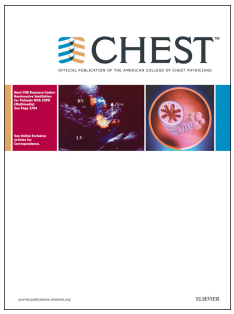
Accepted Manuscript



Translating Basic Research into Clinical Practice: Vitamin D in Asthma – Mechanisms of Action and Considerations for Clinical Trials

Paul E. Pfeffer, Catherine M. Hawrylowicz

PII: S0012-3692(17)32697-1

DOI: 10.1016/j.chest.2017.09.005

Reference: CHEST 1328

To appear in: *CHEST*

Received Date: 9 May 2017

Revised Date: 15 August 2017

Accepted Date: 6 September 2017

Please cite this article as: Pfeffer PE, Hawrylowicz CM, Translating Basic Research into Clinical Practice: Vitamin D in Asthma – Mechanisms of Action and Considerations for Clinical Trials, *CHEST* (2017), doi: 10.1016/j.chest.2017.09.005.

Translating Basic Research into Clinical Practice:

Vitamin D in Asthma – Mechanisms of Action and Considerations for Clinical Trials

Paul E Pfeffer [1,2]

Catherine M Hawrylowicz [2]

[1] William Harvey Research Institute, Queen Mary University of London, London UK, EC1M 6BQ

[2] MRC and Asthma UK Centre for Allergic Mechanisms of Asthma, King's College London, Guy's Hospital, London UK, SE1 9RT

Corresponding author:
Dr Catherine M Hawrylowicz,
MRC and Asthma UK Centre for Allergic Mechanisms of Asthma,
Division of Asthma, Allergy and Lung Biology,
5th Floor Tower Wing, Guy's Hospital,
King's College London,
London SE1 9RT, UK.
Email:catherine.hawrylowicz@kcl.ac.uk

Keywords:
asthma, vitamin D, airway immunology, T helper cells

Acknowledgements:

This review was written jointly by both authors.

The authors have no conflicts of interest to declare.

Abbreviations:

$1,25(OH)_2D3$	1,25 di-hydroxyvitamin D3
25(OH)D	25-hydroxyvitamin D
DC	dendritic cell
IL-	interleukin-
ILC	innate lymphoid cell
Th	helper CD4+ T lymphocyte

Abstract

There is increasing interest in the therapeutic utility of vitamin D in asthma, supported by a significant body of evidence on epidemiological associations between vitamin D insufficiency and worse asthma control. In support of a causal relationship, vitamin D beneficially modulates diverse immunological pathways in heterogeneous asthma endotypes, regulating the actions of lymphocytes, mast cells, antigen-presenting cells and structural cells to dampen excessive inflammatory responses.

Allergic asthma is characterised by a failure of immune tolerance, and development of pathological responses to inhaled aeroallergens, and vitamin D has been extensively shown to support immune regulation. Alarmin cytokines are increasingly implicated in non-allergic eosinophilic inflammation, which vitamin D also regulates. Steroid-resistance and pathological IL-17 responses are features of severe asthma, and vitamin D beneficially enhances the response to steroids in these individuals. Additionally, vitamin D enhances anti-microbial pathways, of relevance to infection-precipitated asthma exacerbations. These mechanisms support a role for vitamin D as secondary prevention to reduce exacerbations and inflammation in asthma. Similar mechanisms, and effects on fetal lung development, likely underlie a primary prevention therapeutic role in pregnancy for vitamin D to reduce the development of asthma in children.

However, randomised controlled trials of variable design show inconsistent positive outcomes for vitamin D interventions in asthma. Increased understanding of vitamin D biology reveals methodological issues that might explain certain negative outcomes. Importantly, on systematic review of the trials to-date, vitamin D is beneficial in asthma. The evidence

discussed in this review supports the importance of optimising vitamin D in holistic asthma care.

Manuscript

The importance of vitamin D in human health is well established, however, for much of the 20th century its actions were only considered influential in calcium and phosphate homeostasis. We have now come to realise that vitamin D has profound paracrine actions throughout the body and in particular to have major effects on the immune system. [1 2] There is a current epidemic of vitamin D insufficiency [3] - in humans the most important source for vitamin D precursors starts with conversion in the skin of 7-dehydrocholesterol to D3 in a reaction requiring solar ultraviolet B radiation, and reduced exposure to sunlight in our modern lifestyle is thought responsible for the high prevalence of vitamin D insufficiency. [1 3] As part of evolving vitamin D research over the last 15 years there has been intense interest in the role vitamin D may play in airway health and homeostasis, and the effect of vitamin D deficiency on airways pathology. In this review we discuss evidence for vitamin D insufficiency / deficiency contributing to asthma pathology and evidence that vitamin D supplementation should be considered part of holistic treatment of asthma.

Epidemiological research has revealed strong, significant associations between diverse aspects of asthma pathology and reduced levels of serum 25-hydroxyvitamin (25(OH)D) vitamin D, the major circulating precursor, which is commonly used as a measure of vitamin D status. Cross-sectional studies have consistently found more severe asthma in patients with lower vitamin D levels (Table of studies and discussion in Mann *et al.* 2014 [4]). Similar associations seen in other diseases has led to vigorous discussion about whether these associations are due to causation or reverse-causation. Three streams of evidence strongly support causation i.e. that reduced levels of circulating vitamin D can contribute to more severe asthma disease: prospective epidemiological evidence, mechanistic research and vitamin D supplementation studies.

Nevertheless reverse-causation (that asthma itself leads to decreased vitamin D levels) is also likely to be true with reduced time spent outside in more severe asthma and the likelihood that asthmatic inflammation is vitamin D consumptive, as has been shown for other types of inflammation. [5] We propose that there is a complex relationship between vitamin D insufficiency and asthmatic pathology, with a vicious circle of causation and reverse-causation, leading to progressive worsening of asthma, and that this cycle may be broken by including vitamin D supplementation in asthma care. As discussed below this evidence supports a therapeutic role for vitamin D as secondary prevention in asthmatic patients to reduce asthma exacerbations and also airway inflammation with remodelling.

Prospective epidemiological evidence

Most vitamin D epidemiological studies have been cross-sectional, comparing vitamin D levels to recent asthma control, and therefore more vulnerable to reverse-causation. Few studies have compared vitamin D levels to future asthma control. However, for example, Brehm *et al.* (2010) have reported an association between baseline serum vitamin D status and risk of a severe exacerbation over the following 4 years in children enrolled into the Childhood Asthma Management Program study. [6] After adjustment for other variables, including physician judged asthma severity, patients with baseline vitamin D insufficiency (< 30 ng/ml) had higher odds of an asthma exacerbation requiring hospitalisation or Emergency Department attendance over the follow-up period.

Mechanistic Research

Translational research over the last 15 years has shown vitamin D to have a major role in regulating immunological responses. Such a role is not surprising given the high frequency of vitamin D response elements in cells of the immune system such as CD4 T lymphocytes. [7] The

immunology of asthma is complex and the disease heterogeneous – we are increasingly aware that multiple independent pathological mechanisms can lead to asthmatic inflammation and that the relative contribution of the pathways will differ from individual to individual patient. These pathways interact and subdivisions described below are to aid discussion. Importantly, vitamin D has beneficial actions at multiple steps in all these proposed pathways. Furthermore, chronic uncontrolled asthmatic inflammation leads to airway remodelling - this is also reduced by vitamin D, likely through its actions on structural cells, as further discussed below.

Aero-allergen triggered asthma

Classically asthmatic inflammation was thought primarily due to antigen-dependent immune responses to aeroallergens, with Th2 lymphocytes, IgE-secreting B lymphocytes and mast cells featuring prominently in this patho-mechanism (Figure 1). Naive T lymphocytes become inappropriately primed to respond to otherwise-innocuous inhaled aeroallergens with Th2 polarity, and upon re-stimulation by inhaled aeroallergens produce the Th2 cytokines IL-4, IL-5 and IL-13 that promote asthma pathology. IL-4 promotes class-switching of B lymphocytes to IgE production. Aero-allergen specific IgE is produced by these B lymphocytes and coats mast cells. Then on inhalation of aeroallergens the IgE upon mast cells becomes cross-linked leading to rapid release of further pro-inflammatory asthma mediators such as leukotrienes and histamine that cause bronchoconstriction and airway mucus production.

At the core of this mechanism is a failure of suppression of inappropriate antigen-dependent immune responses to aeroallergens. Adaptive immune responses are regulated by diverse classes of regulatory T lymphocyte (Treg), for example Foxp3 expressing and IL-10 secreting Tregs, and their coordinated action in healthy individuals ensures tolerance to non-harmful

147 antigens. There is an abundance of evidence that vitamin D plays a vital role in Treg responses.
148 [8] Our laboratory has previously shown *in vivo* vitamin D status positively correlates with the
149 frequency of Foxp3+ Tregs [9] and airway levels of IL-10 [10] in asthma patients. *In vitro*
150 $1,25(OH)_2D3$ promotes distinct IL-10 and Foxp3 expressing CD4+ T cell populations, which is
151 influenced by the local cytokine milieu. [9] In addition to Tregs, adaptive immune responses are
152 also regulated through other mechanisms. In particular, the quality of antigen presentation
153 and expression of co-stimulatory signals by dendritic cells (DCs) is important in determining
154 tolerogenic versus inflammatory immune responses. Multiple facets of DC activity are
155 regulated by vitamin D, including antigen presentation and co-stimulatory signals, to promote
156 tolerogenic DCs. [11]
157
158 As well as promoting appropriate antigen tolerance, vitamin D also modulates other aspects of
159 allergen-stimulated immune responses. Vitamin D can suppress production of IgE by human B
160 lymphocytes *in vitro* and increase IL-10 production, promoting a regulatory B lymphocyte
161 phenotype. [12 13] Notably, in children vitamin D deficiency is associated with increased levels of
162 aeroallergen specific IgE. [14 15] Additionally, vitamin D has been shown to have the capacity to
163 suppress mast cell activation, reducing histamine and TNFα release for example, including in
164 the context of IgE-dependent activation. [16 17] Vitamin D can also promote mast cell production
165 of anti-inflammatory IL-10. [18]
166
167 In support of these actions of vitamin D being significant, Heine and colleagues have shown in a
168 ovalbumin-sensitisation model that vitamin D deficient mice have higher levels of ovalbumin-
169 specific IgE. [19] Addition of vitamin D to a desensitisation protocol further reduced airways
170 concentrations of IL-4 and IL-13 post aerosolised ovalbumin challenge, and also further
171 reduced airways hyper-responsiveness. [19]

Epithelial damage and Alarmin cytokine elicited asthmatic inflammation

There is increasing interest in the mechanisms underpinning non-allergic asthma. Epithelial damage is now understood to prompt release of cytokines known as Alarmins – e.g. IL-25, IL-33 and TSLP – that directly stimulate multiple cell types including type-2 innate lymphoid cells (ILC2s) and mast cells. [20] These stimulated ILC2s then produce Th2-type cytokines including IL-5, which in turn promotes eosinophilic inflammation (Figure 2). Of these Alarmins, IL-33 appears particularly important in asthma with both its gene (*IL33*) and the gene for its receptor (*1L1RL1*) identified in asthma GWAS studies. [21]

Vitamin D has been shown to modulate the epithelial response to stimulation with a potentially anti-inflammatory role for this action. [22] However, of specific relevance to asthma is the capacity of vitamin D to stimulate bronchial epithelial cell production of sST2, a soluble decoy blocker for IL-33, and for this epithelial-produced sST2 to decrease the pro-inflammatory effect of IL-33 on target cells such as mast cells. [23]

There is relatively little evidence as to the effect of vitamin D on ILC2s and eosinophils. Ethier *et al.* have shown vitamin D to be able to enhance eosinophil viability with reduced production of pro-inflammatory necrotic granules. [24] Ruiter and colleagues have shown vitamin D to reduce pro-inflammatory cytokine production by stimulated ILC2s. [25]

Viral infections in particular trigger epithelial IL-33 release, [26] which is important given that Th2-biased asthmatic pathology has been shown to impair appropriate anti-viral immune responses. [27] Indeed asthma exacerbations are frequently associated with viral respiratory tract infections. Vitamin D, however, enhances antimicrobial immune responses through many

197 mechanisms. It enhances cellular production of antimicrobial peptides (such as cathelicidin)
198 and autophagy, important in the response to both bacterial and viral infections. [28] [29] [30]
199 Consistent with these actions of vitamin D, meta-analysis has shown vitamin D
200 supplementation of appropriate patients to reduce acute respiratory tract infections. [31]
201
202 **Steroid-resistant asthma and IL-17**
203 In a proportion of patients with the most severe asthma the pathology appears to have some
204 distinct features, in particular corticosteroid-resistance and an apparent pathological role for
205 IL-17. Multiple pathways to steroid-resistance in airways disease have been described. [32]
206 Airway colonisation with pro-inflammatory bacteria such as Haemophilus influenza, oxidative
207 stress (for example from air pollution) and vitamin D deficiency itself are major contenders for
208 causing acquired steroid-resistance in asthma. Vitamin D enhances anti-microbial pathways
209 and vitamin D also promotes anti-oxidant responses. [33] Additionally vitamin D ameliorates
210 steroid-resistant inflammation through its direct actions on lymphocytes (as discussed below)
211 and monocytes. [34] Steroid-insensitive asthmatics have impaired steroid-induced production
212 of anti-inflammatory IL-10. *In vitro* vitamin D enhances steroid-induced lymphocyte
213 production of IL-10 and supplementation of steroid-resistant asthmatic patients with the
214 calcitriol form of vitamin D restores both the clinical and immunological IL-10 response to
215 corticosteroids. [35] [36] [37] Furthermore, we have shown that corticosteroids actually promote in
216 steroid-resistant individuals production of IL-17, thought in this context to cause pathological
217 neutrophilic inflammation, with amelioration after vitamin D supplementation. [37]
218 Subramanian and colleagues have recently reported vitamin D to reduce production of pro-
219 inflammatory cytokines by stimulated neutrophils. [38] However vitamin D did enhance the
220 anti-bacterial activity of neutrophils, via enhanced production of antimicrobial peptides rather
221 than increased production of elastase or reactive oxygen species. [38] These findings are

222 consistent with earlier findings of vitamin D responsiveness of neutrophils reported by
223 Takahashi and colleagues. [39]

224

225 **Vitamin D and airway remodelling**

226 The downstream effect of asthmatic immune responses, regardless of the mechanisms
227 precipitating those responses, is airway narrowing. Over the short-term this is due to smooth
228 muscle constriction and mucus secretion, whilst over the longer-term airway remodelling and
229 fibrosis occur. Importantly, vitamin D has actions on airway smooth muscle including
230 inhibiting airway smooth muscle cell proliferation. [40] [41] Consistent with this Gupta and
231 colleagues have previously shown airway smooth muscle volume fraction in endobronchial
232 biopsies to negatively correlate with serum vitamin D concentrations in steroid-refractory
233 severe paediatric patients with asthma. [14]

234

235 **Randomised controlled trials (RCTs) of vitamin D to treat asthma**

236 Over the last few years several randomised controlled clinical trials of vitamin D therapies to
237 improve asthma control have completed and published their findings. These studies of vitamin
238 D as secondary prevention to reduce asthma exacerbations have reported a mixture of positive
239 and negative results in their primary outcome measures with, nevertheless, multiple positive
240 secondary outcome measures in some trials with negative primary outcomes. For example, the
241 VIDA trial (Castro *et al.*), despite a negative primary outcome of time to first exacerbation,
242 found in a responder analysis that for each 10ng/ml increase in serum 25(OH)D there was a
243 significant reduction in the rate of treatment failures and of exacerbations. [42] Furthermore, in
244 a recent Cochrane Review of vitamin D for secondary prevention to reduce exacerbations in
245 asthmatic patients, meta-analysis shows vitamin D supplementation to significantly reduce the
246 rate of severe exacerbations in asthmatic patients. [43]

One possible explanation for the divergent results is under-powering in some studies and smaller effect sizes than anticipated. However, we believe that differences and limitations in study design partly explain the differences in study outcome (see Table 1). These methodological issues in vitamin D treatment trials are not specific to asthma and are worthy of detailed discussion.

254 **Table 1: Summary of major vitamin D secondary prevention RCTs in asthma**

255 ACT; Asthma Control Test. ATAQ; Asthma Therapy Assessment Questionnaire. FEV1; Forced

256 Expiratory Volume in 1 second.

257

Trial	
Study Design: Population & Intervention	Primary (1ry) and major Secondary (2ndry) Outcomes
Majak *et al.* 2011 [44]	
Population: 48 children (5-18 years old) with newly diagnosed asthma. Intervention: 500 IU D3 daily (versus placebo). In addition to inhaled steroid.	Outcomes: Significantly lower percentage of participants experiencing an asthma exacerbation in the D3 treated group. No significant difference in improvements in ATAQ score and FEV1 between the two groups.
Lewis *et al.* 2012 [45]	
Population: 30 children (6-17 years age) with asthma; 97% with serum 25(OH)D < 30 ng/ml. 20 completed study (33% drop-out in both groups). Intervention: 1000 IU D3 daily (versus placebo).	Outcomes: No significant effect of D3 therapy on either ACT score or FEV1.
Yadav *et al.* 2013 [46]	
Population: 100 children (5-13 years old) with moderate to severe asthma. Intervention: 60 000 IU D3 per month or placebo.	1ry Outcome: Significantly greater improvement in severity of asthma according to GINA guidelines at 6 months in D3 treated group. 2ndry Outcome: Significantly fewer exacerbations in the D3 treated group.
Castro *et al.* 2014 (VIDA Study) [42]	
Population: 408 adult asthmatics all with serum 25(OH)D < 30 ng/ml. Intervention: Placebo versus 100 000 IU D3 bolus dose followed by 4000 IU daily.	1ry Outcome: No significant effect on rate of first asthma treatment failure. 2ndry Outcomes: No significant effect on rate of first asthma exacerbation. Significantly greater taper of inhaled

Tapering inhaled steroids dependent on asthma control in both groups.	steroids in the D3 treated group. In a vitamin D3 responder analysis: Rate of first exacerbation and overall exacerbation rate were both significantly lower in the D3 incremented subgroup.
Martineau *et al.* 2015 (ViDiAs Study) [47]	
Population: 250 adult asthmatics; 82% with serum 25(OH)D < 75 nmol/l and 58% with serum 25(OH)D < 50 nmol/l. Intervention: 120 000 IU D3 every two months (versus placebo).	1ry Outcome: No significant effect of D3 treatment on time to first severe asthma exacerbation or time to first upper respiratory infection. No effect modification of baseline vitamin D status on either co-primary outcome.
Tachimoto *et al.* 2016 [48]	
Population: 89 schoolchildren (6-15 years age) with asthma. Median average serum 25(OH)D 29 ng/ml. Intervention: 800 IU D3 /day or placebo.	1ry Outcome: Significantly better changes in asthma control levels as defined by GINA in D3 treated arm.

258

The first issue is whether vitamin D supplementation is beneficial in all patients or only beneficial where it corrects low vitamin D levels in deficient patients. Unlike in trials of pharmacologic drugs, in trials of vitamins patients at baseline have variable circulating levels of the vitamins and will have variable natural intake over the course of the trial through their normal activities of daily living. Subgroup analyses in two studies of vitamin D as a treatment for COPD have shown significant benefit from vitamin D supplementation in the subgroups of patients with marked vitamin D insufficiency (serum 25(OH)D levels < 10ng/ml and < 50 nmol/l respectively for the two studies). [49] [50] Similarly Martineau and colleagues have reported a significant interaction between baseline vitamin D status and benefit of supplementation in reducing acute respiratory tract infections, with greatest benefit in those with serum 25(OH)D < 25 nmol/l. [31] A major limitation of some studies may therefore be that many participants were relatively vitamin D sufficient at baseline and less likely to benefit.

Not all supplemented patients in the treatment arms of these trials actually achieved vitamin D sufficiency during the trials. However, that supplementation does achieve vitamin D sufficiency appears an important determinant of benefit. In the VIDA trial approximately one fifth of participants in the vitamin D3 treatment arm did not achieve a serum 25(OH)D3 >= 30 ng/ml (equivalent to approximately 75 nmol/l). Although the rate of exacerbations was not significantly decreased in the overall treatment group compared to placebo, in an exploratory responder analysis when those not achieving a 25(OH)D3 >= 30 ng/ml in the treatment arm were excluded then there was a significant reduction in exacerbations in the treatment arm compared to placebo. [42]

Furthermore, the optimum vitamin D level in asthma is unclear. A serum 25(OH)D3 target concentration of 75 nmol/l (30ng/ml) is often quoted but this is based on inferences from the actions of vitamin D in calcium-phosphate homeostasis. [51] The responder analysis from the

284 VIDA trial is consistent with this target, [42] however, that does not mean that this is necessarily
285 the optimum target vitamin D level in asthma or for general health. There are both proponents
286 of lower target vitamin D concentrations and high target serum levels (such as 100nmol/l
287 40ng/ml). [51 52] In support of the latter are the higher physiological ranges of vitamin D seen in
288 some populations and emerging evidence from vitamin D trials in other medical fields. [53]
289 However, although in clinical practice we assay serum 25(OH)D concentrations as a measure of
290 patient vitamin D status, whether this is the most appropriate form to measure is uncertain.
291 Vitamin D has a complicated metabolic pathway and it is possible that levels of a precursor or
292 other metabolite are more important. [54] Additionally different actions of vitamin D may
293 require different threshold concentrations of serum vitamin D. Finally, the bioavailability of
294 circulating vitamin D metabolites is dependent on other factors such as circulating vitamin D
295 binding protein (VDBP), and polymorphisms in VDBP and other vitamin D axis genes may
296 affect the supplementation dose necessary to achieve functional vitamin D sufficiency. [55]
297
298 Secondly, the correct form of vitamin D to give as a supplement and correct dosing strategy is
299 uncertain (reviewed in detail by Hollis and Wagner, 2013 [54]). In particular, bolus vitamin D
300 supplementation (as used in some asthma studies) may be immunologically problematic,
301 causing large swings in serum levels and inducible homeostatic enzymes, with dysregulation of
302 other vitamin D regulated pathways. [56] Indeed a recent meta-analysis of trials of vitamin D to
303 prevent acute respiratory tract infections found regimens with regular vitamin D dosing to be
304 associated with a protective action of vitamin D but regimens with high-dose boluses to not
305 show protective effect. [31] The commonest forms of vitamin D to give as supplementation are
306 ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3) – although there is little research
307 in to ergocalciferol supplementation in asthma by extrapolating from other diseases
308 cholecalciferol is more likely to be beneficial than ergocalciferol. [57] Finally, correction of

vitamin D insufficiency through increased exposure to ultraviolet radiation may be more physiological and more effective in asthma than oral supplementation. [58]

Thirdly, vitamin D supplementation may be more beneficial in paediatric asthma [44 48] than in adult asthma. [42 47] Notably although Sharief and colleagues found significant associations between serum 25(OH)D and IgE sensitisation to multiple allergens in children and adolescents, significant associations were much fewer in adults. [15] The recent Cochrane Review of vitamin D as secondary prevention in asthma did not directly address this question, however the recent closely-related systematic review of vitamin D to prevent acute respiratory tract infections did not find a significant interaction between age and benefit of vitamin D. [31] A paediatric versus adult difference in benefit of vitamin D could relate to immunopathological differences in childhood onset, typically atopic asthma, and adult asthma. Different immunological pathways are likely to be of prime importance in childhood-onset asthma compared to adult-onset asthma, and vitamin D may have less effect on the pathways of prime importance in adult-onset asthma. Alternatively, differences in the capacity of vitamin D to regulate immune responses in children and adults may perhaps reflect establishment of epigenetic responses to chronic vitamin D insufficiency in adults after years of vitamin D deficiency, leading to these individuals having less capacity to respond to vitamin D therapies.[59]

Fourthly whilst the above mechanistic studies show the capacity of vitamin D to beneficially modulate the different pathways thought to underlie different asthma endotypes, it is possible that some of these actions of vitamin D have little clinical efficacy. Therefore vitamin D therapies may be particularly beneficial in discrete endotypes of asthma. The larger RCTs have

not selected for specific asthma endotypes, however, we have shown evidence for a particular role for vitamin D therapy in a severe steroid-resistant asthma endotype. [36 37]

Further large studies of daily vitamin D supplementation to improve asthma control in vitamin D deficient asthmatic children and adults are needed to better understand these issues. Nevertheless, there is increasing realisation that vitamin D deficiency negatively impacts on general health status and that vitamin D supplementation rarely has side-effects. Therefore, optimising vitamin D status is increasingly becoming part of standard holistic clinical care in asthma (see Box 1 for Clinical Recommendations).

Box 1: Clinical Recommendations

CLINICAL RECOMMENDATIONS

- Daily supplementation of vitamin D deficient and insufficient patients is safe and has positive benefit to health in asthma and other diseases, but infrequent high-dose bolus supplementation should be avoided.
- Supplementation should be given as Vitamin D3 (cholecalciferol).
- The exact serum 25(OH)D concentration below which supplementation should be started and best dosing regimens remain to be established. We would suggest supplementing those with serum 25(OH)D < 50 nmol/l and starting with a dose of 1000 – 4000 IU/day depending on baseline concentration. Serum vitamin D should be reviewed after a few months of supplementation and if vitamin D sufficiency has not been achieved then the supplementation dose should be increased in order to achieve vitamin D sufficiency.
- Serum vitamin D concentrations should be checked in women planning pregnancy pre-conception and also in all pregnant women, with daily high-dose supplementation given during pregnancy. In those planning pregnancy sufficient vitamin D supplementation should be given to rapidly achieve vitamin D sufficiency.

363

364

365 **Early life vitamin D and primary prevention of asthma**

366 Vitamin D deficiency and insufficiency is extremely common in pregnancy, and is associated
367 with preeclampsia, gestational diabetes mellitus and other co-morbidities of pregnancy. [60] It
368 has also been associated with increased respiratory infections, and asthma in the newborn and
369 children in many studies. [61] Vitamin D is proposed to beneficially impact fetal and
370 neonatal lung maturation, as well as immunity in a manner likely to promote maternal-fetal
371 tolerance, and to protect mother and fetus from infection. [61 62] Importantly these associations
372 between maternal vitamin D insufficiency and asthma in the offspring cannot be explained as
373 due to reverse causation although an effect of unmeasured confounding variables in these
374 studies is possible.

375 The mechanisms by which early life vitamin D could help reduce development of asthma
376 include (i) its capacity to support development of tolerogenic immune responses (as discussed
377 above), (ii) facilitating appropriate antiviral and antibacterial immune responses, (iii)
378 enhancing barrier properties of the epidermis and decreasing eczema, and (iv) enhancing
379 appropriate lung development – all factors important in determining whether an individual
380 develops symptomatic chronic airway disease. [63] In particular *in utero* vitamin D has been
381 shown to affect expression of genes important in early pulmonary development (for example
382 branching morphogenesis) and to influence multiple aspects of later lung development
383 including alveolar development and surfactant secretion. [62 64] These vitamin D regulated
384 developmental processes occur at different times in gestation suggesting multiple vital
385 windows for vitamin D actions at different stages of gestation. Furthermore, as vitamin D
386 research continues an increasing number of asthma-relevant development processes are being
387 revealed to be influenced by vitamin D, for example recent evidence for a likely influence of *in*

utero vitamin D status on the early-life microbiome. [65]

These data, with mechanistic evidence discussed above, has now led to randomised controlled trials of high-dose vitamin D supplementation as primary prevention to avert the development of recurrent wheeze and asthma in offspring.

Two large RCT, using doses of vitamin D known to significantly increase vitamin D status of pregnant women, [61] have recently reported similar and encouraging effects on infant respiratory outcomes. In the COPSAC study, Chawes *et al.*, supplemented pregnant women with 2800 IU/d vitamin D3 in the third trimester of pregnancy, and reported a reduction in episodes of troublesome lung symptoms in the offspring through 3-years though no significant effect on risk of persistent wheeze. [66] In the VDAART trial, Litonjua *et al.*, supplemented with 4400 IU/d vitamin D3 during the second and third trimester, reporting a significant reduction in allergic sensitizations and a trend for a reduction in recurrent wheeze and asthma in offspring through 3-years. [67] Importantly, no increase in adverse effects were reported in these or other comparable pregnancy studies. [60 66 67]

In an ancillary study of cord blood samples from a subset of babies born to women within the VDAART cohort, Hornsby and colleagues show supplementation to significantly enhance pro-inflammatory cytokine responses to mitogen and TLR-agonist stimulation, increase *TLR2* and *TLR9* gene expression, and IL-17A secretion in response to T cell receptor-ligation. [68] Thus, vitamin D supplementation during pregnancy modifies the immune system of the neonate in a manner that is predicted to protect the host against pathogenic infections and reduce development of asthma.

However, similar methodological issues exist with primary prevention trials of vitamin D to those discussed above with respect to secondary prevention trials. Stratification of response by baseline vitamin D status is evident - in secondary analyses of the VDAART study infants born to high-dose supplemented mothers who had circulating levels of vitamin D of 30ng/ml

413 or higher at recruitment (sufficiency range) demonstrated a striking and significant reduction
414 in recurrent wheeze and asthma through 3-years. However, infants of high-dose
415 supplemented mothers who were vitamin D deficient at baseline (10-18 weeks gestation) did
416 not have the same significant benefit from randomisation to high-dose vitamin D. This
417 indicates the likelihood of important effects of vitamin D *throughout* neonatal development,
418 likely to include bone, pulmonary and immune effects (as discussed above), and including
419 during the first trimester. [69] It may also suggest that maternal vitamin D deficiency leads to
420 epigenetic changes in the offspring resulting in relative vitamin D resistance. [59] Therefore
421 achieving vitamin D sufficiency needs to be a clinical priority pre-conception. High-dose
422 supplementation in both these trials did not include what appears to be a critical window for
423 vitamin D actions in early pregnancy / pre-conception. Notably this influence of baseline
424 vitamin D status on clinical benefit of vitamin D supplementation to prevent development of
425 asthma is opposite to that noted in the secondary prevention RCTs discussed above.
426 Secondly, similar to the secondary prevention trials, not all patients in these primary
427 prevention trials achieved vitamin D sufficiency despite high-dose supplementation (75% in
428 the VDAART trial and 81% in the COPSAC trial). [67] [66] This underscores the likelihood that
429 different individuals require different doses of vitamin D to achieve sufficiency, however, both
430 trials did note a significant proportion of participants not to have been adequately adherent to
431 the vitamin D intervention. Previously infrequent bolus dosing of vitamin D supplements has
432 been suggested as a strategy to improve adherence but as we discuss above such infrequent
433 bolus dosing may be immunologically problematic.
434 It is also too early to determine whether high-dose vitamin D supplementation in these
435 primary prevention studies has successfully reduced the proportion of children going on to
436 develop asthma – reported follow-up to date is for up to age 3 whereas asthma is often
437 diagnosed slightly later in childhood. Long-term follow-up of these patients is eagerly awaited.

The longitudinal effects of vitamin D during the first 10 years of life were investigated in a high-risk Australian cohort in order to determine relationships between 25(OH)D levels and susceptibility to allergic sensitization, respiratory tract infections, and asthma. [70] The study showed that 25(OH)D deficiency in early childhood is associated with increased risk for persistent asthma. The authors proposed that vitamin D may act by influencing susceptibility to early allergic sensitization, and/or upper respiratory tract colonization with bacterial pathogens, or both.

Together these very recent studies bring real enthusiasm to investigate strategies that restore vitamin D sufficiency in pregnancy and the first years of life, which offer the potential to reduce both important asthma risk factors, namely allergic sensitizations and respiratory tract infections, as well as asthma itself. A future challenge will be to determine safe and effective regimens of vitamin D3 supplementation in the newborn and infant.

Conclusions

There is a wealth of epidemiological evidence of a detrimental association between vitamin D insufficiency and asthma, and translational studies have shown vitamin D to have the capacity to beneficially regulate diverse aspects of asthma pathology. Methodological considerations with vitamin D randomised controlled trials to-date may underlie less impressive than hoped clinical effects in the trials. Further clinical trials, with revised protocols learning from the issues discussed above, are needed. Nevertheless, the evidence-base increasingly supports vitamin D supplementation being a safe, practical and beneficial part of the comprehensive management of asthma.

Figure Legends

Figure 1: Illustrative schematic of the mechanistic pathway of aero-allergen stimulated asthmatic inflammation, and steps at which vitamin D acts.

Vitamin D can act beneficially at multiple steps along the pathway to prevent / reduce asthmatic inflammation.

Figure 2: Schematic for non-allergic eosinophilic asthmatic inflammation.

Epithelial injury, for instance from proteases or viral infection, stimulates epithelial release of Alarmin cytokines such as IL-25 and IL-33. These cytokines act on immune cells such as ILC2s and mast cells to elicit production of Th2-type cytokines that stimulate eosinophilic inflammation. Vitamin D can inhibit this pathway at multiple steps.

Figure 3: Diverse stimuli can elicit steroid-resistant asthmatic inflammation

Th17-mediated neutrophilic airways inflammation can follow diverse precipitants such as oxidative stress that are sensitive to modulation by vitamin D. Furthermore, Th17 lymphocytes and neutrophils themselves are also beneficially sensitive to vitamin D.

References

1. Holick MF. Vitamin D deficiency. *N Engl J Med.* 2007;357:266-281.
2. Chun RF, Liu PT, Modlin RL, Adams JS, Hewison M. Impact of vitamin D on immune function: lessons learned from genome-wide analysis. *Frontiers in physiology.* 2014;5:151.
3. Hypponen E, Power C. Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. *Am J Clin Nutr.* 2007;85:860-868.
4. Mann EH, Chambers ES, Pfeffer PE, Hawrylowicz CM. Immunoregulatory mechanisms of vitamin D relevant to respiratory health and asthma. *Ann N Y Acad Sci.* 2014;1317:57-69.
5. Reid D, Toole BJ, Knox S, et al. The relation between acute changes in the systemic inflammatory response and plasma 25-hydroxyvitamin D concentrations after elective knee arthroplasty. *Am J Clin Nutr.* 2011;93(5):1006-1011.
6. Brehm JM, Schuemann B, Fuhlbrigge AL, et al. Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. *The Journal of allergy and clinical immunology.* 2010;126(1):52-58 e55.

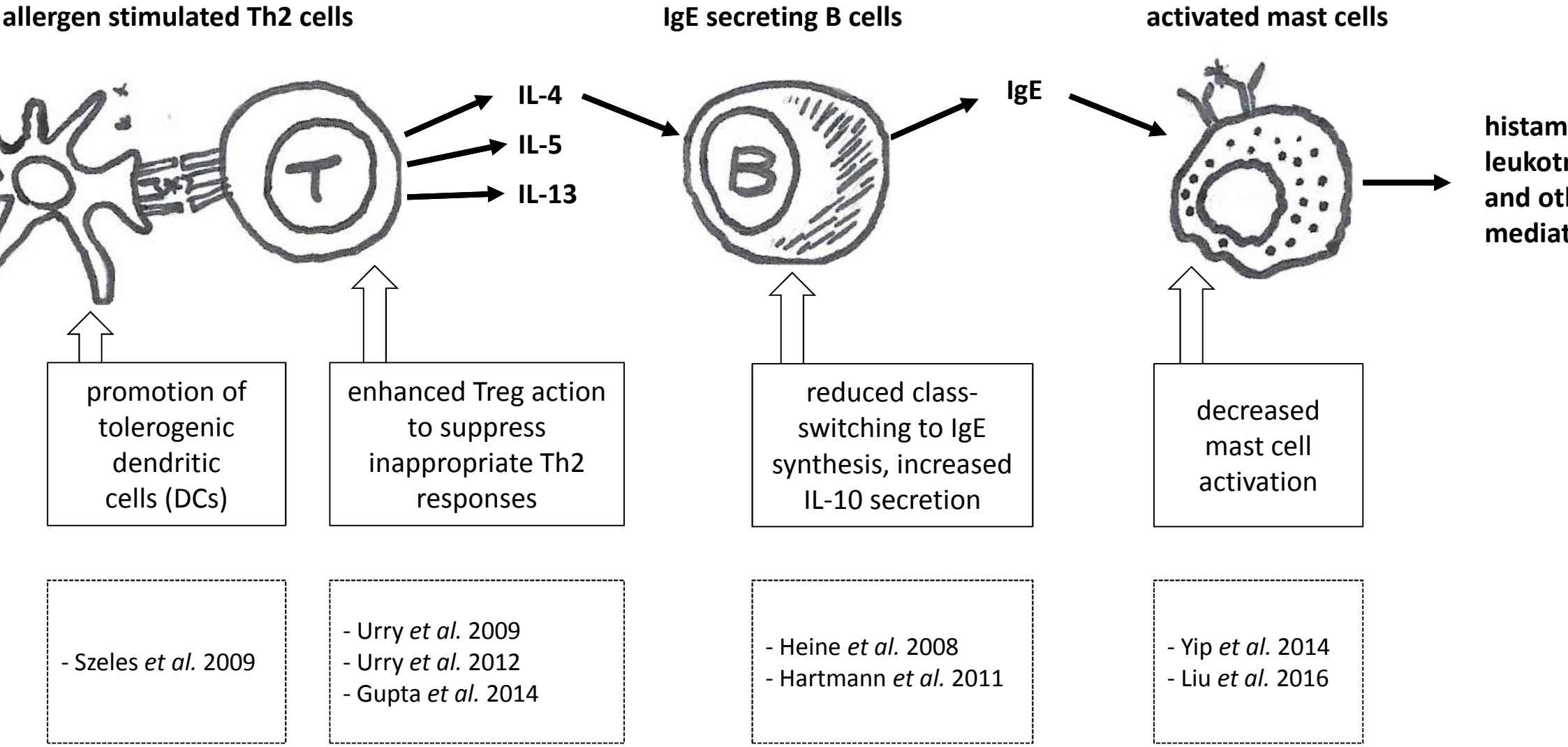
7. Handel AE, Sandve GK, Disanto G, et al. Vitamin D receptor ChIP-seq in primary CD4+ cells: relationship to serum 25-hydroxyvitamin D levels and autoimmune disease. *BMC Medicine.* 2013;11:163.
8. Chambers ES, Hawrylowicz CM. The impact of vitamin D on regulatory T cells. *Current allergy and asthma reports.* 2011;11(1):29-36.
9. Urry Z, Chambers ES, Xystrakis E, et al. The role of 1alpha,25-dihydroxyvitamin D3 and cytokines in the promotion of distinct Foxp3(+) and IL-10(+) CD4(+) T cells. *European journal of immunology.* 2012.
10. Gupta A, Dimeloe S, Richards DF, et al. Defective IL-10 expression and in vitro steroid-induced IL-17A in paediatric severe therapy-resistant asthma. *Thorax.* 2014;69(6):508-515.
11. Szeles L, Keresztes G, Torocsik D, et al. 1,25-dihydroxyvitamin D3 is an autonomous regulator of the transcriptional changes leading to a tolerogenic dendritic cell phenotype. *Journal of immunology.* 2009;182(4):2074-2083.
12. Hartmann B, Heine G, Babina M, et al. Targeting the vitamin D receptor inhibits the B cell-dependent allergic immune response. *Allergy.* 2011;66(4):540-548.
13. Heine G, Niesner U, Chang HD, et al. 1,25-dihydroxyvitamin D(3) promotes IL-10 production in human B cells. *European journal of immunology.* 2008;38(8):2210-2218.
14. Gupta A, Sjoukes A, Richards D, et al. Relationship between Serum Vitamin D, Disease Severity, and Airway Remodeling in Children with Asthma. *American journal of respiratory and critical care medicine.* 2011;184(12):1342-1349.
15. Sharief S, Jariwala S, Kumar J, Muntner P, Melamed ML. Vitamin D levels and food and environmental allergies in the United States: results from the National Health and Nutrition Examination Survey 2005-2006. *The Journal of allergy and clinical immunology.* 2011;127(5):1195-1202.
16. Liu ZQ, Li XX, Qiu SQ, et al. Vitamin D contributes to mast cell stabilization. *Allergy.* 2016.
17. Yip KH, Kolesnikoff N, Yu C, et al. Mechanisms of vitamin D(3) metabolite repression of IgE-dependent mast cell activation. *The Journal of allergy and clinical immunology.* 2014;133(5):1356-1364, 1364 e1351-1314.
18. Biggs L, Yu C, Fedoric B, Lopez AF, Galli SJ, Grimbaldeston MA. Evidence that vitamin D(3) promotes mast cell-dependent reduction of chronic UVB-induced skin pathology in mice. *The Journal of experimental medicine.* 2010;207(3):455-463.
19. Heine G, Tabeling C, Hartmann B, et al. 25-Hydroxvitamin D3 Promotes the Long-Term Effect of Specific Immunotherapy in a Murine Allergy Model. *The Journal of Immunology.* 2014;193(3):1017-1023.
20. Barrett NA, Austen KF. Innate cells and T helper 2 cell immunity in airway inflammation. *Immunity.* 2009;31(3):425-437.
21. Grotenboer NS, Ketelaar ME, Koppelman GH, Nawijn MC. Decoding asthma: translating genetic variation in IL33 and IL1RL1 into disease pathophysiology. *The Journal of allergy and clinical immunology.* 2013;131(3):856-865.
22. Hansdottir S, Monick MM, Lovan N, Powers L, Gerke A, Hunninghake GW. Vitamin D decreases respiratory syncytial virus induction of NF-kappaB-linked chemokines and cytokines in airway epithelium while maintaining the antiviral state. *Journal of immunology.* 2010;184(2):965-974.
23. Pfeffer PE, Chen YH, Woszczek G, et al. Vitamin D enhances production of soluble ST2, inhibiting the action of IL-33. *The Journal of allergy and clinical immunology.* 2015;135(3):824-827 e823.

24. Ethier C, Yu Y, Cameron L, Lacy P, Davoine F. Calcitriol Reduces Eosinophil Necrosis Which Leads to the Diminished Release of Cytotoxic Granules. *International archives of allergy and immunology.* 2016;171(2):119-129.
25. Ruiter B, Patil SU, Shreffler WG. Vitamins A and D have antagonistic effects on expression of effector cytokines and gut-homing integrin in human innate lymphoid cells. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology.* 2015;45(7):1214-1225.
26. Jackson DJ, Makrinioti H, Rana BM, et al. IL-33-dependent type 2 inflammation during rhinovirus-induced asthma exacerbations in vivo. *American journal of respiratory and critical care medicine.* 2014;190(12):1373-1382.
27. Contoli M, Ito K, Padovani A, et al. Th2 cytokines impair innate immune responses to rhinovirus in respiratory epithelial cells. *Allergy.* 2015;70(8):910-920.
28. Liu PT, Stenger S, Li H, et al. Toll-like receptor triggering of a vitamin D mediated human antimicrobial response. *Science.* 2006;311:1770-1773.
29. Beard JA, Bearden A, Striker R. Vitamin D and the anti-viral state. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology.* 2011;50(3):194-200.
30. Fabri M, Stenger S, Shin DM, et al. Vitamin D is required for IFN-gamma-mediated antimicrobial activity of human macrophages. *Science translational medicine.* 2011;3(104):104ra102.
31. Martineau AR, Jolliffe DA, Hooper RL, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *Bmj.* 2017;356:i6583.
32. Barnes PJ. Corticosteroid resistance in patients with asthma and chronic obstructive pulmonary disease. *The Journal of allergy and clinical immunology.* 2013;131(3):636-645.
33. Lan N, Luo G, Yang X, et al. 25-hydroxyvitamin D3-deficiency enhances oxidative stress and corticosteroid resistance in severe asthma exacerbation. *PloS one.* 2014;9(11):e111599.
34. Zhang Y, Leung DY, Goleva E. Anti-inflammatory and corticosteroid-enhancing actions of vitamin D in monocytes of patients with steroid-resistant and those with steroid-sensitive asthma. *The Journal of allergy and clinical immunology.* 2014;133(6):1744-1752 e1741.
35. Xystrakis E, Kusumakar S, Boswell S, et al. Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. *The Journal of clinical investigation.* 2006;116(1):146-155.
36. Nanzer AM, Chambers ES, Ryanna K, et al. The effects of calcitriol treatment in glucocorticoid-resistant asthma. *The Journal of allergy and clinical immunology.* 2014;133(6):1755-1757 e1754.
37. Chambers ES, Nanzer AM, Pfeffer PE, et al. Distinct endotypes of steroid-resistant asthma characterized by IL-17A and IFN-gamma immunophenotypes: Potential benefits of calcitriol. *The Journal of allergy and clinical immunology.* 2015.
38. Subramanian K, Bergman P, Henriques-Normark B. Vitamin D Promotes Pneumococcal Killing and Modulates Inflammatory Responses in Primary Human Neutrophils. *Journal of innate immunity.* 2017.
39. Takahashi K, Nakayama Y, Horiuchi H, et al. Human neutrophils express messenger RNA of vitamin D receptor and respond to 1alpha,25-dihydroxyvitamin D3. *Immunopharmacology and immunotoxicology.* 2002;24(3):335-347.

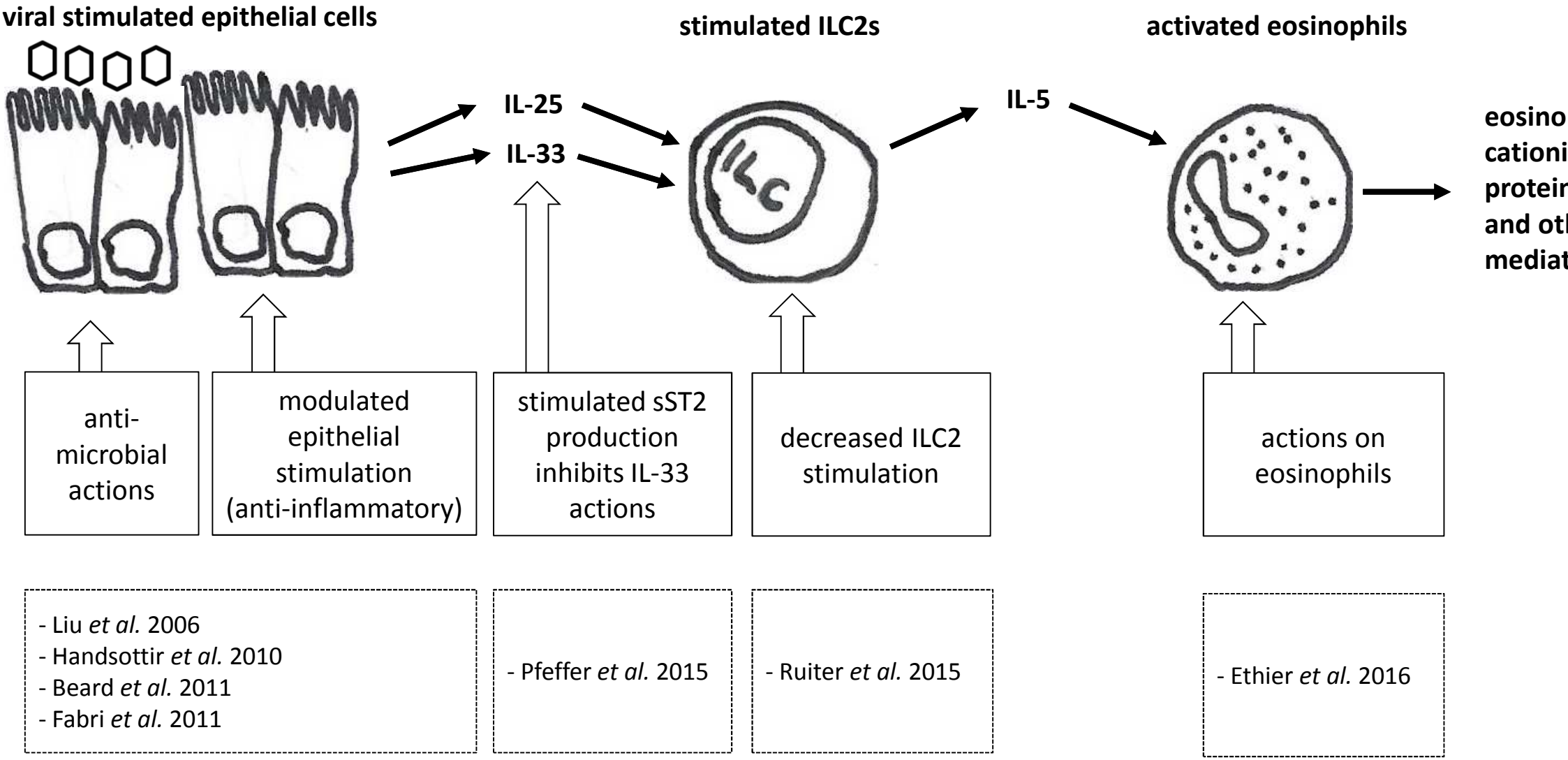
40. Banerjee A, Panettieri R, Jr. Vitamin D modulates airway smooth muscle function in COPD. *Current opinion in pharmacology.* 2012;12(3):266-274.
41. Damera G, Fogle HW, Lim P, et al. Vitamin D inhibits growth of human airway smooth muscle cells through growth factor-induced phosphorylation of retinoblastoma protein and checkpoint kinase 1. *British journal of pharmacology.* 2009;158(6):1429-1441.
42. Castro M, King TS, Kunselman SJ, et al. Effect of vitamin D3 on asthma treatment failures in adults with symptomatic asthma and lower vitamin D levels: the VIDA randomized clinical trial. *Jama.* 2014;311(20):2083-2091.
43. Martineau AR, Cates CJ, Urashima M, et al. Vitamin D for the management of asthma. *The Cochrane database of systematic reviews.* 2016;9:CD011511.
44. Majak P, Olszowiec-Chlebna M, Smejda K, Stelmach I. Vitamin D supplementation in children may prevent asthma exacerbation triggered by acute respiratory infection. *The Journal of allergy and clinical immunology.* 2011;127(5):1294-1296.
45. Lewis E, Fernandez C, Nella A, Hopp R, Gallagher JC, Casale TB. Relationship of 25-hydroxyvitamin D and asthma control in children. *Ann Allergy Asthma Immunol.* 2012;108:280-287.
46. Yadav M, Mittal K. Effect of vitamin D supplementation on moderate to severe bronchial asthma. *Indian journal of pediatrics.* 2014;81(7):650-654.
47. Martineau AR, MacLaughlin BD, Hooper RL, et al. Double-blind randomised placebo-controlled trial of bolus-dose vitamin D3 supplementation in adults with asthma (ViDiAs). *Thorax.* 2015;70(5):451-457.
48. Tachimoto H, Mezawa H, Segawa T, Akiyama N, Ida H, Urashima M. Improved control of childhood asthma with low-dose, short-term vitamin D supplementation: a randomized, double-blind, placebo-controlled trial. *Allergy.* 2016;71(7):1001-1009.
49. Lehouck A, Mathieu C, Carremans C, et al. High Doses of Vitamin D to Reduce Exacerbations in Chronic Obstructive Pulmonary Disease. *Ann Intern Med.* 2012;156:105-114.
50. Martineau AR, James WY, Hooper RL, et al. Vitamin D3 supplementation in patients with chronic obstructive pulmonary disease (ViDiCO): a multicentre, double-blind, randomised controlled trial. *Lancet Respiratory Medicine.* 2015;3(2):120-130.
51. Alshahrani F, Aljohani N. Vitamin D: deficiency, sufficiency and toxicity. *Nutrients.* 2013;5(9):3605-3616.
52. Manson JE, Brannon PM, Rosen CJ, Taylor CL. Vitamin D Deficiency - Is There Really a Pandemic? *N Engl J Med.* 2016;375(19):1817-1820.
53. McDonnell SL, Baggerly KA, Baggerly CA, et al. Maternal 25(OH)D concentrations >/=40 ng/mL associated with 60% lower preterm birth risk among general obstetrical patients at an urban medical center. *PloS one.* 2017;12(7):e0180483.
54. Hollis BW, Wagner CL. Clinical review: The role of the parent compound vitamin D with respect to metabolism and function: Why clinical dose intervals can affect clinical outcomes. *The Journal of clinical endocrinology and metabolism.* 2013;98(12):4619-4628.
55. Yao P, Sun L, Lu L, et al. Effects of Genetic and Nongenetic Factors on Total and Bioavailable 25(OH)D Responses to Vitamin D Supplementation. *The Journal of clinical endocrinology and metabolism.* 2017;102(1):100-110.
56. Weiss ST, Litonjua AA. Vitamin D dosing for infectious and immune disorders. *Thorax.* 2015;70(10):919-920.
57. Chowdhury R, Kunutsor S, Vitezova A, et al. Vitamin D and risk of cause specific death: systematic review and meta-analysis of observational cohort and randomised intervention studies. *Bmj.* 2014;348:g1903.

643 58. Morgan KA, Mann EH, Young AR, Hawrylowicz CM. ASTHMA - comparing the impact of
644 vitamin D versus UVR on clinical and immune parameters. *Photochemical &*
645 *photobiological sciences : Official journal of the European Photochemistry Association and*
646 *the European Society for Photobiology.* 2017;16(3):399-410.
647 59. Xue J, Schoenrock SA, Valdar W, Tarantino LM, Ideraabdullah FY. Maternal vitamin D
648 depletion alters DNA methylation at imprinted loci in multiple generations. *Clinical*
649 *epigenetics.* 2016;8:107.
650 60. Hollis BW, Wagner CL. Vitamin D and pregnancy: skeletal effects, nonskeletal effects,
651 and birth outcomes. *Calcified tissue international.* 2013;92(2):128-139.
652 61. Hollis BW, Wagner CL. Vitamin D supplementation during pregnancy: Improvements in
653 birth outcomes and complications through direct genomic alteration. *Molecular and*
654 *cellular endocrinology.* 2017.
655 62. Lykkedegn S, Sorensen GL, Beck-Nielsen SS, Christesen HT. The impact of vitamin D on
656 fetal and neonatal lung maturation. A systematic review. *American journal of physiology.*
657 *Lung cellular and molecular physiology.* 2015;308(7):L587-602.
658 63. Saglani S, Bush A. The early-life origins of asthma. *Curr Opin Allergy Clin Immunol.*
659 2007;7(1):83-90.
660 64. Foong RE, Bosco A, Jones AC, et al. The effects of in utero vitamin D deficiency on airway
661 smooth muscle mass and lung function. *American journal of respiratory cell and*
662 *molecular biology.* 2015;53(5):664-675.
663 65. Sordillo JE, Zhou Y, McGeachie MJ, et al. Factors influencing the infant gut microbiome at
664 age 3-6 months: Findings from the ethnically diverse Vitamin D Antenatal Asthma
665 Reduction Trial (VDAART). *The Journal of allergy and clinical immunology.*
666 2017;139(2):482-491 e414.
667 66. Chawes BL, Bonnelykke K, Stokholm J, et al. Effect of Vitamin D3 Supplementation
668 During Pregnancy on Risk of Persistent Wheeze in the Offspring: A Randomized Clinical
669 Trial. *Jama.* 2016;315(4):353-361.
670 67. Litonjua AA, Carey VJ, Laranjo N, et al. Effect of Prenatal Supplementation With Vitamin
671 D on Asthma or Recurrent Wheezing in Offspring by Age 3 Years: The VDAART
672 Randomized Clinical Trial. *Jama.* 2016;315(4):362-370.
673 68. Hornsby E, Pfeffer PE, Laranjo N, et al. Vitamin D supplementation during pregnancy:
674 Effect on the neonatal immune system in a randomized controlled trial. *The Journal of*
675 *allergy and clinical immunology.* 2017.
676 69. Wolsk HM, Harshfield BJ, Laranjo N, et al. Vitamin D supplementation in pregnancy,
677 prenatal 25(OH)D levels, race, and subsequent asthma or recurrent wheeze in offspring:
678 Secondary analyses from the Vitamin D Antenatal Asthma Reduction Trial. *The Journal*
679 *of allergy and clinical immunology.* 2017.
680 70. Hollams EM, Teo SM, Kusel M, et al. Vitamin D over the first decade and susceptibility to
681 childhood allergy and asthma. *The Journal of allergy and clinical immunology.*
682 2017;139(2):472-481 e479.

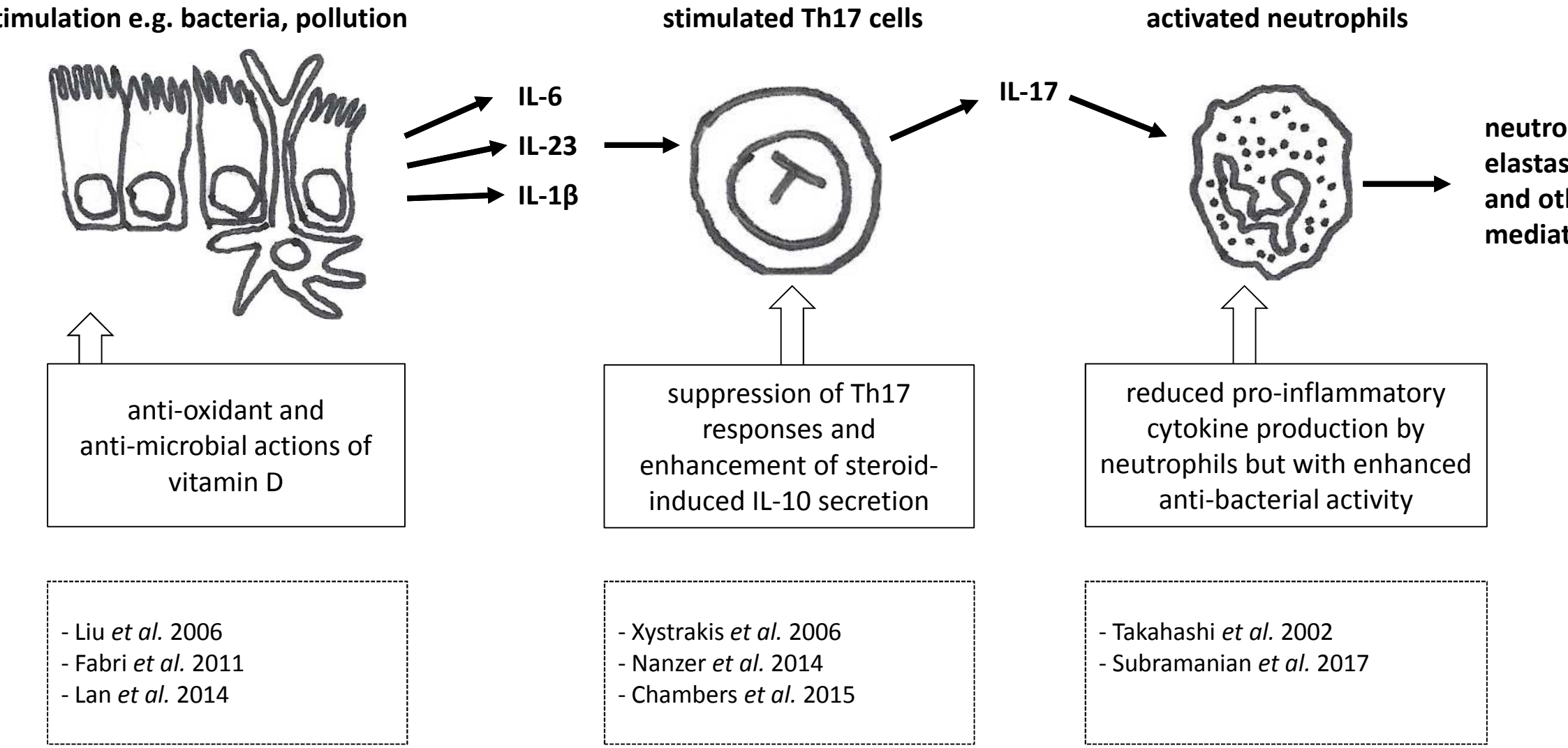
683


allergen stimulated Th2 cells
IgE secreting B cells
activated mast cells
T
IL-4
IL-5
IL-13
B
IgE
histam
leukotr
and ot
mediat
promotion of tolerogenic dendritic cells (DCs)
enhanced Treg action to suppress inappropriate Th2 responses
reduced class-switching to IgE synthesis, increased IL-10 secretion
decreased mast cell activation
- Szeles *et al.* 2009
- Urry *et al.* 2009
- Urry *et al.* 2012
- Gupta *et al.* 2014
- Heine *et al.* 2008
- Hartmann *et al.* 2011
- Yip *et al.* 2014
- Liu *et al.* 2016

gure 1


viral stimulated epithelial cells
stimulated ILC2s
activated eosinophils
IL-25
IL-33
IL-5
ILC
eosino
cationi
protein
and oth
mediat
anti-microbial actions
modulated epithelial stimulation (anti-inflammatory)
stimulated sST2 production inhibits IL-33 actions
decreased ILC2 stimulation
actions on eosinophils
- Liu et al. 2006
- Handsottir et al. 2010
- Beard et al. 2011
- Fabri et al. 2011
- Pfeffer et al. 2015
- Ruiter et al. 2015
- Ethier et al. 2016

gure 2


timulation e.g. bacteria, pollution
stimulated Th17 cells
activated neutrophils
IL-6
IL-23
IL-1β
IL-17
neutro
elastas
and ot
mediat
anti-oxidant and anti-microbial actions of vitamin D
suppression of Th17 responses and enhancement of steroid-induced IL-10 secretion
reduced pro-inflammatory cytokine production by neutrophils but with enhanced anti-bacterial activity
- Liu *et al.* 2006
- Fabri *et al.* 2011
- Lan *et al.* 2014
- Xystrakis *et al.* 2006
- Nanzer *et al.* 2014
- Chambers *et al.* 2015
- Takahashi *et al.* 2002
- Subramanian *et al.* 2017

gure 3