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Review

Regulatory T cells, inflammation and the allergic response—The role of glucocorticoids and Vitamin D☆

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ABSTRACT

Regulatory T cells (TRegs) play a central role in the maintenance of peripheral tolerance. They prevent inappropriate immune responses to ubiquitous allergens in healthy individuals, and contribute to the maintenance of immune homeostasis in the airways. Both Foxp3+ and IL-10+ TReg have been implicated in these functions. Glucocorticoids represent the mainstay of treatment for asthma and other allergic conditions, and evidence that steroids influence TReg function will be reviewed. Growing bodies of epidemiological and immunological data suggest a role for endogenous Vitamin D in immune regulation. This review will discuss the role of glucocorticoids and Vitamin D, and their potential interactions in promoting tolerance in the context of allergic disease and asthma.

Contents

1. Allergy and asthma: disease prevalence

The prevalence of allergic and asthmatic disease has increased dramatically over the past few decades. The prevalence of asthma is currently estimated at 300 million people worldwide with countries including the United Kingdom, Australia and New Zealand reporting prevalence of up to 20% [1,2]. This rise can be demonstrated across the spectrum of allergic disease, with an increase in diseases such as allergic rhinoconjunctivitis causing significant morbidity [3,4]. Whilst there is a strong familial association with the development of allergic disease, the environment also plays an important role with influences such as infectious history, diet, pollution and of interest in the present review, Vitamin D, all proposed to influence the development and severity of disease (Table 1).

2. Immune mechanisms of allergic disease

Allergic sensitization has historically been associated with the development of allergen-specific Th2 responses and IgE produc-

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Table 1
Vitamin D, allergy, asthma and respiratory health: selected epidemiological data.

Reference	Study group and design	Vitamin D status assessed by	Vitamin D supplementation	Primary and secondary outcome	Vitamin D intake: protective or causative?
Pregnancy					
Erkkola et al. [104]	1669, aged 5 *BCS*[c]	Maternal diet (FFQ)[a]	None	Asthma (ISAAC)[b] Allergic rhinitis	Protective
Gale et al. [170]	440, aged 9 months 178, aged 9 years *BCS*	Maternal serum 25(OH)D	None	Eczema Asthma (9 years only)	Causative (non-significant for eczema)
Camargo et al. [95]	1194, aged 3 *BCS*	Maternal diet (FFQ)	None	Asthma (API)[d], Resp infections and eczema	Protective
Devereux et al. [94]	1335, aged 2 1212, aged 5 *BCS*	Maternal diet (FFQ)	None	Wheeze and asthma	Protective (wheeze) Non-significant for atopy or FEV_1
Childhood					
Bäck et al. [171]	123, aged 6 *BCS*	Dietary intake during 1st year of life (FFQ)	None	Atopic dermatitis, allergic rhinitis, asthma	Causative
Brehm et al. [73]	616, aged 6–14 *CSS*[e]	Serum 25(OH)D	None	IgE, eosinophils resp. infections, methacholine test	Protective
Hypponen et al. [172]	7648 adults *BCS*	Not assessed	Vitamin D supplements during 1st year of life	Asthma, allergic rhinitis,	Causative at high doses >2000 IU/d
Adult					
Hypponen et al. [105]	7288, aged 45 *BCS*	Serum 25(OH)D	None	Serum IgE	Dose dependent ↑IgE at very low and at very high 25(OH)
Pinto et al. [173]	86 adult subjects 68 *CCS*[f]	Serum 25(OH)D	None	Severe chronic rhinosinusitis (CRS)	Protective in African-American subjects in winter months only
Black et al. [93]	14076 selected pts. *CCS*[f] (*NHANES III study*)	Serum 25(OH)D	None	FEV1, FEV1/FVC ratio	Protective

[a] Food Frequency Questionnaire.
[b] International Study of Asthma and Allergy in Childhood questionnaire.
[c] Birth Cohort Study.
[d] Asthma Predictive Index.
[e] Cross Sectional Study.
[f] Case Control Study.

tion. IgE binds to the high affinity IgE receptor (FCεRI) present on mast cells. Subsequent challenge with allergen results in cross linking of IgE on the mast cell surface, activation and rapid degranulation of the mast cells, with the release of pre-formed mediators including cysteinyl leukotrienes and histamine [5,6]. The late phase response is denoted by an influx of Th2 lymphocytes and eosinophils leading to a more prolonged response with tissue damage [7]. In recent years a number of additional cells and mediators have been implicated in allergic and asthmatic responses, including basophils, Th17 and iNKT cells, TSLP, IL-25 and IL-33 [1,8,9]. In addition to a range of effector mechanisms proposed to mediate disease, alterations in regulatory T cell activity may also influence disease incidence and progression.

3. Regulatory T cells (TRegs)

A number of different regulatory T cell (TReg) populations have been described although the best understood to date are CD4+ TReg. A major CD4+ TReg population are those deriving from the thymus, which constitute a small percentage of the CD4+ T cell population in humans in the periphery [10]. They are often termed "naturally occurring TReg" and are characterized by the expression of the forkhead winged transcription factor FoxP3 (forkhead box P3), which is constitutively expressed by these cells and programs their development in the thymus [11]. CD4+Foxp3+ TReg also demonstrate high constitutive expression of CD25, the α-chain of the IL-2 receptor [12], cytotoxic T lymphocyte antigen (CTLA)-4 [13,14], and glucocorticoid-induced TNF receptor (GITR) [15,16], although none of these markers is specific to human TReg and can be transiently induced on all effector T cell populations upon activation.

In addition to the naturally occurring compartment, CD4+ TRegs are also induced peripherally. Induced TRegs (iTRegs) may be FoxP3+ or FoxP3− and generally secrete anti-inflammatory cytokines such as IL-10 and TGF-β. It is likely that iTRegs play an important role in the attenuation of autoimmune, infectious, inflammatory and allergic responses to antigens encountered after thymic development as well as to maintain TReg populations with age [17]. De novo extrathymic generation of FoxP3+ iTRegs has been demonstrated in a number of animal models [18–20], including those of oral tolerance [21,22]. *In vitro* studies have identified that the critical requirements for FoxP3+ iTReg induction from naïve CD4+ T cells are TCR stimulation and the cytokines IL-2 and TGF-β [23–25].

The peripheral generation of FoxP3 negative IL-10-secreting TRegs (Tr1 cells or IL-10-Treg) has also been described in mice and in humans *in vivo* following repetitive exposure to antigens such as peptides used for immunotherapy, or bee venom in a population of beekeepers [26–30]. Repeated exposure to antigen has been suggested to induce a switch of Th1 and Th2 cells towards an IL-10-secreting iTReg phenotype [29,31] and it has been proposed that IL-10-secreting TRegs may arise *in vivo* through the deviation from effector phenotypes [26,29,32].

TRegs control effector immune responses through a diverse array of mechanisms including secretion of the anti-inflammatory cytokines IL-10 [33], TGF-β [34] and more recently in mouse studies IL-35 [35], as well as preferential consumption of the T cell growth and survival factor, IL-2 [36]. Additional mechanisms may include direct cytolysis of target cells through perforin [37] or Granzyme B dependent [38] mechanisms. The capacity of antigen presenting cells to prime adaptive immune responses is also impaired by TRegs, through downregulation of their surface expression of the co-stimulatory molecules CD80 and CD86, a mechanism which likely depends upon TReg expression of their ligand, CTLA-4 [39,40]. TRegs may also have a role in the co-ordination and regulation of recruitment of effector T cells to sites of inflammation [41,42].

4. TReg function in allergy and asthma

Evidence that TReg function is impaired in allergic and asthmatic disease has been extensively reviewed elsewhere [43,44]. This is however best highlighted by studies of a mutation of the FoxP3 gene in humans that leads to the loss of this naturally occurring TReg compartment, and a disorder known as immune dysregulation polyendocrinopathy X-linked (IPEX). IPEX is characterized not only by autoimmunity but also severe atopy, manifested as food allergies, atopic dermatitis, hyper-IgE and eosinophilia from a very early age. The afflicted boys die early without intervention in the form of bone marrow transplantation or profound immunosuppression [45]. Early studies also suggested the suppressive activity of CD4+CD25+ T cells from the peripheral blood of allergic patients during the hay fever season was reduced in comparison to healthy controls [46], although caveats in the interpretation of these data remain (reviewed in 42, 43). More directly, Hartl showed that the CD4+CD25+Foxp3+ TReg in the bronchoalveolar lavage, but not in the peripheral blood of children with asthma were decreased in number and suppressive capacity [47].

The anti-inflammatory actions of IL-10 have been extensively documented [48,49]. IL-10 inhibits many functions relevant to asthma. It inhibits mast cell and eosinophil function and favourably modulates IgE to IgG4 ratios. IL-10 acts on macrophages and dendritic cells to inhibit pro-inflammatory cytokine production and the capacity to activate T cells, including Th2 cells. IL-10 is proposed to play a role in maintaining immune homeostasis in the airways [50]. Studies of IL-10 production in the lung and of polymorphisms in the IL-10 gene promoter suggest that individuals who make low IL-10 suffer from more severe asthma [50–52] and similar observations have been made in allergic disease [53,54]. Strikingly, a number of treatments that alleviate the symptoms of allergic and asthmatic disease have been associated with modulation of Foxp3 and/or IL-10+ TReg activity.

5. Current therapies for asthma and allergic disease

At present the mainstay of therapy for allergic disease are antihistamines and glucocorticoids. In asthma, glucocorticoid therapy is generally used in combination with other anti-inflammatory or reliever medications such as beta-agonists [55]. These treatments ameliorate disease but they are not curative. Allergen immunotherapy, the injection of patients with gradually increasing quantities of the allergen to which they are sensitized in order to induce tolerance, is done under carefully controlled clinical conditions. It is efficacious particularly in certain patient groups [56] and the effects will persist after treatment is discontinued, but maximal efficacy requires treatment over several years and there are significant side effects associated with administration, a factor limiting its application in asthmatic patients [57]. Successful allergen immunotherapy is associated with the induction of IL-10-secreting regulatory T cells. This has been extensively reviewed and will not be discussed further here [58,59]. It is noteworthy however that a similar CD4+ IL-10+ T cell population is present in individuals who are not allergic [54] and/or naturally tolerant to allergen [29].

The capacity of glucocorticoids to mediate potent anti-inflammatory actions via inhibition of transcription factors involved in cytokine regulation such as nuclear factor of activated T cells (NFAT), activator protein-1 (AP-1), and nuclear factor kB (NF-kB) has been well documented [60]. However, glucocortioids are increasingly recognised to also beneficially influence both Foxp3+ and IL-10+ TReg function. Adult asthmatic patients receiving either inhaled or systemic glucocorticoid treatment showed increased mRNA expression for Foxp3 and IL-10 in their peripheral blood CD4+ T cells as compared with untreated

asthma patients [61]. In a study with paediatric bronchoalveolar lavage, inhaled glucocorticoid treatment was shown to restore low CD4+CD25+ cell numbers, FoxP3 mRNA and suppressive function [47].

A number of studies demonstrate that glucocorticoids induce IL-10 synthesis both *in vitro* and in patients. CD4+ T cells stimulated in the presence of glucocorticoids such as dexamethasone show a dose dependent induction of IL-10 synthesis and exhibit inhibitory or regulatory activity [62–64]. This induction is enhanced by the additional presence of mediators such as beta-2 agonists [65] or 1α,25-dihydroxyvitamin D3 (calcitriol) [66]. Steroid refractory or insensitive asthma, where patients gain little or no clinical benefit from glucocorticoid therapy, represents a major concern, with those patients most at risk of hospitalization and death from their asthma. The molecular basis of steroid refractory asthma is reviewed in detail elsewhere in this series (see review by P. Barnes). Of relevance here, however, is that CD4+ T cells from steroid resistant asthmatics fail to demonstrate increased IL-10 synthesis following *in vitro* stimulation in the presence of the glucocorticoid dexamethasone [67], suggesting that induction of IL-10 is an important part of the anti-inflammatory effect of glucocorticoids. This defect in steroid-induced IL-10 synthesis was overcome by the addition of calcitriol to the T cell culture and more strikingly, in a small a pilot study, ingestion of calcitriol by steroid refractory asthmatic patients enhanced their response to dexamethasone for the induction of IL-10 *in vitro* [68]. Mechanistic studies suggested calcitriol might act to reduce ligand-induced downregulation of the glucocorticoid receptor. However, these studies were performed *in vitro* with healthy CD4+ T cells, and did not represent direct evidence using patient cells. Both this and a second independent study however suggested that IL-10 increases glucocorticoid receptor expression by CD4+ T cells and monocytes respectively [68,69]. Future studies need to address whether this apparent steroid enhancing property of Vitamin D with respect to IL-10 synthesis equates with any clinical benefit to the patient. Whilst these studies have all involved pharmacological forms and delivery of Vitamin D, a growing literature is identifying a physiological role of the endogenous Vitamin D pathway in immune regulation.

6. Vitamin D physiology

The role of Vitamin D and its metabolites in bone and calcium metabolism is well established and there is increasing awareness of its importance in immune regulation [70,71]. Bioavailability of Vitamin D in the body is greatly influenced by exposure of the skin to sunlight with maximum synthesis achieved when UVB reacts with 7-dihydrocholesterol in the skin at wavelengths of 295 nm. Very little Vitamin D is produced in areas at beyond a latitude of 35° from October to March. Synthesis is greatly influenced by skin pigmentation, sun protection (a sun protection factor of >15 will absorb UVB light to 99%), age and coverage by clothing [72]. Once 7-dehydrocholesterol is photolysed into pre-Vitamin D3 and processed into Vitamin D3 it is converted in the liver to 25-hydroxyvitamin D3 (the major circulating form) and subsequently in the kidneys to its biologically active form 1α,25-dihydroxyvitamin D3 (calcitriol), a mechanism tightly regulated by PTH.

7. Vitamin D epidemiology

The modern lifestyle, which involves working indoors or the use of protective clothing and sunscreen explains why more and more studies emerge reporting hypovitaminosis D in widespread areas of the world including temperate climates [71,73–78]. Serum levels of 25-hydroxyvitamin D3 as low as 25 nmol/L (10 ng/mL) will prevent rickets in children but it is now widely acknowledged that much higher levels, from 75 to 100 nmol/L (30–40 ng/mL) are required for multiple health outcomes including bone and dental health and cancer prevention [79]. Only a few foods, such as oily fish, contain moderate amounts of Vitamin D and unlike countries like the United States of America or Finland, milk and dairy products in the United Kingdom and the rest of Europe are no longer fortified with Vitamin D [72]. It is therefore not surprising that Vitamin D deficiency is common and widespread. Half of the British population has been found to have low levels of Vitamin D during the winter months [80]. Moreover, several reports show Vitamin D deficiency not only in adults but in prepubertal populations: studies conducted in Buenos Aires, Southern Tasmania, the United States of America and Spain found Vitamin D insufficiency (25-hydroxyvitamin D3 < 50 nmol/L) in 10–80% of the children [81–84] with concerns raised over bone health and immune function.

A large number of epidemiological studies show that Vitamin D deficiency is associated with autoimmune disease [85–88] and an increased prevalence of malignancy [89–92]. Moreover, of interest to the present review hypovitaminosis D has been linked to impairment of respiratory health and chronic lung disease (Table 1).

Vitamin D status positively correlates with lung function, as shown in the study by Black in 2005 [93]. The effects of low Vitamin D levels on lung health are seen at an early age and may reflect maternal nutritional intake, with studies demonstrating an association between childhood wheeze and low maternal dietary intake of Vitamin D [94,95]. Observational evidence suggests that Vitamin D deficiency can also be associated with severity of chronic obstructive pulmonary disease although definitive proof of disease causation rather than a simple reflection of inactivity requires larger studies [96,97]. Further evidence of the link between Vitamin D and respiratory health is provided by the association of asthma and COPD with polymorphisms in the Vitamin D receptor [98,99] and Vitamin D binding protein [96]. These studies underline the complex role for Vitamin D and its receptor in the pathogenesis of chronic lung disease.

Vitamin D also plays an important role in protection against respiratory infection. This has been evident since as far back as the early 20th century, with the use of cod liver oil to treat TB and the housing of patients in sanitoriums that provided high levels of sun exposure. Epidemiological studies have now confirmed that there is an increased risk of tuberculosis in patients with Vitamin D deficiency [100]. Vitamin D deficiency also leads to an increased incidence of upper respiratory tract infections in adults [101] and increased prevalence and necessity for hospital admission for acute lower respiratory infection in newborns and children [102,103].

There is controversy in the literature regarding the role of Vitamin D in allergy. Some studies suggest that Vitamin D may provoke allergy or exacerbate allergic disease (Table 1). The most recent studies on asthma in general do not support this association [73,94,95,104]. However, a recent large epidemiological study looking at data from a British cohort, may begin to shed light on these various studies. That study indicated that any positive association between Vitamin D levels and allergy may arise at extremes of intake [105]. Elevated IgE levels in this cohort were seen at very low 25-hydroxyvitamin D3 (<25 nmol/L) and at very high 25-hydroxyvitamin D3 (>135 nmol/L) levels.

8. Immunological effects of Vitamin D

1α-Hydroxylation of 25-hydroxyvitamin D3 occurs at extrarenal sites such as the brain, breast, colon, prostate and cells of the immune system at sites of inflammation, allowing local synthesis of calcitriol (subject to availability of 25-hydroxyvitamin D3 substrate), which can modulate immune responses in a paracrine fashion [72]. Calcitriol binds to the nuclear Vitamin

D receptor (VDR), which is found in many cell types including immune cells such as monocytes, macrophages, dendritic cells and activated T- and B cells [106].

8.1. Effects of Vitamin D on innate immune function

Vitamin D appears to act on innate immune cells to inhibit their inflammatory activity and capacity to prime adaptive immune responses, whilst also promoting direct antimicrobial function. Treatment of human monocytes with calcitriol inhibits their expression of the toll-like receptors (TLRs) TLR2 and TLR4 [107,108]. TLRs recognise non-specific pathogen-associated molecular patterns and are vital for the induction of early inflammatory immune responses. The capacity of calcitriol to downregulate TLR expression has also been demonstrated in monocytes from patients with both latent autoimmune diabetes and Behcet's lymphoma, where basal TLR expression is higher than in healthy individuals [109,110]. Downregulation of monocyte TLR expression by calcitriol leads to a reduced production the pro-inflammatory cytokine tumour necrosis factor-α (TNF-α) in response to stimulation with TLR ligands [107,108].

As well as inhibiting the inflammatory function of innate immune cells, Vitamin D also impairs their capacity to prime adaptive effector immune responses (see below), whilst also promoting synthesis of small antimicrobial peptide products. Human cathelicidin antimicrobial peptide-18 (hCAP-18) is the only member of the cathelicidin family of antimicrobial peptides that is expressed by humans [111] and has been described in neutrophils, alveolar macrophages, epithelial cells and keratinocytes [112–116]. The protein precursor undergoes extracellular cleavage to generate a 37-residue active cationic peptide, LL-37 [112]. The hCAP-18 gene promoter contains a Vitamin D response element (VDRE), and calcitriol has been shown to induce expression in human cell lines [117,118], monocytes [111], neutrophils [117], keratinocytes [116] (an effect which may be enhanced by the cytokine IL-17 [114]), and also respiratory epithelium from both healthy individuals and cystic fibrosis patients [115,119]. Furthermore, it has been shown that induction of 1-α-hydroxylase expression, calcitriol synthesis, and calcitriol-induced expression of hCAP-18 is a downstream effector mechanism of TLR ligation in monocytes [120]. Other antimicrobial products regulated by Vitamin D are the cationic peptides, Defensin-β 2 and 4. Their genes also contain VDREs and their expression is induced in human monocytes by treatment with calcitriol [117,121].

The capacity of calcitriol to induce expression of antimicrobial peptides has been proposed to explain the observed association between Vitamin D insufficiency and infection described above. In one study, Vitamin D status was found to be significantly lower in patients in the intensive care unit with sepsis than healthy controls. In these patients serum 25-hydroxyvitamin D3 directly correlated with serum LL-37 levels [122], suggesting that systemic levels of LL-37 may be regulated by Vitamin D status, and that this may be important in the control of infection. In another study, serum 25-hydroxyvitamin D3 status was not found to correlate with serum LL-37 levels in healthy individuals, but did correlate with the *in vitro* capacity to induce monocyte hCAP-18 expression [123]. *In vitro*, the induction of hCAP-18 in human monocytes, neutrophils and respiratory epithelial cells by calcitriol treatment enhances antimicrobial activity against respiratory pathogens including *Mycobacterium tuberculosis*, *Bordetella bronchiseptica* and *Pseudomonas aeruginosa* [111,115,117,124].

8.2. Effects of vitamin D on effector T lymphocyte function

In addition to its effects on innate immune cells, Vitamin D also appears to modulate effector T lymphocyte function directly. *In vitro* studies have identified that calcitriol inhibits T cell proliferation [106,125] and IL-2 production [126], and production of the Th1-associated cytokine, interferon-γ (IFN-γ) by mouse and human CD4+ T cells [106,125–132]. The IFN-γ gene contains a VDRE and is a recognised target for direct transcriptional repression by ligand-bound VDR [128,133].

The effect of Vitamin D on CD4+ T cell production of the signature Th2 cytokines, IL-4, IL-5 and IL-13 is less clear. One early murine *in vitro* study found that whilst calcitriol treatment reduced the frequency of IFN-γ-secreting CD4+ T cells, it also appeared to promote IL-4-, IL-5- and IL-10-secreting cells, suggesting that Vitamin D enhanced the development of Th2 cells [127]. However, subsequent mouse studies and a study in human cord blood cells found calcitriol to reduce the number of IFN-γ-secreting cells, whilst either not affecting or reducing numbers of IL-4-secreting cells, indicating no favouring of the Th2 phenotype [129,130,132]. It appears that calcitriol concentration *in vitro* may be critical in determining the effect on Th2 cell differentiation and function. A study examining the effect of a high concentration of calcitriol (1×10^{-6} M) on whole human peripheral blood mononuclear cell responses to allergen found that whilst IFN-γ secretion was suppressed, IL-5 and IL-13 secretion were enhanced [134]. However, studies using lower concentrations of calcitriol (1×10^{-7} M to 1×10^{-9} M) find suppression of both Th1- and Th2-associated cytokines [130,135]. Interestingly, an *in vivo* correlate of this model has recently been demonstrated. As described above, in a large cohort of the British population, a significant but non-linear relationship was found between serum 25-hydroxyvitamin D3 status and IgE levels. Both deficient (<25 nmol/L) and excessively high (>135 nmol/L) serum levels of 25-hydroxyvitamin D3 significantly associated with elevated serum IgE levels [105]. It therefore appears that too little or too much Vitamin D may predispose to the development of allergic immune responses, whilst Vitamin D repletion is desirable. Given the very high prevalence of Vitamin D insufficiency, it is unlikely that supplementation would present the problem of raising serum levels excessively. Indeed it has been demonstrated that neither supplementation with Vitamin D precursor [136], nor ingestion of active calcitriol [137] enhances Th2 cytokine levels in human peripheral blood. However, clinical trails of supplementation will need to carefully optimise the dose administered.

The data on inhibition of Th2 responses by Vitamin D in mice *in vivo* also appear somewhat conflicting. For example, VDR knockout mice fail to develop allergic airway disease, despite having elevated levels of the associated circulating mediators (such as IL-5, IL-13, and IgE), suggesting that VDR expression may be necessary for lung development and inflammation to occur [138]. In another murine asthma model, administration of calcitriol was found to inhibit airway inflammation, decrease IL-4 levels in bronchoalveolar lavage fluid and impair T cell migration [139]. Irradiation with a single erythemal dose of UVB light, likely increasing vitamin D levels, prior to sensitization with antigen was also shown protect against airway hyperresponsiveness and inflammation [140]. In a different study, calcitriol enhanced Th2 responses (IL-4 and IL-13) when given early during immunization, but inhibited both IL-5 and airway eosinophilia when administered later [141]. Finally, in mouse models of autoimmune disease, where administration of calcitriol is protective via inhibition of Th1-driven immune responses, variable effects on Th2 cytokine production are reported [142,143]. Such results point to the complexity of the role for vitamin D in the inflammatory responses involved in the pathogenesis of asthma, as well as the importance of timing and dose of exposure.

Another T helper cell subset which appears to be regulated by Vitamin D is Th17 cells. Calcitriol administration has been demonstrated to reduce CD4+ T cell IL-17 production and arrest disease pathology in animal models of Th17-mediated autoimmune uveitis and colitis [144,145]. This is also of relevance to

asthmatic disease, since a role for IL-17 in severe, steroid refractory asthma has recently been proposed. Several studies report the presence and function of Th17 cells in asthma; higher levels of the cytokine IL-17A are found in sputum and BAL of patients with asthma compared to control subjects [146–148] and evidence from both human studies and animal models suggests a predominant role in neutrophil recruitment [146,149,150]. Interestingly, human studies find an association between steroid-resistance and neutrophilic (or non-eosinophilic) asthma [151–155], and recent animal models suggest a link between steroid-resistance and Th17-mediated disease [156]. Therefore the capacity of Vitamin D to regulate Th17 function may confer additional benefit in asthma.

8.3. *Effects of Vitamin D on regulatory T cell (TReg) function*

Human dendritic cells (DCs) differentiated in the presence of calcitriol have reduced surface expression of the antigen presentation molecules CD1a and MHC class II, and the co-stimulatory molecules CD40, CD80 and CD86, and fail to fully mature [157]. Their production of the pro-inflammatory cytokine IL-12 is impaired, and production of the anti-inflammatory IL-10 is enhanced. Consequently, calcitriol-treated DCs cannot stimulate T cell proliferation and instead appear to induce a state of hyporesponsiveness. Indeed, DC pretreatment with calcitriol and subsequent co-culture with CD4+CD25− T cells leads to induction of CD4+FoxP3+ TReg cells with suppressive activity [158]. Calcitriol-treated DCs have therefore been described as being 'tolerogenic' with respect to T cells. A recent report has also demonstrated that calcitriol treatment induces expression of the inhibitory cell-surface molecule Programmed Death Ligand-1 (PDL-1) on DCs, and that this may be critical for the induction of a regulatory T cell phenotype [159].

Calcitriol also appears to act directly on human CD4+ T cells to promote an IL-10-secreting CD4+ regulatory T cell population. Calcitriol induces IL-10 *in vitro* both directly and in concert with glucocorticoids [66,137]. Furthermore ingestion of calcitriol, at standard British National Formulary doses by asthma patients, and subsequent *ex vivo* analysis of their CD3+CD4+ T cells demonstrated an increase in IL-10 gene expression post calcitriol [137]. A separate study demonstrated that Vitamin D supplementation in patients with congestive heart failure had favourable effects on serum cytokine profiles; increasing IL-10 and improving TNF-α/IL-10 ratios [160]. One further study demonstrated that Vitamin D supplementation is associated with increased serum levels of TGF-β [136]. The role of TGF-β in asthma is complex; it may play a dual role in enhancing Foxp3+ TReg populations, whilst also being relevant to wound healing and repair [161].

Studies in a number of animal models have resulted in an emerging consensus supporting a role for Vitamin D in promoting TReg activity *in vivo*. An early study showed that a short treatment of adult non-obese diabetic (NOD) mice with an analogue of calcitriol inhibited IL-12 production, blocked pancreatic infiltration of Th1 cells, enhanced CD4+CD25+ TReg cells, and arrested the progression of type 1 diabetes [162] and is in agreement with a number of studies identifying the capacity of Vitamin D to inhibit autoimmune disease and prevent transplant rejection in animal models [86]. In a second autoimmune disease model, IL-10 signaling was demonstrated to be essential for calcitriol-mediated inhibition of experimental autoimmune encephalomyelitis [163]. In human multiple sclerosis sufferers, Vitamin D status has been found to correlate with peripheral blood regulatory T cell frequency and suppressive function [164,165].

UV light plays an essential role in the biosynthesis of Vitamin D and in asthma relevant models, UVB irradiation of skin, significantly suppressed airway hyperresponsiveness to methacholine, and OVA-specific responses in the lung and lung draining lymph nodes, with a proposed role for UVB-induced TReg [166]. The same group went on to demonstrate that topically applied calcitriol enhances the suppressive activity of CD4+CD25+ cells in skin draining lymph nodes [167]. The calcitriol analogue, calcipotriol, which has low calcaemic activity, has been shown to cause expansion of antigen-specific TReg [168] and these authors

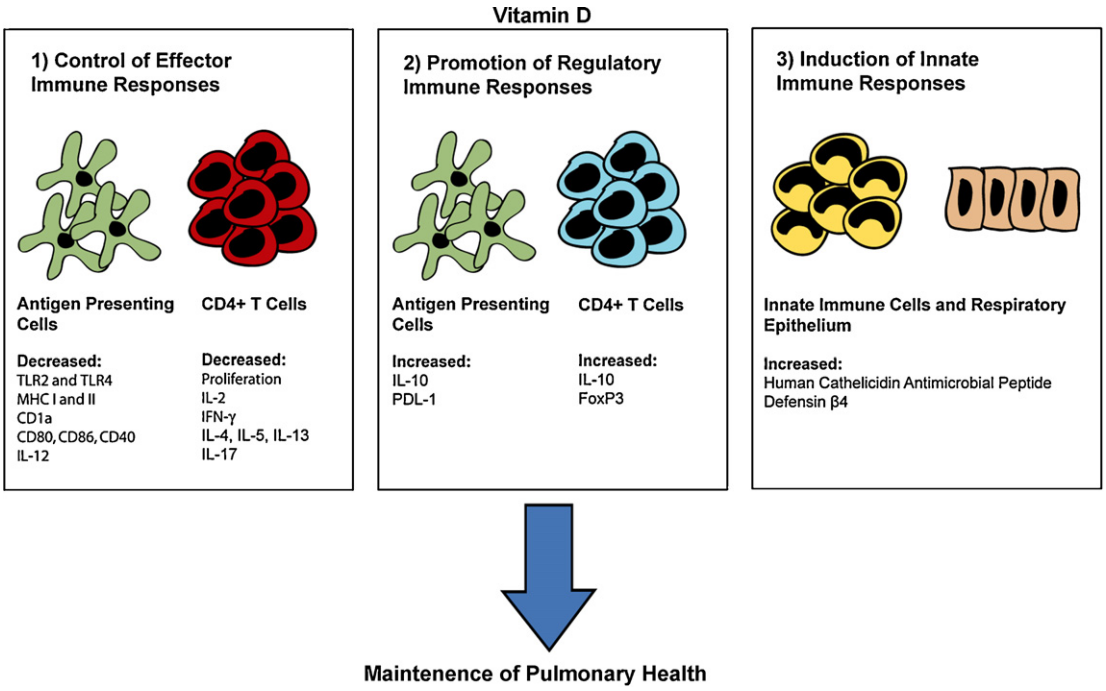

Fig. 1. The role of Vitamin D in the maintenance of pulmonary health. Vitamin D sufficiency is important for optimal pulmonary health and lung function. We propose a model where this occurs via three pathways: (1) the control of inflammatory innate and adaptive effector immune responses; (2) the promotion of regulatory responses, both indirectly via effects on antigen presenting cells and directly via effects on T lymphocytes; (3) the induction of innate antimicrobial mechanisms in immune cells and respiratory epithelium.

went onto demonstrate that Foxp3+ TReg induction by UVB was Vitamin D dependent since UV-irradiated VDR knockout mice failed to develop this subset [168]. Calcitriol has been shown to potentiate the beneficial effects of allergen immunotherapy in a mouse model of allergic asthma [169]. In this model a role for both IL-10 and TGF-β were proposed since antibody blocking of TGF-β and the IL-10 receptor reversed these beneficial effects.

9. Summary and future perspectives

A number of studies, both epidemiological and immunological, are converging to suggest a role for Vitamin D in promoting peripheral tolerance through the inhibition of inflammation, and the induction or maintenance of regulatory T cell populations, both IL-10+ and/or Foxp3+. In the context of infection, in particular respiratory infections, studies have highlighted a role for the Vitamin D pathway in the induction of antimicrobial mechanisms. A model is therefore emerging whereby Vitamin D sufficiency may be important for the overall maintenance of pulmonary health through three different mechanisms. This occurs firstly through the control of inflammatory innate and adaptive immune responses which may cause collateral damage to the delicate gaseous exchange surface of the airways; secondly through the promotion of regulatory immune mechanisms which will also restrain effector immune responses; and finally through the direct induction of innate antimicrobial mechanisms to efficiently resolve infection (Fig. 1).

Our understanding of the role and therapeutic potential of Vitamin D in controlling immune-mediated diseases such as asthma is still developing. Randomised controlled trials are needed and will aid our understanding of its effect on health and disease. Interventional trials in pregnant women and/or infants will be essential to identify whether supplementation might be useful for the primary prevention of asthma. As highlighted by existing epidemiological and immunological studies, a critical consideration with any Vitamin D intervention is likely to be the dose. Interventional trials will address whether Vitamin D supplementation is of benefit in established disease. In addition, trials with calcitriol, the active pharmacological form of Vitamin D will identify whether experimental *in vitro* evidence suggesting a steroid enhancing function in steroid refractory asthma will correlate with clinical benefit.

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