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Inga Wessels, Lothar Rink

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Micronutrients in autoimmune diseases: Possible therapeutic benefits of zinc and vitamin D

Inga Wessels[a], Lothar Rink[a*]

[a]Institute of Immunology, Medical Faculty, RWTH Aachen University, Pauwelsstr. 30, D-52074 Aachen, Germany, iwessels@ukaachen.de; lrink@ukaachen.de

* Corresponding author / Reprint requests:

Lothar Rink, Prof. Dr. rer. nat.

Institute of Immunology

Medical Faculty, RWTH Aachen University

Pauwelsstr. 30

D-52074 Aachen

Germany

Phone: ++49 241 8080209

Fax: ++49 241 8082613

E-mail: lrink@ukaachen.de

Abstract

A functional immune system is essential for healthy life. This is achieved by the coordinate activation and interaction of different immune cells. One should be aware that activation of the immune response is as important as its de-activation when the pathogens are cleared, as otherwise host tissue can be damaged up to life-threatening levels.

Autoimmune diseases (AID) represent a phenomenon of immune cells attacking host cells and tissue. 5 – 8% of the world´s population are currently affected by 80 – 100 AID. In recent years, the incidence has been constantly increasing reaching alarmingly high numbers particularly for type 1 diabetes mellitus, crohn's disease, rheumatoid arthritis, sjogren's syndrome and multiple sclerosis. This indicates a higher societal burden of AID for the future. This article provides an overview of general concepts of triggers and underlying mechanisms leading to self-destruction. Lately, several original concepts of disease etiology were revised and there is a variety of hypotheses on triggers, underlying mechanisms and preventive actions.

This article concentrates on the importance of nutrition, especially zinc and vitamin D, for balancing the immune function. Homespun nutritional remedies seem to re-enter today's therapeutic strategies. Current treatment approaches are largely symptomatic or suppress the immune system. However, recent studies reveal significant benefits of nutrition-related therapeutic approaches including prevention and treatment of established disease, which offers a cost-efficient and trigger-unspecific alternative addressing balancing rather than suppression of the immune system. Zinc and vitamin D are currently the best studied and most promising candidates for therapeutic intervention.

Keywords: Autoimmunity, Nutritional Immunology, Zinc, Vitamin D, tolerance

1. Current concepts of general and nutritional impacts on origin and development autoimmunity

In AID, self-tolerance is disturbed. The immune cells are no longer able to efficiently distinguish between foreign and self and are reacting to antigens, including systemically expressed ones as in SLE (systemic lupus erythematosus, autoantibodies against e.g. dsDNA or hyaluronic acid) and sjogren's syndrome (autoantibodies against e.g. mitochondria or centromer) or organ specific as in MS (against myelin) or T1DM (against pancreatic beta cells) [5]. The classification of psoriasis as AID is discussed but not finally decided. However, it often occurs along with AID and will be included into the discussion here. Generally, organ specific AID are mainly caused by Th1 and Th17 cells. The existence of a mixed Th1/Th17 phenotype has been discussed as well [6]. Systemic AID often involve disturbed Th2 functions, excessive auto-antibody levels and immune-complex deposition, increasing host cell destruction by the complement system. Elevated Th2 responses often come along with highly reactive macrophages, exacerbating tissue destruction.

Although a multitude of triggers have been suggested to elicit AID including hormonal factors, genetic predisposition, pathogens, stress, sunlight and other environmental factors, nutritional deficiencies were shown to be involved in the etiology of but also result from AID. For most AID, decreased levels of certain nutrients, including zinc and vitamin D were detected. The question of cause and consequence is difficult to answer. The loss of nutrients is obvious for diseases such as IBD (inflammatory bowel disease) and especially CD, as the main route of nutrients into the human body is through the intestine. Here, decreased uptake as well as increased loss of nutrients and food refusal play important roles and water- as well as fat-soluble vitamins such as Vitamin D, minerals and trace elements including iron, zinc, selenium, and calcium are affected [7].

Mechanisms underlying the initiation and progression of AID remain poorly understood [8]. Still, some clear similarities between the different autoimmune disorders were observed, in regard to immune-related mechanisms [1]. In a meta-analysis of publicly available gene expression datasets it was found that some common pathway perturbations are shared between the 12 different AID that were tested (T1DM, behcet's disease, sjogren's syndrome, MS, PAPA syndrome (pyogenic arthritis, pyoderma gangrenosum, and acne), CD, cryopyrin-associated periodic syndrome, ulcerative colitis, juvenile idiopathic arthritis, tumor necrosis factor receptor-associated periodic

syndrome, mutation in the gene of mevalonate kinase syndrome, hyper IgD Syndrome and SLE). Especially disturbances in phosphoinositide 3-kinase (PI3K)-Akt, Toll-like receptor (TLR), and NF-kappa B signaling, all known to be involved in guiding immune cell polarization, migration, growth, survival and differentiation were commonly disturbed [1].

2. Zinc in healthy immune reactions and autoimmune diseases

Zinc is an essential trace element that needs to be taken up via the diet. Zinc deficiency is highly prevalent in developing countries, where over 25% of the population revealed an inadequate zinc intake [9]. Thus, supplementation programs have been implemented especially for children, as they are in addition to pregnant and lactating women and the elderly one of the risk groups to develop zinc deficiency [10]. Zinc supplementation of children was beneficial and lowered the incidence of diarrhea and pneumonia, diseases known to be more severe in zinc deficient individuals. In developed countries, up to 15% of the population do not take in adequate amounts of zinc as well, as was recently reported [9]. Therefore, zinc supplementation should be considered in those. Apart from its major effects on the immune function including wound healing, stunted growth, skin problems, and loss of appetite are some major symptoms of zinc deficiency [11]. Zinc deficiency is largely caused by under- or malnutrition. Moreover, diets based on phytate-rich foods, such as corn-products, can result in suboptimal zinc intake, as phytates bind zinc and decrease its bioavailability and thus hinder the uptake of zinc by intestinal cells [12]. Finally, defects in transporters, necessary for the intestinal uptake of zinc, such as zinc import protein (Zip)4 in acrodermatitis enteropatica, or illnesses associated with intestinal leakage can result in the decreased uptake or increased loss of zinc from the body.

All cells require zinc and need to maintain a balanced intracellular zinc homeostasis to ensure proper function. This is done by the co-operative action of 24 zinc transport proteins and several intra- and extracellular zinc binding proteins such as metallothionein (intracellular) as well as albumin, α_2-macroglobulin, transferrin and calprotectin (extracellular). All cells suffer during zinc deficiency, but cells of the immune system are extremely sensitive and strongly affected. Also, some general basic mechanisms on how zinc deficiency causes deregulated immune responses can be named, as zinc is essential for DNA replication, RNA transcription, cell division, cell

proliferation and cell activation, especially due to its regulatory role in intracellular signaling [13]. Moreover, apoptosis is potentiated by zinc deficiency, zinc has antioxidant functions and is able to stabilize membranes. Thus, increased susceptibility to a wide range of infectious agents and less efficient pathogen clearance are the results, as is an increased risk to develop AID, due to disturbed tolerance as will be detailed in later paragraphs.

In a recent meta-analysis, Sanna et al. (2018) found 26,095 articles, including duplicates though, investigating the association of zinc with autoimmunity. 62 of those were compared in detail, to examine the experimental evidence regarding zinc levels in the serum, plasma, urine and hair of patients with autoimmune disorders. The analysis included T1DM (22 studies), RA (18 studies), MS (7 studies) and 15 papers investigating other AID such as alopecia areata, SLE, pemphigus vulgaris, autoimmune hepatitis, celiac disease, hashimoto thyroiditis, sjogren's syndrome and juvenile idiopathic arthritis. The most important and consistent finding in this regard is the significantly lower serum and plasma zinc concentration in autoimmune patients compared to healthy controls [14]. A recent MS study in a big cohort offers one of the latest examples [14,15]. For most diseases, it is not entirely clear if zinc is involved in disease etiology or rather a consequence of the pathology.

It was amongst others noted, that serum zinc was decreased in a rat model for RA, while elevated zinc levels were found in the liver. This can be explained by the activation of Zip14 by pro-inflammatory cytokines, which is known to transfer zinc from serum into the liver [16–18]. If zinc is transiently transferred to certain organs in other AID remains to be investigated, but it seems reasonable that serum zinc is reduced in all AID with increased pro-inflammatory cytokine levels in the blood. Especially thyroid-related diseases, such as hashimoto thyroiditis, often come along with serum hypozincemia [19]. A significant correlation of serum zinc levels with thyroid volume in nodular goiter patients, and with thyroid autoantibodies in autoimmune thyroid disease was shown recently in a human study involving 201 subjects in total [20]. However, no data on possible underlying mechanisms are available, yet and supplementation studies are so far missing.

In IBD patients, zinc deficiency is caused by decreased oral intake, malabsorption, and/or previous small bowel resections. The prevalence of zinc deficiency is between 15 and 40% and serum zinc levels were inversely correlated to disease severity [7].

Similar data are available for CD. Here, patients often suffer from skin problems such as fistulas, which was correlated to a low zinc status [21]. Zinc´s role in wound healing, benefits for immune functions and support of intestinal barrier functions explain why normalization of zinc levels in IBD and CD patients improved the status of the subjects. As a balanced diet provides sufficient zinc and as excessive zinc intake might not be helpful in regard to therapy of disease, zinc status should be assessed to decide if zinc should be supplemented. Examples for studies, where zinc supplementation has been tested in AID therapy can be found in table 1.

Patients with alopecia areata suffer from non-scarring, autoimmune, inflammatory hair loss and were often found to be zinc deficient. Duration and severity of disease was inversely correlated with serum zinc levels. Mechanisms are rather unknown, but as zinc is important for functional activities of hair follicles, this might be a starting point for future research. [22].

The role of zinc for development, polarization and function of immune cell subsets were suggested as possible explanations for the associations of zinc and the immune dysregulation in AID. A multitude of effects are due to the regulatory role of zinc in intracellular signaling. Figure 1 gives an overview on the major effects zinc has on immune cells and their functions, in regard to AID signaling pathways, important for immune cell development and functions and known sensitivity for zinc homeostasis.

3. VitD in healthy and autoimmune response

Vitamin D can be endogenously produced. As long as access to sunlight is provided on a regular basis, there is no dietary requirement for this vitamin [23]. However, vitamin D can also be obtained from food sources. After its uptake, vitamin D (cholecalciferol) is converted into 25(OH)D and then into the biologically active metabolite $1,25(OH)_2D$ (calcitriol) in liver and kidneys, respectively. The nomenclature of vitamin D and its metabolites is well defined: the major form of vitamin D, found in the serum and used to define the vitamin D status is 25(OH)D. For experimental approaches, 25(OH)D and $1,25(OH)_2D$ are usually used [24]. To facilitate reading, those forms will be summarized as VitD henceforth. VitD concentrations in the body are tightly controlled, and excessive VitD can be converted into less active $24,25(OH)_2D$ and $1,24,25(OH)_3D$ [25]. Alternatively to the dietary uptake, VitD can also be synthesized from 7-dehydroxycholesterol in response to ultra violet light [26]. When VitD has entered the

cell, it activates gene expression via a nuclear receptor that is also a transcription factor, named vitamin D receptor (VDR). VDR, which is expressed in almost all human cells, can bind to the DNA at the vitamin D response element (VDRE), after receptor conformation has changed due to substrate binding (i.e. VitD) [27]. Inside the cell, VitD plays a key role in regulating proliferation, cellular growth, differentiation, apoptosis, DNA repair and oxidative stress, membrane transport and in cell adhesion [28]. VitD is also important for maintenance of calcium homeostasis, as it regulates the uptake of calcium in the intestine. During VitD deficiency, calcium can however also be mobilized from the bone for a limited time, before symptoms of deficiency such as osteomalacia and rickets occur [29]. VitD is used to induce maturation of myeloid pre-cursers into mature monocytic cells and is applied to treat myeloid leukemia, indicating the importance of VitD for the development of cells from the innate immune system [30,31].

Recent data indicate that VitD deficiency is associated with AID [32] such as MS, IBD, T1DM, SLE and RA, where VitD supplementation was beneficial and ameliorated disease severity [33–38]. The prevalence of VitD deficiencies in AID patients is high. For all diseases mentioned above, clinical trials found benefits of VitD supplementation of human AID patients as well. Existing trials and their outcomes have recently been discussed in great detail [39] and are summarized in table 1. Thus, we will concentrate on the underlying mechanisms here.

One of the central findings for research in this area was that MS prevalence increases with latitude, probably due to less exposure to sunlight. Similar observations were made for T1DM and IBD [40–42]. For MS in the northern hemisphere, the risk to develop the disease was even higher in April than in October and November [43]. Serum VitD is inversely correlated to AID incidence. Moreover, highest disease severity was correlated with lowest serum VitD values. [44–48]. Finally, polymorphisms in VDR were more often found in patients with AID, including MS, RA, SLE, IBD, T1DM and CD, than in healthy controls [49–53]. Among the common risk single nucleotide polymorphisms found for AID, several are linked with the vitamin D metabolism, such as CYP24A1, CYP27B1, and CYP27B1 as was recently summarized by Lu et al. [54].

All these data support the association of serum VitD levels, VitD intake, and UV light exposure to AID and coined the idea, that VitD supplementation might lower disease susceptibility and severity or improve the responsiveness to current treatment strategies

for all kinds of AID. Indeed, early childhood VitD supplementation reduced the risk for T1DM by up to 30% [38,55].

The immunomodulatory effect of VitD has been known for ages. Thus, molecular mechanisms underlying the association of VitD to AID are so far mainly postulated to rely on VitD's importance for balancing the immune response, similar to what was described for zinc earlier. T and B cells as well as monocytes/macrophages and dendritic cells can convert 25(OH)D into $1,25(OH)_2D$ which supports the supply of the active metabolite at the side of infection. To explain the molecular basis for the role of VitD in AID, the next paragraphs will describe effects of VitD on the different immune cells, which is also summarized in Figure 2, providing implications for optimizing the therapeutic use of VitD for AID.

4. The adaptive immune system

4.1 T lymphocytes

Self-tolerance is chiefly required from cells belonging to the adaptive immune system, namely B cells and T cells, as they recognize specific antigens through their membrane bound receptors (T cell receptor / TCR, B cell receptor / BCR). T cells can be roughly grouped into $CD8^+$, cytotoxic T lymphocytes (CTL) and $CD4^+$, T helper (Th) cells. The latter can be further sub-grouped into Th1, Th2, Th17 and regulatory T (Treg) cells, to name the most important ones [56]. Phenotyping of the T cell subsets is primarily done by measuring lineage specific transcription factors: Th1 cells: T-box–containing protein expressed in T cells (T-bet), Th2 cells: GATA-3 (GATA-binding protein 3 to DNA sequence [A/T]GATA[A/G]), Th17 cells: RORC2 (Retinoid acid receptor (RAR)-related orphan receptor, RORγt in mice), Treg cells: forkhead box P3 (Foxp3) [56].

During healthy immune cell development, autoimmunity is prevented by deletion, anergy and suppression of auto-reactive cells. Other cells that bind moderately to MHC complexes receive survival signals and enter the thymic medulla. During deletion T cells are irreversibly eliminated by apoptosis, induced through interaction of Fas and Fas ligand, presenting two of the first molecules found to be altered in patients with AID [57]. Breaking any of those mechanisms of tolerance can cause AID [58].

In general, lymphopenia was often observed pre-ceding AID [59]. Thus, it is important that the numbers of circulating lymphocytes are stable. Especially dysfunctions during early development of T and B cells were suggested as pre-requisites of AID [60,61].

Interestingly, lymphopenia and dysregulated lymphopoiesis are regularly observed during zinc deficiency. The whole set of T cells is strongly depending on sufficient zinc supply. In mice, even a modest zinc deficiency altered the expression of 1,200 genes related to the survival, proliferation, and the response of T cells [62]. This was partly explained by the strong impact of zinc on interleukin (IL)-2 production and the signal transduction induced by this cytokine, as IL-2 is the central factor in T cell proliferation and development [63–65]. The effect of different zinc compounds on the proliferation, differentiation, and activation of human T cells has been established in multiple *in vitro* studies and was reviewed elsewhere [66]. Zinc was reported to affect components of the TCR signaling pathway. As TCR signaling is indispensable for the survival as well as for cytokine production, this offers another explanation for the strong malfunction of T cells during zinc deficiency [63,67,68]. It was shown that zinc plays a vital part in intracellular signaling, either by affecting the activity of phosphatases and kinases or as second messenger. Various studies revealed zinc´s effect on Mitogen-Activated Protein Kinases (MAPK), TLR and TCR signaling pathways. Zinc acts at several steps in TCR-mediated T cell activation: 1. Zinc directly activates lymphocyte-specific c-src family protein tyrosine kinase (Lck) which is located upstream of all TCR-dependent signaling pathways [69]. Subsequently, Lck is recruited to the TCR signaling complex, where it is auto-phosphorylated [70]. Via zeta-chain associated protein kinase 70 kDa (ZAP70) p38 is subsequently activated, without involvement of MAP kinases, through phosphorylation of tyrosine 323. Consequently, p38 is autophosphorylated at Thr180/Tyr182 residues [71]. Moreover, zinc was shown to inhibit a whole set of phosphotyrosine phosphatases (PTP), which may result in sustained phosphorylation of other signaling pathways involved in the complex network of signaling pathways involved in T cell activation and differentiation of which not all have been successfully studied, yet [13,72]. Therapeutic zinc supplementation is therefore under consideration as a possible option to treat T cell-mediated autoimmunity [73–75].

Similarly, VitD deficiency decreases lymphocyte quantities [76], affects TCR signaling and was related to leukopenia in SLE patients [77]. VDR expressing T cells, especially from Th cell type, seem to play a vital part in MS, as VitD treatment of Experimental Autoimmune Encephalomyelitis (EAE) mice was beneficial in the presence of VDR in T cells, but not in their absence [78]. The finding that VitD was also able to inhibit T cell migration to the central nervous system in EAE mice strengthens this hypothesis as well

[79]. Interestingly, a lot of single nucleotide polymorphisms associated with T-cell-mediated AID are in close proximity to VDR binding motives which underlines that VitD treatment is a promising option for AID treatment [80]. In summary, zinc and VitD deficiency-related lymphopenia are risk factors for AID.

4.2 Th1/Th2 balance

Th1 over-representation and reaction against self-antigens causes tissue destruction, which plays a vital role especially in organ-specific AID. There is evidence that autoimmune diseases such as MS, CD, RA and T1DM are primarily Th1 cell mediated and that often the shift of the Th1/Th2 balance alleviates these diseases. This is underlined by the fact that T-bet deficient mice were protected from EAE [81]. Along with this, transfer of myelin-specific Th1 cells induced EAE [82]. However, since the discovery of Th17, it is debated, that detriments are not caused by Th1 alone but rather by cooperative effects of Th1 and Th17 cells, as detailed later.

Th2 cells on the other hand stimulate the production of antibodies in response to extracellular pathogens and were associated largely with systemic autoimmune disease such as SLE and other chronic conditions [83].

Early studies showed that Th1 cytokines are decreased during zinc deficiency, while Th2 cytokines remained constant, resulting in a decreased Th1/Th2 ratio [84,85]. Moreover, zinc chloride ($ZnCl_2$), Zinc oxide (ZnO) or zinc sulfate ($ZnSO_4$) decreased proliferation and cytokine production (IL-6, IL-2, IL-10) by isolated T cells and peripheral blood mononuclear cells (PBMC), activated using mitogens [86]. Zinc aspartate reduced proliferation and poke weed mitogen- and anti-CD3/CD28-induced production and secretion of several T cell cytokines relevant to EAE and MS *in vitro*, including Th1 (e.g. IL-2, interferon (IFN)-γ; tumor necrosis factor (TNF)-α) and Th2 (e.g. IL-4, IL-5, IL-13) as well as Th17 (IL-17, granulocyte macrophage-colony stimulating factor (GM-CSF)) cytokines. Here, effects that were initially found in EAE mice were verified when human primary PBMCs and murine primary splenocytes were tested [87]. $ZnSO_4$ also decreased IFNγ levels in human mixed lymphocyte culture [88]. Proliferation of freshly stimulated T cells was inhibited by zinc aspartate (up to 150 µM) as was the production of lineage specific cytokines for Th1, Th2 and also Th17 cells [89]. Results from this study were also compared with those for the immunosuppressive drugs cyclosporin A, dexamethasone, and rapamycin, often used in AID therapy. Only zinc aspartate and rapamycin were able to inhibit proliferation and cytokine production of pre-activated T

cells in this particular study. Zinc aspartate might thus be able to suppress proliferation and excessive cytokine secretion of pre-activated human T cells *in vitro* and might be beneficial as treatment option for T cell-mediated autoimmune diseases [89], which was not tested *in vivo* in this extend so far. Moreover, the immunosuppressive mechanism (cell cycle arrest, DNA damage, and/or apoptosis) and the signal transduction pathways involved should be explored in more detail.

Similarly, VitD can inhibit IFNγ production in T cells via the silencer region BED within the IFNγ promoter [90]. In addition, treatment of activated T cells with VitD inhibited the IL-12-induced tyrosine phosphorylation of Janus kinase (JAK)2, Tyrosine kinase (TYK)2, Signal Transducer and Activator of Transcription (STAT)3, and STAT4 in association with a decrease in T cell proliferation *in vitro* [91]. However, VitD's effect might depend on the differentiation status and IL-2 availability as was found in later studies and thus this mechanism might not be the most important in regard to AID treatment [92–96].

Th2 cells were suggested to protect against Th1 and Th17-related AID [97]. VitD induces Th2 cell polarization via GATA-3. It thus increases IL-4 expression, regulated via STAT6 and GATA3 explaining the positive effects of VitD in diseases such as EAE and IBD, while IL-2 expression was inhibited [98]. In STAT6 knock out (KO) mice, no benefits of VitD on EAE development were seen [35,99,100]. Th2 numbers could successfully be increased by VitD treatment in SLE and MS patients [101,102]. However, deviating results were found for the association of VitD, Th2 and AID, when polarizing stimuli were altered. VitD increased IL-4 expression by naïve $CD4^+$ T cells and PBMC in some studies, while VitD had no effect, when external IL-4 was added to support polarization, which is in line with results from RA patients, where no increase in IL-4 was detected after VitD treatment. Low IL-4 levels might therefore be an indicator for benefits of VitD treatment for increased Th2 polarization, but the exact mechanisms need to be explored [93,101,103–105].

4.3 Th17/Treg cell balance

While Th1 and Th2 were long described as main mediators in AID, more recent data point to Th17 cells and their pro-inflammatory armor including IL-17A, IL-17F, TNFα and GM-CSF. Polarization into Th17 cells is induced by transforming growth factor (TGF)β combined with IL-6 in mice and TGFβ, IL-1β and IL-23 in humans. IL-23, together with IL-17, was suggested to result in their pathogenicity in EAE and in collagen-induced

arthritis [106,107]. Pathogenic Th17 cells revealed a different expression profile including IFNγ and T-bet, usually found in Th1 cells [108]. In addition to EAE, significantly elevated Th17 cell numbers were observed in RA and SLE patients [109,110]. Especially memory Th17 cells were associated with activation of synovial fibroblasts, augmenting AID such as RA [111]. Detriments of Th17-driven AID are based on a pro-inflammatory feedback loop, amplifying levels of IL-6, IL-17A and tissue destructing enzymes [111].

In contrast to the previously discussed Th types, that increase AID, Tregs´ purpose is to maintain tolerance and prevent or balance overshooting pro-inflammatory immune responses. Via IL-10 and TGFβ, they can downregulate activation of DCs, CTL, macrophages and the remaining Th cell types. Patients with mutations in Foxp3, the central Treg cell transcription factor, suffer from massive autoimmune reactions summarized as IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome [112]. In patients with AID such as MS, Treg cells revealed impaired function and reduced Foxp3 expression at the single cell level and in some cases also reduced numbers [113].

Our understanding of the importance of zinc in regulating the Treg/Th17 balance has much improved in the last decade and results are summarized in Figure 3. A disturbed Treg/Th17 balance was amongst others observed in the elderly, a population known to suffer from zinc deficiency [114]. Zinc was shown to inhibit the development of Th17 cells via its effect on the IL-6/STAT3 signaling cascade. Here, the α-helical secondary structure of STAT3 was altered after binding of zinc. This disrupted the association of STAT3 with the JAK2 kinase and also with glycoprotein (gp)130 [115]. In line with this, Th17 polarization was supported by zinc deficient conditions. Here, significantly augmented activation of IL-6-induced JAK/STAT3 signaling was found to underlie the effects of zinc deficiency [116].

It was found that TGFβ1 is able to induce a short zinc influx into the cellular cytoplasm. No such zinc flux was observed for other Treg cell-associated cytokines such as IL-10. Additional zinc supplementation is capable of enhancing the TGFβ1 signaling pathways in T cells and hereby increasing the number of regulatory T cells, which was found investigating PBMC from single donors but also an allogeneic immune reaction using mixed-lymphocyte culture [117,118]. An appropriate intracellular free zinc level in Th cells might be important for proper cellular functions of the cells and intracellular free

zinc levels were suggested as a biomarker to discriminate Treg cells from other Th cell subsets [119]. Zinc supplementation inhibits the histone deacetylase Sirt-1, resulting in increased acetylation of Foxp3, thereby preventing its degradation [118]. Underlying mechanisms of zinc's effects on Treg cells also include the elevated activation of the Smad2/3 signaling pathway in cells from mixed-lymphocyte cultures where zinc was added in combination with TGFβ1, compared to a treatment with TGFβ1 alone. Since Smad-binding elements were found in the conserved non-coding sequence (CNS)1 region of the Foxp3 promoter and zinc prevents Foxp3 degradation, zinc might affect Treg polarization in multiple ways. In regard to the activation of Foxp3 expression, p38-mediated signaling is important. As the p38 signaling pathway is a target of zinc, this might play a role in zinc-induced Treg cell polarization, adding one more mechanism for the zinc-induced Foxp3 expression [63,120,121]. In addition to augmenting TGFβ1-induced zinc flux and thus signaling,

Zinc and TGFβ1 co-treatment also reduced the secretion of IFNγ, underlining that a rather anti-inflammatory milieu is created [122]. In contrast to TGFβ1, which also induces Th17 development, zinc was specific for Treg cell differentiation, not increasing Th17, Th1 or Th2 polarization, and might thus be a great option for treatment of immune-dysbalance [117]. Surprisingly, zinc deficiency also increased IL-2- and TGFβ-induced Treg cell polarization, causing elevated Treg cell numbers. However, those Treg cells are possibly non-functional, as a low expression of miR146, essential for their activity, was found [116]. In combination with the increased polarization of T cells into Th17 cell subtype during zinc deficiency, benefits of zinc supplementation for AID, where the Treg/Th17 cell balance is disturbed, can be expected, as is also underlined by the positive effects of zinc supplementation in EAE [87,123–125].

Similar to zinc, VitD is a major regulator of the Th17 to Treg cell balance as is illustrated in Figure 4. VitD decreases Th17 cell differentiation which is at least in part due to the autocrine effects of IL-17A and IL-17F, but also due to direct effects of VitD on decreasing RORC2 and chemokine receptor (CCR)6 expression as shown *in vitro* [92,96,126]. Decreased CCR6 expression explains why less autoreactive Th17 cells are recruited to the central nervous system in VitD treated EAE mice [98]. Cells that were differentiated under high VitD conditions were even less potent in eliciting EAE if transferred between mice [127].

The inhibition of EAE progression and anti-retinal autoimmunity is at least partly due to suppression of Th17 activity by VitD, which disturbs the pro-inflammatory loop between immune and synovial cells [128,129]. *In vitro*, VitD decreased expression of IL-22, IL-17A, and IL-17F by $CD4^+$ T cells including memory cells [105,127,129]. This was even the case in the presents of Th17 polarizing factors [127,130]. In regard to the IL-17A gene, the VDR competes with NFAT for promotor binding and thus blocks activation by the latter, as VDR recruits Runt-related transcription factor (RUNX)1 and histone deacetylase (HDAC) [127]. VitD might also regulate IL-17A in a more indirect way, as VDR forms a complex with retinoid X receptor (RXR), HDAC2, and Smad3 to inhibit Smad7 transcription, thereby preventing IL-17A production [131]. Similar mechanisms have been suggested for inhibition of IL-2 expression. Therefore, this mechanism might play a role in other VitD regulated genes that are regularly activated by Nuclear Factor of Activated T cell (NFAT) [94].

Recently, the existence of a non-classic Th1 cell type, denoted Th17.1 cells was suggested [132,133]. As their functions in AID in relation to VitD are probably similar or a mix of what was suggested for Th1 and Th17 cells, this will not be detailed in here, but results point to an even higher pathogenicity of those cells in AID [134,135].

VitD does not only decrease Th17 polarization, but similar to zinc also supports Treg cell development. When EAE mice were supplemented with VitD, elevated numbers of Treg cells were detected in spinal cord, spleen and lymph nodes. Overall disease status of the patients was improved as well. In this study, IL-10 and its signaling where found as central mechanisms conferring VitD´s effects on Treg cells. Performing Treg cell polarization under high VitD conditions, improved Treg cell differentiation, even in the presence of additional Th17 polarizing agents [130]. In line with this, the suppressive capacity of Treg cells in MS patients was positively correlated to VitD serum [103,126,127,130,131,136,137].

Several suggestions were made on the underlying mechanisms for VitD-induced Treg cell development: The Foxp3 promotor has three binding sites for the VDR, explaining its increased expression during high VitD conditions [127,136]. Moreover, the inhibitory effect of Th17 polarizing cytokines on cytotoxic T lymphocyte associated protein (CTLA)4 might be reversed by VitD, so that CTLA4 expression is activated, favoring Treg cell polarization [130]. The enzyme indoleamine 2,3 dioxygenase (IDO), which catabolizes tryptophan and triggers cellular stress responses, is expressed in dendritic

cells (DC) and involved in the activation of Treg cells by DCs. As a third mechanism, the activation of IDO by VitD was shown, explaining increased Treg cell numbers [138,139]. Although data showing positive effects on Treg development are convincing, there are also some studies, where no effect of VitD on Treg cell polarization was found [102,140]. However, trends were also observed there and the overall effect of VitD treatment in regard to disease outcome was positive.

It can be concluded, that VitD and zinc support differentiation of Treg cells in healthy controls and patients with AID, which might be at least one mechanism of how zinc and VitD are able to re-balance the immune response in the latter group.

4.4 Cytotoxic T cells

CTL support the immune reaction by secreting TNFα and IFNγ [141]. CTL malfunctioning was associated with EAE, where myelin-specific CTL were found. Interestingly, symptoms elicited by CTL during EAE were different from those observed when myelin-specific Th cells were transferred to mice and resembled MS more closely than Th cell associated EAE [142,143]. Increasing evidence exists on the role of regulatory, IL-17-secreting CTL in MS in humans [144]. Moreover, it was suggested that myelin-specific CTL cells are involved in the differentiation of encephalitogenic $CD4^+$ T cells in Lewis rats. Interestingly, myelin-specific CTL had far less impact on CD4+ T cell priming in dark agouti rats [145,146]. However, the role of CTL in the autoimmune pathology of MS has been both understudied and controversial [147]. In case of T1DM, the importance of CTL in addition to Th cells during pathology has been noticed [148,149]. Autoimmune intestinal inflammation was induced by transferring hsp60-specific CTL into mice [150] and numbers of IL-17 expressing T cells (both Th and CTL) in synovial fluid were correlated to disease severity in patients with psoriatic arthritis, distinguishing this AID from RA, where Th cells are enriched [151].

The number of CTL is decreased in zinc deficient humans [152]. Moreover, the cytotoxic activity of CTL is ameliorated, resulting in decreased anti-allogeneic tumor activity in mice [153]. A direct association between zinc, CTL and AID was so far not investigated, but zinc´s strong effects on IL-2R- and TCR-induced signaling [63,64] suggest that AID, related to dys-functioning CTL in AID might benefit from zinc supplementation.

VDR expression by CTL is even higher than by Th cells. Lack of VDR in CTL contributed to the development of IBD in mice. Interestingly, CTL from VDR KO mice proliferated without antigen stimulation expressed more IL-2 and were more sensitive to

IL-2. Transferring VDR-deficient CTL into Rag KO mice as AID models, also induced IBD. This indicates that expression of the VDR, and thus probably also sufficient levels of VitD, are required to prevent that quiescent $CD8^+$ T cells including auto-reactive ones, proliferate and become pathogenic $CD8^+$ T cells that contribute to the development of AID such as IBD and CD [154].

In line with this, VitD inhibited the secretion of TNFα and IFNγ by activated CTL from healthy donors and MS patients [155] and topic treatment of psoriatic lesions with calcipotriol lowered the amount of IL-17A secreting CTL, resulting in clinical and histological improvement [156]. $CD8\alpha\alpha^+$ T cells, intestinal intraepithelial lymphocytes, are involved in suppressing the immune response against intestinal antigens. Numbers of $CD8\alpha\alpha^+$ T cells were reduced in VDR KO mice, offering one more connection between VitD and AID, especially affecting the intestine [157].

The role of zinc and VitD in CTL-mediated AID needs to be explored in more detail, but first studies point to benefits of VitD and zinc supplementation during CTL dysfunction.

4.5 Unconventional T cells

Most human T cells express the TCR together with CD8 or CD4. However, there are also cells expressing the TCR but neither CD4 nor CD8 [158] which were denoted as double negative or unconventional T cells. Those cells have a less diverse TCR repertoire, have no major histocompatibility complex (MHC) restriction and amongst others include natural killer T cells (NKT cells), mucosal-associated invariant T (MAIT) cells and γδ T cells. Unconventional T cells do not bind classic peptide antigens but lipids or small metabolites, such as phosphoantigens, alkylamines, isoprenoid pyrophosphates, thus rather “public” antigens [159], and do not necessarily require antigen processing and MHC presentation.

In EAE, collagen induced arthritis, and experimental autoimmune uveitis, enriched numbers of γδ T cells were detected that supported disease development largely by IL-17, IL-23 and GM-CSF production and by activating Th17 cells [160–163]. An association of γδ T cells with various other autoimmune diseases, such as RA, autoimmune thyroid disease, and autoimmune liver disease was described as well [164–166]. Their role in T1DM is discussed controversially and studies showed support of disease progression but also its suppression by γδ T cells [167,168]. γδ T cells are also involved in controlling development of germinal center and autoreactive IgG formation. Details are summarized in a recent review by Paul et al. [169].

No direct connection of zinc to γδ T cell-related AID has been described, so far. One single study was found, investigating an association of VitD, γδ T cells and AID. Here, it was shown that VitD can inhibit phospholigand-induced γδ T cell expansion. IFNγ production and CD25 expression were reduced and Akt as well as Extracellular Signal-Regulated Kinases (ERK) signaling pathways were compromised, resulting in potentiation of antigen-induced cell death of the cells when cells were stimulated under high VitD conditions [170].

NKT can be grouped into type I or invariant (i)NKT cells and type II or variant NKT cells due to the expression of invariant and variant TCRs, respectively. Both types secrete IL-4, IL-17A, IFNγ and other pro-inflammatory mediators upon ligation of their TCR. While NKT cells were suggested as being involved in the pathology of collagen induced arthritis, they were protective in EAE, T1DM and SLE [99,171]. Underlying mechanisms have only been weakly investigated so far and the issue if they are protective or detrimental in regard to AID is discussed controversially [172].

iNKT cell develop in the thymus. To differentiate into functionally active cells, VitD-induced T-bet expression is required during their maturation as was shown in VDR KO mice. The response of iNKT cells from VDR KO mice to TCR ligation was disturbed, even when antigen was presented by antigen presenting cells (APC) from wild type mice [173]. Results from another study showed, that in CD1d KO mice, depleted of NKT cells, VitD treatment was less efficient in preventing EAE development than in mice with normal NKT cell development and functions. The authors also showed that VitD altered the cytokine profile of wild type iNKT cells and suggest that especially the decrease of IL-4 in mice lacking iNKT cells might be responsible for the lower protection against EAE. This was underlined by the finding, that VitD did not have any effect in regard to EAE in IL-4 KO mice [99].

As the role of unconventional T cells in AID is not clear yet, detriments or benefits of zinc and VitD supplementation are highly speculative.

4.6 B cells

During B cell development, several check-points can be found that hinder the generation of auto-reactive B cells [174,175]. Suggestions for triggers that disturb those check-points are manifold, but underlying mechanisms are understudied.

Via the B cell receptor, B cells can detect antigens, differentiate into plasma cells and secrete large amounts of antibodies against the detected antigen, which might be a self-

antigen as well, if tolerance is disturbed. This was observed in SLE and RA, where for unknown reasons high amounts of anti-nuclear antibodies (ANA) or anti-citrullinated peptide antibodies (ACPA) were detected in over 95% and 70% of the patients, respectively [176,177]. Sjogren's syndrome, Grave's disease and T1DM show a high frequency of autoantibodies as well [178]. In case of the latter antibodies targeting the zinc transporter ZnT8 belong to the four most frequent auto-antibodies [179]. However, as it is rather viewed as a marker, targeting ZnT8 expression does not appear to be a relevant approach in regard to therapy.

B cell receptor-mediated signaling is affected by the zinc status, similar to what was described for TCR-induced signaling. Zinc´s importance for intracellular signaling in B cells includes a variety of pathways and signaling molecules including PTP1B, Lyn, STAT-6, receptor PTPase β, and Src homology region 2 domain-containing phosphatase (SHP)-1, which has recently been investigated and reviewed in great detail [10,66,180]. However, no association between zinc status, B cell receptor signaling and AID has been described so far and an association of zinc with antigen-presentation by B cells was also not addressed.

It was suggested that RA may be induced by B lymphocyte stimulator (BlyS)-mediated B-lymphocyte dysplasia and dysfunction, which was correlated to decreased serum zinc levels [181]. Again, if the alteration in B cell activity causes the loss or redistribution of zinc, or if zinc deficiency comes first is not clear, but in general, correction of the dysbalanced zinc homeostasis is promising. Zinc deficiency results in decreased maturation and function of B cells, affecting especially early developmental stages [182], while B cell activation was recently suggested to come along with increased intracellular zinc levels [183]. Apoptosis is also strongly affected by zinc homeostasis. Here, low but also abnormally high zinc conditions induced cell death in B cell lymphoma lines [184]. Investigations on effects of zinc deficiency on the antibody production did not produce entirely clear data. Studies investigating per-cell production of residual B cells in a limited area of an organ revealed only minor decreases, while most other studies, investigating T cell-dependent and T cell independent antibody production on whole organ bases found decreased antibody production [185–188]. Also, if changes in antibody production are due to an effect of zinc on the B cells or on the T cells, inducing antibody production remains to be finally tested.

Response elements for the VDR were found in key immune response genes of B cells, affecting B cell differentiation, antibody production, cytokine expression and presentation of antigen. B cells undergo distinct developmental stages including class switch and somatic hypermutation. Here, VitD was shown to act rather inhibitory in cells from early developmental stages as it decreases proliferation and class switch but induces apoptosis [189,190]. In contrast, VitD increased differentiation of mature B cells into plasma cells and CCR10 expression, important for mucosal migration towards the site of infection. On a molecular basis this might involve VitD-induced inhibition of nuclear factor kappa B (NFκB) translocation, and thereby blocking CD40-induced signaling [191].

Studies on the association between VitD, AID and B cells are scarce. In regard to cytokine production, VitD-induced VDR binding to the IL-10 promoter induced its gene expression by B cells [192]. However, there was no correlation of VitD serum levels and IL-10 expression in healthy controls or relapsing-remitting MS patients [193]. On the other hand, there are a lot of studies suggesting decreased antibody production including anti-ANA after VitD supplementation, which is in line with low VitD serum levels in patients with high anti-ANA titers and even non-SLE patients with low VitD serum levels had elevated anti-ANA titers [104,189,190,194–197].

The effects of VitD on antigen presentation have only been a topic in one study, where authors found decreased CD86 expression on VitD-primed B cells, which are thus less potent T cell stimulants [198].

The limited amount of studies on B cells in autoimmunity in general and little knowledge on an association between B cell-involving AID with serum zinc and VitD levels, prevents drawing a real conclusion. However, zinc and VitD might be interesting candidates in regard to the development of new therapeutic approaches for AID and normalizing B cell activities.

5. The innate immune system

During the last decades, evidence accumulated that not only adaptive immune cells cause AID but that AID are frequently based on malfunctioning innate immune cells as well. Here, symptoms such as fevers and multi-organ inflammation are common. The term “auto-inflammatory diseases” is often used to distinguish those from diseases elicited primarily by adaptive immune cells [199], but as the general symptoms of self-

tissue directed inflammation are the same as in classic AID, no difference in terminology was chosen here. In addition, often AID cannot easily be categorized. In psoriasis for example, autoreactive Th17 cells cause dysregulation of innate immune cells [200].

5.1 Dendritic cells

Dendritic cells are important for bridging the innate and the adaptive immune system. They are able to take up and process antigens and to present them to T cells via MHC. Deletion of DCs in experimental AID models for psoriasis, SLE, and T1DM ameliorated or even prevented disease, highlighting their importance for AID development [201–203].

Fine tuning of the development and function of DCs is strongly affected by their intracellular zinc levels. Moreover, zinc was suggested to be involved in lipopolysaccharide (LPS)-induced effects, as LPS stimulation caused the downregulation of zinc importer ZIP6 and the upregulation of zinc exporters via TLR4. The resulting reduction in intracellular zinc was associated with increased DC maturation. In line with this, reducing intracellular zinc levels via incubation with a zinc chelator, triggered murine DC maturation *in vitro*, as monitored via MHC expression [204]. MHC II molecules are located in lysosomal and endosomal compartments in immature DCs and it was shown that export of those molecules is inhibited by zinc. The reduction of intracellular zinc might thus enable translocation of MHC II molecules to the surface and thereby facilitate antigen presentation and subsequent interaction with the adaptive immune system [205]. By presenting (auto-) antigens to T cells, DCs have the potential to induce AID. If zinc deficiency favors the presentation of autoantigens by DCs remains to be tested, but a general increase in presentation might increase the chance that not only foreign antigens are chosen.

It was however shown that exposure of murine bone marrow derived DCs to zinc *in vitro* induced a tolerogenic phenotype. Here, the expression of programmed death-ligand (PD-L)1, PD-L2, IDO, and surface MHC II were diminished. Furthermore, tryptophan degradation and kynurenine production by DCs was triggered. This resulted in strong suppression of the pro-inflammatory response after stimulation by TLR ligands. The results were verified *in vivo,* showing that zinc supplementation inhibited presentation of fungal antigen via MHC II on DCs, increased PD-L1 and PD-L2 expression on MHC II^{low} DCs and altered the Treg - Th17 balance favoring Treg cell polarization.

Numbers of Th17 cells were decreased. Thus, zinc can shape the tolerogenic potential of DCs *in vitro* and *in vivo* and promotes Treg polarization during fungal infection [206].
In vitro studies revealed that human and murine DCs, differentiated from monocytes under high VitD conditions *in vitro*, remain immature and tolerogenic. For this, VitD has to be added before differentiation is induced. Those DCs express less pro-inflammatory IL-12 and TNFα and have also a potential to induce Treg polarization. It was shown that those tolerogenic DCs induce apoptosis in auto-reactive T cells, but not in T cells specific for foreign antigens. Similar effects of VitD were found for maturation of dermal DCs and plasmocytoid DCs [207,208]. Activation of the PI3K-Akt-mTOR (mammalian target of rapamycin) pathway and thus expression of IDO, immunoglobulin-like transcript(ILT)3 and CC-chemokine ligand (CCL)-2, together with a switch towards glycolysis were suggested to underlie VitD-induced tolerogenic DCs [209,210]. In another study, VitD-mediated IDO induction was suggested to result in DC development [211]. To explain the DC-mediated Treg polarization either induction of herpesvirus entry mediator (HVEM), secretion of TGFβ1 or IL-10 are discussed [207,212]. Figure 5 summarizes so far suggested theories, but more research is needed, to clarify those points.
However, zinc and VitD seem to favor a tolerogenic DC phenotype.

5.2 Macrophages

Monocytes, circulating in the blood, differentiate into macrophages when they are activated and infiltrate infected tissue. In addition, especially tissues that are exposed to the environment, harbor tissue specific macrophages, constituting the mononuclear phagocytic system together with the DCs and monocytes [213]. Similar to DCs, macrophages can phagocytose microorganisms, process and present foreign antigens. Depending on the pathogen and cytokine environment, macrophages can polarize into pro-inflammatory M1 or anti-inflammatory M2 macrophages [214]. In contrast to most other cell types, polarized macrophages retain the ability to alter their phenotype in response to changes in the environment [215], which has a critical therapeutic value.
Unbalanced M1/M2 polarization was often associated with AID. Especially an increase in the M1/M2 ratio was associated with SLE, IBD, T1DM and RA. [216] [217–219]. In case of RA for example, IL-1β is one of the central factors and also one of the major products of M1 macrophages, explaining the connection [220].

It has long been suggested, that while adaptive immune cells are severely compromised in number and function during zinc deficiency, innate immune cells remain largely constant in numbers and their basal activity is increased, causing a constant low grade inflammatory milieu in zinc deficient individuals [10,221]. Numerous studies have provided evidence that zinc is essential for the differentiation and normal function of monocytes and macrophages [222,223]. For a plethora of pro-inflammatory mediators such as IL-β, TNFα, calprotectin, IL-6 and reactive oxygen species (ROS) zinc deficiency was shown to increase their expression, amongst other by zinc-induced epigenetic alterations [120,224–226]. Zinc deficiency augmented the effect of LPS resulting in the secretion of large amounts of IL-1β, whereas high zinc concentrations diminished secretion by suppressing the LPS-induced monocytic response via inhibition of cyclic nucleotide phosphodiesterases (PDE). Subsequently, cellular cyclic guanosine monophosphate (cGMP) levels increased which suppressed LPS-induced TNF-α and IL-1β production in human monocytes [227,228]. Serum zinc deficiency was also inversely correlated to the severity of inflammatory diseases and the grade of hyper-inflammation as monitored via levels of TNFα and IL-6 [18]. Zinc deficiency augmented synthesis of inducible nitric oxide synthase (iNOS) causing elevated production of NO, which was reversed by zinc supplementation [229]. If this is associated to the hyper-inflammatory response observed in AID has not directly been explored, however, various symptoms in regard to innate immune cells, found during zinc deficiency, closely resemble those found in AID patients.

Dierichs et al. identified zinc as a decisive factor during lineage decision. Interestingly, zinc supplementation inhibited the differentiation into M2 macrophages as did zinc deficiency [230]. This suggests that zinc status should be assessed before considering zinc as treatment strategy for M1-mediated AID. Normalization of the patient´s zinc status might re-balance M1/M2 polarization, whereas elevating serum zinc levels might even support the M1 response. However, current results are derived from *in vitro* experiments using a cell line and should be evaluated *in vivo*.

More recent studies have revealed a role of zinc transporters in regulation of the inflammatory response. Stimulation of macrophages with LPS caused an increased expression of Zip8 and zinc influx into the cell resulting in elevation of intracellular zinc levels. As zinc serves as a negative regulator of the NF-κB signaling pathways this suppressed activity of monocytes and macrophages [231]. Zip10 in contrast was

decreased after stimulation of bone marrow derived-macrophages with LPS. Using Zip10 KO mice, it was shown that macrophage survival during an inflammatory response depends on Zip10-mediated zinc influx, as cells with missing Zip10 and thus zinc influx, die by apoptosis. Generally, cells from Zip10-KO mice were not able to respond significantly to LPS and thus expression of inflammatory cytokines was ameliorated. This however, prevented subsequent liver damage and lowered mortality, when sepsis was induced [232]. Effects of other transporters might be interesting to study.

In summary, zinc deficiency causes increased production of pro-inflammatory cytokines, reactive oxygen species and reactive nitrogen species by M1 macrophages, all shown to be highly elevated in tissues affected during AID. Thus, zinc supplementation should be considered for AID patients with low serum zinc values and high inflammatory parameters.

VitD can be used to induce differentiation of myeloid precursor into monocytic cells and this process is enhanced during zinc deficiency [222]. In the activation and polarization of macrophages, VitD plays a dual role. During early infection stages, it stimulate differentiation of monocytes into macrophages, important to reach sufficient cell numbers for an efficient immune response [233]. Moreover, VitD is important for cathelicidin expression by monocytes and macrophages, as well as for IL-1β production via CCAAT/enhancer binding protein (C/EBP)β or ERK1/2 signaling pathways [30,234,235], all important to enable pathogen clearance.

At later stages, VitD is involved in the resolution of the inflammatory response. VDR deficient mice for example responded to LPS with a hyper-inflammatory reaction [236]. Also, mature macrophages responded to VitD treatment with an increase in IL-10 production and a decrease in expression of Receptor activator of NFκB ligand (RANKL), cyclooxygenase (COX)-2, NO, IL-6, IL-1β and TNFα [236–240]. This suggests that VitD might be able to restore the balance of M1/M2 polarization disturbed during disease, by promoting the M2 phenotype on cost of the M1 phenotype. Moreover, this results in the capability of VitD treated macrophages to activate T cells [240].

Regarding underlying mechanisms, which are described in figure 5, the thioester superfamily member 4 (THEM4) was found to be affected by VitD. THEM4 is an inhibitor of NFκB and prevents its binding to the DNA as was shown for the COX-2 promoter [238]. In addition, induction of Suppressor of Cytokine Signalling (SOCS) via

NFκB-induced miR155 is blocked by THEM4, resulting in decreased IL-6 and TNFα expression [239]. No information on the pathways involved in dampening production of the other pro-inflammatory mediators is available so far.
Targeting M1 derived, pro-inflammatory mediators in therapy of AID has already been proven to be successful, indicating that VitD and zinc might also be applicable.

5.3 Innate lymphoid cells (ILC)

Due to advanced technologies, knowledge and highly sensitive instruments, the so called innate lymphoid cells (ILC) were uncovered recently and grouped into ILC1, ILC2, ILC3 and NK cells [241]. Interestingly, the expression profiles of ILCs match certain types of Th subtypes. All subtypes are important for tissue homeostasis by regulating tissue repair. ILC1 and NK cell polarization is T-bet dependent as is development of Th1 cells and they express IFNγ, indicating their importance during viral infections. ILC2 cells are characterized by their GATA3-dependent development and expression of type 2 cytokines including IL-5 and IL-13, resembling Th2 cells. The development of ILC3 is induced via RORC2 and cells secrete IL-17A, similar for what was described for Th17 cells [241].
ILC were identified in peripheral blood of MS patients and in inflamed intestine of IBD patients, amongst others via IL-23-induced IL-17A and IFNγ expression [242–244]. In addition, ILC3 cells were enriched in skin lesions and blood from psoriasis patients, promoting the disease by secretion of IL-22 and IL-17A [171,245]. In patients with sjogren´s syndrome, IL-22 producing ILC3 were enriched in bone marrow, peripheral blood, synovial fluid and in the gut [246]. So far, it is not clear, if ILCs are rather supporting the development of AID or suppressing it, which was recently reviewed [247]. Also, as in most cases all subtypes of ILCs were found in AID patients, the exact assignment of one type to a certain disease is so far barely possible.
The role of zinc in NK cell development and function just recently started to emerge [248–250]. Moreover, so far data suggest that lytic and killing activity of NK cells is decreased during zinc deficiency due to decreased MHC-I recognition and increased when zinc is added to cells *in vitro* [250].
Results on the association between NK cells, VitD and AID are quite contradictory as well. Investigation of a NK cell line showed that VitD incubation lead to increased activities of benzyloxycarbonyl-L-lysine thiobenzyl (BLT) esterase and protein kinase C (PKC) and thereby stimulation of NK cell activity including cytotoxicity [251]. However,

this was not reproduced, when primary human blood cells were investigated [252,253]. Development of NK cells from hematopoietic stem cells, their IFNγ expression and cytotoxicity were even impaired under high VitD conditions [253]. When over-reactive NK cells were isolated from women with recurrent pregnancy loss, VitD could reduce their activity, decrease cytotoxic capacity and the production of pro-inflammatory cytokines [252].

ILC1 and ILC3 cells were enriched in VDR KO mice and it was suggested that expression of the VDR regulates ILC frequencies, IL-22 levels, and susceptibility to *Citrobacter rodentium*-induced colitis [254]. VitD treatment for two weeks did not alter the amount of ILCs in patients with psoriasis in another study, but VitD still caused clinical improvement by reducing the frequency of IL17-expressing CTL [156]. However, knowledge and data available in this area of research is far too little to draw conclusions and studies should address this field in the future, including not only analysis of cell numbers, but also on their activity.

Collectively, the role of ILCs in AID needs to be clarified before assumptions on effects of zinc and VitD on their functions should be made.

6. Non-immune cells

The damage during AID is mostly done by immune cells and their products. However, the activation and survival of other cells within the body, especially in the area that is affected by the autoimmune reaction add to the severity of the disease. Thus, in some cases, the host cells are not only the target, but take part in disease development themselves. As examples, damage of brain, intestine and pancreas are discussed in the next paragraphs.

6.1 Neuronal cells in MS

The brain is usually protected from the immune cells by the blood brain barrier (BBB). However, excessive activation of matrix-degrading enzymes such as matrix metalloproteinase 9 (MMP-9) can cause BBB disruption and subsequent immune cell infiltration from peripheral blood (figure 7.1) [255]. Other factors, causing the destruction of neurons, subsequently leading to motor dysfunction are primarily excessive levels of reactive oxygen and nitrogen species in the brain [256].

In contrast to what was reported in previous chapters, high zinc concentrations were found in the brains of EAE mice and MS patients, while serum zinc levels are still decreased compared to healthy controls. Similar results were found for non-AID neuronal diseases, such as Alzheimer's disease, dementia and Parkinson's diseases to name a few [257]. In the brain, zinc is usually packed into synaptic vesicles and released by ZnT3 to act as a neurotransmitter. However, if zinc concentrations exceed a certain threshold, neurotoxicity is the consequence, which was broadly discussed in a recent review [258]. In a study by Choi et al. (2013), abnormal vesicular zinc release and intracellular zinc accumulation were found and associated with activation of MMP-9, the disruption of the BBB and thus the increased recruitment of immune cells from peripheral blood, responsible for destruction of spinal cord white matter and subsequent motor deficits (figure 7.1). MOG (myelin oligodendrocyte glycoprotein)-induced EAE mice were treated with the metal chelator clioquinol via gavage, which ameliorated disease scores, the disruption of the BBB, and thus immune cell infiltration and myelin destruction. The same effects were found, when zinc release from vesicles was abolished in ZnT3 KO mice (Figure 7.2). Clioquinol gavage was still effective, when disease was already established, resulting in a reduction of clinical scores and less destruction of myelin compared to vehicle treated EAE animals [259]. This led to the hypothesis that excessive zinc, released into the extracellular space, supports destruction of the myelin sheath in the spinal cord white matter and the generation of motor deficits in MS patients, similar to what was observed in mice.

Subsequent studies found that zinc activates the Nicotinamide Adenine Dinucleotide Phosphate Hydrogen (NADPH) oxidase in neuronal cells via protein kinase C thus increasing ROS production [260]. Also, increased synaptic zinc was associated with death of oligodendrocytes, which in healthy subjects built the myelin sheath, but are destroyed during MS. Furthermore, intracellular zinc release increased the toxic effects of peroxynitrite, contributing to the death of oligodendrocytes [261,262]. Peroxynitrite results from the reaction of superoxide with nitric oxide. In line with this, elevated levels of iNOS were found in MS lesions [261,263]. Apoptosis as induced by peroxynitrite is mediated via ERK1/2 signaling, which was shown to be affected by intracellular zinc levels in numerous studies and proven as underlying mechanism in death of oligodendrocytes here as well. In this regard, it was postulated that AMPA receptor activation results in intracellular zinc release [264]. 12-lipoxygenase was identified as a

zinc target involved in ROS production and oligodendrocyte death as well [261]. In another study, cell death was reported to be mediated by protein kinase C, which was also related to neurotoxicity and its activity is affected by the zinc status of the cell [265–267]. A role of zinc in inducing autophagy in neuronal cells was suggested as well [259]. Finally, zinc induces COX-2 in microglia, resulting in their-activation, increased expression of pro-inflammatory cytokines and oxidative stress. Subsequently, this results in the death of dopaminergic neurons through Bax-mediated apoptosis [268]. Together, those data create an obstacle for MS treatment by zinc, as serum levels should be re-balanced by supplementation, but brain zinc not further increased. However, zinc supplementation was still a successful treatment strategy in animal models.

Interestingly, VitD was shown to support the BBB integrity. In a recent study using a human brain microvascular endothelial cell line, it was observed that VitD attenuated the damage of barrier function after exposure to either sera from MS patients (both relapse–remitting and secondary progressive MS) or TNFα. Upregulation of tight junction proteins and downregulation of cell adhesion molecules were found as underlying mechanisms [269]. Therefore, combining zinc with vitamin D might offer a solution.

6.2 Intestinal cells and IBD/CD

Similar to what was just described for neuronal cells, intestinal cells are targets of AID and known to be sensitive to altered zinc homeostasis. Sufficient zinc supply is important to preserve intestinal barrier function and to prevent trans-mucosal leakage [270]. In line with this, the leaky gut syndrome was associated with zinc deficiency. As intestinal injury can cause intestinal loss of zinc and malabsorption causing zinc deficiency, zinc deficiency-induced barrier dysfunctions are self-perpetuating. Literature here is convincing and a lot of great reviews are available [271–274]. Thus, only the most important points will be included here.

In a zinc supplementation study for pigs during weaning, which often develop diarrhea, clear impacts of zinc on colonic morphology, mucin profiles and immune response were observed. It was suggested that those changes might support local defense mechanisms and affect colonic physiology, contributing to the reduction of post-weaning diarrhea, which might be transferrable to AID-induced stress on the intestine [275]. Culturing human intestinal Caco-2 cells under zinc deficient conditions in conjunction

with TNFα exposure, to model existing intestinal inflammation, higher rates of apoptosis were observed compared to cells growing under zinc adequate conditions, explaining the loss of epithelial barrier integrity [276]. This epithelial barrier dysfunction then facilitates recruitment of leukocytes and unregulated leakage of luminal antigens across the epithelial barrier. This leak does normally not take place and causes exposure of the immune cells to auto-antigens thus triggering AID [277]. On a molecular basis, zinc is necessary for the expression of tight junction proteins [278,279]. During zinc deficiency, enterocyte peptidase activity is also altered, resulting in decreased absorption of proteins [280]. Figure 8A summarizes the effects of zinc that were suggested regarding intestinal cells.

Along with what was just described for zinc, VitD is an important player during intestinal epithelial defense against infectious agents as is illustrated in figure 8B. VitD deficiency was revealed to predispose to increased severity of intestinal injury in an infectious model of colitis in mice [281]. In line with this, VitD supplementation ameliorated intestinal epithelial turnover and thereby improved the integrity as well as the function of the intestinal barrier during liver cirrhosis in rats. One of the underlying mechanisms of those beneficial effects include the effect of VitD on heme oxygenase 1 signaling activation [282]. Moreover, VitD is involved in activating expression of TLR2, NOD2, β defensin, Claudin-2, Claudin-12, ZO1 and ZO2 [283–285].

6.3 Zinc and pancreatic alpha and beta cells in T1DM

In comparison to other cells, pancreatic β cells contain very high zinc concentrations, especially within the insulin secretory granules [286]. ZnT8, one of 24 zinc transport proteins identified in humans, is located in the granule membrane and can increase granular zinc, thus providing sufficient zinc levels for insulin synthesis, storage and stability, as can be seen in figure 9A [287,288].

As early as 1966, publications on decreased secretion of insulin and insulin sensitivity in zinc-deficient rats occurred [289]. Also, zinc plays an important role in the biochemistry of insulin and glucagon and stimulates lipogenesis and glucose uptake in isolated adipocytes [290]. For example, high levels of ZnT5 were detected in insulin secretory granules, while ZnT3 and Znt7 are suggested to support insulin secretion [291–293]. In a recent *in vitro* study using INS-1E beta cells, Zip14 was found to be important for their functions, which remains to be verified [294]. Alpha cells require ZnT8 for glucagon secretion during hypoglycemia [179,295]. Interestingly, mutations in the gene encoding

ZnT8 were associated with increased risk to develop diabetes, however, majorly T2D [296]. Also, auto-antibodies against ZnT8 were detected in patients with T1DM, but were not directly related to the disease as indicated earlier [179]. High zinc levels were also shown to increase insulin sensitivity in target tissues. In this, zinc inhibits PTP1B, increasing phosphorylation of the insulin receptor and thereby downstream signaling and gene activation [297]. Thus, effects of zinc are similar to those of reactive oxygen species, but mechanisms are different, as was investigated and described in great detail [298,299]. Signaling pathways affected include PI3K and protein kinase B (associated with enhanced glucose uptake), as revealed in 3T3-L1 fibroblasts and adipocytes [300]. This might explain the association of zinc deficiency with increased incidence of diabetes. On the other hand, insulin itself can regulate intracellular zinc levels through NADPH oxidase-generated hydrogen peroxide production, which causes release of zinc from metallothionein or the release of zinc from the endoplasmic reticulum by the zinc transporter Zip7 [301,302]. Finally, zinc is directly involved in regulating insulin function and a part of its structure. The insulin hexamer contains two zinc ions and one calcium ion. Zinc is involved in the transition from inactive “T” state of insulin into “R” state after interaction with phenolic ligands and zinc is co-secreted with insulin [303]. The complex has paracrine effects and induces glucagon secretion from alpha cells and might also be involved in inhibition of hepatic insulin clearance. Zinc ions themselves might affect alpha cells as well [304,305]. Also, zinc inhibits fibrillation of monomeric insulin and dimer formation with amyloid polypeptide *in vitro*, which remains to be proven *in vivo* [306].

VitD affects pancreatic beta cell function in various ways, as depicted in figure 9B. After entering the cell, VitD binds to the VDR-RXR complex which subsequently binds to the insulin promoter. Thereby, transcription is activated and insulin is synthesized. In addition, it was suggested that VitD promotes survival of beta cells via downregulation of the Fas-related pathways (Fas/Fas-L). Calbindin, a cytosolic calcium-binding protein found in beta cells, is also regulated by VitD and acts as a modulator of depolarization-stimulated insulin release. Furthermore, calbindin may also protect against apoptosis via its ability to buffer intracellular calcium (figure 10). VitD is also able to regulate extracellular calcium concentrations, which alters calcium flux through the beta cell. Alterations in calcium flux can directly influence insulin secretion, as this is a calcium-dependent process [307]. Recently, VitD was suggested to induce autophagy of

pancreatic β-cells, as well as prevented insulitis in a streptozotocin induced T1DM mouse model and in MIN6 mouse insulinoma β-cells [308], which remains to be explored in more detail.

The presented data indicate that the roles of zinc and VitD especially in MS and T1DM are complex. However, although a multitude of different mechanisms were proposed for zinc´s effects, they are all supporting the usage of zinc to prevent and treat AID, especially in patients that reveal zinc deficiency.

7 Current treatment strategies

If an AID is not treated, severe progression culminating in the destruction of the affected organ and ultimately death can be anticipated. Therefore, early diagnosis and start of therapy are of high priority. However, this often involves interdisciplinary analysis, until the diagnosis is clear as all kinds of organs, organ systems and combinations of organs can be affected. Some AID share several symptoms, but do not respond to the same treatment, whereas others, that produce different signs of disease can be perfectly treated using the same approach such as glucocorticoids [309]. Some current strategies are described in Table 2. However, treatment decision is quite complex and more global strategies are highly warranted, to overcome trial-and-error approaches. Moreover, current treatment regimens are often costly, as is represented especially by the costs for medication used for MS patients and thus alternatives such as zinc or vitamin D, which can be provided by a certain diet, and are also affordable supplements, offer a perfect support or even alternative.

There is a complex interaction of genetic and environmental factors so that in most cases clinical symptoms appear delayed from eliciting events. Also, the same disease might show a variety of symptoms and phenotypes adding another diagnostic obstacle. Although it was found that one immune cell type is largely responsible for the individual AID, it is in most cases the combination of the dysbalance of various immune cells that underlies the pathology. Thus, instead of finding therapeutic options addressing one cell type or even one of its functions, more general approaches for rebalancing should be preferred. Therefore, traditionally, the most common way of treating AID is by unspecific immunosuppressive or anti-inflammatory agents, which has the disadvantage of increased susceptibility to pathogen-induced inflammatory diseases and of significant morbidity.

Targeting intracellular signaling pathways involved in the inflammatory response has shown success, as for example IL-1 blockade in cryopyrinopathies, juvenile idiopathic arthritis and gout [310]. For psoriasis, addressing IL-17, TNFα and IL-12/23 have been successful strategies and RA was controlled by depleting B cells or using janus kinases, IL-6, IL-1β or TNFα as targets [311,312].

Unfortunately, the response of patients to those treatments has been heterogeneously efficient, leaving a lot of unresponsive patients with their symptoms as was the case of 30 – 40% of IBD, RA and psoriasis patients treated with agents blocking TNFα [312]. Thus, alternative strategies are warranted or at least biomarkers that help to predict responsiveness to a certain agent, so that different personalized strategies are used instead.

This might be achieved applying advanced strategies such as transcriptome analysis [313]. Especially new technologies such as next-generation sequencing already generated promising data but are so far rather helpful to increase our knowledge on mechanisms underlying diseases. Gene expression profiling for example uncovered therapeutic targets such as IL-1 in AID characterized by innate immunity and enabled targets for psoriasis analyzing the skin transcriptome [314,315]. As those techniques are quite costly approaches, including them in routine diagnostics might rather be done at a later point, especially for smaller laboratories [316]. Alternatively, defining as many environmental triggers as possible and addressing them, is quite promising if nutritional triggers are involved in disease etiology and deficiencies of certain elements have been found in a patient. Although the use of biologicals such as TNFα inhibitors has much improved the success of AID treatment, there is still a far too high number of non-responding patients. New therapeutic approaches should preferably involve oral drugs with little side effects. In this regard, the afore mentioned data strongly suggest taking nutritional strategies into account when developing new treatment schemes for AID. First promising results from mouse experiments but also some human studies are available, especially for MS/EAE, T1DM, IBD and AID affecting the gastro-intestinal tract in regard to zinc as well as VitD for prevention and treatment. If those strategies are combined with novel diagnostic approaches, individualized therapy might be possible and effective in the future. However, prevention using nutritional approaches would be an advantage in multiple regards to decrease the incidence of AID (Table 1)

Summary and concluding remarks

The incidence of AID is rising, resulting in high amounts of people suffering from diseases that are difficult to treat. Thus patients are subjected to severe symptoms and death. Generally, AID predisposition cannot simply be related to one factor, such as limited sunlight and thus VitD deficiency or being vegan and thus at risk for zinc deficiency. Also, not every VitD or zinc deficient person will develop an AID, as genetics and the total lifestyle play a vital part here as well. However, deficiencies of nutritional elements such as zinc and vitamin D are often found in patients with AID. Their origin as well as the symptoms and complications they cause are manifold, which impedes diagnosis and management. Thus, during clinical care of AID patient's, possible micronutrient deficiencies should be considered and if observed consequently be treated by monitored supplementation of the deficient element(s). In this regard, more studies concentrating on nutritional element interactions are strongly warranted to be able to optimize treatment strategies for AID patients and stopping the increase in incidence using simple and cost-effective nutritional approaches.

Figure legends

Figure 1: Effects of Zinc on auto-immune-related functions of immune cells

An illustration of the zinc-regulated processes in immune cells, which can be related to autoimmune diseases, as detailed in the text. CTL: cytotoxic lymphocytes; BCR: B cell receptor; DC: Dendritic Cells, GM-CSF: Granulocyte Macrophage-Colony Stimulating Factor; IL: Interleukin; LPS: lipopolysaccharide; Mφ: macrophages; MHC: Major Histocompatibility Complex: MLC: Mixed Lymphocyte Culture; NK: Natural Killer, TCR: T cell receptor; TNF: Tumor Necrosis Factor

Figure 2: Effects of vitamin D on auto-immune-related functions of immune cells

An overview of Vitamin D´s effects immune cells, which can be related to autoimmune diseases and which are described in the text. CD: cluster of differentiation; COX: Cyclooxygenase; CTL: cytotoxic lymphocytes; DC: Dendritic Cells, IFN: Interferon; IL: Interleukin; ILC: innate lymphoid cells; Mφ: macrophages; MHC: Major Histocompatibility Complex: NK: Natural Killer; NO: nitrogen oxide; TCR: T cell receptor; TNF: Tumor Necrosis Factor; VDR: Vitamin D Receptor

Figure 3: Depending on its concentration, zinc exerts inhibitory or stimulatory effects on signal transduction in Treg (A) and Th17 (B) cells.

(A) For Treg cells, so far effects have been described on transforming growth factor β receptor (TGFβR)-dependent (A) or –independent, but CD28, T cell receptor (TCR) or interleukin (IL-)2R –dependent signaling.
Zinc inhibits mammalian target of rapamycin (mTOR) activity in response to CD28, TCR or IL-2R stimulation in Treg cells, which abolishes TGF-induced Smad signaling. On the other hand, zinc increases the phosphorylation of Smad by inhibiting phosphatases, resulting in increased Smad binding to the forkhead box protein (Foxp)3 promoter and a net increase in its expression. Also, zinc blocks calcineurin-mediated Nuclear Factor of Activated T Cell (NFAT)-activation. Zinc increases Akt phosphorylation and thus mTOR induced chromatin remodeling within the Foxp3 promoter. On the other hand, zinc inhibits Protein Kinase A (PKA) and p38-mediated signaling, resulting in decreased Cyclic Adenosine Monophosphate (cAMP)-response-element-binding protein (CREB) and Activator Protein (AP)-1 binding to the Foxp3 promoter. Still the net effect of zinc

are increased cytoplasmic Foxp3 levels, amongst others due to the zinc-mediated decrease in Sirtuin (Sirt)-1 activity and therefore decreased acetylation-induced proteasomal destruction of Foxp3 protein. Finally, zinc activates micro(mi)RNA146a resulting in stronger Treg cell activation. In summary zinc inhibits interferon regulatory factor (IRF)-1, Sirt-1 and Smad, while it induces Kruppel-like factor (KLF)-10, Foxp3, cytotoxic T-lymphocyte-associated protein (CTLA)4 and CD25 in Treg cells.
(B) Th17 cells are induced by IL-6 and TGFβ. Zinc binds STAT3, inhibiting its activation by IL-6
CCL: Chemokine Ligand; ERK: extracellular signal–regulated kinases; GM-CSF: Granulocyte Macrophage-Colony Stimulating Factor; HAT: histone acetylase; IL: Interleukin; JNK: Janus Kinase; P: phosphorylation; PI3K: phosphatidylinositol-3-kinase; STAT: Signal Transducer and Activator of Transcription.

Figure 4: Vitamin D´s effects on Treg (A) and Th17 (B) cells

(A) Vitamin D increases Foxp3 expression via increased binding of the vitamin D receptor (VDR) to the Foxp3 promoter within the calcitriol-dependent enhancer region. In summary, Vitamin D induces Foxp3, cytotoxic T-lymphocyte-associated protein (CTLA)4, Interleukin (IL-)10, Helios and transforming growth factor (TGF)β expression via its receptor (VDR).
(B) After binding of vitamin D its receptor, retinoid X receptor (RXR) is recruited as well as phosphorylated (P) Smad3. The complex binds to the DNA and recruits histone deacetylase (HDAC2), resulting in blockade of Smad7 expression. Moreover, VitD inhibits the translocation of Nuclear Factor of Activated T Cell (NFAT) to the nucleus and transcription of IL-17 mRNA. Translation of IL-17 mRNA to IL-17 protein is also decreased by VitD. In summary, VitD increases expression of Interleukin (IL)-17, IL-23 receptor, IL-22, Interferon (IFN)γ, Retinoic-acid-orphan-receptor-C (RORc), chemokine receptor (CCR)6, extracellular signal–regulated kinase (ERK), Smad3, VDR, IL-10 and IL-4 in Th17 cells.

Figure 5: Proposed effect of Zinc and Vitamin D supplementation on T cell activation by dendritic cells (DC)

Zinc (Zn) (A) as well as Vitamin D (VitD) (B) shape the tolerogenic potential of dendritic cells, which affects Th cell polarization, favoring Treg differentiation over the

development of Th17 cells. Shown are the mechanisms underlying the effects of zinc and Vitamine D on DCs proposed so far, which needs to be evaluated in more detail, especially as the amount of available *in vivo* data is limited.
AG: antigen; CCL: CC-chemokine ligand; CD: cluster of differentiation; IDO: indoleamine 2,3 dioxygenase; IL: interleukin; ILT: immunoglobulin-like transcript; MHC: Major Histocompatibility Complex; mTOR: mammalian target of rapamycin; PI3K: phosphatidylinositol-3-kinase; RXR: retinoid X receptor; TCR: T cell receptor; TLR: Toll-like receptor, TNF: tumor necrosis factor; PD-L: programmed death-ligand; VDR: vitamin D receptor

Figure 6: Vitamin D alters Cytokine expression in macrophages

After ligand binding, Vitamin D receptor (VDR) is recruited to the THEMA4 promoter, resulting in expression of the adjacent gene. THEMA4 then blocks NFκB-induced expression of Interleukin (IL-)6, Tumor Necrosis Factor (TNF)α and COX)-2. In summary, VitD treatment results in decreased expression of the pro-inflammatory mediators IL-1β, IL-6, TNFα and cyclooxygenase (COX)-2, while the expression of anti-inflammatory IL-10 is increased.

Figure 7: Zinc´s Effect on the Blood Brain Barrier and neuronal communication

The effects of zinc within the brain are manifold. 1): matrix metalloprotease (MMP)-9, responsible for degradation of extracellular matrix and thus involved in blood brain barrier (BBB) maintenance, acts zinc dependent. 2) Moreover it was shown that zinc release by ZnT3 into the synaptic space is involved in communication of pre- and post-synaptic neuronal terminals. 3) Within the postsynaptic neurons zinc is involved in activation of proteinkinase C (PKC), and thus via NADPH oxidase (NOX), reactive oxygen species (ROS) and Poly(ADP-Ribose)-Polymerase 1 (PARP-1), effects apoptosis. 4) Recently, it was suggested that α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) activation results in intracellular zinc release, inducing apoptosis in oligodendrocytes. 5) Zinc also alters autophagy and 6) activates microglia to produce pro-inflammatory interleukin (IL-)6, IL-1β and tumor necrosis factor (TNF)α as well as ROS, adding to apoptosis of neighboring cells.

Figure 8: Altered zinc or Vitamin D homeostasis can affect gene expression of intestinal epithelial cells

A) Intestinal epithelial cells react to changes in zinc homeostasis with altered expression and phosphorylation of tight junction proteins and membrane location of zona occludens (ZO)-1. This is amongst others associated with zinc´s effects on phospholipase C (PLC) and inositol triphosphate (IP_3).
MAP kinase kinase (MKK), AMP-activated protein kinase (AMPK), endoplasmic reticulum (ER).
B) Via the vitamin D receptor (VDR), VitD affects the expression of several genes including toll-like receptor (TLR)-2, nucleotide-binding oligomerization domain-containing protein (NOD)-2, and zona occludens (ZO)-1 and ZO-2 as well as the phosphorylation of occludin.

Figure 9: Illustration of effects of zinc and Vitamin D on insulin-induced signaling and metabolism

A) Zinc is well known for its effect on intracellular signaling. Amongst others, zinc alters activation of protein tyrosine phosphatase (PTEN), tribbles 3 (TRB3), Protein-tyrosine phosphatase (PTP1), subsequently altering phosphatidylinositol-3-kinase (PI3K) and Akt signaling pathways, which affects expression of glucose transporter (Glut)4. Regarding levels of free zinc, available for signaling, the zinc-binding protein metallothionein (MT) plays a major role. Binding of zinc to MT is redox sensitive and thus affected by H_2O_2, produced by the NADPH oxidase (NOX).
B) It was found that the genes for insulin and calbindin have vitamin D receptor (VDR) binding sites in their promoters. Thus, the binding of VitD to its receptor can alter their expression leading to altered release of insulin or apoptosis.

References

[1] Arakelyan A, Nersisyan L, Poghosyan D, Khondkaryan L, Hakobyan A, Löffler-Wirth H et al. Autoimmunity and autoinflammation: A systems view on signaling pathway dysregulation profiles. PLoS ONE 2017;12(11):e0187572.

[2] Lerner A, Jeremias P, Matthias T. The World Incidence and Prevalence of Autoimmune Diseases is Increasing. IJCD 2015;3(4):151–5.

[3] Lerner A, Matthias T. Changes in intestinal tight junction permeability associated with industrial food additives explain the rising incidence of autoimmune disease. Autoimmun Rev 2015;14(6):479–89.

[4] Lerner A, Matthias T. Rheumatoid arthritis-celiac disease relationship: Joints get that gut feeling. Autoimmun Rev 2015;14(11):1038–47.

[5] Marrack P, Kappler J, Kotzin BL. Autoimmune disease: why and where it occurs. Nature Medicine 2001;7(8):899.

[6] Damsker JM, Hansen AM, Caspi RR. Th1 and Th17 cells: Adversaries and collaborators. Ann N Y Acad Sci 2010;1183:211–21.

[7] Clarke K, Chintanaboina J. Allergic and Immunologic Perspectives of Inflammatory Bowel Disease. Clin Rev Allergy Immunol 2018.

[8] Ermann J, Fathman CG. Autoimmune diseases: genes, bugs and failed regulation. Nat Immunol 2001;2(9):759–61.

[9] Wessells KR, Brown KH. Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting. PLoS ONE 2012;7(11):e50568.

[10] Wessels I, Maywald M, Rink L. Zinc as a Gatekeeper of Immune Function. Nutrients 2017;9(12).

[11] Prasad AS. Effects of zinc deficiency on Th1 and Th2 cytokine shifts. J Infect Dis 2000;182 Suppl 1:S62-8.

[12] Lönnerdal B. Dietary factors influencing zinc absorption. J Nutr 2000;130(5S Suppl):1378S-83S.

[13] Haase H, Maret W. Intracellular zinc fluctuations modulate protein tyrosine phosphatase activity in insulin/insulin-like growth factor-1 signaling. Exp Cell Res 2003;291(2):289–98.

[14] Sanna A, Firinu D, Zavattari P, Valera P. Zinc Status and Autoimmunity: A Systematic Review and Meta-Analysis. Nutrients 2018;10(1).

[15] Pawlitzki M, Uebelhör J, Sweeney-Reed CM, Stephanik H, Hoffmann J, Lux A et al. Lower Serum Zinc Levels in Patients with Multiple Sclerosis Compared to Healthy Controls. Nutrients 2018;10(8):967.

[16] Salgueiro MJ, Krebs N, Zubillaga MB, Weill R, Postaire E, Lysionek AE et al. Zinc and Diabetes Mellitus Is There a Need of Zinc Supplementation in Diabetes Mellitus Patients? BTER 2001;81(3):215–28.

[17] Sujatha P, Pasula S, Sameera K. Trace elements in diabetes mellitus. J Clin Diagn Res 2013;7(9):1863–5.

[18] Wessels I, Cousins RJ. Zinc dyshomeostasis during polymicrobial sepsis in mice involves zinc transporter Zip14 and can be overcome by zinc supplementation. Am J Physiol Gastrointest Liver Physiol 2015;309(9):G768-78.

[19] Nishi Y, Kawate R, Usui T. Zinc metabolism in thyroid disease. Postgrad Med J 1980;56(662):833–7.

[20] Ertek S, Cicero AF, Caglar O, Erdogan G. Relationship between serum zinc levels, thyroid hormones and thyroid volume following successful iodine supplementation. Hormones (Athens) 2010;9(3):263–8.

[21] Kruis W, Phuong Nguyen G. Iron Deficiency, Zinc, Magnesium, Vitamin Deficiencies in Crohn's Disease: Substitute or Not? Dig Dis 2016;34(1-2):105–11.

[22] Abdel Fattah NSA, Atef MM, Al-Qaradaghi SMQ. Evaluation of serum zinc level in patients with newly diagnosed and resistant alopecia areata. Int J Dermatol 2016;55(1):24–9.

[23] Norman AW. Cholecalciferol | Physiology. In: Caballero B, editor. Encyclopedia of food sciences and nutrition, 2nd ed. Amsterdam: Academic Press; 2003, p. 1213–1220.

[24] Vieth R. Why “Vitamin D” is not a hormone, and not a synonym for 1,25-dihydroxy-vitamin D, its analogs or deltanoids. J Steroid Biochem Mol Biol 2004;89-90:571–3.

[25] Ross AC, Taylor CL, Yaktine AL, Del Valle HB (eds.). Dietary Reference Intakes for Calcium and Vitamin D. Washington (DC); 2011.

[26] Engelsen O. The relationship between ultraviolet radiation exposure and vitamin D status. Nutrients 2010;2(5):482–95.

[27] Pike JW, Meyer MB. The vitamin D receptor: new paradigms for the regulation of gene expression by 1,25-dihydroxyvitamin D(3). Endocrinol Metab Clin North Am 2010;39(2):255-69, table of contents.

[28] Zahoor I, Haq E. Vitamin D and Multiple Sclerosis: An Update: Codon Publications; 2017. doi:10.15586/codon.multiplesclerosis.2017.ch5.

[29] Horst RL, Goff JP, Reinhardt TA. Role of vitamin D in calcium homeostasis and its use in prevention of bovine periparturient paresis. Acta Vet Scand Suppl 2003;97:35–50.

[30] Wessels I, Fleischer D, Rink L, Uciechowski P. Changes in chromatin structure and methylation of the human interleukin-1beta gene during monopoiesis. Immunology 2010;130(3):410–7.

[31] Seyedalipour F, Mansouri A, Vaezi M, Gholami K, Heidari K, Hadjibabaie M et al. High Prevalence of Vitamin D Deficiency in Newly Diagnosed Acute Myeloid Leukemia Patients and Its Adverse Outcome. Int J Hematol Oncol Stem Cell Res 2017;11(3):209–16.

[32] Carmeliet G, Dermauw V, Bouillon R. Vitamin D signaling in calcium and bone homeostasis: a delicate balance. Best Pract Res Clin Endocrinol Metab 2015;29(4):621–31.

[33] Lemire JM, Archer DC. 1,25-dihydroxyvitamin D3 prevents the in vivo induction of murine experimental autoimmune encephalomyelitis. J Clin Invest 1991;87(3):1103–7.

[34] Zwerina K, Baum W, Axmann R, Heiland GR, Distler JH, Smolen J et al. Vitamin D receptor regulates TNF-mediated arthritis. Ann Rheum Dis 2011;70(6):1122–9.

[35] Cantorna MT, Munsick C, Bemiss C, Mahon BD. 1,25-Dihydroxycholecalciferol prevents and ameliorates symptoms of experimental murine inflammatory bowel disease. J Nutr 2000;130(11):2648–52.

[36] Mathieu C, Waer M, Laureys J, Rutgeerts O, Bouillon R. Prevention of autoimmune diabetes in NOD mice by 1,25 dihydroxyvitamin D3. Diabetologia 1994;37(6):552–8.

[37] Lemire JM, Ince A, Takashima M. 1,25-Dihydroxyvitamin D3 attenuates the expression of experimental murine lupus of MRL/l mice. Autoimmunity 1992;12(2):143–8.

[38] Song GG, Bae S-C, Lee YH. Association between vitamin D intake and the risk of rheumatoid arthritis: a meta-analysis. Clin Rheumatol 2012;31(12):1733–9.

[39] Dankers W, Colin EM, van Hamburg JP, Lubberts E. Vitamin D in Autoimmunity: Molecular Mechanisms and Therapeutic Potential. Front Immunol 2016;7:697.

[40] Tao C, Simpson S, van der Mei I, Blizzard L, Havrdova E, Horakova D et al. Higher latitude is significantly associated with an earlier age of disease onset in multiple sclerosis. J Neurol Neurosurg Psychiatry 2016;87(12):1343–9.

[41] Mohr SB, Garland CF, Gorham ED, Garland FC. The association between ultraviolet B irradiance, vitamin D status and incidence rates of type 1 diabetes in 51 regions worldwide. Diabetologia 2008;51(8):1391–8.

[42] Szilagyi A, Leighton H, Burstein B, Xue X. Latitude, sunshine, and human lactase phenotype distributions may contribute to geographic patterns of modern disease: the inflammatory bowel disease model. Clin Epidemiol 2014;6:183–98.

[43] Dobson R, Giovannoni G, Ramagopalan S. The month of birth effect in multiple sclerosis: systematic review, meta-analysis and effect of latitude. J Neurol Neurosurg Psychiatry 2013;84(4):427–32.

[44] Shen L, Zhuang Q-S, Ji H-F. Assessment of vitamin D levels in type 1 and type 2 diabetes patients: Results from metaanalysis. Mol Nutr Food Res 2016;60(5):1059–67.

[45] Duan S, Lv Z, Fan X, Le Wang, Han F, Wang H et al. Vitamin D status and the risk of multiple sclerosis: a systematic review and meta-analysis. Neurosci Lett 2014;570:108–13.

[46] Lin J, Liu J, Davies ML, Chen W. Serum Vitamin D Level and Rheumatoid Arthritis Disease Activity: Review and Meta-Analysis. PLoS ONE 2016;11(1):e0146351.

[47] Lu C, Yang J, Yu W, Li D, Xiang Z, Lin Y et al. Association between 25(OH)D Level, Ultraviolet Exposure, Geographical Location, and Inflammatory Bowel Disease Activity: A Systematic Review and Meta-Analysis. PLoS ONE 2015;10(7):e0132036.

[48] Sahebari M, Nabavi N, Salehi M. Correlation between serum 25(OH)D values and lupus disease activity: an original article and a systematic review with meta-analysis focusing on serum VitD confounders. Lupus 2014;23(11):1164–77.

[49] Tizaoui K, Kaabachi W, Hamzaoui A, Hamzaoui K. Association between vitamin D receptor polymorphisms and multiple sclerosis: systematic review and meta-analysis of case-control studies. Cell Mol Immunol 2015;12(2):243–52.

[50] Lee YH, Bae S-C, Choi SJ, Ji JD, Song GG. Associations between vitamin D receptor polymorphisms and susceptibility to rheumatoid arthritis and systemic lupus erythematosus: a meta-analysis. Mol Biol Rep 2011;38(6):3643–51.

[51] Xue L-N, Xu K-Q, Zhang W, Wang Q, Wu J, Wang X-Y. Associations between vitamin D receptor polymorphisms and susceptibility to ulcerative colitis and Crohn's disease: a meta-analysis. Inflamm Bowel Dis 2013;19(1):54–60.

[52] Wang L, Wang ZT, Hu JJ, Fan R, Zhou J, Zhong J. Polymorphisms of the vitamin D receptor gene and the risk of inflammatory bowel disease: a meta-analysis. Genet Mol Res 2014;13(2):2598–610.

[53] Tizaoui K, Kaabachi W, Hamzaoui A, Hamzaoui K. Contribution of VDR polymorphisms to type 1 diabetes susceptibility: Systematic review of case-control studies and meta-analysis. J Steroid Biochem Mol Biol 2014;143:240–9.

[54] Lu M, Taylor BV, Körner H. Genomic Effects of the Vitamin D Receptor: Potentially the Link between Vitamin D, Immune Cells, and Multiple Sclerosis. Front Immunol 2018;9.

[55] Zipitis CS, Akobeng AK. Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis. Arch Dis Child 2008;93(6):512–7.

[56] Zhu J, Paul WE. CD4 T cells: fates, functions, and faults. Blood 2008;112(5):1557–69.

[57] Nagata S, Suda T. Fas and Fas ligand: Lpr and gld mutations. Immunol Today 1995;16(1):39–43.

[58] Delgoffe GM, Powell JD. Feeding an army: The metabolism of T cells in activation, anergy, and exhaustion. Mol Immunol 2015;68(2 Pt C):492–6.

[59] Fischer HJ, Witte A-K, Walter L, Gröne H-J, van den Brandt J, Reichardt HM. Distinct roles of T-cell lymphopenia and the microbial flora for gastrointestinal and CNS autoimmunity. FASEB J 2016;30(5):1724–32.

[60] Marleau AM, Sarvetnick N. T cell homeostasis in tolerance and immunity. J Leukoc Biol 2005;78(3):575–84.

[61] Shlomchik MJ, Craft JE, Mamula MJ. From T to B and back again: Positive feedback in systemic autoimmune disease. Nat Rev Immunol 2001;1(2):147–53.

[62] Moore JB, Blanchard RK, McCormack WT, Cousins RJ. cDNA array analysis identifies thymic LCK as upregulated in moderate murine zinc deficiency before T-lymphocyte population changes. J Nutr 2001;131(12):3189–96.

[63] Honscheid A, Rink L, Haase H. T-lymphocytes: a target for stimulatory and inhibitory effects of zinc ions. Endocr Metab Immune Disord Drug Targets 2009;9(2):132–44.

[64] Kaltenberg J, Plum LM, Ober-Blöbaum JL, Hönscheid A, Rink L, Haase H. Zinc signals promote IL-2-dependent proliferation of T cells. Eur J Immunol 2010;40(5):1496–503.

[65] Plum LM, Brieger A, Engelhardt G, Hebel S, Nessel A, Arlt M et al. PTEN-inhibition by zinc ions augments interleukin-2-mediated Akt phosphorylation. Metallomics 2014;6(7):1277–87.

[66] Gammoh NZ, Rink L. Zinc in Infection and Inflammation. Nutrients 2017;9(6).

[67] Bao B, Prasad A, Beck FWJ, Suneja A, Sarkar F. Toxic effect of zinc on NF-kappaB, IL-2, IL-2 receptor alpha, and TNF-alpha in HUT-78 (Th(0)) cells. Toxicol Lett 2006;166(3):222–8.

[68] Yu M, Lee W-W, Tomar D, Pryshchep S, Czesnikiewicz-Guzik M, Lamar DL et al. Regulation of T cell receptor signaling by activation-induced zinc influx. Journal of Experimental Medicine 2011;208(4):775–85.

[69] Pernelle JJ, Creuzet C, Loeb J, Gacon G. Phosphorylation of the lymphoid cell kinase p56lck is stimulated by micromolar concentrations of Zn2+. FEBS Lett 1991;281(1-2):278–82.

[70] Romir J, Lilie H, Egerer-Sieber C, Bauer F, Sticht H, Muller YA. Crystal structure analysis and solution studies of human Lck-SH3; zinc-induced homodimerization competes with the binding of proline-rich motifs. J Mol Biol 2007;365(5):1417–28.

[71] Ashwell JD. The many paths to p38 mitogen-activated protein kinase activation in the immune system. Nat Rev Immunol 2006;6(7):532–40.

[72] Haase H, Maret W. Protein tyrosine phosphatases as targets of the combined insulinomimetic effects of zinc and oxidants. Biometals 2005;18(4):333–8.

[73] Wellinghausen N, Kirchner H, Rink L. The immunobiology of zinc. Immunol Today 1997;18(11):519–21.

[74] Rink L, Haase H. Zinc homeostasis and immunity. Trends Immunol 2007;28(1):1–4.

[75] Faber C, Gabriel P, Ibs K-H, Rink L. Zinc in pharmacological doses suppresses allogeneic reaction without affecting the antigenic response. Bone Marrow Transplant 2004;33(12):1241–6.

[76] Höck AD. Vitamin D3 Deficiency Results in Dysfunctions of Immunity with Severe Fatigue and Depression in a Variety of Diseases. In Vivo 2014;28(1):133–45.

[77] Simioni JA, Heimovski F, Skare TL. On lupus, vitamin D and leukopenia. Revista Brasileira de Reumatologia (English Edition) 2016;56(3):206–11.

[78] Mayne CG, Spanier JA, Relland LM, Williams CB, Hayes CE. 1,25-Dihydroxyvitamin D3 acts directly on the T lymphocyte vitamin D receptor to inhibit experimental autoimmune encephalomyelitis. Eur J Immunol 2011;41(3):822–32.

[79] Grishkan IV, Fairchild AN, Calabresi PA, Gocke AR. 1,25-Dihydroxyvitamin D3 selectively and reversibly impairs T helper-cell CNS localization. Proc Natl Acad Sci U S A 2013;110(52):21101–6.

[80] Handel AE, Sandve GK, Disanto G, Berlanga-Taylor AJ, Gallone G, Hanwell H 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(1):163.

[81] Nath N, Prasad R, Giri S, Singh AK, Singh I. T-bet is essential for the progression of experimental autoimmune encephalomyelitis. Immunology 2006;118(3):384–91.

[82] Waldburger KE, Hastings RC, Schaub RG, Goldman SJ, Leonard JP. Adoptive transfer of experimental allergic encephalomyelitis after in vitro treatment with recombinant murine interleukin-12. Preferential expansion of interferon-gamma-producing cells and increased expression of macrophage-associated inducible nitric oxide synthase as immunomodulatory mechanisms. Am J Pathol 1996;148(2):375–82.

[83] Crane IJ, Forrester JV. Th1 and Th2 lymphocytes in autoimmune disease. Crit Rev Immunol 2005;25(2):75–102.

[84] Sprietsma JE. Zinc-controlled Th1/Th2 switch significantly determines development of diseases. Medical Hypotheses 1997;49(1):1–14.

[85] Cakman I, Rohwer J, Schütz RM, Kirchner H, Rink L. Dysregulation between TH1 and TH2 T cell subpopulations in the elderly. Mech Ageing Dev 1996;87(3):197–209.

[86] Reinhold D, Ansorge S, Grüngreiff K. Zinc regulates DNA synthesis and IL-2, IL-6, and IL-10 production of PWM-stimulated PBMC and normalizes the periphere cytokine concentration in chronic liver disease. The Journal of Trace Elements in Experimental Medicine 1997;10(1):19–27.

[87] Schubert C, Guttek K, Grüngreiff K, Thielitz A, Bühling F, Reinhold A et al. Oral zinc aspartate treats experimental autoimmune encephalomyelitis. BioMetals 2014;27(6):1249–62.

[88] Campo CA, Wellinghausen N, Faber C, Fischer A, Rink L. Zinc inhibits the mixed lymphocyte culture. Biol Trace Elem Res 2001;79(1):15–22.

[89] Guttek K, Wagenbrett L, Reinhold A, Grüngreiff K, Reinhold D. Zinc aspartate suppresses proliferation and Th1/Th2/Th17 cytokine production of pre-activated human T cells in vitro. J Trace Elem Med Biol 2018;49:86–90.

[90] Cippitelli M, Santoni A. Vitamin D3: a transcriptional modulator of the interferon-γ gene. Eur J Immunol 1998;28(10):3017–30.

[91] Muthian G, Raikwar HP, Rajasingh J, Bright JJ. 1,25 Dihydroxyvitamin-D3 modulates JAK-STAT pathway in IL-12/IFNgamma axis leading to Th1 response in experimental allergic encephalomyelitis. J Neurosci Res 2006;83(7):1299–309.

[92] Palmer MT, Lee YK, Maynard CL, Oliver JR, Bikle DD, Jetten AM et al. Lineage-specific Effects of 1,25-Dihydroxyvitamin D3 on the Development of Effector CD4 T Cells. J. Biol. Chem. 2011;286(2):997–1004.

[93] Staeva-Vieira TP, Freedman LP. 1,25-Dihydroxyvitamin D3 Inhibits IFN-γ and IL-4 Levels During In Vitro Polarization of Primary Murine CD4+ T Cells. The Journal of Immunology 2002;168(3):1181–9.

[94] Takeuchi A, Reddy GS, Kobayashi T, Okano T, Park J, Sharma S. Nuclear factor of activated T cells (NFAT) as a molecular target for 1alpha,25-dihydroxyvitamin D3-mediated effects. J Immunol 1998;160(1):209–18.

[95] Peelen E, Thewissen M, Knippenberg S, Smolders J, Muris A-H, Menheere P et al. Fraction of IL-10+ and IL-17+ CD8 T cells is increased in MS patients in remission and during a relapse, but is not influenced by immune modulators. Journal of Neuroimmunology 2013;258(1):77–84.

[96] Chang SH, Chung Y, Dong C. Vitamin D Suppresses Th17 Cytokine Production by Inducing C/EBP Homologous Protein (CHOP) Expression. J. Biol. Chem. 2010;285(50):38751–5.

[97] van Hamburg JP, Mus A-M, Bruijn, Marjolein J. W. de, Vogel Ld, Boon L, Cornelissen F et al. GATA-3 protects against severe joint inflammation and bone erosion and reduces differentiation of Th17 cells during experimental arthritis. Arthritis & Rheumatism 2009;60(3):750–9.

[98] Chang J-H, Cha H-R, Lee D-S, Seo KY, Kweon M-N. 1,25-Dihydroxyvitamin D3 Inhibits the Differentiation and Migration of TH17 Cells to Protect against Experimental Autoimmune Encephalomyelitis. PLOS ONE 2010;5(9):e12925.

[99] Waddell A, Zhao J, Cantorna MT. NKT cells can help mediate the protective effects of 1,25-dihydroxyvitamin D3 in experimental autoimmune encephalomyelitis in mice. Int Immunol 2015;27(5):237–44.

[100] Sloka S, Silva C, Wang J, Yong VW. Predominance of Th2 polarization by Vitamin D through a STAT6-dependent mechanism. J Neuroinflammation 2011;8(1):56.

[101] Piantoni S, Andreoli L, Scarsi M, Zanola A, Dall'Ara F, Pizzorni C et al. Phenotype modifications of T-cells and their shift toward a Th2 response in patients with systemic lupus erythematosus supplemented with different monthly regimens of vitamin D. Lupus 2015;24(4-5):490–8.

[102] Smolders J, Peelen E, Thewissen M, Tervaert JWC, Menheere P, Hupperts R et al. Safety and T Cell Modulating Effects of High Dose Vitamin D3 Supplementation in Multiple Sclerosis. PLOS ONE 2010;5(12):e15235.

[103] Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HFJ, O'Garra A. 1α,25-Dihydroxyvitamin D3 Has a Direct Effect on Naive CD4+ T Cells to Enhance the Development of Th2 Cells. The Journal of Immunology 2001;167(9):4974–80.

[104] Terrier B, Derian N, Schoindre Y, Chaara W, Geri G, Zahr N et al. Restoration of regulatory and effector T cell balance and B cell homeostasis in systemic lupus erythematosus patients through vitamin D supplementation. Arthritis Research & Therapy 2012;14(5):R221.

[105] Colin EM, Asmawidjaja PS, van Hamburg JP, Mus AMC, van Driel M, Hazes JMW et al. 1,25-dihydroxyvitamin D3 modulates Th17 polarization and interleukin-22 expression by memory T cells from patients with early rheumatoid arthritis. Arthritis & Rheumatism 2010;62(1):132–42.

[106] Murphy CA, Langrish CL, Chen Y, Blumenschein W, McClanahan T, Kastelein RA et al. Divergent Pro- and Antiinflammatory Roles for IL-23 and IL-12 in Joint Autoimmune Inflammation. Journal of Experimental Medicine 2003;198(12):1951–7.

[107] Lubberts E, Joosten LAB, van de Loo, F. A. J., Schwarzenberger P, Kolls J, van den Berg, W. B. Overexpression of IL-17 in the knee joint of collagen type II

immunized mice promotes collagen arthritis and aggravates joint destruction. Inflammation Research 2002;51(2):102–4.

[108] Hirota K, Duarte JH, Veldhoen M, Hornsby E, Li Y, Cua DJ et al. Fate mapping of IL-17-producing T cells in inflammatory responses. Nat Immunol 2011;12(3):255.

[109] Yang J, Chu Y, Yang X, Di Gao, Zhu L, Yang X et al. Th17 and natural Treg cell population dynamics in systemic lupus erythematosus. Arthritis Rheum 2009;60(5):1472–83.

[110] Leipe J, Grunke M, Dechant C, Reindl C, Kerzendorf U, Schulze-Koops H et al. Role of Th17 cells in human autoimmune arthritis. Arthritis Rheum 2010;62(10):2876–85.

[111] van Hamburg JP, Asmawidjaja PS, Davelaar N, Mus AMC, Colin EM, Hazes JMW et al. Th17 cells, but not Th1 cells, from patients with early rheumatoid arthritis are potent inducers of matrix metalloproteinases and proinflammatory cytokines upon synovial fibroblast interaction, including autocrine interleukin-17A production. Arthritis & Rheumatism 2011;63(1):73–83.

[112] Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of *FOXP3*. Nature Genetics 2001;27(1):20.

[113] Venken K, Hellings N, Thewissen M, Somers V, Hensen K, Rummens J-L et al. Compromised CD4+ CD25(high) regulatory T-cell function in patients with relapsing-remitting multiple sclerosis is correlated with a reduced frequency of FOXP3-positive cells and reduced FOXP3 expression at the single-cell level. Immunology 2008;123(1):79–89.

[114] Schmitt V, Rink L, Uciechowski P. The Th17/Treg balance is disturbed during aging. Exp Gerontol 2013;48(12):1379–86.

[115] Kitabayashi C, Fukada T, Kanamoto M, Ohashi W, Hojyo S, Atsumi T et al. Zinc suppresses Th17 development via inhibition of STAT3 activation. Int Immunol 2010;22(5):375–86.

[116] Kulik L, Maywald M, Kloubert V, Weßels I, Rink L. Zinc deficiency drives Th17 polarization and promotes loss of Treg cell-function. Journal of Nutritional Biochemistry 2018;under revision.

[117] Maywald M, Meurer SK, Weiskirchen R, Rink L. Zinc supplementation augments TGF-beta1-dependent regulatory T cell induction. Mol Nutr Food Res 2017;61(3).

[118] Rosenkranz E, Metz CHD, Maywald M, Hilgers R-D, Weßels I, Senff T et al. Zinc supplementation induces regulatory T cells by inhibition of Sirt-1 deacetylase in mixed lymphocyte cultures. Mol Nutr Food Res 2016;60(3):661–71.

[119] Maywald M, Wang F, Rink L. The Intracellular Free Zinc Level Is Vital for Treg Function and a Feasible Tool to Discriminate between Treg and Activated Th Cells. Int J Mol Sci 2018;19(11).

[120] Wessels I, Haase H, Engelhardt G, Rink L, Uciechowski P. Zinc deficiency induces production of the proinflammatory cytokines IL-1β and TNFα in promyeloid cells via epigenetic and redox-dependent mechanisms. J Nutr Biochem 2013;24(1):289–97.

[121] Daaboul D, Rosenkranz E, Uciechowski P, Rink L. Repletion of zinc in zinc-deficient cells strongly up-regulates IL-1beta-induced IL-2 production in T-cells. Metallomics 2012;4(10):1088–97.

[122] Maywald M, Rink L. Zinc supplementation induces CD4+CD25+Foxp3+ antigen-specific regulatory T cells and suppresses IFN-γ production by upregulation of Foxp3 and KLF-10 and downregulation of IRF-1. Eur J Nutr 2017;56(5):1859–69.

[123] Rosenkranz E, Maywald M, Hilgers R-D, Brieger A, Clarner T, Kipp M et al. Induction of regulatory T cells in Th1-/Th17-driven experimental autoimmune encephalomyelitis by zinc administration. J Nutr Biochem 2016;29:116–23.

[124] Stoye D, Schubert C, Goihl A, Guttek K, Reinhold A, Brocke S et al. Zinc aspartate suppresses T cell activation in vitro and relapsing experimental autoimmune encephalomyelitis in SJL/J mice. Biometals 2012;25(3):529–39.

[125] Straubel D, Thielitz A, Reinhold A, Grüngreiff K, Reinhold D. Combined Treatment with Zinc Aspartate and Intravenous Immunoglobulins (IVIGs) Ameliorates Experimental Autoimmune Encephalomyelitis (EAE). J Immunol Res 2018;2018:5982169.

[126] Tian Y, Wang C, Ye Z, Xiao X, Kijlstra A, Yang P. Effect of 1,25-Dihydroxyvitamin D3 on Th17 and Th1 Response in Patients with Behçet's Disease. Invest. Ophthalmol. Vis. Sci. 2012;53(10):6434–41.

[127] Joshi S, Pantalena L-C, Liu XK, Gaffen SL, Liu H, Rohowsky-Kochan C et al. 1,25-dihydroxyvitamin D(3) ameliorates Th17 autoimmunity via transcriptional modulation of interleukin-17A. Mol Cell Biol 2011;31(17):3653–69.

[128] Tang J, Zhou R, Luger D, Zhu W, Silver PB, Grajewski RS et al. Calcitriol Suppresses Antiretinal Autoimmunity through Inhibitory Effects on the Th17 Effector Response. The Journal of Immunology 2009;182(8):4624–32.

[129] van Hamburg JP, Asmawidjaja PS, Davelaar N, Mus AMC, Cornelissen F, Leeuwen, Johannes P T M van et al. TNF blockade requires 1,25(OH)2D3 to control human Th17-mediated synovial inflammation. Ann Rheum Dis 2012;71(4):606–12.

[130] Jeffery LE, Qureshi OS, Gardner D, Hou TZ, Briggs Z, Soskic B et al. Vitamin D Antagonises the Suppressive Effect of Inflammatory Cytokines on CTLA-4 Expression and Regulatory Function. PLOS ONE 2015;10(7):e0131539.

[131] Nanduri R, Mahajan S, Bhagyaraj E, Sethi K, Kalra R, Chandra V et al. The Active Form of Vitamin D Transcriptionally Represses Smad7 Signaling and Activates Extracellular Signal-regulated Kinase (ERK) to Inhibit the Differentiation of a Inflammatory T Helper Cell Subset and Suppress Experimental Autoimmune Encephalomyelitis. J. Biol. Chem. 2015;290(19):12222–36.

[132] Maggi L, Santarlasci V, Capone M, Rossi MC, Querci V, Mazzoni A et al. Distinctive features of classic and nonclassic (Th17 derived) human Th1 cells. Eur J Immunol 2012;42(12):3180–8.

[133] Ramstein J, Broos CE, Simpson LJ, Ansel KM, Sun SA, Ho ME et al. IFN-gamma-Producing T-Helper 17.1 Cells Are Increased in Sarcoidosis and Are More Prevalent than T-Helper Type 1 Cells. Am J Respir Crit Care Med 2016;193(11):1281–91.

[134] Cosmi L, Liotta F, Maggi E, Romagnani S, Annunziato F. Th17 and Non-Classic Th1 Cells in Chronic Inflammatory Disorders: Two Sides of the Same Coin. IAA 2014;164(3):171–7.

[135] Paulissen SMJ, van Hamburg JP, Dankers W, Lubberts E. The role and modulation of CCR6+ Th17 cell populations in rheumatoid arthritis. Cytokine 2015;74(1):43–53.

[136] Kang SW, Kim SH, Lee N, Lee W-W, Hwang K-A, Shin MS et al. 1,25-Dihyroxyvitamin D3 promotes FOXP3 expression via binding to vitamin D response elements in its conserved noncoding sequence region. J Immunol 2012;188(11):5276–82.

[137] Jeffery LE, Burke F, Mura M, Zheng Y, Qureshi OS, Hewison M et al. 1,25-Dihydroxyvitamin D3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. J Immunol 2009;183(9):5458–67.

[138] Correale J, Ysrraelit MC, Gaitán MI. Immunomodulatory effects of Vitamin D in multiple sclerosis. Brain 2009;132(5):1146–60.

[139] Baban B, Chandler PR, Sharma MD, Pihkala J, Koni PA, Munn DH et al. IDO activates regulatory T cells and blocks their conversion into TH17-like T cells. J Immunol 2009;183(4):2475–83.

[140] Gabbay MAL, Sato MN, Finazzo C, Duarte AJS, Dib SA. Effect of Cholecalciferol as Adjunctive Therapy With Insulin on Protective Immunologic Profile and Decline of Residual β-Cell Function in New-Onset Type 1 Diabetes Mellitus. Arch Pediatr Adolesc Med 2012;166(7):601–7.

[141] Zhang N, Bevan MJ. CD8+ T Cells: Foot Soldiers of the Immune System. Immunity 2011;35(2):161–8.

[142] Huseby ES, Liggitt D, Brabb T, Schnabel B, Öhlén C, Goverman J. A Pathogenic Role for Myelin-Specific Cd8+ T Cells in a Model for Multiple Sclerosis. Journal of Experimental Medicine 2001;194(5):669–76.

[143] Sun D, Whitaker JN, Huang Z, Di Liu, Coleclough C, Wekerle H et al. Myelin Antigen-Specific CD8+ T Cells Are Encephalitogenic and Produce Severe Disease in C57BL/6 Mice. The Journal of Immunology 2001;166(12):7579–87.

[144] Denic A, Wootla B, Rodriguez M. CD8(+) T cells in multiple sclerosis. Expert Opin Ther Targets 2013;17(9):1053–66.

[145] Camara M, Beyersdorf N, Fischer HJ, Herold MJ, Ip CW, van den Brandt J et al. $CD8^+$ T cell help is required for efficient induction of EAE in Lewis rats. Journal of Neuroimmunology 2013;260(1-2):17–27.

[146] Fischer HJ, van den Brandt J, Lingner T, Odoardi F, Flügel A, Weishaupt A et al. Modulation of CNS autoimmune responses by CD8(+) T cells coincides with their oligoclonal expansion. Journal of Neuroimmunology 2016;290:26–32.

[147] Sinha S, Boyden AW, Itani FR, Crawford MP, Karandikar NJ. CD8+ T-Cells as Immune Regulators of Multiple Sclerosis. Front Immunol 2015;6.

[148] Nagata M, Santamaria P, Kawamura T, Utsugi T, Yoon JW. Evidence for the role of CD8+ cytotoxic T cells in the destruction of pancreatic beta-cells in nonobese diabetic mice. The Journal of Immunology 1994;152(4):2042–50.

[149] Walker LSK, Herrath M von. CD4 T cell differentiation in type 1 diabetes. Clin Exp Immunol 2016;183(1):16–29.

[150] Steinhoff U, Brinkmann V, Klemm U, Aichele P, Seiler P, Brandt U et al. Autoimmune Intestinal Pathology Induced by hsp60-Specific CD8 T Cells. Immunity 1999;11(3):349–58.

[151] Menon B, Gullick NJ, Walter GJ, Rajasekhar M, Garrood T, Evans HG et al. Interleukin-17+CD8+ T Cells Are Enriched in the Joints of Patients With Psoriatic Arthritis and Correlate With Disease Activity and Joint Damage Progression. Arthritis & Rheumatology 2014;66(5):1272–81.

[152] Beck FW, Prasad AS, Kaplan J, Fitzgerald JT, Brewer GJ. Changes in cytokine production and T cell subpopulations in experimentally induced zinc-deficient humans. Am J Physiol 1997;272(6 Pt 1):E1002-7.

[153] Frost P, Rabbani P, Smith J, Prasad A. Cell-mediated cytotoxicity and tumor growth in zinc-deficient mice. Proc Soc Exp Biol Med 1981;167(3):333–7.

[154] Chen J, Bruce D, Cantorna MT. Vitamin D receptor expression controls proliferation of naïve CD8 + T cells and development of CD8 mediated gastrointestinal inflammation. BMC Immunology 2014;15(1):6.

[155] Lysandropoulos AP, Jaquiéry E, Jilek S, Pantaleo G, Schluep M, Du Pasquier RA. Vitamin D has a direct immunomodulatory effect on CD8+ T cells of patients with early multiple sclerosis and healthy control subjects. Journal of Neuroimmunology 2011;233(1):240–4.

[156] Dyring-Andersen B, Bonefeld CM, Bzorek M, Løvendorf MB, Lauritsen JPH, Skov L et al. The Vitamin D Analogue Calcipotriol Reduces the Frequency of CD8+IL-17+ T Cells in Psoriasis Lesions. Scandinavian Journal of Immunology 2015;82(1):84–91.

[157] Bruce D, Cantorna MT. Intrinsic Requirement for the Vitamin D Receptor in the Development of CD8αα-Expressing T Cells. The Journal of Immunology 2011;186(5):2819–25.

[158] Brooks EG, Balk SP, Aupeix K, Colonna M, Strominger JL, Groh-Spies V. Human T-cell receptor (TCR) alpha/beta + CD4-CD8- T cells express oligoclonal

TCRs, share junctional motifs across TCR V beta-gene families, and phenotypically resemble memory T cells. Proc Natl Acad Sci U S A 1993;90(24):11787–91.

[159] Godfrey DI, Uldrich AP, McCluskey J, Rossjohn J, Moody DB. The burgeoning family of unconventional T cells. Nat Immunol 2015;16(11):1114.

[160] Rajan AJ, Gao YL, Raine CS, Brosnan CF. A pathogenic role for gamma delta T cells in relapsing-remitting experimental allergic encephalomyelitis in the SJL mouse. J Immunol 1996;157(2):941–9.

[161] Roark CL, French JD, Taylor MA, Bendele AM, Born WK, O'Brien RL. Exacerbation of collagen-induced arthritis by oligoclonal, IL-17-producing gamma delta T cells. J Immunol 2007;179(8):5576–83.

[162] Nian H, Shao H, O'Brien RL, Born WK, Kaplan HJ, Sun D. Activated gammadelta T cells promote the activation of uveitogenic T cells and exacerbate EAU development. Invest Ophthalmol Vis Sci 2011;52(8):5920–7.

[163] Edwards SC, McGinley AM, McGuinness NC, Mills KHG. γδ T Cells and NK Cells – Distinct Pathogenic Roles as Innate-Like Immune Cells in CNS Autoimmunity. Front. Immunol. 2015;6:455.

[164] Keystone EC, Rittershaus C, Wood N, Snow KM, Flatow J, Purvis JC et al. Elevation of a gamma delta T cell subset in peripheral blood and synovial fluid of patients with rheumatoid arthritis. Clin Exp Immunol 1991;84(1):78–82.

[165] Roura-Mir IC, Alcalde L, Vargas F, Tolosa E, Obiols G, Foz M et al. Gamma delta lymphocytes in endocrine autoimmunity: evidence of expansion in Graves' disease but not in type 1 diabetes. Clin Exp Immunol 1993;92(2):288–95.

[166] Wen L, Peakman M, Mieli-Vergani G, Vergani D. Elevation of activated gamma delta T cell receptor bearing T lymphocytes in patients with autoimmune chronic liver disease. Clin Exp Immunol 1992;89(1):78–82.

[167] Han G, Wang R, Chen G, Wang J, Xu R, Wang L et al. Interleukin-17-producing gammadelta+ T cells protect NOD mice from type 1 diabetes through a mechanism involving transforming growth factor-beta. Immunology 2010;129(2):197–206.

[168] Markle JGM, Mortin-Toth S, Wong ASL, Geng L, Hayday A, Danska JS. γδ T cells are essential effectors of type 1 diabetes in the nonobese diabetic mouse model. J Immunol 2013;190(11):5392–401.

[169] Paul S, Shilpi, Lal G. Role of gamma-delta (γδ) T cells in autoimmunity. J Leukoc Biol 2015;97(2):259–71.

[170] Chen L, Cencioni MT, Angelini DF, Borsellino G, Battistini L, Brosnan CF. Transcriptional profiling of gamma delta T cells identifies a role for vitamin D in the immunoregulation of the V gamma 9V delta 2 response to phosphate-containing ligands. J Immunol 2005;174(10):6144–52.

[171] Villanova F, Flutter B, Tosi I, Grys K, Sreeneebus H, Perera GK et al. Characterization of Innate Lymphoid Cells in Human Skin and Blood Demonstrates Increase of NKp44+ ILC3 in Psoriasis. Journal of Investigative Dermatology 2014;134(4):984–91.

[172] Torina A, Guggino G, La Manna MP, Sireci G. The Janus Face of NKT Cell Function in Autoimmunity and Infectious Diseases. Int J Mol Sci 2018;19(2).

[173] Yu S, Cantorna MT. The vitamin D receptor is required for iNKT cell development. PNAS 2008;105(13):5207–12.

[174] Hippen KL, Schram BR, Tze LE, Pape KA, Jenkins MK, Behrens TW. In vivo assessment of the relative contributions of deletion, anergy, and editing to B cell self-tolerance. J Immunol 2005;175(2):909–16.

[175] Pillai S, Mattoo H, Cariappa A. B cells and autoimmunity. Curr Opin Immunol 2011;23(6):721–31.

[176] Greidinger EL, Hoffman RW. Antinuclear Antibody Testing: Methods, Indications, and Interpretation. Laboratory Medicine 2003;34(2):113–7.

[177] van Venrooij WJ, Beers, Joyce J. B. C. van, Pruijn GJM. Anti-CCP antibodies: the past, the present and the future. Nature Reviews Rheumatology 2011;7(7):391.

[178] Wang J, Wen Y, Zhou M, Shi X, Jiang L, Li M et al. Ectopic germinal center and megalin defect in primary Sjogren syndrome with renal Fanconi syndrome. Arthritis Research & Therapy 2017;19(1):120.

[179] Wenzlau JM, Juhl K, Yu L, Moua O, Sarkar SA, Gottlieb P et al. The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. Proc Natl Acad Sci U S A 2007;104(43):17040–5.

[180] Gruber K, Maywald M, Rosenkranz E, Haase H, Plumakers B, Rink L. Zinc deficiency adversely influences interleukin-4 and interleukin-6 signaling. J Biol Regul Homeost Agents 2013;27(3):661–71.

[181] Li J, Liang Y, Mao H, Deng W, Zhang J. Effects of B-lymphocyte dysfunction on the serum copper, selenium and zinc levels of rheumatoid arthritis patients. Pak J Med Sci 2014;30(5):1064–7.

[182] Fraker PJ, Osati-Ashtiani F, Wagner MA, King LE. Possible roles for glucocorticoids and apoptosis in the suppression of lymphopoiesis during zinc deficiency: a review. J Am Coll Nutr 1995;14(1):11–7.

[183] Ollig J, Kloubert V, Taylor KM, Rink L. B cell activation and proliferation increase intracellular zinc levels. J Nutr Biochem 2019;64:72–9.

[184] Lecane PS, Karaman MW, Sirisawad M, Naumovski L, Miller RA, Hacia JG et al. Motexafin gadolinium and zinc induce oxidative stress responses and apoptosis in B-cell lymphoma lines. Cancer Res 2005;65(24):11676–88.

[185] Shankar AH, Prasad AS. Zinc and immune function: the biological basis of altered resistance to infection. Am J Clin Nutr 1998;68(2 Suppl):447S-463S.

[186] Luecke RW, Simonel CE, Fraker PJ. The effect of restricted dietary intake on the antibody mediated response of the zinc deficient A/J mouse. J Nutr 1978;108(5):881–7.

[187] Fraker PJ, Hildebrandt K, Luecke RW. Alteration of antibody-mediated responses of suckling mice to T-cell-dependent and independent antigens by maternal marginal zinc deficiency: restoration of responsivity by nutritional repletion. J Nutr 1984;114(1):170–9.

[188] Kreft B, Fischer A, Krüger S, Sack K, Kirchner H, Rink L. The impaired immune response to diphtheria vaccination in elderly chronic hemodialysis patients is related to zinc deficiency. Biogerontology 2000;1(1):61–6.

[189] Chen S, Sims GP, Chen XX, Gu YY, Chen S, Lipsky PE. Modulatory Effects of 1,25-Dihydroxyvitamin D3 on Human B Cell Differentiation. The Journal of Immunology 2007;179(3):1634–47.

[190] Lemire JM, Adams JS, Sakai R, Jordan SC. 1 alpha,25-dihydroxyvitamin D3 suppresses proliferation and immunoglobulin production by normal human peripheral blood mononuclear cells. J Clin Invest 1984;74(2):657–61.

[191] Geldmeyer-Hilt K, Heine G, Hartmann B, Baumgrass R, Radbruch A, Worm M. 1,25-dihydroxyvitamin D3 impairs NF-κB activation in human naïve B cells. Biochemical and Biophysical Research Communications 2011;407(4):699–702.

[192] Heine G, Niesner U, Chang H-D, Steinmeyer A, Zügel U, Zuberbier T et al. 1,25-dihydroxyvitamin D3 promotes IL-10 production in human B cells. Eur J Immunol 2008;38(8):2210–8.

[193] Knippenberg S, Peelen E, Smolders J, Thewissen M, Menheere P, Cohen Tervaert JW et al. Reduction in IL-10 producing B cells (Breg) in multiple sclerosis is accompanied by a reduced naïve/memory Breg ratio during a relapse but not in remission. Journal of Neuroimmunology 2011;239(1):80–6.

[194] Iho S, Takahashi T, Kura F, Sugiyama H, Hoshino T. The effect of 1,25-dihydroxyvitamin D3 on in vitro immunoglobulin production in human B cells. The Journal of Immunology 1986;136(12):4427–31.

[195] Chen WC, Vayuvegula B, Gupta S. 1,25-Dihydroxyvitamin D3-mediated inhibition of human B cell differentiation. Clin Exp Immunol 1987;69(3):639–46.

[196] Heine G, Anton K, Henz BM, Worm M. 1alpha,25-dihydroxyvitamin D3 inhibits anti-CD40 plus IL-4-mediated IgE production in vitro. Eur J Immunol 2002;32(12):3395–404.

[197] Ritterhouse LL, Crowe SR, Niewold TB, Kamen DL, Macwana SR, Roberts VC et al. Vitamin D deficiency is associated with an increased autoimmune response in healthy individuals and in patients with systemic lupus erythematosus. Ann Rheum Dis 2011;70(9):1569–74.

[198] Drozdenko G, Scheel T, Heine G, Baumgrass R, Worm M. Impaired T cell activation and cytokine production by calcitriol-primed human B cells. Clinical & Experimental Immunology 2014;178(2):364–72.

[199] Stoffels M, Kastner DL. Old Dogs, New Tricks: Monogenic Autoinflammatory Disease Unleashed. Annu Rev Genomics Hum Genet 2016;17:245–72.

[200] Arakawa A, Siewert K, Stöhr J, Besgen P, Kim S-M, Rühl G et al. Melanocyte antigen triggers autoimmunity in human psoriasis. J Exp Med 2015;212(13):2203–12.

[201] Wohn C, Ober-Blöbaum JL, Haak S, Pantelyushin S, Cheong C, Zahner SP et al. Langerin(neg) conventional dendritic cells produce IL-23 to drive psoriatic plaque formation in mice. Proc Natl Acad Sci U S A 2013;110(26):10723–8.

[202] Sisirak V, Ganguly D, Lewis KL, Couillault C, Tanaka L, Bolland S et al. Genetic evidence for the role of plasmacytoid dendritic cells in systemic lupus erythematosus. J Exp Med 2014;211(10):1969–76.

[203] Ferris ST, Carrero JA, Mohan JF, Calderon B, Murphy KM, Unanue ER. A minor subset of Batf3-dependent antigen-presenting cells in islets of Langerhans is

essential for the development of autoimmune diabetes. Immunity 2014;41(4):657–69.

[204] Kitamura H, Morikawa H, Kamon H, Iguchi M, Hojyo S, Fukada T et al. Toll-like receptor-mediated regulation of zinc homeostasis influences dendritic cell function. Nat Immunol 2006;7(9):971–7.

[205] Chow A, Toomre D, Garrett W, Mellman I. Dendritic cell maturation triggers retrograde MHC class II transport from lysosomes to the plasma membrane. Nature 2002;418(6901):988–94.

[206] George MM, Vignesh KS, Landero Figueroa JA, Caruso JA, Deepe GS. Zinc Induces Dendritic Cell Tolerogenic Phenotype and Skews Regulatory T cell – Th17 Balance. J Immunol 2016;197(5):1864–76.

[207] van der Aar AMG, Sibiryak DS, Bakdash G, van Capel TMM, van der Kleij HPM, Opstelten D-JE et al. Vitamin D3 targets epidermal and dermal dendritic cells for induction of distinct regulatory T cells. J Allergy Clin Immunol 2011;127(6):1532-40.e7.

[208] Karthaus N, van Spriel AB, Looman MWG, Chen S, Spilgies LM, Lieben L et al. Vitamin D controls murine and human plasmacytoid dendritic cell function. J Invest Dermatol 2014;134(5):1255–64.

[209] Ferreira GB, Vanherwegen A-S, Eelen G, Gutiérrez ACF, van Lommel L, Marchal K et al. Vitamin D3 Induces Tolerance in Human Dendritic Cells by Activation of Intracellular Metabolic Pathways. Cell Rep 2015.

[210] Penna G, Roncari A, Amuchastegui S, Daniel KC, Berti E, Colonna M et al. Expression of the inhibitory receptor ILT3 on dendritic cells is dispensable for induction of CD4+Foxp3+ regulatory T cells by 1,25-dihydroxyvitamin D3. Blood 2005;106(10):3490–7.

[211] Farias AS, Spagnol GS, Bordeaux-Rego P, Oliveira COF, Fontana AGM, Paula RFO de et al. Vitamin D3 induces IDO+ tolerogenic DCs and enhances Treg, reducing the severity of EAE. CNS Neurosci Ther 2013;19(4):269–77.

[212] Huang Y, Zhao Y, Ran X, Wang C. Increased expression of herpesvirus entry mediator in 1,25-dihydroxyvitamin D3-treated mouse bone marrow-derived dendritic cells promotes the generation of $CD4^+CD25^+Foxp3^+$ regulatory T cells. Mol Med Rep 2014;9(3):813–8.

[213] Haniffa M, Bigley V, Collin M. Human mononuclear phagocyte system reunited. Semin Cell Dev Biol 2015;41:59–69.

[214] Funes SC, Rios M, Escobar-Vera J, Kalergis AM. Implications of macrophage polarization in autoimmunity. Immunology 2018;154(2):186–95.

[215] Liu H, Wu X, Gang N, Wang S, Deng W, Zan L et al. Macrophage functional phenotype can be consecutively and reversibly shifted to adapt to microenvironmental changes. Int J Clin Exp Med 2015;8(2):3044–53.

[216] Jun HS, Yoon CS, Zbytnuik L, van Rooijen N, Yoon JW. The role of macrophages in T cell-mediated autoimmune diabetes in nonobese diabetic mice. J Exp Med 1999;189(2):347–58.

[217] Chalmers S, Doerner J, Wen J, Putterman C. Macrophage depletion attenuates skin and kidney disease in lupus mice (BA7P.143). The Journal of Immunology 2015;194(1 Supplement):115.3-115.3.

[218] Triantafyllopoulou A, Franzke C-W, Seshan SV, Perino G, Kalliolias GD, Ramanujam M et al. Proliferative lesions and metalloproteinase activity in murine lupus nephritis mediated by type I interferons and macrophages. Proc Natl Acad Sci U S A 2010;107(7):3012–7.

[219] Orme J, Mohan C. Macrophage subpopulations in systemic lupus erythematosus. Discov Med 2012;13(69):151–8.

[220] Wang Y, Han C-C, Cui D, Li Y, Ma Y, Wei W. Is macrophage polarization important in rheumatoid arthritis? Int Immunopharmacol 2017;50:345–52.

[221] Kahmann L, Uciechowski P, Warmuth S, Plümäkers B, Gressner AM, Malavolta M et al. Zinc supplementation in the elderly reduces spontaneous inflammatory cytokine release and restores T cell functions. Rejuvenation Res 2008;11(1):227–37.

[222] Dubben S, Honscheid A, Winkler K, Rink L, Haase H. Cellular zinc homeostasis is a regulator in monocyte differentiation of HL-60 cells by 1 alpha,25-dihydroxyvitamin D3. J Leukoc Biol 2010;87(5):833–44.

[223] Haase H, Ober-Blöbaum JL, Engelhardt G, Hebel S, Heit A, Heine H et al. Zinc signals are essential for lipopolysaccharide-induced signal transduction in monocytes. J Immunol 2008;181(9):6491–502.

[224] Poplutz MK, Wessels I, Rink L, Uciechowski P. Regulation of the Interleukin-6 gene expression during monocytic differentiation of HL-60 cells by chromatin remodeling and methylation. Immunobiology 2014;219(8):619–26.

[225] Lienau S, Rink L, Wessels I. The role of zinc in calprotectin expression in human myeloid cells. J Trace Elem Med Biol 2018;49:106–12.

[226] King JC, Brown KH, Gibson RS, Krebs NF, Lowe NM, Siekmann JH et al. Biomarkers of Nutrition for Development (BOND)-Zinc Review. J Nutr 2016.

[227] Bulow V von, Rink L, Haase H. Zinc-Mediated Inhibition of Cyclic Nucleotide Phosphodiesterase Activity and Expression Suppresses TNF- and IL-1 Production in Monocytes by Elevation of Guanosine 3',5'-Cyclic Monophosphate. The Journal of Immunology 2005;175(7):4697–705.

[228] Driessen C, Hirv K, Kirchner H, Rink L. Divergent Effects of Zinc on Different Bacterial Pathogenic Agents. J Infect Dis 1995;171(2):486–9.

[229] Brieger A, Rink L, Haase H. Differential regulation of TLR-dependent MyD88 and TRIF signaling pathways by free zinc ions. J Immunol 2013;191(4):1808–17.

[230] Dierichs L, Kloubert V, Rink L. Cellular zinc homeostasis modulates polarization of THP-1-derived macrophages. Eur J Nutr 2017.

[231] Liu M-J, Bao S, Gálvez-Peralta M, Pyle CJ, Rudawsky AC, Pavlovicz RE et al. ZIP8 regulates host defense through zinc-mediated inhibition of NF-κB. Cell Rep 2013;3(2):386–400.

[232] Gao H, Zhao L, Wang H, Xie E, Wang X, Wu Q et al. Metal transporter Slc39a10 regulates susceptibility to inflammatory stimuli by controlling macrophage survival. PNAS 2017;114(49):12940–5.

[233] Xu H, Soruri A, Gieseler RKH, Peters JH. 1,25-Dihydroxyvitamin D3 Exerts Opposing Effects to IL-4 on MHC Class-II Antigen Expression, Accessory Activity, and Phagocytosis of Human Monocytes. Scand J Immunol 1993;38(6):535–40.

[234] Liu PT, Stenger S, Tang DH, Modlin RL. Cutting Edge: Vitamin D-Mediated Human Antimicrobial Activity against Mycobacterium tuberculosis Is Dependent on the Induction of Cathelicidin. The Journal of Immunology 2007;179(4):2060–3.

[235] Lee B-N-R, Kim T-H, Jun J-B, Yoo D-H, Woo J-H, Choi SJ et al. Upregulation of interleukin-1β production by 1,25-Dihydroxyvitamin D3 in activated human macrophages. Mol Biol Rep 2011;38(3):2193–201.

[236] Chen Y, Liu W, Sun T, Huang Y, Wang Y, Deb DK et al. 1,25-Dihydroxyvitamin D Promotes Negative Feedback Regulation of TLR Signaling via Targeting MicroRNA-155–SOCS1 in Macrophages. The Journal of Immunology 2013;190(7):3687–95.

[237] Neve A, Corrado A, Cantatore FP. Immunomodulatory effects of vitamin D in peripheral blood monocyte-derived macrophages from patients with rheumatoid arthritis. Clinical and Experimental Medicine 2014;14(3):275–83.

[238] Wang Q, He Y, Shen Y, Zhang Q, Di Chen, Zuo C et al. Vitamin D Inhibits COX-2 Expression and Inflammatory Response by Targeting Thioesterase Superfamily Member 4. J. Biol. Chem. 2014;289(17):11681–94.

[239] Zhang X, Zhou M, Guo Y, Song Z, Liu B. 1,25-Dihydroxyvitamin D3 Promotes High Glucose-Induced M1 Macrophage Switching to M2 via the VDR-PPAR Signaling Pathway. BioMed Research International 2015;2015.

[240] Korf H, Wenes M, Stijlemans B, Takiishi T, Robert S, Miani M et al. 1,25-Dihydroxyvitamin D3 curtails the inflammatory and T cell stimulatory capacity of macrophages through an IL-10-dependent mechanism. Immunobiology 2012;217(12):1292–300.

[241] Spits H, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G et al. Innate lymphoid cells — a proposal for uniform nomenclature. Nature Reviews Immunology 2013;13(2):145.

[242] Perry JSA, Han S, Xu Q, Herman ML, Kennedy LB, Csako G et al. Inhibition of LTi Cell Development by CD25 Blockade Is Associated with Decreased Intrathecal Inflammation in Multiple Sclerosis. Science Translational Medicine 2012;4(145):145ra106-145ra106.

[243] Geremia A, Arancibia-Cárcamo CV, Fleming MPP, Rust N, Singh B, Mortensen NJ et al. IL-23–responsive innate lymphoid cells are increased in inflammatory bowel disease. Journal of Experimental Medicine 2011;208(6):1127–33.

[244] Buonocore S, Ahern PP, Uhlig HH, Ivanov II, Littman DR, Maloy KJ et al. Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. Nature 2010;464(7293):1371.

[245] Teunissen MBM, Munneke JM, Bernink JH, Spuls PI, Res PCM, te Velde A et al. Composition of Innate Lymphoid Cell Subsets in the Human Skin: Enrichment of

NCR+ ILC3 in Lesional Skin and Blood of Psoriasis Patients. Journal of Investigative Dermatology 2014;134(9):2351–60.

[246] Ciccia F, Guggino G, Giardina A, Ferrante A, Carrubbi F, Giacomelli R et al. The role of innate and lymphoid IL-22-producing cells in the immunopathology of primary Sjögren's syndrome. Expert Rev Clin Immunol 2014;10(4):533–41.

[247] Poggi A, Zocchi MR. NK Cell Autoreactivity and Autoimmune Diseases. Front. Immunol. 2014;5:27.

[248] Rajagopalan S, Long EO. Zinc bound to the killer cell-inhibitory receptor modulates the negative signal in human NK cells. J Immunol 1998;161(3):1299–305.

[249] Kumar S, Rajagopalan S, Sarkar P, Dorward DW, Peterson ME, Liao H-S et al. Zinc-Induced Polymerization of Killer-Cell Ig-like Receptor into Filaments Promotes Its Inhibitory Function at Cytotoxic Immunological Synapses. Mol Cell 2016;62(1):21–33.

[250] Rolles B, Maywald M, Rink L. Influence of zinc deficiency and supplementation on NK cell cytotoxicity. Journal of Functional Foods 2018;48:322–8.

[251] Balogh G, Boland AR de, Boland R, Barja P. Effect of 1,25(OH)2-Vitamin D3 on the Activation of Natural Killer Cells: Role of Protein Kinase C and Extracellular Calcium. Experimental and Molecular Pathology 1999;67(2):63–74.

[252] Ota K, Dambaeva S, Kim MW-I, Han A-R, Fukui A, Gilman-Sachs A et al. 1,25-Dihydroxy-vitamin D3 regulates NK-cell cytotoxicity, cytokine secretion, and degranulation in women with recurrent pregnancy losses. Eur J Immunol 2015;45(11):3188–99.

[253] Weeres MA, Robien K, Ahn Y-O, Neulen M-L, Bergerson R, Miller JS et al. The Effects of 1,25-Dihydroxyvitamin D3 on In Vitro Human NK Cell Development from Hematopoietic Stem Cells. The Journal of Immunology 2014;193(7):3456–62.

[254] Chen J, Waddell A, Lin Y-D, Cantorna MT. Dysbiosis caused by vitamin D receptor deficiency confers colonization resistance to *Citrobacter rodentium* through modulation of innate lymphoid cells. Mucosal Immunology 2015;8(3):618.

[255] Choi BY, Jung JW, Suh SW, Choi B, Jung J, Suh S. The Emerging Role of Zinc in the Pathogenesis of Multiple Sclerosis. Int J Mol Sci 2017;18(10):2070.

[256] Gilgun-Sherki Y, Melamed E, Offen D. The role of oxidative stress in the pathogenesis of multiple sclerosis: the need for effective antioxidant therapy. J Neurol 2004;251(3):261–8.

[257] Nuttall JR, Oteiza PI. Zinc and the aging brain. Genes Nutr 2014;9(1).

[258] Morris DR, Levenson CW. Neurotoxicity of Zinc. Adv Neurobiol 2017;18:303–12.

[259] Choi BY, Jang BG, Kim JH, Seo J-N, Wu G, Sohn M et al. Copper/zinc chelation by clioquinol reduces spinal cord white matter damage and behavioral deficits in a murine MOG-induced multiple sclerosis model. Neurobiology of Disease 2013;54:382–91.

[260] Kim Y-H, Koh J-Y. The role of NADPH oxidase and neuronal nitric oxide synthase in zinc-induced poly(ADP-ribose) polymerase activation and cell death in cortical culture. Exp Neurol 2002;177(2):407–18.

[261] Zhang Y, Aizenman E, DeFranco DB, Rosenberg PA. Intracellular zinc release, 12-lipoxygenase activation and MAPK dependent neuronal and oligodendroglial death. Mol Med 2007;13(7-8):350–5.

[262] Li S, Vana AC, Ribeiro R, Zhang Y. Distinct role of nitric oxide and peroxynitrite in mediating oligodendrocyte toxicity in culture and in experimental autoimmune encephalomyelitis. Neuroscience 2011;184:107–19.

[263] Liu JS-H, Zhao M-L, Brosnan CF, Lee SC. Expression of Inducible Nitric Oxide Synthase and Nitrotyrosine in Multiple Sclerosis Lesions. Am J Pathol 2001;158(6):2057–66.

[264] Kiedrowski L. Cytosolic acidification and intracellular zinc release in hippocampal neurons. J Neurochem 2012;121(3):438–50.

[265] Noh KM, Kim YH, Koh JY. Mediation by membrane protein kinase C of zinc-induced oxidative neuronal injury in mouse cortical cultures. J Neurochem 1999;72(4):1609–16.

[266] Li S, Lin W, Tchantchou F, Lai R, Wen J, Zhang Y. Protein kinase C mediates peroxynitrite toxicity to oligodendrocytes. Mol Cell Neurosci 2011;48(1):62–71.

[267] Chou SS, Clegg MS, Momma TY, Niles BJ, Duffy JY, Daston GP et al. Alterations in protein kinase C activity and processing during zinc-deficiency-induced cell death. Biochem J 2004;383(Pt 1):63–71.

[268] Chauhan AK, Mittra N, Patel DK, Singh C. Cyclooxygenase-2 Directs Microglial Activation-Mediated Inflammation and Oxidative Stress Leading to Intrinsic Apoptosis in Zn-Induced Parkinsonism. Mol Neurobiol 2018;55(3):2162–73.

[269] Takahashi S, Maeda T, Sano Y, Nishihara H, Takeshita Y, Shimizu F et al. Active form of vitamin D directly protects the blood-brain barrier in multiple sclerosis. Clin Exp Neuroimmunol 2017;8(3):244–54.

[270] Weisshof R, Chermesh I. Micronutrient deficiencies in inflammatory bowel disease. Curr Opin Clin Nutr Metab Care 2015;18(6):576–81.

[271] Goh J, O'Morain CA. Review article: nutrition and adult inflammatory bowel disease. Aliment Pharmacol Ther 2003;17(3):307–20.

[272] Hering NA, Schulzke J-D. Therapeutic options to modulate barrier defects in inflammatory bowel disease. Dig Dis 2009;27(4):450–4.

[273] Scrimgeour AG, Condlin ML. Zinc and micronutrient combinations to combat gastrointestinal inflammation. Curr Opin Clin Nutr Metab Care 2009;12(6):653–60.

[274] Skrovanek S, DiGuilio K, Bailey R, Huntington W, Urbas R, Mayilvaganan B et al. Zinc and gastrointestinal disease. World J Gastrointest Pathophysiol 2014;5(4):496–513.

[275] Liu P, Pieper R, Rieger J, Vahjen W, Davin R, Plendl J et al. Effect of dietary zinc oxide on morphological characteristics, mucin composition and gene expression in the colon of weaned piglets. PLoS ONE 2014;9(3):e91091.

[276] Ranaldi G, Ferruzza S, Canali R, Leoni G, Zalewski PD, Sambuy Y et al. Intracellular zinc is required for intestinal cell survival signals triggered by the inflammatory cytokine TNFalpha. J Nutr Biochem 2013;24(6):967–76.

[277] Pedersen J, Coskun M, Soendergaard C, Salem M, Nielsen OH. Inflammatory pathways of importance for management of inflammatory bowel disease. World J Gastroenterol 2014;20(1):64–77.

[278] Blanchard RK, Cousins RJ. Upregulation of rat intestinal uroguanylin mRNA by dietary zinc restriction. Am J Physiol 1997;272(5 Pt 1):G972-8.

[279] Koo SI, Turk DE. Effect of zinc deficiency on intestinal transport triglyceride in the rat. J Nutr 1977;107(5):909–19.

[280] Moran JR, Lyerly A. The effects of severe zinc deficiency on intestinal amino acid losses in the rat. Life Sci 1985;36(26):2515–21.

[281] Assa A, Vong L, Pinnell LJ, Avitzur N, Johnson-Henry KC, Sherman PM. Vitamin D deficiency promotes epithelial barrier dysfunction and intestinal inflammation. J Infect Dis 2014;210(8):1296–305.

[282] Wang P-F, Yao D-H, Hu Y-Y, Li Y. Vitamin D Improves Intestinal Barrier Function in Cirrhosis Rats by Upregulating Heme Oxygenase-1 Expression. Biomol Ther (Seoul) 2018.

[283] Wang T-T, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 2004;173(5):2909–12.

[284] Clark A, Mach N. Role of Vitamin D in the Hygiene Hypothesis: The Interplay between Vitamin D, Vitamin D Receptors, Gut Microbiota, and Immune Response. Front. Immunol. 2016;7:627.

[285] Zhang Y-G, Lu R, Xia Y, Zhou D, Petrof E, Claud EC et al. Lack of Vitamin D Receptor Leads to Hyperfunction of Claudin-2 in Intestinal Inflammatory Responses. Inflamm Bowel Dis 2019;25(1):97–110.

[286] Hutton JC, Penn EJ, Peshavaria M. Low-molecular-weight constituents of isolated insulin-secretory granules. Bivalent cations, adenine nucleotides and inorganic phosphate. Biochem J 1983;210(2):297–305.

[287] Chimienti F, Devergnas S, Favier A, Seve M. Identification and Cloning of a -Cell-Specific Zinc Transporter, ZnT-8, Localized Into Insulin Secretory Granules. Diabetes 2004;53(9):2330–7.

[288] Dodson EJ, Dodson GG, Hodgkin DC, Reynolds CD. Structural relationships in the two-zinc insulin hexamer. Can. J. Biochem. 1979;57(6):469–79.

[289] Quarterman J, Mills CF, Humphries WR. The reduced secretion of, and sensitivity to insulin in zinc-deficient rats. Biochemical and Biophysical Research Communications 1966;25(3):354–8.

[290] Coulston L, Dandona P. Insulin-like Effect of Zinc on Adipocytes. Diabetes 1980;29(8):665–7.

[291] Kambe T, Narita H, Yamaguchi-Iwai Y, Hirose J, Amano T, Sugiura N et al. Cloning and characterization of a novel mammalian zinc transporter, zinc transporter 5, abundantly expressed in pancreatic beta cells. J Biol Chem 2002;277(21):19049–55.

[292] Smidt K, Jessen N, Petersen AB, Larsen A, Magnusson N, Jeppesen JB et al. SLC30A3 responds to glucose- and zinc variations in beta-cells and is critical for insulin production and in vivo glucose-metabolism during beta-cell stress. PLoS ONE 2009;4(5):e5684.

[293] Huang L, Yan M, Kirschke CP. Over-expression of ZnT7 increases insulin synthesis and secretion in pancreatic beta-cells by promoting insulin gene transcription. Exp Cell Res 2010;316(16):2630–43.

[294] Maxel T, Smidt K, Petersen CC, Honoré B, Christensen AK, Jeppesen PB et al. The zinc transporter Zip14 (SLC39a14) affects Beta-cell Function: Proteomics, Gene expression, and Insulin secretion studies in INS-1E cells. Sci Rep 2019;9(1):8589.

[295] Solomou A, Meur G, Bellomo E, Hodson DJ, Tomas A, Li SM et al. The Zinc Transporter Slc30a8/ZnT8 Is Required in a Subpopulation of Pancreatic α-Cells for Hypoglycemia-induced Glucagon Secretion. J Biol Chem 2015;290(35):21432–42.

[296] Davidson HW, Wenzlau JM, O'Brien RM. Zinc transporter 8 (ZnT8) and β cell function. Trends Endocrinol Metab 2014;25(8):415–24.

[297] Haase H, Maret W. Fluctuations of cellular, available zinc modulate insulin signaling via inhibition of protein tyrosine phosphatases. J Trace Elem Med Biol 2005;19(1):37–42.

[298] Bellomo E, Massarotti A, Hogstrand C, Maret W. Zinc ions modulate protein tyrosine phosphatase 1B activity. Metallomics 2014;6(7):1229–39.

[299] Maret W. Zinc in Pancreatic Islet Biology, Insulin Sensitivity, and Diabetes. Prev Nutr Food Sci 2017;22(1):1–8.

[300] Tang X, Shay NF. Zinc has an insulin-like effect on glucose transport mediated by phosphoinositol-3-kinase and Akt in 3T3-L1 fibroblasts and adipocytes. J Nutr 2001;131(5):1414–20.

[301] Mahadev K, Motoshima H, Wu X, Ruddy JM, Arnold RS, Cheng G et al. The NAD(P)H Oxidase Homolog Nox4 Modulates Insulin-Stimulated Generation of H2O2 and Plays an Integral Role in Insulin Signal Transduction. Mol. Cell. Biol. 2004;24(5):1844–54.

[302] Taylor KM, Hiscox S, Nicholson RI, Hogstrand C, Kille P. Protein kinase CK2 triggers cytosolic zinc signaling pathways by phosphorylation of zinc channel ZIP7. Sci Signal 2012;5(210):ra11.

[303] Dunn MF. Zinc-ligand interactions modulate assembly and stability of the insulin hexamer -- a review. Biometals 2005;18(4):295–303.

[304] Ishihara H, Wollheim CB. Is zinc an intra-islet regulator of glucagon secretion? Diabetol Int 2016;7(2):106–10.

[305] Tamaki M, Fujitani Y, Hara A, Uchida T, Tamura Y, Takeno K et al. The diabetes-susceptible gene SLC30A8/ZnT8 regulates hepatic insulin clearance. J Clin Invest 2013;123(10):4513–24.

[306] Brender JR, Hartman K, Nanga RPR, Popovych N, La Salud Bea R de, Vivekanandan S et al. Role of zinc in human islet amyloid polypeptide aggregation. J Am Chem Soc 2010;132(26):8973–83.

[307] Mitri J, Pittas AG. Vitamin D and diabetes. Endocrinol Metab Clin North Am 2014;43(1):205–32.

[308] Wang Y, He D, Ni C, Zhou H, Wu S, Xue Z et al. Vitamin D induces autophagy of pancreatic β-cells and enhances insulin secretion. Mol Med Rep 2016;14(3):2644–50.

[309] Ciccarelli F, Martinis M, Ginaldi L. An Update on Autoinflammatory Diseases. CMC 2013;21(3):261–9.

[310] Dinarello CA. An expanding role for interleukin-1 blockade from gout to cancer. Mol Med 2014;20 Suppl 1:S43-58.

[311] Langley RG, Elewski BE, Lebwohl M, Reich K, Griffiths CEM, Papp K et al. Secukinumab in plaque psoriasis--results of two phase 3 trials. N Engl J Med 2014;371(4):326–38.

[312] Siebert S, Tsoukas A, Robertson J, McInnes I. Cytokines as therapeutic targets in rheumatoid arthritis and other inflammatory diseases. Pharmacol Rev 2015;67(2):280–309.

[313] Banchereau R, Cepika A-M, Banchereau J, Pascual V. Understanding Human Autoimmunity and Autoinflammation Through Transcriptomics. Annu Rev Immunol 2017;35:337–70.

[314] Pascual V, Allantaz F, Arce E, Punaro M, Banchereau J. Role of interleukin-1 (IL-1) in the pathogenesis of systemic onset juvenile idiopathic arthritis and clinical response to IL-1 blockade. J Exp Med 2005;201(9):1479–86.

[315] Noda S, Krueger JG, Guttman-Yassky E. The translational revolution and use of biologics in patients with inflammatory skin diseases. J Allergy Clin Immunol 2015;135(2):324–36.

[316] Raza K, Ahmad S. Recent advancement in nextgerneration sequencing techniques and its computational analysis. International Journal of Bioinformatics Research and Applications 2018;in press.

[317] Sena KCM de, Arrais RF, Almeida MdG, Araujo DM de, dos Santos MM, Lima VT de et al. Effects of Zinc Supplementation in Patients with Type 1 Diabetes. BTER 2005;105(1-3):1–10.

[318] Faure P, Benhamou PY, Perard A, Halimi S, Roussel AM. Lipid peroxidation in insulin-dependent diabetic patients with early retina degenerative lesions: effects of an oral zinc supplementation. Eur J Clin Nutr 1995;49(4):282–8.

[319] Shidfar F, Aghasi M, Vafa M, Heydari I, Hosseini S, Shidfar S. Effects of combination of zinc and vitamin A supplementation on serum fasting blood sugar, insulin, apoprotein B and apoprotein A-I in patients with type I diabetes. Int J Food Sci Nutr 2010;61(2):182–91.

[320] Cunningham JJ, Fu A, Mearkle PL, Brown RG. Hyperzincuria in individuals with insulin-dependent diabetes mellitus: concurrent zinc status and the effect of high-dose zinc supplementation. Metab Clin Exp 1994;43(12):1558–62.

[321] Tang Y, Yang Q, Lu J, Zhang X, Di Suen, Tan Y et al. Zinc supplementation partially prevents renal pathological changes in diabetic rats. J Nutr Biochem 2010;21(3):237–46.

[322] Li X, Liao L, Yan X, Huang G, Lin J, Lei M et al. Protective effects of 1-α-hydroxyvitamin D3 on residual β-cell function in patients with adult-onset latent autoimmune diabetes (LADA). Diabetes/Metabolism Research and Reviews 2009;25(5):411–6.

[323] Walter M, Kaupper T, Adler K, Foersch J, Bonifacio E, Ziegler A-G. No effect of the 1alpha,25-dihydroxyvitamin D3 on beta-cell residual function and insulin requirement in adults with new-onset type 1 diabetes. Diabetes Care 2010;33(7):1443–8.

[324] Ataie-Jafari A, Loke S-C, Rahmat AB, Larijani B, Abbasi F, Leow MKS et al. A randomized placebo-controlled trial of alphacalcidol on the preservation of beta cell

function in children with recent onset type 1 diabetes. Clinical Nutrition 2013;32(6):911–7.

[325] Spach KM, Nashold FE, Dittel BN, Hayes CE. IL-10 signaling is essential for 1,25-dihydroxyvitamin D3-mediated inhibition of experimental autoimmune encephalomyelitis. J Immunol 2006;177(9):6030–7.

[326] Cantorna MT, Hayes CE, DeLuca HF. 1,25-Dihydroxyvitamin D3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. Proc Natl Acad Sci U S A 1996;93(15):7861–4.

[327] Xie Z, Chen J, Zheng C, Wu J, Cheng Y, Zhu S et al. 1,25-dihydroxyvitamin D3-induced dendritic cells suppress experimental autoimmune encephalomyelitis by increasing proportions of the regulatory lymphocytes and reducing T helper type 1 and type 17 cells. Immunology 2017;152(3):414–24.

[328] Salari S, Khomand P, Arasteh M, Yousefzamani B, Hassanzadeh K. Zinc sulphate: A reasonable choice for depression management in patients with multiple sclerosis: A randomized, double-blind, placebo-controlled clinical trial. Pharmacological Reports 2015;67(3):606–9.

[329] Burton JM, Kimball S, Vieth R, Bar-Or A, Dosch H-M, Cheung R et al. A phase I/II dose-escalation trial of vitamin D3 and calcium in multiple sclerosis. Neurology 2010;74(23):1852–9.

[330] Mosayebi G, Ghazavi A, Ghasami K, Jand Y, Kokhaei P. Therapeutic effect of vitamin D3 in multiple sclerosis patients. Immunol Invest 2011;40(6):627–39.

[331] Soilu-Hänninen M, Aivo J, Lindström B-M, Elovaara I, Sumelahti M-L, Färkkilä M et al. A randomised, double blind, placebo controlled trial with vitamin D3 as an add on treatment to interferon β-1b in patients with multiple sclerosis. J Neurol Neurosurg Psychiatry 2012;83(5):565–71.

[332] Kampman MT, Steffensen LH, Mellgren SI, Jørgensen L. Effect of vitamin D3 supplementation on relapses, disease progression, and measures of function in persons with multiple sclerosis: exploratory outcomes from a double-blind randomised controlled trial. Mult Scler 2012;18(8):1144–51.

[333] Derakhshandi H, Etemadifar M, Feizi A, Abtahi S-H, Minagar A, Abtahi M-A et al. Preventive effect of vitamin D3 supplementation on conversion of optic neuritis to clinically definite multiple sclerosis: a double blind, randomized, placebo-controlled pilot clinical trial. Acta Neurol Belg 2013;113(3):257–63.

[334] Sadeghian G, Ziaei H, Nilforoushzadeh MA. Treatment of localized psoriasis with a topical formulation of zinc pyrithione. Acta Dermatovenerol Alp Pannonica Adriat 2011;20(4):187–90.

[335] Clemmensen OJ, Siggaard-Andersen J, Worm AM, Stahl D, Frost F, Bloch I. Psoriatic arthritis treated with oral zinc sulphate. Br J Dermatol 1980;103(4):411–5.

[336] Simkin PA. Zinc sulphate in rheumatoid arthritis. Prog Clin Biol Res 1977;14:343–56.

[337] Simkin PA. Treatment of rheumatoid arthritis with oral zinc sulfate. Agents Actions Suppl 1981;8:587–96.

[338] Mattingly PC, Mowat AG. Zinc sulphate in rheumatoid arthritis. Ann Rheum Dis 1982;41(5):456–7.

[339] Rasker JJ, Kardaun SH. Lack of beneficial effect of zinc sulphate in rheumatoid arthritis. Scand J Rheumatol 1982;11(3):168–70.

[340] Herold A, Bucurenci N, Mazilu E, Szegli G, Sidenco L, Baican I. Zinc aspartate in vivo and in vitro modulation of reactive oxygen species production by human neutrophils and monocytes. Roum Arch Microbiol Immunol 1993;52(2):101–8.

[341] Peretz A, Cantinieaux B, Nève J, Siderova V, Fondu P. Effects of zinc supplementation on the phagocytic functions of polymorphonuclears in patients with inflammatory rheumatic diseases. J Trace Elem Electrolytes Health Dis 1994;8(3-4):189–94.

[342] Salesi M, Farajzadegan Z. Efficacy of vitamin D in patients with active rheumatoid arthritis receiving methotrexate therapy. Rheumatol Int 2012;32(7):2129–33.

[343] Dehghan A, Rahimpour S, Soleymani-Salehabadi H, Owlia MB. Role of vitamin D in flare ups of rheumatoid arthritis. Z Rheumatol 2014;73(5):461–4.

[344] Hansen KE, Bartels CM, Gangnon RE, Jones AN, Gogineni J. An evaluation of high-dose vitamin D for rheumatoid arthritis. J Clin Rheumatol 2014;20(2):112–4.

[345] Ohkawara T, Takeda H, Kato K, Miyashita K, Kato M, Iwanaga T et al. Polaprezinc (N-(3-aminopropionyl)-L-histidinato zinc) ameliorates dextran sulfate sodium-induced colitis in mice. Scand J Gastroenterol 2005;40(11):1321–7.

[346] Tran CD, Ball JM, Sundar S, Coyle P, Howarth GS. The Role of Zinc and Metallothionein in the Dextran Sulfate Sodium-Induced Colitis Mouse Model. Dig Dis Sci 2007;52(9):2113–21.

[347] Jørgensen SP, Agnholt J, Glerup H, Lyhne S, Villadsen GE, Hvas CL et al. Clinical trial: vitamin D3 treatment in Crohn's disease - a randomized double-blind placebo-controlled study. Aliment Pharmacol Ther 2010;32(3):377–83.

[348] Wingate KE, Jacobson K, Issenman R, Carroll M, Barker C, Israel D et al. 25-Hydroxyvitamin D concentrations in children with Crohn's disease supplemented with either 2000 or 400 IU daily for 6 months: a randomized controlled study. J Pediatr 2014;164(4):860–5.

[349] Raftery T, Martineau AR, Greiller CL, Ghosh S, McNamara D, Bennett K et al. Effects of vitamin D supplementation on intestinal permeability, cathelicidin and disease markers in Crohn's disease: Results from a randomised double-blind placebo-controlled study. United European Gastroenterol J 2015;3(3):294–302.

[350] Aranow C, Kamen DL, Dall'Era M, Massarotti EM, Mackay MC, Koumpouras F et al. Randomized, Double-Blind, Placebo-Controlled Trial of the Effect of Vitamin D3 on the Interferon Signature in Patients With Systemic Lupus Erythematosus. Arthritis & rheumatology (Hoboken, N.J.) 2015;67(7):1848–57.

[351] Kamen DL, Oates JC. A Pilot Study to Determine if Vitamin D Repletion Improves Endothelial Function in Lupus Patients. Am J Med Sci 2015;350(4):302–7.

[352] Lima GL, Paupitz J, Aikawa NE, Takayama L, Bonfa E, Pereira RMR. Vitamin D Supplementation in Adolescents and Young Adults With Juvenile Systemic Lupus Erythematosus for Improvement in Disease Activity and Fatigue Scores: A Randomized, Double-Blind, Placebo-Controlled Trial. Arthritis Care Res (Hoboken) 2016;68(1):91–8.

[353] Fink G, Heitner J. Evaluating the cost-effectiveness of preventive zinc supplementation. BMC Public Health 2014;14.

[354] Weaver CM, Bischoff–Ferrari HA, Shanahan CJ. Cost-benefit analysis of calcium and vitamin D supplements. Archives of Osteoporosis 2019;14(1):50.

Table 1:

(a) Recommended Daily allowance (RDA, WHO[1]), population reference intake (PRI, EFSA[2]) and upper limits (UL) for zinc and vitamin D. (b) Summary of the most relevant supplementation studies.

a)

element	**sex**	**UL per day**	**LPI[3]**	**RDA/PRI per day**	
VitD	m	100 µg / 4000 IU	n. r.[4]	15 µg / 600 IU	WHO & EFSA
VitD	f	100 µg / 4000 IU	n. r.	15 µg / 600 IU	WHO & EFSA
zinc	m	25 mg	300 mg 600 mg 900 mg 1200 mg	9.4 mg 11.7 mg 14.0 mg 16.3 mg	EFSA
zinc	f	25 mg	300 mg 600 mg 900 mg 1200 mg	7.5 mg 9.3 mg 11.0 mg 12.7 mg	EFSA
zinc	m	40 mg	n. s.[5]	11 mg	WHO
zinc	f	40 mg	n. s.	8 mg	WHO

Abbreviations: [1]WHO: World Health Organization, [2]EFSA: European Food Safety Authority, [3]LPI: level of phytate intake, [4]n. r.: not relevant, [5]n.s. not specified

b)

Disease	Element	dosage	Period	Effect	Reference
T1DM	Zn glycine	7.5-15 mg/d (4 – 17y)	4 we	Increased erythrocyte zinc & HbA1c	[317]
T1DM	ZnGlc	30 mg/d	3 mo	Reduced lipid peroxidation	[318]
T1DM	$ZnSO_4$ +VitA	10 mg/d +25,000 IU (7 – 20y)	12 we	Increase in serum Apo-A1, decrease in Apo-B/Apo-A1 ratio	[319]
T1DM	ZnGlc	50 mg/d	1 mo	Increased HbA1c	[320]
T1DM (rat)	$ZnSO_4$	5 mg/kg/d	3 mo	Reductions in: oxidative stress, kidney/glomeru-lar damage, lymphocytic infiltration,blood glucose & urinary protein levels, urinary albumin excretion,	[321]
T1DM	α-calcidol	10 IU/2x/d	12 mo	stable FCP	[322]
T1DM	rocaltrol	10 IU/d	18 mo	no changes	[323]
T1DM	Cholecal-ciferol	2000 IU/d	18 mo	decrease CRP	[140]
T1DM	α-calcidol	10 IU / 1 – 2x/d (8 – 12y)	6 mo	lower requirement for insulin	[324]
EAE (mouse)	ZnAsp	0.3 - 1.5 mg/kg	d1 - 22	lower EAE score, decrease in Th17 cells, increase in Treg cells	[123]
EAE (mouse)	ZnAsp	1.5 mg/kg	d1-10 or d11-19	reduction of the clinical severity (during first relapse)	[124]
EAE (mouse)	ZnAsp	6 – 12 µg/d	during relaxing remitting phase	reduced clinical and histopathological signs	[87]
EAE (mouse)	ZnAsp, IVIG, ZnAsp +IVIG	ZnAsp (30 µg/d), IVIG (10 mg/d) or both (i.p.)	from d11 to d15 or d11 to d19 after immuni-zation	Zn: diminishes clinical signs, IVIG: no effect Zn+IVIG: less severity during acute & relapsing-remitting phase	[125]
EAE (mouse)	calcitriol	4 IU/every 2ndd	d2 – 15	less EAE development, abrogated rise in antibody titer to myelin basic protein, less histopathology	[33]
EAE (mouse)	calcitriol	Prevention 2 IU/d (f), 4 IU/d (m) Treatment 8 IU/d	d1 - 30	IL-10 dependent protec-tion from EAE develop-ment/ progression	[325]

EAE (mouse)	calcitriol	1 IU/d	d1 - 34	complete EAE preven-tion (pre-treatment), prevention of pro-gression (treatment after onset)	[326]
EAE (mouse)	calcitriol - treated DCs	1 – 2 nM (*in vitro*)	8d pre-treating DCs d1 – 20 of EAE	prevents EAE, upregu-lates Treg, down-regulates Th1, Th17	[327]
MS-related depressi on	$ZnSO_4$	50 mg/d	12 we	decreased depression score, unaltered neuro-logical state	[328]
MS	Cholecal-ciferol	up to 280,000 IU/we	52 we	trend towards reduced relapse	[329]
MS	Cholecal-ciferol	300,000 IU/mo	6 mo	no effect on EDSS	[330]
MS	Cholecal-ciferol	20,000 IU/we	12 mo	trend towards reduced EDSS	[331]
MS	Cholecal-ciferol	20,000 IU/we	96 we	no effect on EDSS, relapse, fatigue	[332]
MS	Cholecal-ciferol	50,000 IU/we	12 mo	decreased incidence ratio of demyelination plaques, reduced progression risk	[333]
MS ($CD4^+$ T cells, *in vitro)*	VitD	10 nM (*in vitro*)	12h – d7	decreased proliferation of patient´s CD4+ T cells & MBP-specific T cells *in vitro*; enhanced IL-10 producing cells, reduced IL-6 & IL-17 secreting cells	[138]
PS	zinc (not defined)	topical: 0.25% Zn + Pyr in an emollient base, 2x/d	3 mo	Decreased plaques / PASI score	[334]
Psoriat-ric arthritis	$ZnSO_4$	50 mg/3x/d	6 mo	Reduction of joint pain, increase of mobility, , decrease of swelling, reduction of serum immunoglobulins, increase of serum albumin	[335]
RA	$ZnSO_4$	50 mg/3x/d	12 we	Decreased joint swelling, stiffness, walking time	[336,337]
RA	$ZnSO_4$	50 mg/3x/d	6 mo	no effect on RA, increased alkaline phosphatase	[338]
RA	$ZnSO_4$	45 mg/3x/d	5 – 6 mo	no effect	[339]
RA	ZnAsp	260 mg/d	d1 - 15	Decreased ROS of patient´s monocytes *in*	[340]

				vitro	
RA	ZnGlc	45 mg/d	2 mo	Increased phagocytosis	[341]
RA	VitD	50,000 IU/we	12 we	Modest improve in tender joint count, swollen joint count, ESR, VAS	[342]
RA	VitD	50,000 IU/we	6 mo	No effect on relapse rate	[343]
RA	VitD	50,000 IU/3x/we - 2x mo	12 mo	No effect on RA, no increase in pain	[344]
CIA/ EAE	Zn (not defined)	≈ 12 – 21 mg/d (3000 ppm in drinking water)	30d before disease induction	inhibits development of CIA & EAE involving blockade of Th17 cell development	[115]
Colitis (mouse)	Zn carno-sine	1.7 mg/kg/d	d1 - 7	Reduced pro-inflam-matory cytokine levels and neutrophils accu-mulation, cytoprotection by overexpression of HSPs	[345]
Colitis (mouse)	ZnO	2.4 mg/d (gavage)	d1 – 6	Reduction of disease activity index (50%), less colon damage	[346]
CD	VitD	1,200 IU/ d	12 mo	Trend to reduced relapse	[347]
CD	VitD	400 - 2000 IU/d (children)	6 mo	No difference in CDAI, ESR, CRP	[348]
CD	VitD	2,000 IU/d	3 mo	Stabilized intestinal permeability, reduced CRP, trend to decreased CDAI	[349]
SLE	VitD	2,000 – 4,000 IU/d	12 we	Increased serum VitD, no change in IFN	[350]
SLE	VitD	5,000 IU/d	16 we	Tendency of increased flow-mediated dilation	[351]
SLE	VitD	50,000 IU/we	24 we	Decreased disease activity, improved fatigue	[352]

Abbreviations:

AID: Autoimmune Diseases

Apo: Apolipoprotein

VAS: visual analogue scale

CD: crohn´s disease

CDAI: crohn´s disease activity index

CIA: collagen induced arthritis

CRP: C reactive protein

DC: dendritic cells

EDSS: Expanded Disability Status Scale

EAE: Experimental autoimmune encephalomyelitis

ESR: erythrocyte sedimentation rate

FCP: fasting C peptide

Hb: Hemoglobin

HSP: heat shock protein

IFN: interferone

IVIG: *in vitro* immune globulins

MBP: myelin basic protein

PASI: Psoriasis Area Severity Index

PS: psoriasis

RA: Rheumatoid Arthritis

ROS: reactive oxygen species

T1DM: type 1 diabetes

VAS: visual analogue scale

ZnGlc: zinc glucinate

$ZnSO_4$: zinc sulfate

$ZnCl_2$: zinc chloride

Zn+Pyr: zinc and pyrithione

Table 2:

Example costs for current treatment strategies in US dollar, comparing numbers for Europe (represented by the National Institute for Health and Care Excellence (NICE), UK), Canada (showing numbers from the Canadian Agency for Drugs and Technologies in Health (CADTH) and for the USA as found in recent studies. Supplementation with 10 mg zinc and 1,000 IU Vitamin D will cost less than 22$ (developing countries) and 45 – 58.5$ per year, respectively [353,354].

Disease	Medication	Cost / year [$]	Country	Reference
CD	Infliximab, Adalimumab, Golimumab	12,393 – 13,772	UK	NICE, 2015
	Infliximab, Adalimumab Vedolizumab	46,415 18,000 - 20,700 21,458	Canada	CADTH 2013 2007
	Infliximab, Adalimumab, Ustekinumab	50,510 54,985 72,921	USA	Aliyev et al., 2018
RA	Tocilizumab, Infliximab	5,095- 11,320 9,974- 11,400	UK	NICE 2017
	Tocilizumab IV, Infliximab	8,154 - 102,706	Canada	CADTH, 2018
	Tocilizumab, Infliximab	14,334 - 24,916	USA	Gu et al., 2016
MS	Ocrelizumab β Interferon Alemtuzumab Natalizumab Glatiramer acetate	24,096 9,055 70,880 18,525 8,404	UK	NICE, 2014, 2007, 2017
	β Interferon Natalizumab, Glatiramer acetate	18,677 - 25,458 42,847 16,286	Canada	CADTH, 2018
	β Interferon Natalizumab, Glatiramer acetate	10,583 – 53,032 36,485 – 51,306 34,635 – 47,253	USA	Hartung et al., 2015

HIGHLIGHTS

- Immune function during zinc deficiency
- Immune function during zinc supplementation
- Immune function during vitamin D deficiency
- Immune function during vitamin D supplementation
- Clinical trials of zinc and vitamin D supplementation to treat auto immune diseases