRS with nasal polyps (CRSwNP) which is heavily Th2 skewed [1]. Elevated levels of Th2 cytokines contribute to the symptoms of CRS by stimulating mucus production and recruitment of eosinophils [2].

Dispersed throughout the nasal and sinus mucosa are antigen-presenting cells (APC), among which are dendritic cells (DCs) and macrophages, that play a critical role in regulating Th1/Th2 skewing. Additionally, monocytes are recruited from the peripheral blood to the site of exposure, where they are differentiated into DCs or macrophages under the influence of mediators such as granulocyte–macrophage colony-stimulating factor (GM-CSF) [3]. Once matured, DCs direct naive T cells towards either a Th1 or Th2 phenotype, based on the type of stimulus inducing maturation and cues from the external environment. For example, DCs matured in the presence of prostaglandin E_2 (PGE_2) promote Th2 responses [4]. Furthermore, DC expression of $CD86^+$ has been shown to be elevated in Th2-skewed respiratory diseases such as asthma and allergic rhinitis [5,6]. Macrophages represent another class of APC that regulate inflammation. In response to cytokines and microbial products, macrophages produce proinflammatory and anti-inflammatory mediators [7,8]. Elevated numbers of

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macrophages are observed in asthma [9], yet it is unclear if they are elevated systemically in sinusitis. Like DCs, their ability to regulate downstream immune responses suggests that they may contribute to the inflammatory response in sinusitis.

Vitamin D_3 (VD_3) is an immunomodulatory steroid hormone that regulates DC, monocyte, macrophage and T cell functions. VD_3 plays an important role as an immune regulator through its ability to block monocyte to DC differentiation and maturation, thereby diminishing DCs ability to stimulate T cell Th1/Th2 differentiation [10]. Several studies have also shown that exposure of DCs to VD_3 re-programs them to support a tolerogenic phenotype [11–13]. In macrophages, VD_3 has been shown to exert an opposite effect, promoting monocyte to macrophage differentiation and proliferation [14]. Therefore, VD_3 may play an important role in inflammatory diseases such as CRS.

Increasing evidence suggests that VD_3 plays an important role in respiratory health. For example, in a study of 6–14-year-old children with asthma, 28% were determined to have severe VD_3 deficiencies. Furthermore, increased VD_3 levels were associated with reduced likelihood for being hospitalized and reduced use of anti-inflammatory medications [15]. In steroid-resistant asthmatics it has been shown that VD_3 administration can down-regulate Th2 skewing [16]. Data from the Third National Health and Nutrition Examination Survey (NHANES III) showed that VD_3 levels are associated inversely with the occurrence of upper respiratory tract infections, and this association was even stronger in those with asthma [17].

In the upper airway, two reports have examined the role of VD_3 in allergic rhinitis. Using data from the NHANES III, Wjst and Hypponen found that the prevalence of allergic rhinitis increased across quartile groups of VD_3 serum levels [18]. Pinto *et al*. observed that African Americans with allergic rhinitis have lower VD_3 levels than race- and age-matched controls, suggesting that VD_3 has a potential role in upper respiratory disease in African Americans [19]. Previous studies by our group have reported that some forms of Th2 skewed CRS, such as allergic fungal rhinosinusitis (AFRS), occur more commonly in African Americans, who are more susceptible to VD_3 deficiency, raising the possibility of a role in AFRS. Additionally, African Americans with AFRS demonstrate more bone erosion than Caucasians, further supporting a potential role of VD_3 [20,21]. Therefore, in these studies we examined if VD_3 deficiency may contribute to immune dysfunction and bone erosion in CRS.

Methods

Clinical evaluation

Studies were conducted retrospectively at the Medical University of South Carolina with Institutional Review Board approval. The Medical University of South Carolina Institutional Review Board granted approval prior to initiation of the study and informed written consent was obtained from all participants. Patients were divided among four diagnostic groups: AFRS, CRSwNP, CRSsNP and control. AFRS patients met the classic Bent and Kuhn criteria, with immunoglobulin (Ig)E hypersensitivity to fungi demonstrated by either skin testing or elevated serum IgE [22]. CRSsNP patients were diagnosed through clinical and radiographic examinations that revealed inflammatory sinus disease without frank nasal polyposis and no subjective history of atopy. Control patients were undergoing repair of spontaneous cerebrospinal fluid leak and had no history of sinusitis and no radiographic or endoscopic evidence of inflammatory sinus disease at time of surgery. Patients who had taken oral steroids or immunotherapy within 30 days of surgery were excluded from the study.

Determination of VD_3 deficiency

Levels of 25-dihydroxy VD_3 were measured by enzyme-linked immunosorbent assay (ELISA) (Alpco Immunoassays, Salem, NH, USA) according to the manufacturer's instructions. VD_3 insufficiency was defined as <32 ng/ml and deficiency as ≤ 20 ng/ml [23–25]. Samples analysed in these studies were collected from mid-March to late August 2009 and March to May 2010 at latitude 32°N (spring/summer) to minimize the impact of seasonal variation in VD_3 levels.

Analysis of circulating immune cells and immune regulatory products

Peripheral blood was collected at time of sinus surgery and used as the source of plasma and peripheral blood mononuclear cells (PBMCs). Circulating levels of DCs and monocytes were determined by immunostaining followed by flow cytometric analysis. Prior to staining, PBMCs were incubated in phosphate-buffered saline (PBS) with 1% bovine serum albumin (BSA) to block non-specific binding. DCs were identified by positive staining for CD209 (DC-SIGN), CD1a and CD1c. CD209 is expressed in a small number of circulating DCs [26]; it has been shown to up be up-regulated in the sinuses of patients with CRS and has been shown to support Th2 skewing [27–29]. CD86 was examined to identify macrophages and DCs and for its role in initiation of Th2 responses [30,31]. CD14 was used to identify monocytes. Expression of the co-stimulatory molecule CD86 was also examined on DCs and macrophages. Macrophages were identified by staining for CD68, after treatment with Cytofix/Perm. CD209, CD1c and $CD1a^+$ cells were confirmed as DCs by staining lineage cocktail 1 (CD3, CD14, CD16, CD19, CD20 and CD56) and CD68-negative. Live cell gating was determined by propidium iodide staining and marker channels were set using the isotype controls specific to each antibody. Analysis was performed on a BD fluorescence activated cell sorter (FACS) FACSCantos using

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FACS Diva software. All reagents for immunostaining were from BD Biosciences (San Diego, CA, USA).

Plasma levels of GM-CSF (BD Biosciences) and PGE_2 (R&D Systems, Minneapolis, MN, USA) were measured by ELISA and performed according to the manufacturers' instructions.

Bone erosion scoring

Degree of bone erosion was analysed by two graders using a previously published staging system [32]. A computed tomography (CT) bone remodelling score was assigned by both graders and then averaged to yield a mean CT bone erosion score for each patient. Graders were blinded to age, race, gender and VD_3 status of the patients.

Statistical analysis

Statistical analysis was conducted using GraphPad Prism version 5.02 software (La Jolla, CA, USA). Values were first determined to follow a normal distribution using a D'Agostino and Pearson omnibus normality test. A one-way analysis of variance (ANOVA) with *post-hoc* unpaired Student's *t*-test was then used to determine statistically significant differences between patient cohorts and indicated parameters. A Pearson's correlation analysis was used to determine if there was a correlation between VD_3 levels and the aforementioned immune parameters. Two-way ANOVA was conducted to determine if differences observed in VD_3 levels were influenced by age, gender, body mass index (BMI) or race.

Within the subset of patients whose mean CT bone remodelling score was greater than 0, an unpaired *t*-test was used to determine statistical significance those with adequate VD_3 (greater than or equal to 32 ng/ml) or insufficient VD_3 levels (<32 ng/ml) on the CT bone remodelling score. An unpaired Student's *t*-test was used to determine differences in bone erosion scores between VD_3-deficient and -insufficient patients. A Pearson's correlation analysis was used to determine if there was a correlation between VD_3 levels and bone erosion severity.

Results

CRSwNP and AFRS patients have elevated numbers of circulating DCs and DC regulatory products, while CRSsNP displays elevated numbers of circulating macrophages

In these retrospective studies, we examined PBMCs from patients with CRSsNP, CRSwNP or AFRS to determine if there were differences in circulating numbers of APCs and monocytes compared to controls. First, expression of CD86 was assessed due to its role in Th2 initiation [5,6]. Compared to controls, we found elevated numbers of $CD86^+$ PBMCs in CRSsNP ($P = 0{\cdot}007$), CRSwNP ($P < 0{\cdot}0001$) and AFRS ($P < 0{\cdot}0001$) (Fig. 1a). There was no statistically significant difference between CRSsNP and CRSwNP ($P = 0{\cdot}368$) or AFRS ($P = 0{\cdot}190$).

Next, staining for CD209 and CD68 was conducted to identify circulating DCs and macrophages, respectively, more definitively. Only CRSwNP and AFRS displayed elevated levels of $CD209^+$ DCs (Fig. 1b) compared to control ($P < 0{\cdot}0001$ for each group). CRSwNP and AFRS circulating DC numbers were also elevated compared to CRSsNP ($P = 0{\cdot}0001$ and $P = 0{\cdot}0014$, respectively). Similar to the CD209 results, circulating numbers of $CD1c^+$ DCs (Fig. 1c) were elevated in CRSwNP and AFRS *versus* control ($P < 0{\cdot}0001$ for both analyses). No differences were observed between control and CRSsNP levels of $CD1c^+$ DCs ($P = 0{\cdot}15$). Unlike changes in DC numbers, only CRSsNP had increased numbers of circulating $CD68^+$ macrophages (Fig. 1d) compared to control ($P = 0{\cdot}003$), CRSwNP ($P = 0{\cdot}004$) and AFRS ($P = 0{\cdot}03$). Lastly, we measured circulating monocyte levels (Fig. 1e). Compared to control there were elevated numbers of $CD14^+$ cells in CRSsNP ($P = 0{\cdot}01$), CRSwNP ($P = 0{\cdot}0013$) and AFRS ($P = 0{\cdot}0002$). There was no significant difference in levels between the three sinusitis subclasses. Taken together, these results demonstrate that all three sinusitis subclasses have increased circulating monocytes. However, only CRSwNP and AFRS have increased numbers of circulating DCs, while only CRSsNP has increased circulating macrophages. These differences in immune cell composition may help to account for differences in Th1/Th2 skewing observed in the various sinusitis subclasses.

CRSwNP and AFRS, but not CRSsNP, have insufficient circulating levels of vitamin D_3

After observing increased numbers of circulating DCs in CRSwNP and AFRS, we next determined if these patients were VD_3-deficient, as VD_3 has been shown to block monocyte to DC differentiation and DC maturation. Mean plasma 25-OH VD_3 levels for controls (51 ± 4 ng/ml) and CRSsNP (45 ± 2 ng/ml) were well above the recommended minimum level of 32 ng/ml (Fig. 2). Mean 25-OH VD_3 levels for CRSwNP (18 ± 4 ng/ml) and AFRS (21 ± 5 ng/ml) were significantly lower when compared to either control or CRSsNP ($P \leq 0{\cdot}0001$ for all comparisons).

Two-way ANOVA analysis was used to determine if differences in VD_3 were influenced by gender, race or BMI, all of which are known to effect VD_3 levels (summarized in Table 1). It was determined that gender ($P = 0{\cdot}58$), race ($P = 0{\cdot}12$) and BMI ($P = 0{\cdot}18$) did not influence significantly the differences in VD_3 observed among the various patient cohorts. *Post-hoc t*-test analysis identified that overweight patients with AFRS have significantly lower VD_3 than AFRS patients, whose BMI was in the healthy range ($P = 0{\cdot}03$), suggesting that weight can contribute further to VD_3 insufficiency associated with AFRS. These results demonstrate that CRSwNP and AFRS are VD_3-insufficient compared to

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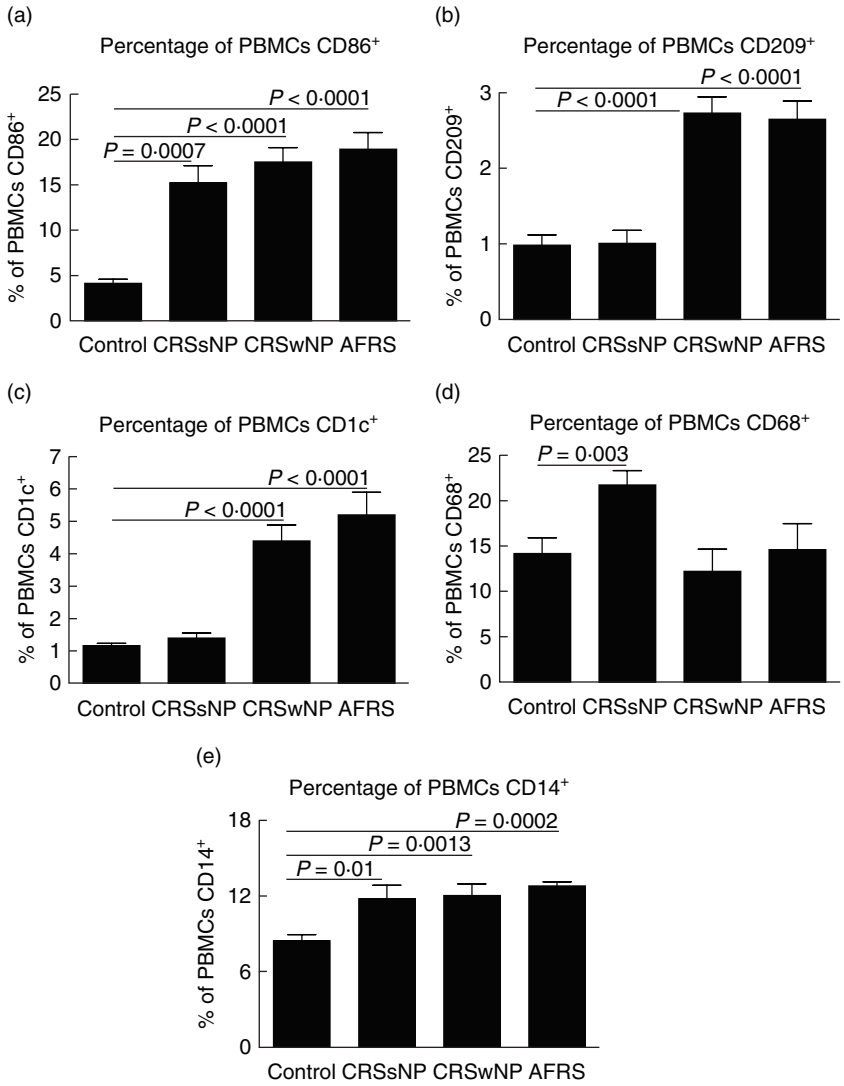

Fig. 1. Circulating levels of antigen-presenting cells (APCs) and monocytes are altered in chronic rhinosinusitis (CRS). Immunostaining and flow cytometric analysis of peripheral blood mononuclear cells (PBMCs) in patients with CRS for (a) $CD86^+$ dendritic cells (DCs) and macrophages, (b) $CD209^+$ DCs, (c) $CD1c^+$ DCs, (d) $CD68^+$ macrophages and (e) $CD14^+$ monocytes. Statistics shown represent Student's *t*-test between indicated patient groups.

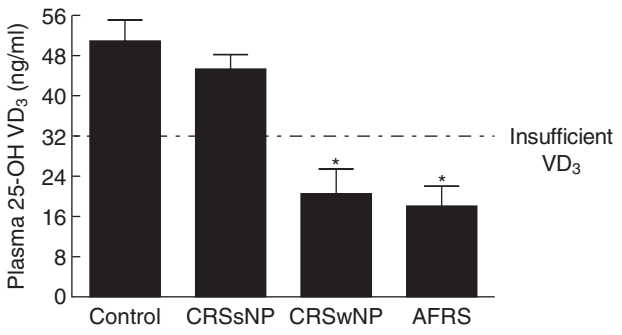

Fig. 2. Chronic rhinosinusitis with nasal polyps (CRSwNP) and allergic fungal rhinosinusitis (AFRS) have insufficient levels of circulating 25-OH vitamin D_3 (VD_3). Compared to control and CRSwNP, AFRS and CRSwNP have significantly lower plasma VD_3 levels with means below the recommended level of 32 ng/ml. (*) $P \leq 0{\cdot}0001$ *versus* control or chronic rhinosinusitis without nasal polyps (CRSsNP).

control. Conversely, CRSsNP was found to be VD_3-sufficient, implicating VD_3 in the pathophysiology of the different subtypes of chronic sinusitis.

Vitamin D_3 levels correlate inversely with numbers of circulating mature DCs and DC regulatory factors

After determining that CRSwNP and AFRS have lower VD_3 levels, we next determined if there was an association between VD_3 and elevated numbers of circulating DCs. First, we examined the impact VD_3 on circulating $CD86^+$ and $CD209^+$ PBMCs. VD_3-insufficient patients had double the number of circulating $CD86^+$ cells than those with healthy VD_3 levels ($P = 0{\cdot}01$) (Fig. 3a). Those who were VD_3-deficient had nearly four times as many $CD86^+$ cells as control ($P < 0{\cdot}0001$) and twice as many as those who were insufficient ($P = 0{\cdot}01$). $CD209^+$ DCs (Fig. 3b) followed a similar trend, with increased numbers of cells in those whose VD_3 levels were healthy compared to insufficient ($P = 0{\cdot}008$) or deficient ($P < 0{\cdot}0001$). Patients who were deficient also had

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Table 1. Vitamin D_3 stratification by diagnosis, race,† gender and body mass index (BMI).‡

	Control ($n = 14$)	CRSsNP ($n = 20$)	CRSwNP ($n = 9$)	AFRS ($n = 14$)
	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.
Male	53·3 ± 14	50·2 ± 30	18·0 ± 17	26·4 ± 24
Female	45·3 ± 15	58·3 ± 28	15·8 ± 7	15·5 ± 9
P-value	0·33	0·15	0·79	0·32
Caucasian	56·0 ± 14	43·8 ± 13	21·3 ± 17	31·2 ± 22
African American	41·2 ± 15	59·0 ± 4	8·5 ± 9	13·0 ± 7
P-value	0·09	0·11	0·35	0·07
Healthy	53·0 ± 15	39·7 ± 14	21·7 ± 5	32·0 ± 2
Overweight	50·0 ± 18	45·3 ± 13	18·5 ± 10	8·9 ± 6·8
P-value	0·78	0·44	0·64	0·03*

*Statistically significant. †For stratification by race, data from one Hispanic individual was excluded. ‡Using the classification system defined by the US National Institute of Health, individuals were stratified by BMI as healthy (≥18·5 to 24·9) or overweight (≥25). CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps; AFRS, allergic fungal rhinosinusitis; s.d., standard deviation.

significantly more $CD209^+$ DCs than those who were insufficient ($P = 0{\cdot}003$). Furthermore, those who were VD_3-insufficient or -deficient also had significantly higher circulating levels of $CD1c^+$ DCs compared to healthy controls ($P = 0{\cdot}0003$ and $P < 0{\cdot}0001$, respectively). As shown in Fig. 3d, a strong inverse correlation exists between circulating $CD86^+$ DCs and VD_3 status ($R^2 = 0{\cdot}8501$, $P < 0{\cdot}0001$). VD_3 also correlated inversely with PBMC expression of $CD209^+$ (Fig. 3e) ($R^2 = 0{\cdot}7977$, $P < 0{\cdot}0001$), CD1c (Fig. 3f) ($R^2 = 0{\cdot}8404$, $P < 0{\cdot}0001$) and CD1a ($R^2 = 0{\cdot}9197$, $P < 0{\cdot}0001$, data not shown). Of the nine CRSwNP patients with $CD209^+$ measurement, five had negative allergy testing, three had positive allergy testing and one was untested. Further evaluation determined that there were no significant differences between circulating $CD209^+$ DCs levels in atopic *versus* non-atopic CRSwNP individuals (data not shown, $P = 0{\cdot}88$). This would suggest that while atopic status may contribute to elevated numbers of DCs, such as in AFRS, there are mechanisms such as VD_3 deficiency that result in an altered immune profile independent of atopy.

While the CRSsNP cohort was overall VD_3-sufficient, a correlation analysis was conducted between VD_3 and $CD68^+$. As expected, there was no association between VD_3 and circulating numbers of $CD68^+$ cells (data not shown; $R^2 = 0{\cdot}08$, $P = 0{\cdot}72$). Similarly, there was no correlation between VD_3 plasma levels and circulation $CD14^+$ monocyte levels among any of the cohorts (data not shown; $R^2 = 0{\cdot}015$, $P = 0{\cdot}71$).

Next we assessed plasma levels of macrophage and DC regulatory products, GM-CSF and PGE_2. Figure 4a,b demonstrates that compared to control, GM-CSF and PGE_2 were increased in CRSsNP ($P = 0{\cdot}02$ and $P = 0{\cdot}0011$, respectively), CRSwNP ($P < 0{\cdot}0001$ and $P = 0{\cdot}0004$, respectively) and AFRS ($P = 0{\cdot}0067$ and $P = 0{\cdot}0057$, respectively). Levels of GM-CSF were also significantly higher in CRSwNP and AFRS compared to CRSsNP ($P = 0{\cdot}03$ and $P = 0{\cdot}01$, respectively) and levels of PGE_2 were significantly higher in AFRS compared to CRSsNP ($P = 0{\cdot}005$). There was no statistically significant difference between CRSsNP and CRSwNP plasma PGE_2 levels ($P = 0{\cdot}08$). Similar to the DCs/VD_3 correlation, VD_3 correlated inversely with GM-CSF ($R^2 = 0{\cdot}7039$, $P = 0{\cdot}0012$) (Fig. 4c) and PGE_2 (Fig. 4d) ($R^2 = 0{\cdot}7401$, $P = 0{\cdot}0081$). These results demonstrate that VD_3 deficiency is associated with elevated levels of circulating DCs and DC regulatory products in CRSwNP and AFRS.

VD_3 deficiency is associated with increased bone erosion in CRS

VD_3 has long been known as a regulator of bone health due to its ability to stimulate calcium absorption. Therefore we measured the severity of bone erosion on preoperative CT scans in patients with varying levels of VD_3. As shown in Fig. 5a, the average CT bone remodelling score in patients with insufficient levels (<32 ng/ml) of serum VD_3 was significantly greater than in patients with adequate (≥32 ng/ml) VD_3 ($P = 0{\cdot}016$) levels. Furthermore, as in our immune studies, a strong inverse relationship was identified between reduced plasma levels of VD_3 and an increase in the CT bone remodelling score ($R^2 = 0{\cdot}553$ and $P = 0{\cdot}009$) (Fig. 5b). Figure 5c is a representative CT scan from an AFRS patient with a bone erosion score of 22 and VD_3 level of 11 ng/ml. These results support the role of VD_3 in the exacerbation of CRS-associated bone erosion.

Discussion

In these retrospective studies we investigated circulating levels of APCs in chronic rhinosinusitis. Patients with CRSwNP and AFRS displayed elevated numbers of circulating DCs, while CRSsNP had increased numbers of macrophages. In other respiratory diseases, such as asthma, DC numbers are elevated and make a significant contribution to disease pathogenesis, including the initiation of Th2 skewing [5,6,31].

Investigation into the potential mechanism driving elevated numbers of DCs led us to examine VD_3. Both CRSwNP and AFRS patients were identified as being VD_3-insufficient (<32 ng/ml) compared to control and CRSsNP. Furthermore, a strong association between VD_3 deficiency and increased levels of circulating DCs in CRSwNP and AFRS was identified. Atopic status was examined as additional mechanism accounting for elevated numbers of DCs, although it was determined that there was no difference in circulating DC numbers between atopic and non-atopic CRSwNP individuals. It is hypothesized that lack of VD_3 allows the elevated numbers of monocytes in CRSwNP and AFRS to proceed systemically to DC differentiation and

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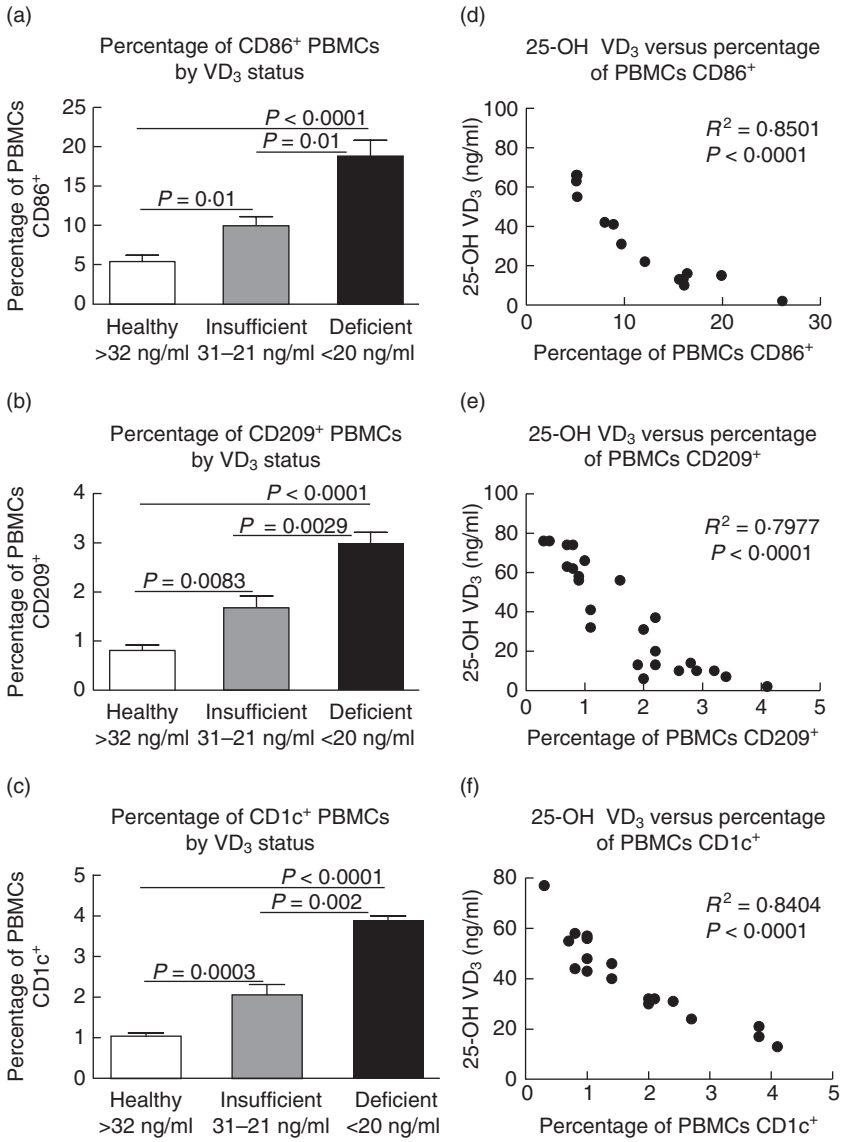

Fig. 3. 25-OH vitamin D_3 (VD_3) levels correlate inversely with levels of circulating $CD86^+$, $CD209^+$ and $CD1c^+$ cells. Clinical stratification based upon VD_3 levels and corresponding levels of (a) $CD86^+$, (b) $CD209^+$ cells and (c) $CD1c^+$. Pearson's correlation analysis demonstrating (d) 25-OH VD_3 *versus* percentage of peripheral blood mononuclear cells (PBMCs) $CD86^+$, (e) 25-OH VD_3 *versus* percentage of PBMC $CD209^+$ and (f) 25-OH VD_3 *versus* percentage of PBMC $CD1c^+$.

maturation more freely. While a large body of literature supports VD_3 as promoting Th1 or Th2 skewing in various disease states [33], ultimately all these demonstrate a failure of DCs to be kept in a tolerogenic state. In studies by Penna *et al.* it was shown that the 1,25 VD_3 promoted myeloid DCs to promote a tolerogenic state [34]. The lack of the 1,25 VD_3 precursor, 25-OH VD_3, observed in CRSwNP and AFRS may therefore allow DCs to mature with other environmental or host signals driving DCs to promote Th2 inflammation.

VD_3 did not correlate with all the changes in immune parameters observed in these studies. No correlation was observed between VD_3 and $CD14^+$ monocytes, suggesting that the presence of DC and macrophage precursors is not dependent upon VD_3. Additionally, elevations in $CD68^+$ macrophages did not correlate with VD_3. This was not entirely unexpected, because in contrast to its inhibitory effects upon DC maturation, VD_3 promotes monocyte to macrophage differentiation. Thus, patients with CRSsNP who had normal VD_3 levels had higher macrophage levels than CRSwNP and AFRS patients who were VD_3-insufficient.

Our studies also identified that plasma levels of PGE_2 and GM-CSF were up-regulated in CRSsNP and to an even greater extent in CRSwNP and AFRS. Moreover, both of these factors were found to correlate inversely with VD_3 in CRSwNP and AFRS. These results are consistent with reports in asthma showing elevated PGE_2 [35]. One cellular source of PGE_2 identified previously in asthmatics is macrophages [36], which may account for the slight increase in CRSsNP, as these patients possess elevated numbers of circulating macrophages. As with PGE_2, GM-CSF has also been identified as being elevated in asthma [37] and has been shown to be a contributor to airway inflammation and hyperresponsiveness [38]. While our studies are the first to identify GM-CSF as being elevated systemically, previous studies have shown

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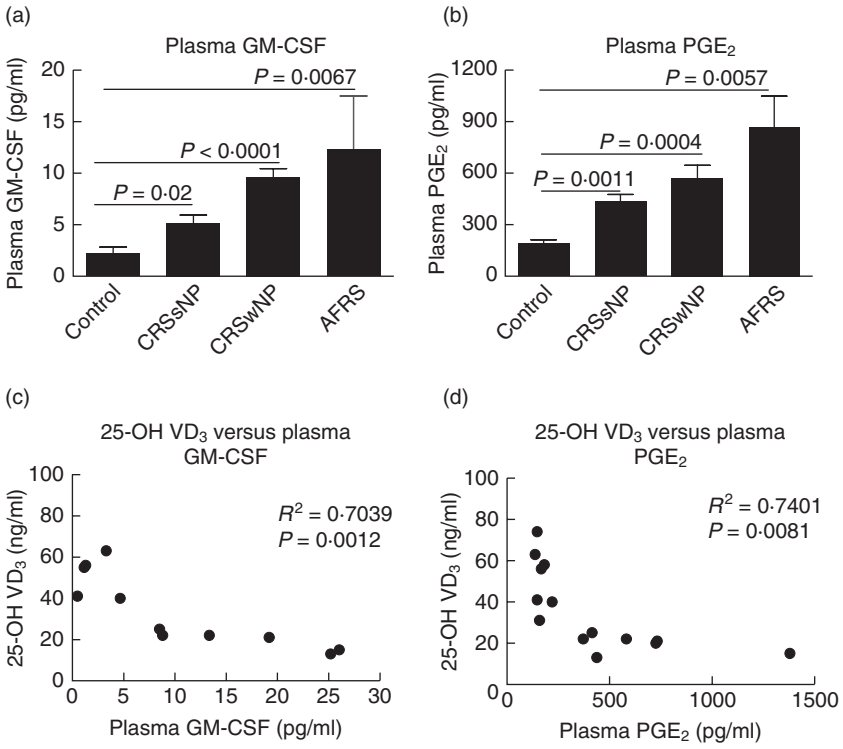

Fig. 4. Circulating levels of dendritic cell (DC) regulatory factors are up-regulated in chronic rhinosinusitis (CRS) correlated inversely with 25-OH vitamin D_3 (VD_3). Plasma levels of (a) granulocyte–macrophage colony-stimulating factor (GM-CSF) and (b) prostaglandin E_2 (PGE_2) as measured by enxyme-linked immunosorbent assay (ELISA). Statistics shown represent Student's *t*-test between indicated patient groups. Pearson's correlation analysis of plasma levels for GM-CSF (c) and PGE_2 (d) as determined by ELISA *versus* plasma 25-OH VD_3.

GM-CSF up-regulation locally in allergic and non-allergic polyp tissue compared to turbinate [39]. However, the role of both of these factors in CRSsNP and CRSwNP remains to be identified.

In addition to examination of immune parameters, the impact of VD_3 on bone erosion in CRS was investigated. Patients with more severe forms of CRS that present with bone erosion into the orbit and/or skull base demonstrated more severe VD_3 deficiencies. These results echo similar findings in other diseases, such as rheumatoid arthritis, that report a relationship between VD_3 receptor polymorphisms and accelerated bone loss [40]. It is unclear if VD_3 deficiencies

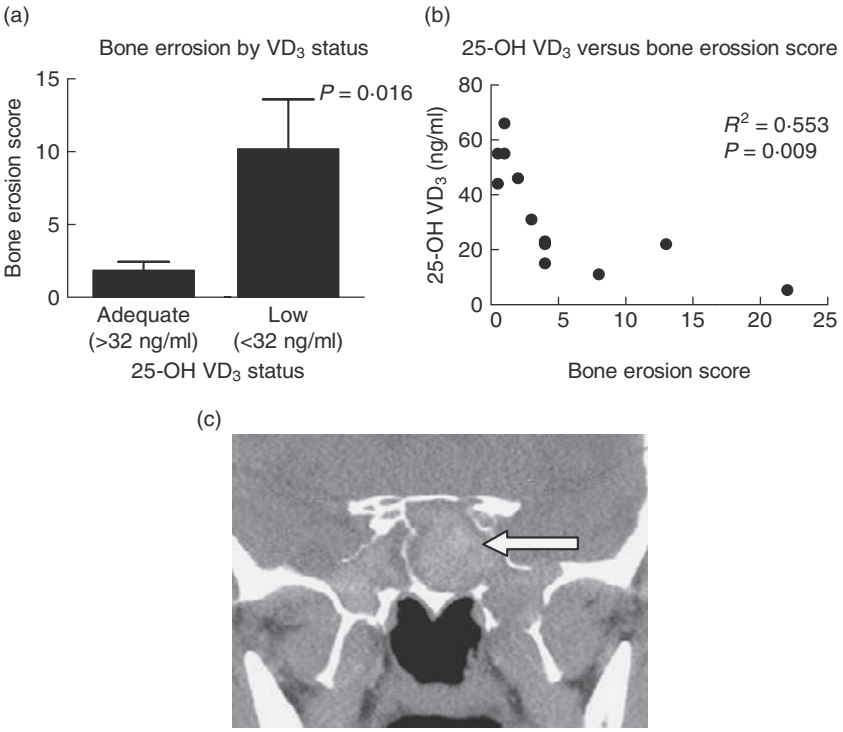

Fig. 5. Vitamin D_3 (VD_3) deficiency is associated with increased bone erosion. (a) Patients with sufficient VD_3 had significantly less bone erosion than those with insufficient VD_3. (b) Pearson's correlation examining 25-OH VD_3 *versus* bone erosion score. (c) Representative coronal computed tomography (CT) from allergic fungal rhinosinusitis (AFRS) patient demonstrates bone erosion bilaterally along the lateral and inferior walls of the sphenoid sinus (arrow).

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lead to systemic abnormalities of bone metabolism or if they even play a major role in localized bone loss within the sinonasal cavity.

VD_3 targets many of the same DC regulatory pathways as corticosteroids, such as prednisone, one of the most commonly prescribed treatments for CRS. Based on this, it could be suggested that supplementation with VD_3 in CRSwNP and AFRS may be analogous to replacing one's natural prednisone. Based on the results of the above-mentioned studies and the results presented here, there is increasing evidence to support a role for VD_3 as a key player in the immunopathology of CRSwNP and AFRS.

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Disclosure

None of the authors listed have any potential conflicts to disclose related to the research presented herein.

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