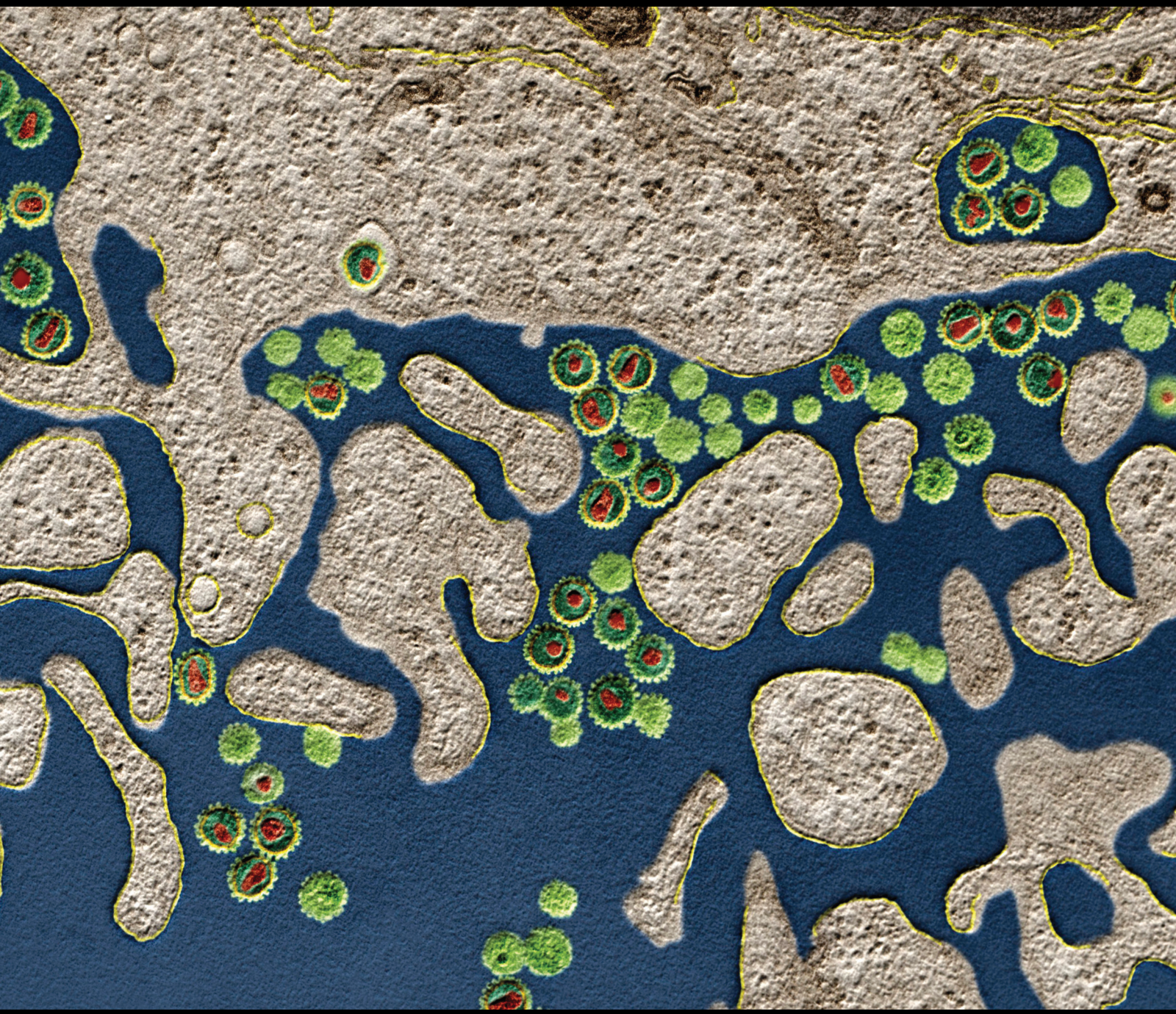
Journal of Immunology Research

Roles of zinc and zinc mediators in immunity

Lead Guest Editor: Shintaro Hojyo
Guest Editors: Toshiyuki Fukada and Ananda S. Prasad



Roles of zinc and zinc mediators in immunity

Journal of Immunology Research

Roles of zinc and zinc mediators in immunity

Lead Guest Editor: Shintaro Hojyo
Guest Editors: Toshiyuki Fukada and Ananda S. Prasad

Contents

Hindawi
Journal of Immunology Research
Volume 2020, Article ID 9207279, 12 pages
https://doi.org/10.1155/2020/9207279

Review Article

Lessons Learned from Experimental Human Model of Zinc Deficiency

Ananda S. Prasad

Department of Oncology, Wayne State University School of Medicine and Barbara Ann Karmanos Cancer Center, Detroit, Michigan 48201, USA

Correspondence should be addressed to Ananda S. Prasad; prasada@karmanos.org

Received 24 January 2019; Accepted 5 September 2019; Published 9 January 2020

Academic Editor: Alexandre Keller

Zinc is an essential element for humans, and its deficiency was documented in 1963. Nutritional zinc deficiency is now known to affect over two billion subjects in the developing world. Conditioned deficiency of zinc in many diseases has also been observed. In zinc-deficient dwarfs from the Middle East, we reported growth retardation, delayed sexual development, susceptibility to infections, poor appetite, and mental lethargy. We never found a zinc-deficient dwarf who survived beyond the age of 25 y. In an experimental model of human mild zinc deficiency, we reported decreased thymulin (a thymopoietic hormone) activity in Th1 cells, decreased mRNAs of IL-2 and IFN-gamma genes, and decreased activity of natural killer cells (NK) and T cytotoxic T cells. The effect of zinc deficiency on thymulin activity and IL-2 mRNA was seen within eight to twelve weeks of the institution of zinc-deficient diet in human volunteers, whereas lymphocyte zinc decreased in 20 weeks and plasma zinc decreased in 24 weeks after instituting zinc-deficient diet. We hypothesized that decreased thymulin activity, which is known to proliferate Th1 cells, decreased the proliferation differentiation of Th1 cells. This resulted in decreased generation of IL-2 and IFN-gamma. We observed no effect in Th2 cell function; thus, zinc deficiency resulted in an imbalance of Th1 to Th2 function resulting in decreased cell-mediated immunity. Zinc therapy may be very useful in many chronic diseases. Zinc supplementation improves cell-mediated immunity, decreases oxidative stress, and decreases generation of chronic inflammatory cytokines in humans. Development of sensitive immunological biomarkers may be more sensitive than an assay of zinc in plasma and peripheral blood cells for diagnosis of marginal zinc deficiency in human.

1. Introduction

Raulin in 1869 [1] showed for the first time that zinc was required for the growth of Aspergillus niger. Essentiality of zinc for growth of plants in 1926 and the growth of rats in 1934 was reported [2, 3]. In 1955, a disease called parakeratosis in swine due to zinc deficiency was observed [4]. Zinc was shown to be essential for chickens in 1958 [5]. In animals, the manifestations of zinc deficiency included growth failure, loss of hair, thickening and hyperkeratinization of the epidermis, and testicular atrophy. Deficiency of zinc in breeding hens resulting in decreased hatchability, gross embryonic anomalies characterized by abnormal skeletal development, and weakness in chicks that hatched were observed [6]. Although the essentiality of zinc for animals was established, its ubiquity made it seem improbable that zinc deficiency in humans could lead to significant problems in humans. During the past 50 years, however, it has become apparent that deficiency of zinc in humans is quite prevalent.

I went to Shiraz, Iran, in 1958. I had finished my training as a Medical Scientist under the guidance of Prof. C.J. Watson. I accepted an invitation from Prof. H.A. Reimann to help him set up a medical curriculum and training program at the Shiraz Medical School, Shiraz, Iran.

Within three weeks of my arrival in Shiraz, my Chief Resident showed me a 21 yr old male who was extremely growth retarded, had no secondary sexual characteristics, and had hypogonadism, hepatosplenomegaly, rough skin, and mental lethargy [7]. He was severely anemic, and the anemia was due to iron deficiency. There was no blood loss. Severe iron

deficiency in males does not occur without loss of blood. His diet consisted of only bread made of unleavened flour, and animal protein intake was negligible.

Iron deficiency does not cause growth retardation and hypogonadism. An examination of the periodic table suggested to me that perhaps this syndrome was due to both iron and zinc deficiency [7]. We suggested that perhaps high content of phosphate in the diet and geophagia, which was very common in villagers of Iran, may have impaired absorption of both iron and zinc. We studied 10 additional cases in Nemazee Hospital, Shiraz, Iran, within a short period of time, suggesting that perhaps zinc deficiency in the Middle East may be common.

I subsequently went to US Naval Medical Research Unit No. 3 in Cairo, Egypt, and studied zinc metabolism extensively in Egyptian dwarfs.

We were able to document that growth retardation and hypogonadism in these subjects were due to zinc deficiency. Zinc concentration in plasma, red cells, urine, and hair was decreased; the Zn 65 plasma turnover rate was increased, and the 24 h exchangeable pool of Zn 65 was decreased in all the dwarfs [8, 9].

In Egypt, the rate of growth was greater in patients who received supplemental zinc as compared with those receiving iron instead or those receiving only an adequate animal protein diet. Pubic hair appeared in all subjects within 7-12 wk after zinc supplementation. Genitalia size increased to normal size, and secondary sexual characteristics developed within 12-24 wk in all subjects following zinc supplementation. In contrast, no such changes were observed in a comparable length of time in the iron-supplemented group or in the group on an animal protein diet alone [10]. The growth retardation and gonadal hypofunction in these subjects were related to zinc deficiency. The anemia was due to iron deficiency and responded to oral iron treatment. These studies clearly showed that severe anemia and iron deficiency were not causative factors for growth retardation and hypogonadism in these subjects.

The diet of the Middle Eastern subjects (both Iran and Egypt) consisted of mainly cereal protein high in phytate (an organic phosphate compound) which complexes both zinc and iron resulting in decreased availability of both zinc and iron.

We concluded that excess content of dietary phytate was responsible for deficiencies of both iron and zinc in Middle Eastern dwarfs [7, 8, 10].

The recommended dietary allowance for zinc was established in 1974 by the US National Academy of Science, National Research Council [11]. The current estimate of WHO is that nearly two billion subjects in the developing world may have nutritional deficiency of zinc.

Childhood malnutrition is a common problem in the developing world. A 1993 report based on WHO's global data of children's growth showed that approximately 42% of children less than 5 y of age have low height compared to the international reference standard [12, 13].

The causes of growth failure are not fully understood; inadequate food nutrition and poor nutritional quality of many traditional foods may be important factors.

The high intake of cereal protein rich in phytate prevents absorption of zinc and iron. Zinc is extremely important for growth, and our studies in the Middle East in the sixties showed that the dwarfs had adequate intake of caloric and vitamins but were zinc deficient [7–10].

There have been several studies, which document the effect of zinc supplementation on increase in height and gain in weight in children who were supplemented with zinc.

Brown et al. [13] published a meta-analysis of 33 studies published on the effect of zinc supplementation in children.

There was a highly significant effect of zinc supplementation on increase in height [13]. These studies indicate that zinc deficiency is a global health problem, which affects growth and development in children.

It is now evident that nutritional as well as conditioned deficiency of zinc may complicate many diseases in human subjects.

2. Zinc Deficiency in Human

2.1. Severe Zinc Deficiency. A severe deficiency of zinc may be life-threatening as has been reported in patients with acrodermatitis enteropathica (AE) [14] (a genetic disorder), after use of total parenteral nutrition without zinc, after penicillamine therapy, and with acute alcoholism. The clinical manifestations of severely zinc-deficient subjects include bullous-pustular dermatitis, diarrhea, alopecia, mental disturbances, and intercurrent infections due to cell-mediated immune deficiency; if untreated, the zinc deficiency becomes fatal. AE is caused by a mutation in a zinc transporter, ZIP-4, which results in malabsorption of zinc [15].

2.2. Moderate Level of Zinc Deficiency. Growth retardation, hypogonadism, skin changes, poor appetite, mental lethargy, abnormal dark adaptation, and delayed wound healing are some of the manifestations of moderate zinc deficiency in human subjects. Moderate deficiency of zinc due to nutritional factors, malabsorption, sickle cell disease, chronic renal disease, and other debilitating conditions has now been well documented [7–10].

During my stay in the Middle East, I never saw a dwarf older than 25 years of age. Local physicians told us that the dwarfs died because of infections, suggesting that zinc deficiency may have affected their immunity adversely.

Females are also affected by zinc deficiency. Although this was not appreciated in earlier studies, later studies documented zinc deficiency in females. Growth retardation and ovarian dysfunction due to zinc deficiency were observed in zinc-deficient females [16].

2.3. Marginal Deficiency of Zinc. A mild zinc deficiency was induced by an experimental soybean-based protein diet in normal human volunteers. The diet met all recommended dietary allowances for protein and essential macro- and micronutrient except for zinc. The daily intake of zinc was restricted to 3 to 5 mg daily. The RDA for zinc in adults is 12-15 mg/d. [16, 17].

The volunteers were admitted to a Clinical Research Center metabolic ward at the University of Michigan Hospital.

They were ambulatory and encouraged to do daily exercises. They were studied for a total of 24 weeks.

The details of our protocol have been published [17].

We observed neurosensory changes, oligospermia in males, decreased serum testosterone concentration, hyperammonemia, decreased lean body mass, decreased serum thymulin activity, decreased IL-2 activity, decreased NK cell activity, and alterations in T cell subpopulations in our marginally zinc-deficient subjects. All the above manifestations were correctable by supplementation with zinc [17–19].

3. Zinc and Immunity

Bach et al. [20] described the biochemical characteristics of a thymic hormone protein called facteur thymique serique (FTS). This was later called thymulin. In 1983, it was reported that zinc was essential for its activity [21].

At that time in Detroit, we were working with a human experimental mild zinc deficiency model. The reports from France were very exciting. Our studies in the Middle East showed that the dwarfs were susceptible to infections, and they died before they reached 25 years of age. The thymulin zinc study suggested to me that zinc was involved in immunity. The report that a thymic hormone required zinc for its activity stimulated me to collaborate with Prof. Bach and Dardene.

I met them in Paris, and they were equally excited to study thymulin in a human zinc deficiency model. Together, we published several papers on these subjects. We observed that thymulin was required for T helper cell differentiation and proliferation. We reported that zinc was required for gene expression of IL-2 and IFN-gamma [18]; IL-2 generation upregulated natural killer cell activity and T cytotoxic cell activity [18, 19, 22]. IFN-gamma along with IL-12 generated by monocytes-macrophages was important for the activity of monocytes-macrophages for killing viruses, bacteria, and parasites.

In mild deficiency of zinc in the human model, we observed decreased serum thymulin activity, which was corrected by in vivo and in vitro zinc supplementation, suggesting that this index was a sensitive indicator of mild zinc deficiency in humans [18]. It is probable that because of zinc deficiency, host-defense mechanisms were compromised in a large segment of population in the developing world.

The effect of zinc on lymphocytes also appears to be that of a mitogen, and the kinetics of these influences most closely approximate the effects of antigen stimulation on lymphocyte in culture [23]. It is suggested that zinc directly stimulates DNA synthesis, either by activating enzymes or by altering the binding of F1 and F3 histones to DNA, which affects RNA synthesis. Direct cell-surface effects of zinc cannot be ruled out. It is conceivable that zinc could be operating at several different levels in influencing lymphocyte monoclonal proliferation [23].

Iwata et al. in 1979 [24] showed that one obvious effect of zinc deficiency was decrease in thymocytes in mice and humans which resulted in reduction of thymic hormone activity.

DNA polymerase, RNA polymerase, reverse transcription, and deoxythymidine kinase are zinc-dependent enzymes and are involved in DNA synthesis [25].

We reported that zinc is required for the gene expression of deoxythymidine kinase, an enzyme essential for DNA synthesis and cell division [26–29].

4. Nucleoside Phosphorylase (NPase) and Zinc

Giblett et al. in 1975 [30] reported a case of a 5 y old girl with severely defective T cell immunity, normal B cell, and nucleoside phosphorylase deficiency.

Nucleoside phosphorylase catalyzes the conversion of inosine and deoxyinosine to hypoxanthine and guanosine and deoxyguanosine to xanthine. Accumulation of deoxyguanosine is believed to be toxic to T cells.

In our experimental model of human zinc deficiency, we observed that NPase activity in lymphocytes was decreased in zinc-deficient subjects and that decreased NPase may partly account for abnormal T cell functions in zinc deficiency [31, 32].

5. Zinc and Neutrophils

Briggs et al. in 1982 reported that granulocytes in zinc-deficient uremic subjects showed impaired mobility and decreased both chemotactic and chemokinetic activities, in comparison with subjects who were supplemented with zinc. Others also observed abnormal granulocyte chemotaxis, corrected by zinc supplementation in patients with acrodermatitis enteropathica and in chronic renal disease patients without uremia [33]. Thus, it appears that zinc is essential for chemotaxis.

6. Zinc Modulates Cell-Mediated Immune Functions and Participates in T Helper Cell Differentiation

Zinc is essential for cell-mediated innate immunity and activities of natural killer cells. Macrophages are also affected by zinc deficiency. Phagocytosis, intracellular killing, and cytokine production by these cells are affected by zinc deficiency. The growth and function of T and B cells are affected adversely due to zinc deficiency. Zinc is needed for DNA synthesis, RNA transcription, and cell division [25]. Zinc deficiency adversely affects the generation and functions of cytokines, the basic messengers of the immune system [18, 19].

Figure 1 shows the landscape of action of zinc on immune cells.

Our studies have shown that serum thymulin activity is zinc dependent [18]. Thymulin, a thymopoietic hormone, is required for proliferation and differentiation of T helper cells. We observed that Th1 (T helper 1) functions in human zinc deficiency were affected adversely. Zinc was also required for gene expression of IL-2 and IFN-γ from Th1 cells, and the generation of these cytokines was adversely affected due to zinc deficiency. IL-2 deficiency decreased adversely NK cell lytic activity and decreased T cytotoxic cells [22]. Decreased

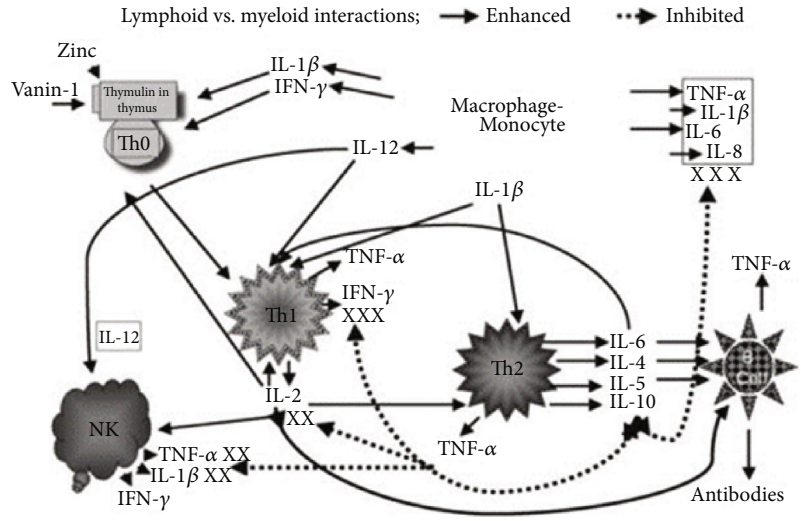


Figure 1: The landscape of zinc action on immune cells. Zinc is an essential component of thymulin, a thymic hormone involved in maturation and differentiation of T cells. The gene expression of IL-2 and IFN-γ (Th1 cytokines) is zinc dependent. IL-2 is involved in the activation of NK and T cytolytic cells. IL-12 is generated by stimulated macrophages-monocytes and is zinc dependent. IFN-γ and IL-12 together play a major role in the killing of parasites, viruses, and bacteria by macrophages-monocytes. Th2 cytokines are not affected by zinc deficiency except for IL-10 production, which is increased in the zinc-deficient elderly subjects. This is corrected by zinc supplementation. Increased IL-10 affects adversely Th1 and macrophage functions.

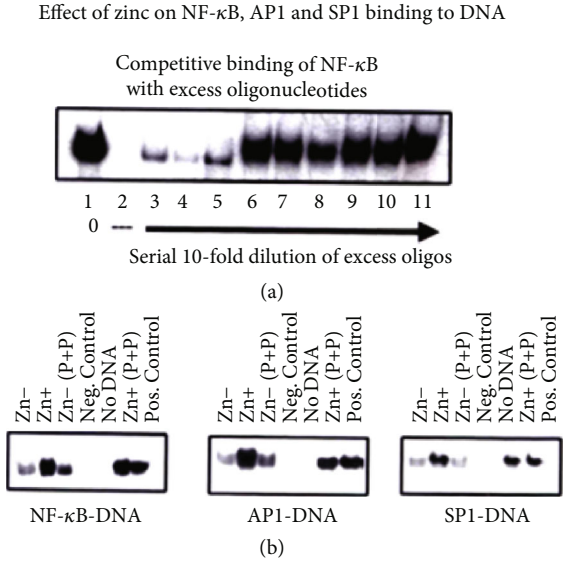


Figure 2: In a cell culture model HUT-78, the binding of NF-κB to DNA, an essential transcription factor for gene expression of Th1 cytokines (IL-2 and IFN-γ), is regulated by zinc as shown in this figure. We also show that the other transcription factors essential for gene expression of Th1 cytokines, AP1 and SP1, are also zinc dependent and their binding to DNA is regulated by zinc [35].

IFN-γ affected macrophages and monocyte function affecting killing of viruses, bacteria, and other infective agents. Zinc deficiency in humans resulted in decreased Th1 functions but did not affect Th2 cells; thus, there was an imbalance between Th1 and Th2 functions, resulting in adverse effect on cell-mediated immunity.

Zinc deficiency resulted in activating monocytes-macrophages, which resulted in increased generation of inflammatory cytokines such as TNF-α, IL-1β, and IL-6 and increased oxidative stress [34].

Figure 2 shows the effect of zinc on NF-κB, AP1, and SP1 binding to DNA. Zinc deficiency decreases the binding of NF-κB, AP1, and SP-1 to DNA [35].

Figure 3 summarizes the effects of zinc on NF-κB activation in HUT-78 cells. Zinc is involved at several steps in the activation of NF-κB in HUT-78 cells. Zinc is required for gene expression of NF-κB and its activation.

Figure 4 shows our experiment in HUT-78 cells. HUT-78 (Th0) cells were incubated under zinc-deficient and zinc-sufficient conditions for 4 days and then exposed to

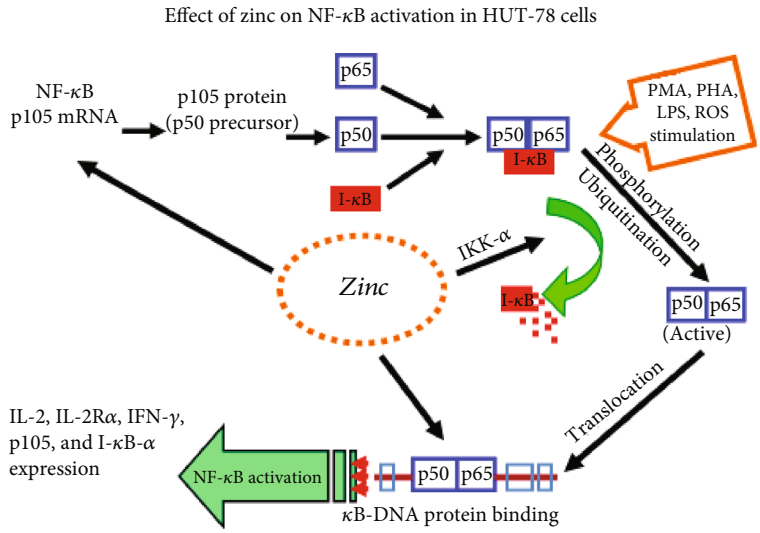


Figure 3: Our results of activation of NF-κB by zinc in HUT-78, a cell culture model. We observed that zinc was required for the expression of p105 mRNA, a precursor of p50 NF-κB protein. Once expressed, p50 NF-κB binds to Iκ B in the plasma. Following phosphorylation of Iκ B by zinc, NF-κB 50 is released for binding to DNA and gene expression of various proteins such as IL-2, IL-2Rα and β, IFN-γ, and Iκ B-α.

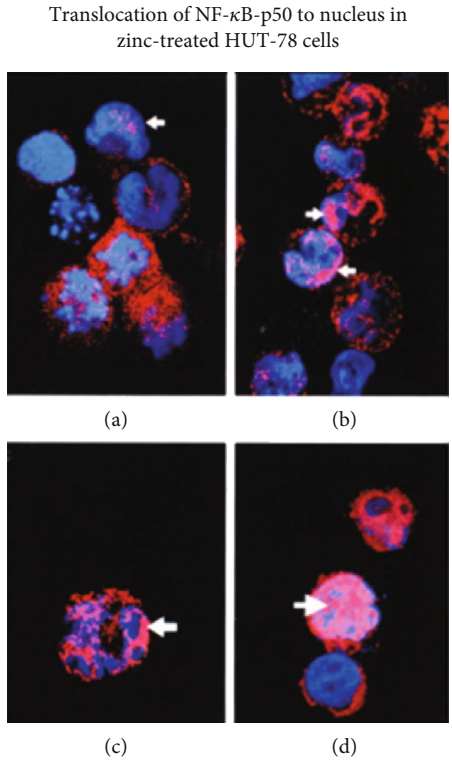


Figure 4: The translocation of NF-κB to DNA for binding and gene expression. Translocation of NF-κB-p50 to the nucleus under zinc-deficient and zinc-sufficient conditions. HUT-78 cells were incubated under Zn- and Zn+ conditions for 4 days and then exposed to PMA/PHA for 3 hours. Confocal images were prepared to show cytosolic NF-κB and nuclear material and the colocalization of NF-κB in the nucleus: (a) nonstimulated Zn- cells; (b) nonstimulated Zn+ cells; (c) PMA/PHA-stimulated Zn- cells; (d) PMA/PHA-stimulated Zn+ cells. Arrows indicate areas of colocalization. PMA/PHA-stimulated Zn+ cells showed the greatest translocation of NF-κB-p50 to the nucleus [36].

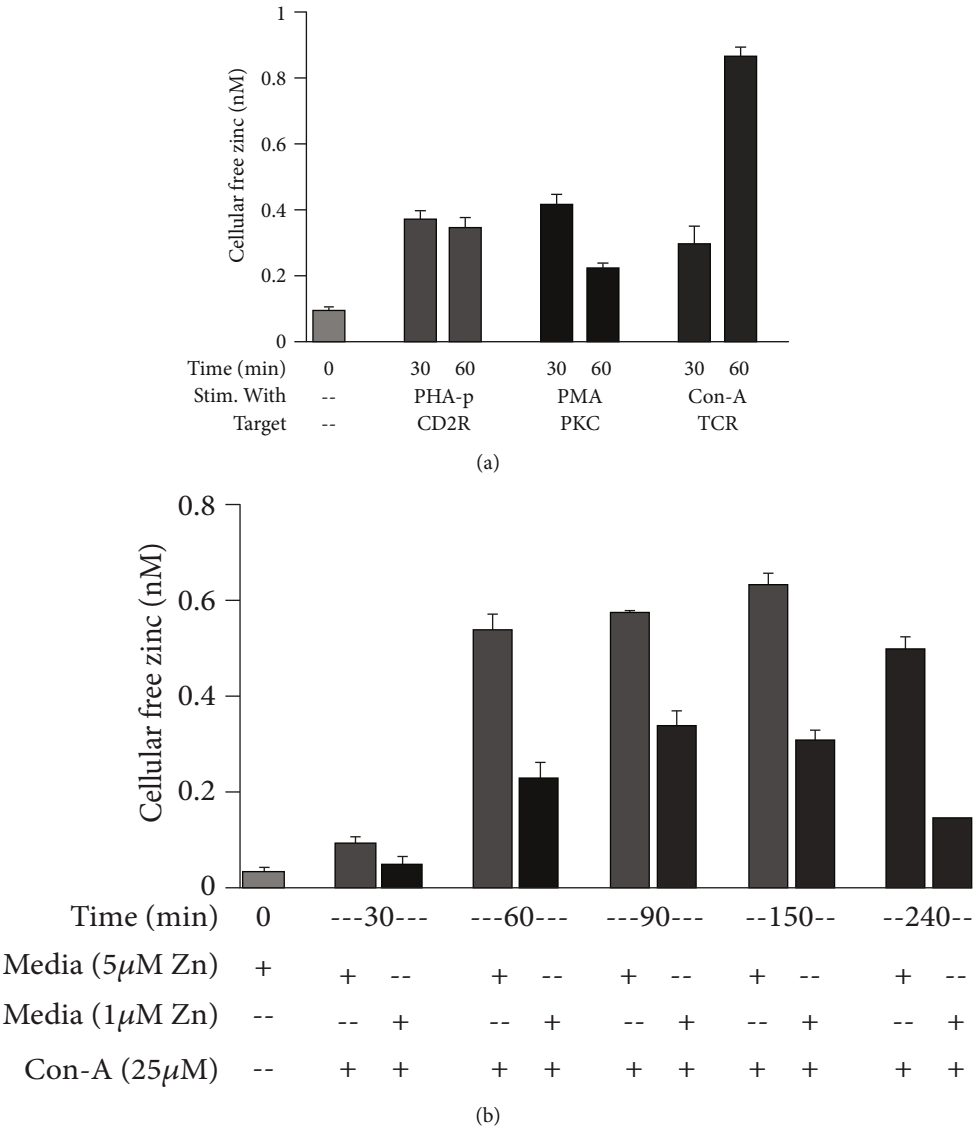


Figure 5: Activation of TCR increases intracellular free zinc. (a) Cells were incubated for 24 h in normal media to which had been added 15 μM zinc (to load cells with zinc) and then stimulated in normal media (5 μM zinc) in the presence of PHA, PMA, or Con-A for 30 and 60 min to determine which pathway was involved in differentiation or activation of Th0 or naïve cells and resulted in an increase in cellular free zinc. (b) To determine the time course for an increase in cellular free zinc, HUT-78 cells were stimulated in normal or in zinc-deficient medium. Stimulation in normal vs. zinc-deficient media allowed us to determine if the increase in cellular free zinc was due to an influx of extracellular free zinc or only a release of free zinc within the cellular compartment under Con-A stimulation. It appears that the increase in cellular free zinc is a result of both extracellular zinc influx and the release of free zinc from intracellular sources following Con-A stimulation ($n = 3$) [37].

PMA/PHA for 3 hours. This figure shows confocal images of cytosolic NF-κB and nuclear NF-κB. This figure shows that PMA/PHA-stimulated Zn+ cells showed greater localization of NF-κB to nuclear DNA compared to zinc-deficient cells [36].

Figure 5 shows that intracellular free zinc increased in HUT-78 (Th0) cells following Con-A stimulation of cells via TCR receptor, releasing intracellular free zinc which functions as a signal molecule for generation of IFN-γ, T-bet, and IL-12Rβ2, mRNAs required for Th1 cell differentiation [37].

Figure 6 shows schematically the role of various factors involved in Th1 cell differentiation. The increase in intracellular free zinc follows Th0 stimulation with Con-A, which functions as the upregulator of various transcription factors. It upregulates IFN-γ, T-bet, and IL-12Rβ2 receptors and STAT4 for Th1 cell differentiation [36].

7. Zinc and Allergy

In one study, the influence of zinc on allergen-induced cell growth, CD4+ regulatory T (Treg) cells, and cytokine expression during allergic immune reaction was investigated [38].

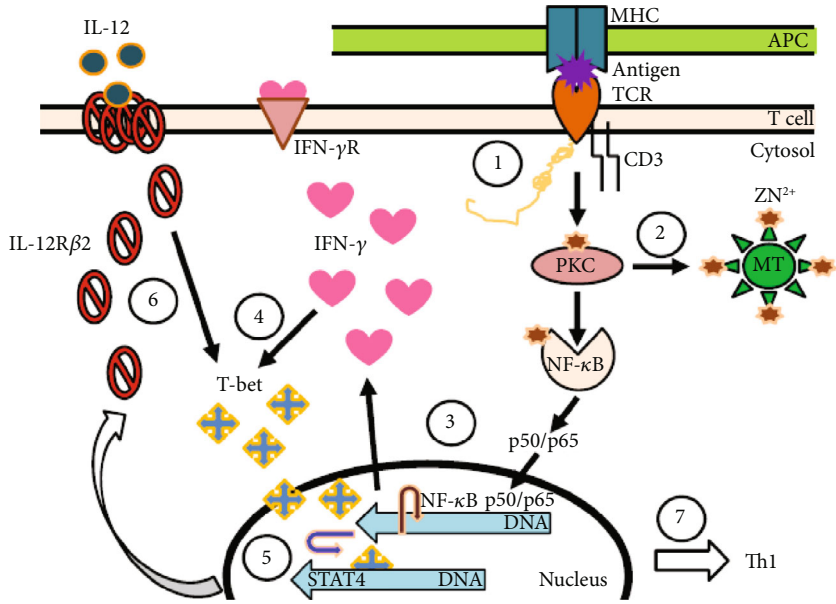


Figure 6: Role of zinc in differentiating Th0 T cells to the Th1 subtype. The differentiation of Th0 or CD4+ naïve T cells to the Th1 subtype is a two-stage process involving many transcription factors and events which are zinc-dependent. (1) Engagement of TCR by CD3 antibodies or Con-A or an antigen by APC is the first step in the differentiation process. Here, zinc acts as a bridge between the CD4 or CD8 receptor and Lck, a tyrosine kinase activated during differentiation. (2) Once the initial process begins, zinc-dependent PKC-h is phosphorylated and able to activate the release of free zinc from metallothionein, the endoplasmic reticulum, or the Golgi. (3) An increase in free zinc is then used for binding of activated NF-κB-p50/p65 to DNA, which initiates the transcription and production of IFN-γ. (4) IFN-γ enhances the expression of T-bet. IFN-γ and T-bet expression then becomes autocrine/paracrine. During the second stage, after TCR is disengaged. (5) T-bet associates with STAT4 for transcription and expression of IL-12Rβ2 which is a zinc-dependent process. (6) Once the expression of IL-12Rβ2 is enhanced, T-bet expression then depends upon IL-12. (7) Once the Th1 cells are differentiated and stabilized, they function in the absence of IFN-γ. Full expression of T-bet also inhibits the expression of GATA3 which is responsible for Th2 differentiation, thus, locking in the Th1 phenotype [37].

PBMC from nonatopic and atopic subjects were treated with timothy green allergen preincubated with or without (50 μM) zinc.

In CD3+ T cells, combination of zinc 50 and allergen significantly reduced PBMC proliferation of atopic subjects. Zinc 50 plus allergen enhanced Th1 cytokines as shown by increased IFN-γ/IL-10 ratios, and enhanced generation of Treg cells was observed. Zinc appeared to downregulate unwanted hyperresponsive cells. There was a significant shift from IL-10 to Th1 cytokine IFN-γ and enhanced generation of Treg cells.

This study suggests that zinc supplementation may be useful as a therapy for allergies without negatively affecting the immune system.

T lymphocytes regulate and coordinate immune responses in allergic diseases. Treg cells suppress CD4+ effector T cells, CD8+ T cells, antigen-presenting cells (APCs), NK cells, and also B cells. A decreased expression of Tregs was observed in patients with asthma and allergic rhinitis, and this results in expansion of Th2 cells [39].

Allergies belong to the Th2-driven diseases and are often accompanied by zinc deficiency and are therefore targets for zinc-induced modulation of the allergic immune reactions [40, 41].

Th1 and Th17 cells also contribute to allergic inflammation and hyperresponsiveness [42–44].

Treg inhabits T cell activation following allergen exposure, but this process is not optimal in atopic individuals.

Allergic asthmatic patients have impaired Treg-mediated suppression, and this correlates well with decreased serum zinc levels as reported in many studies.

Zinc supplementation is able to restore Treg function. Zinc deficiency in pregnancy is known to increase the incidence of allergies in children [45].

8. Zinc Decreases Oxidative Stress and Decreases the Generation of Chronic Inflammatory Cytokines in Humans

Figure 7 summarizes our concept regarding the role of zinc as a proantioxidant and anti-inflammatory agent.

ROS is known to activate NF-κB. Zinc decreases NADPH oxidase activity, and SOD is a zinc-copper-containing enzyme which is upregulated by zinc. These effects of zinc decrease oxidative stress. Zinc also upregulates MT synthesis, and MT decreases $^{\cdot}$OH burden.

Zinc upregulates A20, a transcription factor which inhibits NF-κB activation [34, 46].

Downregulation of NF-κB decreases generation of inflammatory cytokines which decreases ROS.

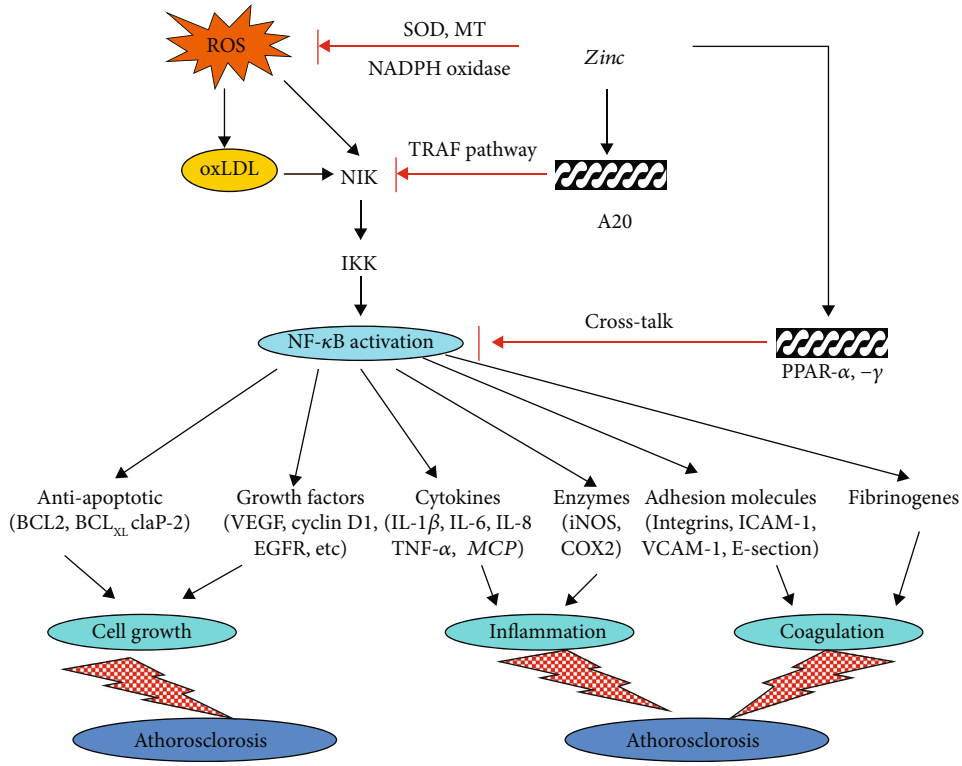


Figure 7: Our concept regarding the role of zinc as an antioxidant and anti-inflammatory agent. Reactive oxygen species (ROS) is known to activate NF-κB. Zinc decreases ROS generation. NADPH oxidase is inhibited by zinc and SOD, which is both a zinc and copper-containing enzyme that is upregulated. SOD is known to decrease oxidative stress. Metallothionein (MT) is induced by zinc and MT, which contains 26 moles of cysteine per mole of protein, decreasing OH burden. Zinc via A20 inhibits NF-κB activation, and this results in a decrease in generation of inflammatory cytokines and adhesion molecules. This figure also shows that zinc may have a preventive role in some cancers such as colon and prostate and in atherosclerosis inasmuch as chronic inflammation has been implicated in the development of these disorders [46, 47].

The anti-inflammatory role of zinc may have protective effect on atherosclerosis, prostate cancer, and colon cancer.

9. Effect of Marginal Zinc Deficiency in Human on Sensitive Immunological Parameters

Zinc deficiency increases oxidative stress and upregulates generation of inflammatory cytokines in humans [46].

Zinc supplementation decreases NADPH oxidase in monocytes-macrophages, which decreases generation of free radicals. Superoxide dismutase is both a zinc- and copper-dependent enzyme, which is essential for downregulating free radicals. Metallothionein is a zinc protein very effective in decreasing $^{\cdot}$OH; thus, zinc is an effective agent to decrease oxidative stress. Zinc supplementation increases A20 (a zinc-dependent transcription factor) activity in monocytes-macrophages, and A20 downregulates generation of TNF-α. Thus, zinc is an effective agent in decreasing oxidative stress and decreasing generation of inflammatory cytokines [46, 47].

Figure 8 shows the effect of marginal zinc deficiency in humans on plasma zinc [48].

Figure 9 shows the effect of marginal zinc deficiency in the experimental model [48].

Figure 10 shows the assay of ecto-5′-nucleotidase, an enzyme that is a marker of lymphocyte maturity. Ecto-5′ NT is a very sensitive biomarker of human marginal zinc deficiency [48].

Figure 11 shows the serum active thymulin (thymic hormone) in marginal human zinc deficiency. This also illustrates that the assay of active serum thymulin may be a very good biomarker of marginal human zinc deficiency [18].

Our studies have documented that the assay of immunological parameters may be more sensitive biomarkers of marginal zinc deficiency in humans than the assay of zinc in plasma or blood cells. Serum thymulin activity, IL-2 mRNA generation after PHA-PMA activation of peripheral blood mononuclear cells, and assay of activity of ecto-5′NT, a marker for lymphocyte maturity, are more sensitive biomarkers of zinc deficiency in comparison to the assay of zinc in plasma or peripheral blood cells.

10. Conclusion

Zinc deficiency is very prevalent worldwide. Cell-mediated immune dysfunction leading to increased infection is common in zinc-deficient subjects. Zinc is essential for the serum thymulin activity. Thymulin, a thymopoietic hormone, is essential for proliferation of Th0 cells. T-bet (a zinc-

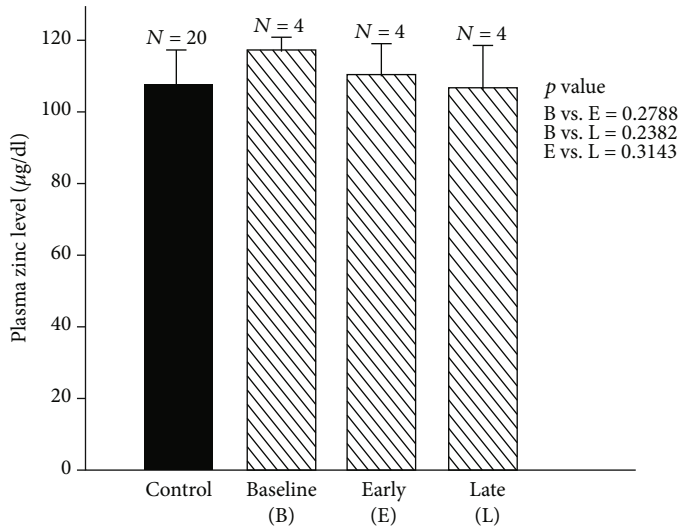


Figure 8: Changes in plasma zinc during early and late zinc deficiency periods in marginal deficiency of zinc in humans. Plasma zinc levels (mean ± SD) during baseline (B) vs. early zinc deficiency period (E) and late zinc deficiency period (L) were as follows: B vs. E, 116.20 ± 3.51 μg/dl vs. 109.10 ± 8.30 μg/dl, $p = 0.27$; B vs. L, 116.20 ± 3.51 μg/dl vs. 105 ± 11.38 μg/dl, $p = 0.23$; and E vs. L, 109.10 ± 8.30 μg/dl vs. 105.53 ± 11.38 μg/dl, $p = 0.31$. The values for plasma zinc in normal control subjects (mean ± SD) are also shown (107.26 ± 8.92 μg/dl) [48].

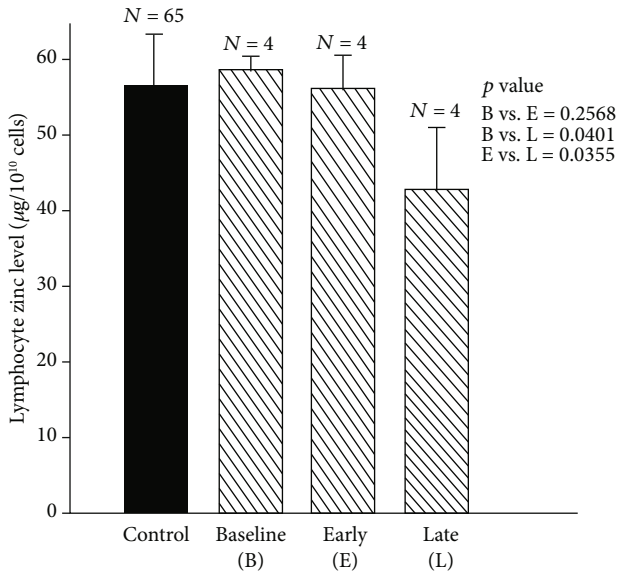


Figure 9: Changes in lymphocyte zinc level during early and late zinc deficiency periods in the experimental model of human zinc deficiency. Lymphocyte zinc levels ((mean ± SD) $\mu g/10^{10}$ cells) during baseline (B) vs. early zinc deficiency period (E) and late zinc deficiency period (L) were as follows: B vs. E, 58.36 ± 1.64 $\mu g/10^{10}$ cells vs. 55.29 ± 4.20 $\mu g/10^{10}$ cells, $p = 0.25$; B vs. L, 58.36 ± 1.64 $\mu g/10^{10}$ cells vs. 41.67 ± 8.26 $\mu g/10^{10}$ cells, $p = 0.04$; E vs. L, 55.29 ± 4.20 $\mu g/10^{10}$ cells vs. 41.67 ± 8.26 $\mu g/10^{10}$ cells, $p = 0.03$. The lymphocyte zinc level (mean ± SD) for control subjects is also shown (56.56 ± 6.42 $\mu g/10^{10}$ cells [48].

dependent transcription factor), INF-γ, and STAT4 are required for differentiation of Th1 cells from Th0 cells. Zinc-dependent transcription factors, NF-κB, AP1, and SP-1 are required for generation for IL-2 mRNA from Th1 cells. Zinc deficiency in humans results in downregulation of IL-2 cytokine from Th1 cells. IL-2 is essential for NK cell lytic activity and activation of T cytolytic cells, which participate in killing of viruses, bacteria, and cancer cells.

In zinc deficiency, although Th1 cells are affected adversely, Th2 cells remain unaffected. This results in a shift from Th1 to Th2 functions and results in cell-mediated immune dysfunction in zinc deficiency.

Zinc deficiency in humans activates monocytes-macrophages, which generate free radicals leading to oxidative stress and upregulate generation of inflammatory cytokines such as TNF-α, IL-1β, and IL-6. Inasmuch as many chronic diseases in humans including cancer of the prostate and colon and atherosclerosis have been related to increased oxidative stress and chronic inflammation, zinc supplementation to decrease oxidative stress and inflammatory cytokines may prove to be very useful in management of a few chronic diseases.

In our studies in the experimental model of mild human zinc deficiency, we observed that serum thymulin activity declined within eight to twelve weeks of institution of zinc-deficient diet in human volunteers [18]. At the same time, we observed that IL-2 mRNA and IL-2 generation from lymphocytes decreased the lymphocyte zinc decreased at the end of twenty weeks, and the decrease in plasma zinc was noted at the end of 24 weeks [48]. These observations suggested to us that the assay of immunological parameters was more sensitive than the assay of zinc in plasma and peripheral blood cells.

We published later that the assay of IL-2 mRNA may be a specific and sensitive biomarker of human zinc deficiency [49].

Immunological parameters such as assays of thymulin activity and lymphocyte ecto-5′-nucleotidase, a marker of lymphocyte maturity, and determination of mRNAs of IL-2 and IFN-γ after PHA and PMA stimulation of mononuclear cells in peripheral blood may be very sensitive and useful biomarkers of human zinc deficiency. These assays are more

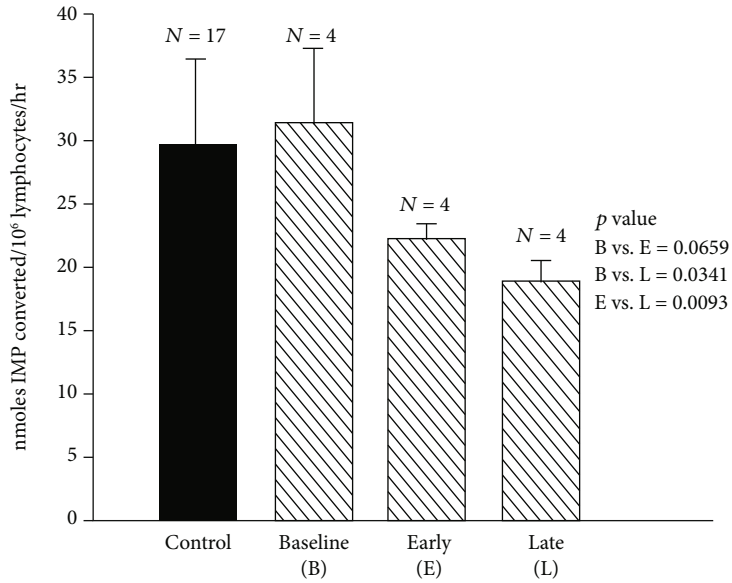


Figure 10: Changes in lymphocyte 5′NT activity during baseline, early zinc deficiency, and late zinc deficiency periods in the experimental model of human zinc deficiency IL 5′NT activity (mean ± SD) nmoles IMP converted/10^6 lymphocytes/hour) during baseline (B) vs. early deficiency period (E) and late deficiency period (L) were as follows: B vs. E, 31.13 ± 5.56 nmol IMP converted per 10^6 lymphocytes per hour vs. 21.95 ± 0.92 nmol IMP converted per 10^6 lymphocytes per hour, $p = 0.06$; B vs. L, 31.13 ± 5.56 nmol IMP converted per 10^6 lymphocytes per hour vs. 18.50 ± 1.58 nmol IMP converted per 10^6 lymphocytes per hour, $p = 0.03$; E vs. L, 21.95 ± 0.92 nmol IMP converted per 10^6 lymphocytes per hour vs. 18.50 ± 1.58 nmol IMP converted per 10^6 lymphocytes per hour, $p = 0.009$. The values for 5′NT in normal control subjects are also shown (29.5 ± 6.53 nmol IMP converted per 10^6 lymphocytes per hour) [48].

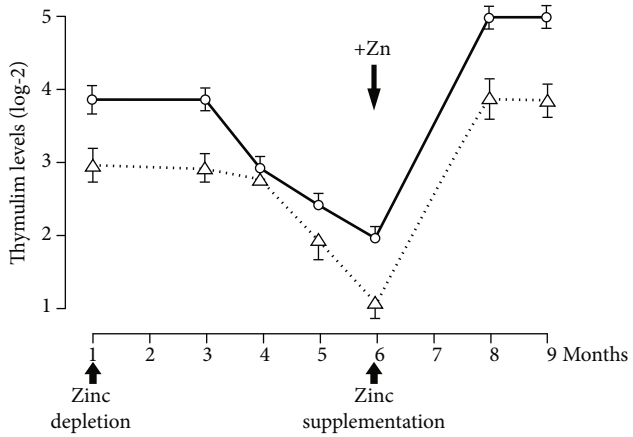


Figure 11: Thymulin activity—levels of thymulin activity in a sequential study of young human volunteers submitted to a zinc-restricted diet for 6 mo followed by zinc supplementation are shown here. Results are expressed as log-2 reciprocal titers (mean ± SEM). Each determination was performed in triplicate [18].

sensitive than determination of zinc in plasma, lymphocytes, neutrophils, or platelets.

Abbreviations

A20: Zinc-containing transcription factor
DNA: Deoxyribonucleic acid
HUT-78: Human malignant lymphoblastoid cell line of Th0 phenotype
ICAM-1: Soluble intercellular adhesion molecule-1
IFN-γ: Interferon-γ
IκB: Inhibitory protein of NF-κB
IL-1β: Interleukin-1β
IL-2: Interleukin 2
IL-6: Interleukin 6
IL-10: Interleukin 10
IL-12Rβ2: Interleukin 12 receptor, beta 2
mRNA: Messenger RNA
MT: Metallothionein
MyD88: Myeloid differentiation primary response 88
NADPH: Nicotinamide adenine dinucleotide phosphate
NF-κB: Nuclear factor kappa B
NK cell: Natural killer cells
PHA: Phytohemagglutinin-p
PMA: Phorbol myristate acetate
RNA: Ribonucleic acid
ROS: Reactive oxygen species
SOD: Superoxide dismutase
STAT4: Transcription factor STAT4
T-bet: Transcription factor involved in T cell differentiation
T cell: Thymic-dependent cell
TCR: T cell receptor
Th: T helper cell
Th1: T helper one
TNF-α: Tumor necrosis factor-α
TRIF: Domain containing adapter-inducing interferon-β
TTP: Thymidine triphosphate
VCAM-1: Soluble vascular cell adhesion molecule

WHO: World Health Organization
ZIP-4: SLC 39 a solute carrier.

Conflicts of Interest

The author declares no conflict of interest.

Acknowledgments

This study was supported by the National Institutes of Health, USA, and Labcatal Laboratories, Paris, France.

References

[1] J. Raulin, "Chemical studies on vegetation," *Annals of Science Nature*, vol. XI, pp. 93–99, 1869.

[2] A. L. Sommer and C. B. Lipman, "Evidence on the indispensable nature of zinc and boron for higher green plants," *Plant Physiology*, vol. 1, no. 3, pp. 231–249, 1926.

[3] W. R. Todd, C. A. Elvehjem, and E. B. Hart, "Zinc in the nutrition of the rat," *American Journal of Physiology*, vol. 107, no. 1, pp. 146–156, 1933.

[4] H. F. Tucker and W. D. Salmon, "Parakeratosis or zinc deficiency disease in the Pig," *Proceedings of the Society for Experimental Biology and Medicine*, vol. 88, no. 4, pp. 613–616, 1955.

[5] B. L. O'Dell, P. M. Newberne, and J. E. Savage, "Significance of dietary zinc for the growing chicken," *Journal of Nutrition*, vol. 65, no. 4, pp. 503–523, 1958.

[6] D. L. Blamberg, U. B. Blackwood, W. C. Supplee, and G. F. Combs, "Effect of zinc deficiency in hens on hatchability and embryonic development," *Proceedings of the Society for Experimental Biology and Medicine*, vol. 104, no. 2, pp. 217–220, 1960.

[7] A. S. Prasad, J. A. Halsted, and M. Nadimi, "Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia," *The American Journal of Medicine*, vol. 31, no. 4, pp. 532–546, 1961.

[8] A. S. Prasad, A. Miale Jr., Z. Farid, H. Sandstead, and A. Schulert, "Zinc metabolism in patients with the syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism, and hypognadism," *Journal of Laboratory and Clinical Medicine*, vol. 61, pp. 537–549, 1963.

[9] A. S. Prasad, A. Miale Jr., Z. Farid, H. H. Sandstead, A. R. Schulert, and W. J. Darby, "Biochemical studies on dwarfism, hypogonadism, and anemia," *Archives of Internal Medicine*, vol. 111, no. 4, p. 407, 1963.

[10] H. H. Sandstead, A. S. Prasad, A. R. Schulert et al., "Human zinc deficiency, endocrine manifestations and response to treatment," *American Journal of Clinical Nutrition*, vol. 20, no. 5, pp. 422–442, 1967.

[11] National Research Council (US), *Recommended dietary allowances*, National Academy Press (US), Washington (DC), third edition edition, 1974.

[12] World Health Organization and Department of Nutrition for Health Development, "WHO global database on child growth and malnutrition," March 2002, http://www.who.int/nutgrowthdb/.

[13] K. H. Brown, J. M. Peerson, J. Rivera, and L. H. Allen, "Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials," *American Journal of Nutrition*, vol. 75, no. 6, pp. 1062–1071, 2002.

[14] P. M. Barnes and E. J. Moynahan, "Zinc deficiency in acrodermatitis enteropathica: multiple dietary intolerance treated with synthetic diet," *Proceedings of the Royal Society of Medicine*, vol. 66, no. 4, pp. 327–329, 1973.

[15] S. P. Meftah, H. Kuivaniemi, G. Tromp et al., "A new mutation in exon 3 of the SCL39A4 gene in a Tunisian family with severe acrodermatitis enteropathica," *Nutrition*, vol. 22, no. 10, pp. 1067–1070, 2006.

[16] A. S. Prasad, "Clinical spectrum of human zinc deficiency," in *Biochemistry of Zinc*, pp. 219–224, Plenum, New York, 1993.

[17] A. S. Prasad, P. Rabbani, A. Abbasii, E. Bowersox, and M. R. Fox, "Experimental zinc deficiency in humans," *Annals of Internal Medicine*, vol. 89, no. 4, pp. 483–490, 1978.

[18] A. S. Prasad, S. Meftah, J. Abdallah et al., "Serum thymulin in human zinc deficiency," *Journal of Clinical Investigation*, vol. 82, no. 4, pp. 1202–1210, 1988.

[19] F. W. Beck, A. S. Prasad, J. Kaplan, J. T. Fitzgerald, and G. J. Brewer, "Changes in cytokine production and T cell subpopulations in experimentally induced zinc-deficient humans," *American Journal of Physiology-Endocrinology and Metabolism*, vol. 272, no. 6, pp. E1002–E1007, 1997.

[20] J. F. Bach, M. Dardenne, J. M. Pleau, and J. Rosa, "Biochemical characterisation of a serum thymic factor," *Nature*, vol. 266, no. 5597, pp. 55–57, 1977.

[21] L. Gastinel, M. Dardenne, J. Pleau, and J. Bach, "Studies on the zinc binding site to the serum thymic factor," *Biochimica et Biophysica Acta (BBA) - General Subjects*, vol. 797, no. 2, pp. 147–155, 1984.

[22] E. Tapazolglou, A. S. Prasad, H. Gretchen, G. J. Brewera, and J. Kaplana, "Decreased natural killer cell activity in patients with zinc deficiency with sickle cell disease," *Journal of Laboratory and Clinical Medicine*, vol. 105, pp. 19–22, 1985.

[23] P. J. Fraker, M. E. Gershwin, R. A. Good, and A. Prasad, "Interrelationships between zinc and immune function," *Federation Proceedings*, vol. 45, no. 5, pp. 1474–1479, 1986.

[24] T. Iwata, G. S. Incefy, T. Tanaka et al., "Circulating thymic hormone levels in zinc deficiency," *Cellular Immunology*, vol. 47, no. 1, pp. 100–105, 1979.

[25] A. S. Prasad, "Zinc and enzymes," in *Biochemistry of Zinc*, pp. 17–53, 1993.

[26] F. Fernandez-Madrid, A. S. Prasad, and D. Oberleas, "Effect of zinc deficiency on nucleic acids, collagen, and noncollagenous protein of the connective tissue," *Journal of Laboratory and Clinical Medicine*, vol. 82, no. 6, pp. 951–961, 1973.

[27] A. S. Prasad and D. Oberleas, "Thymidine kinase activity and incorporation of thymidine into DNA in zinc-deficient tissue," *Journal of Laboratory and Clinical Medicine*, vol. 83, no. 4, pp. 634–639, 1974.

[28] A. S. Prasad, F. Fernandez-Madrid, and J. R. Ryan, "Deoxythymidine kinase activity of human implanted sponge connective tissue in zinc deficiency," *American Journal of Physiology*, vol. 236, no. 3, pp. E272–E275, 1979.

[29] A. S. Prasad, F. W. J. Beck, L. Endre, W. Handschu, M. Kukuruga, and G. Kumar, "Zinc deficiency affects cell cycle and deoxythymidine kinase gene expression in HUT-78 cells," *Journal of Laboratory and Clinical Medicine*, vol. 128, no. 1, pp. 51–60, 1996.

[30] E. R. Giblett, A. J. Ammann, R. Sandman, D. W. Wara, and L. K. Diamond, "Nucleoside-phosphorylase deficiency in a

child with severely defective T-cell immunity and normal B-cell immunity," *The Lancet*, vol. 305, no. 7914, article S0140673675919509, pp. 1010–1013, 1975.

[31] A. S. Prasad and P. Rabbani, "Nucleoside phosphorylase in zinc deficiency," *Transactions of the Association of American Physicians*, vol. 94, pp. 314–321, 1981.

[32] S. Meftah and A. S. Prasad, "Nucleotides in lymphocytes of human subjects with zinc deficiency," *Journal of Laboratory and Clinical Medicine*, vol. 114, no. 2, pp. 114–119, 1989.

[33] W. A. Briggs, M. M. Pedersen, S. K. Mahajan, D. H. Sillix, A. S. Prasad, and F. D. McDonald, "Lymphocyte and granulocyte function in zinc-treated and zinc-deficient hemodialysis patients," *Kidney International*, vol. 21, no. 6, pp. 827–832, 1982.

[34] B. Bao, A. S. Prasad, F. W. J. Beck et al., "Zinc decreases C-reactive protein, lipid peroxidation, and inflammatory cytokines in elderly subjects: a potential implication of zinc as an atheroprotective agent," *The American Journal of Clinical Nutrition*, vol. 91, no. 6, pp. 1634–1641, 2010.

[35] A. S. Prasad, B. Bao, F. W. J. Beck, and F. H. Sarkar, "Zinc enhances the expression of interleukin-2 and interleukin-2 receptors in HUT-78 cells by way of NF-κB activation," *Journal of Laboratory and Clinical Medicine*, vol. 140, no. 4, pp. 272–289, 2002.

[36] A. S. Prasad, B. Bao, F. W. J. Beck, and F. H. Sarkar, "Zinc activates NF-κB in HUT-78 cells," *Journal of Laboratory and Clinical Medicine*, vol. 138, no. 4, pp. 250–256, 2001.

[37] B. Bao, A. S. Prasad, F. W. J. Beck et al., "Intracellular free zinc up-regulates IFN-γ and T-bet essential for Th_1 differentiation in Con-A stimulated HUT-78 cells," *Biochemical and Biophysical Research Communications*, vol. 407, no. 4, pp. 703–707, 2011.

[38] E. Rosenkranz, R. D. Hilgers, P. Uciechowski, A. Petersen, B. Plümäkers, and L. Rink, "Zinc enhances the number of regulatory T cells in allergen-stimulated cells from atopic subjects," *European Journal of Nutrition*, vol. 56, no. 2, pp. 557–567, 2017.

[39] S. Provoost, T. Maes, Y. M. van Durme et al., "Decreased FOXP3 protein expression in patients with asthma," *Allergy*, vol. 64, no. 10, pp. 1539–1546, 2009.

[40] L. Rink, *Zinc in Human Health*, IOS Press, Amsterdam, 2009.

[41] M. Richter, R. Bonneau, M. A. Girard, C. Beaulieu, and P. Larivée, "Zinc status modulates bronchopulmonary eosinophil infiltration in a murine model of allergic inflammation," *Chest*, vol. 123, no. 3, p. 446S, 2003.

[42] A. Barczyk, W. Pierzchala, G. Caramori et al., "Decreased percentage of $CD4^+Foxp3^+TGF\text{-}\beta^+$ and increased percentage of $CD4^+IL\text{-}17^+$ cells in bronchoalveolar lavage of asthmatics," *Journal of Inflammation*, vol. 11, no. 1, p. 22, 2014.

[43] J. K. Krishnaswamy, A. C. Jirmo, A. M. Baru et al., "Toll-like receptor-2 agonist-allergen coupling efficiently redirects Th2 cell responses and inhibits allergic airway eosinophilia," *American Journal of Respiratory Cell and Molecular Biology*, vol. 47, no. 6, pp. 852–863, 2012.

[44] N. Hayashi, T. Yoshimoto, K. Izuhara, K. Matsui, T. Tanaka, and K. Nakanishi, "T helper 1 cells stimulated with ovalbumin and IL-18 induce airway hyperresponsiveness and lung fibrosis by IFN-γ and IL-13 production," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 37, pp. 14765–14770, 2007.

[45] U. Nurmatov, B. I. Nwaru, G. Devereux, and A. Sheikh, "Confounding and effect modification in studies of diet and childhood asthma and allergies," *Allergy*, vol. 67, no. 8, pp. 1041–1059, 2012.

[46] A. S. Prasad, B. Bao, F. W. J. Beck, and F. H. Sarkar, "Zinc-suppressed inflammatory cytokines by induction of A20-mediated inhibition of nuclear factor-κB," *Nutrition*, vol. 27, no. 7-8, pp. 816–823, 2011.

[47] A. S. Prasad, B. Bao, F. W. J. Beck, O. Kucuk, and F. H. Sarkar, "Antioxidant effect of zinc in humans," *Free Radical Biology and Medicine*, vol. 37, no. 8, pp. 1182–1190, 2004.

[48] S. Meftah, A. S. Prasad, D. Y. Lee, and G. J. Brewer, "Ecto 5' nucleotidase (5'NT) as a sensitive indicator of human zinc deficiency," *Journal of Laboratory and Clinical Medicine*, vol. 118, no. 4, pp. 309–316, 1991.

[49] A. S. Prasad, B. Bao, F. W. J. Beck, and F. H. Sarkar, "Correction of interleukin-2 gene expression by *in vitro* zinc addition to mononuclear cells from zinc-deficient human subjects: A specific test for zinc deficiency in humans," *Translational Research*, vol. 148, no. 6, pp. 325–333, 2006.

Hindawi
Journal of Immunology Research
Volume 2019, Article ID 8396878, 11 pages
https://doi.org/10.1155/2019/8396878

Review Article

Contribution of Zinc and Zinc Transporters in the Pathogenesis of Inflammatory Bowel Diseases

Wakana Ohashi[1] **and Toshiyuki Fukada**[2]

[1]*Department of Molecular and Medical Pharmacology, Graduate School of Medicine and Pharmaceutical Sciences for Research, University of Toyama, Toyama, Japan*
[2]*Molecular and Cellular Physiology, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro, Tokushima, Japan*

Correspondence should be addressed to Wakana Ohashi; wohashi@med.u-toyama.ac.jp
and Toshiyuki Fukada; fukada@ph.bunri-u.ac.jp

Received 7 November 2018; Accepted 29 January 2019; Published 10 March 2019

Academic Editor: Patrice Petit

Intestinal epithelial cells cover the surface of the intestinal tract. The cells are important for preserving the integrity of the mucosal barriers to protect the host from luminal antigens and pathogens. The mucosal barriers are maintained by the continuous and rapid self-renewal of intestinal epithelial cells. Defects in the self-renewal of these cells are associated with gastrointestinal diseases, including inflammatory bowel diseases and diarrhea. Zinc is an essential trace element for living organisms, and zinc deficiency is closely linked to the impaired mucosal integrity. Recent evidence has shown that zinc transporters contribute to the barrier function of intestinal epithelial cells. In this review, we describe the recent advances in understanding the role of zinc and zinc transporters in the barrier function and homeostasis of intestinal epithelial cells.

1. Introduction

The gastrointestinal tract absorbs nutrients from digested foods and protects against luminal antigens and invading pathogens, including commensal bacteria and food. For this protective function, the intestines have developed a robust system of physical, chemical, and biological mucosal barriers. Defects in the mucosal barriers lead to the translocation of luminal antigens into the host, which induces host immune and inflammatory responses. These responses increase the susceptibility to various gastrointestinal diseases. Maintenance of the mucosal barrier system is therefore a critical issue in intestinal health [1]. The intestinal epithelial cells generate and maintain the mucosal barriers by continuous renewal [2]. Any impairment in the renewal cycle perturbs the mucosal barriers. Maintenance of the intestinal epithelial homeostasis is essential for preserving mucosal barrier functions.

Zinc is an essential trace element for all living organisms and is involved in a variety of important biological processes. It is a nonredox transition metal that serves as a catalytic cofactor and has structural functions in numerous proteins. Bioinformatics analyses have revealed that approximately 10% and 6% of the genes in the genomes of humans and bacteria, respectively, encode products with zinc-binding potential [3–5]. The functions of zinc-binding proteins are highly divergent and include transcription factors, DNA synthase, ubiquitin ligase, receptors, and kinases [3]. Zinc acts as a signaling molecule, such as second messengers, to mediate signaling pathways [6–9]. Zinc deficiency dysregulates cellular functions. Excess zinc is also toxic to cells. Thus, the level and distribution of zinc must be tightly fine-tuned. Zinc transporters regulate the distribution of zinc by controlling zinc influx and efflux via organelle membranes, thereby contributing to the maintenance of zinc homeostasis [10, 11]. Zinc transporters consist of two families: solute carrier (SLC) 39A and SLC30. The SLC39A/Zrt- and Irt-related protein (ZIP) family has 14 members and functions to transport zinc from the extracellular/organelle region into the cytosol. The SCL30A/Zn transporter (ZnT) is composed of 10

members and participates in exporting zinc from the cytosol. By regulating the flux of zinc, zinc transporters are involved in the regulation of various zinc-mediated biological functions. The physiological and pathological functions of zinc transporters have been explored through genetic approaches [10–12].

Several lines of evidence suggest that zinc deficiency causes diarrhea and mucosal barrier dysfunction, while zinc supplementation improves symptoms [13]. Thus, in the intestine, zinc is essential to maintain intestinal homeostasis and regulate intestinal disorder. In this review, we focus on the roles of zinc and zinc transporters in intestinal epithelial homeostasis and disorder.

2. Intestinal Epithelial Cell: A Critical Player in the Mucosal Barriers

The small intestine is composed of villi that protrude into the lumen and crypts that penetrate the mucosa. In contrast, the large intestines have no protruding villi, consisting only of crypts that allow water absorption. The surface of the intestinal mucosa is covered by a monolayer of intestinal epithelial cells. These cells consist of differentiated and undifferentiated cells [14]. Differentiated epithelial cells include absorptive enterocytes, mucin-producing goblet cells, and Paneth cells, which secrete antibacterial factors and constitute a niche for intestinal stem cells [15–19]. All intestinal epithelial linages are derived from intestinal stem cells. Intestinal stem cells that feature leucine-rich repeat-containing G-protein-coupled receptor 5 (Lgr5) as a marker protein constantly self-renew and produce daughter cells that are designated transit-amplifying (TA) cells [20]. TA cells undergo vigorous proliferation to increase the number of the cells, which differentiate into specialized epithelial lineages. Most differentiated epithelial cells migrate from the crypts to the tip of the villi as they differentiate. Intestinal epithelial cells reaching the tips of the villi undergo apoptosis. The renewal of intestinal epithelial cells takes 3-5 days in mice or 1 week in humans [21, 22].

Intestinal stem cells are cells that are capable of both self-renewal and multipotency. The number of the intestinal stem cells is dynamically regulated. In the steady state, the stem cell number is maintained at a certain level. During development or tissue repair, the stem cells actively self-renew, contributing to increased tissue size or repair [23]. Crypt base columnar (CBC) cells are distributed between Paneth cells at the crypt base and express Lgr5. *Lgr5* is the target gene of canonical Wingless-Int (Wnt) signaling, wherein Lgr5 functions as the receptor and mediates R-spondin signaling, thus potentiating Wnt signaling [24–26]. The $Lgr5^+$ CBC cells represent actively cycling stem cells that divide every 24 h [21]. The cells that reside at the +4 position immediately above the Paneth cells in the crypts also represent a stem cell population. The +4 cells dominantly express Hopx and display the slow cycling and DNA label-retention characteristics of quiescent stem cells. Recent studies suggest that they are a reverse stem cell population that can be rapidly recruited to maintain epithelial homeostasis following injury [27, 28]. Indeed, when $Lgr5^+$ stem cells are lost, $Hopx^+$ quiescent stem cells express Lgr5, leading to their active self-renewal, thereby compensating for the loss of $Lgr5^+$ stem cells. Furthermore, $Hopx^+$ quiescent stem cells and $Lgr5^+$ active cells can compensate for one another when the other cell type is depleted [27].

Goblet cells are a secretory lineage that has an important role in the generation of the mucus layer by secreting O-glycosylated mucin proteins [15]. Mucus gel layers on the intestinal surface present a physicochemical barrier that prevents the invasion of microorganisms [29]. The mucus layer is composed of an outer layer colonized by microorganisms, and a sterile dense inner layer firmly attached to the epithelial surface that contains antibacterial molecules [29]. The mucus layer also contains immunoglobulin A (IgA) produced by B cells in the lamina propria. Secreted IgA in the lumen has a role in neutralizing invading pathogens, antigen presentation to microfold cells (M cells) on the follicular-associated epithelium surface, and disturbance of the attachment of microorganisms on the epithelial surface [30]. In addition to the barrier function, the mucus layer also serves as a nutrient source for intestinal microbes [31]. Polymerized MUC2 in the inner layer is proteolytically degraded to generate the outer layer that is colonized by microorganisms. The mucus layer is highly glycosylated and can be utilized as an energy source by intestinal microbes during dietary fiber-depleted conditions. This leads to the erosion of the mucus barrier, which induces intestinal inflammation [31], implying that the nutritional state could influence the strength of the mucus barrier.

Paneth cells secrete various antibacterial molecules, such as α-defensins and RegIII-γ, and participate in innate immunity in the gastrointestinal tract [32, 33]. α-Defensins are cationic peptides consisting of 18-45 amino acid residues. Upon binding to the cellular surface of a microorganism, α-defensins form cationic pores in the membrane. Disruption of the cell membrane integrity leads to an efflux of nutrients or ions, which kills the bacteria [34]. The secretion of antimicrobial peptides depends on bacterial sensing [32]. Paneth cells express pathogen recognition receptors (PRRs), including Toll-like receptors and nucleotide oligomerization domain 2 (NOD2). Therefore, Paneth cells play a role in innate immunity by sensing bacteria and bacterial antigens. Paneth cells also store a large amount of zinc in their granules [35, 36]. Bacteria or bacterial components bind to PRRs, inducing the secretion of antibacterial molecules and zinc from Paneth cells [32, 33, 37, 38]. Additionally, Paneth cells also secrete maintenance factors, such as epidermal growth factors (EGFs) and Wnt3a, to form a niche for intestinal stem cells [39]. Thus, Paneth cells contribute to intestinal stem cell homeostasis and innate immunity by serving as biological barriers.

Cell junctions, which include the tight and adherens junction, firmly link intestinal epithelial cells, thereby forming a physical barrier that inhibits microbial invasion by the paracellular pathway. Pathogen-associated molecular pattern signaling regulates physical barrier functions. TLR2 signaling can enhance intestinal integrity by preserving the expression of zonula occludins-1 (ZO-1), a critical component of the tight junction in the intestinal epithelial

cells [40]. The NOD-like receptor family is a component of inflammasomes. In the intestines, molecules that include adenosine triphosphate (ATP), DNA, and RNA are secreted from injured cells. These secreted molecules act as danger signals that result in the activation of inflammasomes. It has been reported that NLRP3- or NLRP6-dependent secretion of interleukin- (IL-) 1β and IL-18 contributes to the maintenance of intestinal epithelial homeostasis by promoting the maturation and regeneration of intestinal epithelial cells [41, 42]. Thus, the innate immune signaling regulates both the physiological and biological barrier formations.

3. Intestinal Epithelial Cells during Intestinal Inflammation

Emerging evidence suggests that impaired integrity of the mucosal barrier is associated with the pathogenesis of intestinal inflammation [43, 44]. Tight junctions restrict the translocation of luminal materials into the host, thereby protecting the host from luminal antigens and pathogens. Defects in tight junctions (e.g., increased apoptosis of intestinal epithelial cells) cause focal leakages that allow water and small molecules to enter the mucosa, thus inducing a mucosal immunological response [45]. The dysfunction of cell junctions causes intestinal inflammation. Inflammatory bowel diseases (IBDs), which include ulcerative colitis (UC) and Crohn's disease (CD), involve the chronic inflammation of the gastrointestinal tract characterized by epithelial barrier dysfunctions and alterations in immune regulation. IBD patients display increased paracellular permeability with tight junction abnormalities. Moreover, decreased expressions of junctional molecules, such as occludins and claudins, have been found in patients with IBDs [46–48]. Mice lacking claudin-7, an important component of tight junctions, have enhanced paracellular organic solute flux and develop spontaneous colitis [49].

Dysfunctions of Paneth cells have been observed with various intestinal inflammatory diseases. Reduced secretion of α-defensin and other antimicrobial peptides was described in patients with CD [50]. In particular, α-defensin 5 is downregulated in these patients [51–53]. This might be related to the alteration in epigenetic modification status. The α-defensin 5 gene in patients with CD is highly methylated compared to the gene in healthy individuals [54]. Thus, epigenetic programming might be a causal factor in the pathogenesis of CD. NOD2 acts as an intracellular bacterial sensor by recognizing muramyl dipeptide (MDP), a structural component of bacteria. Mice deficient in NOD2 display decreased production of α-defensin in Paneth cells and are very susceptible to Listeria infections [37]. Adaptor protein- (AP-) 1B is a polarized sorting factor that mediates the polarized secretion of proteins by regulating intracellular protein sorting. The loss of AP-1B function results in the reduced expression of antimicrobial proteins and impaired IgA secretion, leading to the development of spontaneous chronic colitis by hypoepithelial barrier function [55]. Decreased expressions of AP-1M2 have also been observed in patients with CD [55].

Defective mucin is also involved in the pathogenesis of IBDs. Mucin2 (MUC2) expression is decreased in patients with IBDs [56]. Lack of MUC2 reportedly leads to the development of spontaneous colitis characterized by mucosal thickening and superficial erosions [57]. The same authors reported that mice lacking MUC2 showed severe damage against dextran sodium sulfate- (DSS-) induced experimental colitis.

The pathogenesis of IBDs is multifactorial and includes environmental and genetic factors. Regarding the genetic factors, recent genome-wide association studies (GWAS) have identified multiple genes associated with the risk of IBD [58–61]. Variations in the genes related to epithelial barrier functions, such as *NOD2* and *FUT2*, have been identified as genes associated with the pathogenesis of IBDs [59, 61]. Homeostasis of intestinal epithelial cells is maintained through continuous self-renewal, including rapid proliferation, differentiation, and apoptosis. Wnt/β-catenin and Notch signaling have essential roles for the intestinal epithelial turnover [62]. Defects in these processes also lead to the impairment of barrier functions and IBD pathogenesis. GWAS have identified Wnt signaling-related genes T cell factor 4 (*TCF4*) and lipoprotein receptor-related protein 6 (*LRP6*) as associated with the risk for IBD [63]. The functional variant in *LRP6* has been associated with early-onset ileal CD [64]. Furthermore, a recent study reported specific changes in the DNA methylation status in the intestinal epithelial cells that were associated with the development of IBD, suggesting that intestinal epithelial cell functions are epigenetically regulated along with the disease development [65].

4. Zinc in Intestinal Diseases

Zinc is an essential trace element in living organisms, including mammalians, bacteria, and plants. It has been estimated that approximately 10% of the human genome encode zinc-binding proteins [3]. Consequently, zinc-binding proteins constitute a large proportion of the total proteome [4]. The functions of these zinc-binding proteins are very diverse. Dysregulation of zinc homeostasis is associated with the pathogenesis of gastrointestinal diseases [13].

Zinc deficiency induces to diarrhea [13, 66, 67]. Reduced serum zinc levels or decreased zinc levels in the colorectal mucosa have been found in patients with persistent diarrhea. Zinc supplementation is effective in the prevention or improvement of diarrhea [67–69]. The World Health Organization has recommended zinc supplementation for the treatment of diarrhea as it reduces the duration and severity of symptoms associated with diarrhea and prevents subsequent episodes [70]. Zinc deficiency can also lead to an increased risk of gastrointestinal infectious diseases [71]. In pathogenic bacterial infections, the tissue zinc level was altered in the inflamed intestine. In a mouse model of Salmonella infection, the level of zinc was reduced in the inflamed gut [72]. Zinc also has a beneficial effect on infectious diseases like shigellosis. Malabsorption of nitrogen and the abnormal loss of mucus and transmucosal protein have been reported in patients with shigellosis.

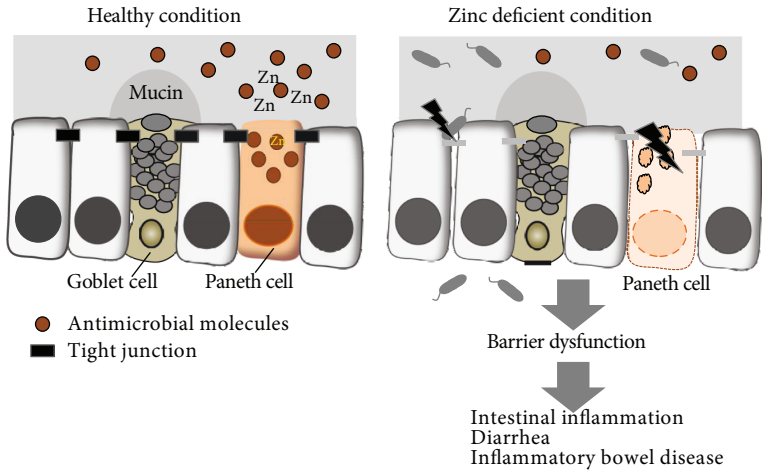


Figure 1: Zinc preserves mucosal barrier functions. In healthy conditions, mucosal barriers, including tight junctions, the mucus layer, and antimicrobial molecules, protect the host from luminal antigens/pathogens. In contrast, in zinc-deficient conditions, mucosal barriers are impaired. Thus, luminal contents can access the mucosal surface and translocate across the epithelial cells, leading to inflammation and intestinal diseases.

Zinc supplementation improves intestinal barrier function, intestinal permeability, nitrogen absorption, and symptoms and also increases immune responses [73–76].

Serum zinc levels in patients with CD are reduced compared to levels in healthy individuals [13, 77]. The absorption of zinc in patients with IBD is also lower compared to healthy controls. Although the relationship between low serum zinc levels and CD development is somewhat controversial, zinc supplementation appears to have beneficial effects on disease outcomes [78, 79]. A prospective study showed that the supplementation of zinc led to improved outcomes for patients with CD [80]. A cohort study of female IBD patients suggested that zinc intake can reduce the risk for CD [81]. The influence of zinc deficiency on colitis has also been examined in animal studies. For example, zinc deficiency exacerbates the severity of experimental colitis in rats [82, 83]. The severity of colitis has been correlated with serum zinc levels in mice [83]. Consistent with these reports, zinc treatment improved the severity of experimental colitis in mice [84–87]. The molecules that mediate the beneficial effect of zinc in colitis have been explored. GPR39 is a specific zinc receptor expressed on the plasma membrane [88]. Upon sensing extracellular zinc, GPR39 activates downstream signal pathways to regulate cellular functions, including proliferation, differentiation, and survival [89]. GPR39 sensing of extracellular zinc appears to affect the severity of colitis. Mice lacking GPR39 showed increased mortality after the induction of experimental colitis [90].

5. Zinc and Zinc Transporters in Intestinal Epithelial Cells

5.1. Mucosal Barriers. Disruption of the physical barrier formed by intestinal epithelial cells is associated with gastrointestinal diseases [45, 91]. Increased apoptosis of the intestinal epithelial cells causes weakened epithelial barrier tightness and induces focal leaks. Focal leaks lead to increased intestinal permeability and leak fluxes, followed by the activation of immunological responses in the mucosa. Decreased barriers lead to leaky gut properties. Emerging evidence has implicated zinc in the maintenance of the mucosal barriers (Figure 1) [85, 92]. Zinc deficiency leads to reduced expressions of occludin and ZO-1 proteins, resulting in decreased tight junctions in Caco-2 cells [93]. Depletion of zinc induces occludin-3 proteolysis and decreased claudin-3 transcription [94]. In contrast, in mice with bacterial infections, zinc supplementation protects the mice from intestinal dysfunction and intestinal leakage induced by bacterial toxins [95]. Zinc supplementation also enhances tight junctions in Caco-2 cells, characterized by an increase in transepithelial electrical resistance (TEER) and induced expressions of claudin-2, claudin-7, and ZO-1 proteins [96, 97]. Furthermore, zinc facilitates tight junction formation via GPR39 by preserving occludin and ZO-1 expressions in Caco2 and HT29 cells (Figure 2(a)) [98]. Zinc also activates the mammalian target of rapamycin pathway via GPR39 in HT29 cells [98]. Thus, the zinc-GPR39 axis seems to have a regulatory role in tight junction strength between intestinal epithelial cells. The ZIP14 zinc transporter is expressed on plasma membranes and mediates zinc influx into the cytosol, thereby regulating cellular signaling (Figure 2(a)). Mice lacking ZIP14 display increased intestinal permeability associated with altered expressions of tight junction proteins of claudin-1 and claudin-2 [99]. Thus, zinc transporter-mediated zinc signaling may affect the intestinal barrier functions. As described above, Paneth cells contain a large amount of zinc in their granules [35, 36]. Considering that Paneth cells are susceptible to zinc deficiency, zinc seems to contribute to the establishment of a biological barrier, since Paneth cells have a critical role in the production of antimicrobial molecules.

5.2. Intestinal Immunity. Intestinal macrophages are abundant in the small and large intestines. In particular, they are found in close proximity to the intestinal epithelial cells [100]. The major functions of intestinal macrophages are

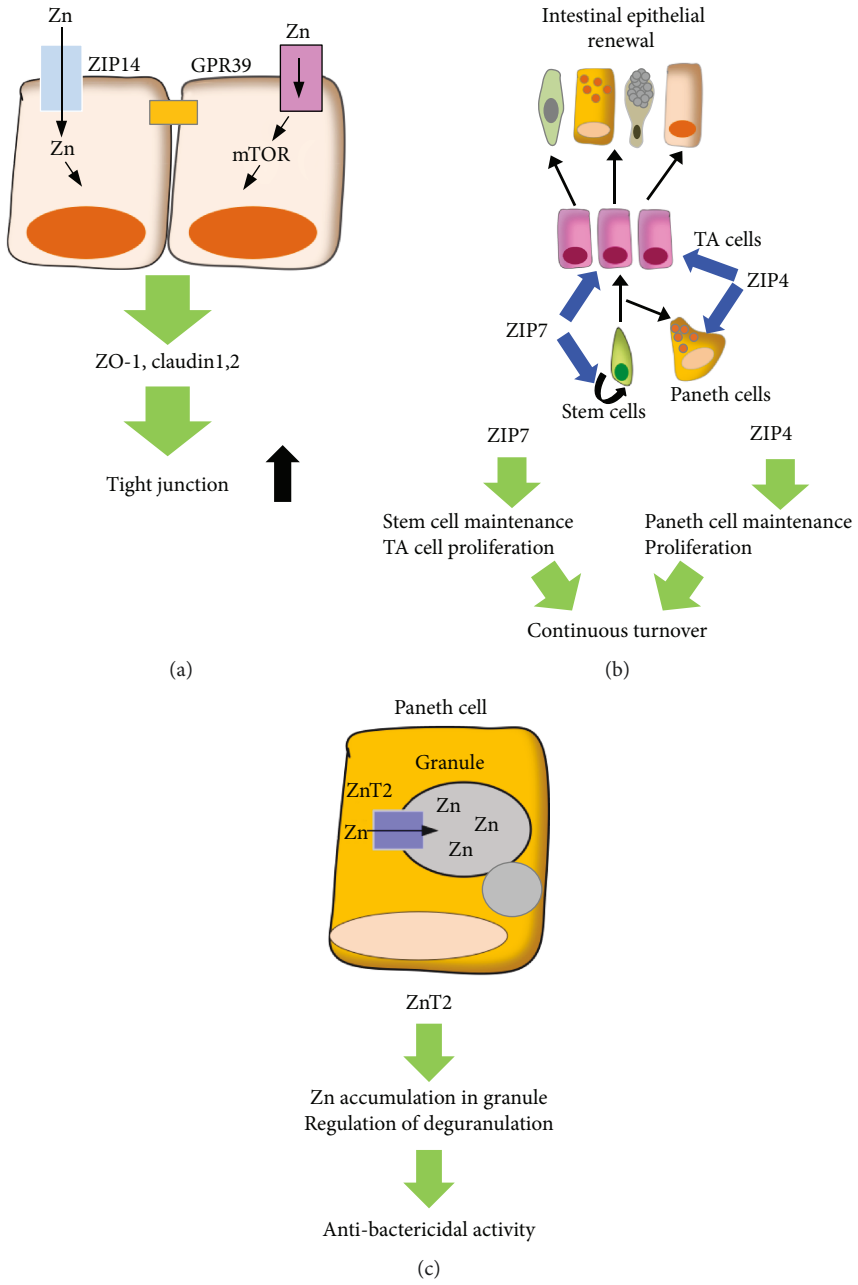


Figure 2: Zinc in intestinal epithelial cells ZIP14 and GPR39 mediates the establishment of the physical barrier through tight junctions (a). ZIP7 and ZIP4 contribute to the maintenance of intestinal homeostasis by securing continuous epithelial turnover (b). ZnT2 facilitates zinc accumulation into the secretory granule, contributing to the antimicrobial function of Paneth cells.

sensing, uptake, and clearance of microorganisms invading across the epithelial barrier. Intestinal macrophages thus play an important role in the maintenance of gut homeostasis by contributing to barrier functions. Compared to peritoneal macrophages, intestinal macrophages are highly bactericidal and show increased expressions of metallothionein 1 (MT-1) associated with increased intracellular zinc level [101]. The increased zinc level in intestinal macrophages is missing in mice treated with antibiotics. Furthermore, NOD2 ligand MDPs can induce the upregulation of MT-1 and intracellular zinc levels in intestinal macrophages [101]. Thus, zinc may have a role in regulating the bactericidal potential of intestinal macrophages, thereby mediating a part of symbiotic relationship in the intestine. Dysbiosis has been proposed as a key feature of IBD [102]. There is experimental evidence of the involvement of zinc in the microflora community, whereby excess dietary zinc alters the gut microbiota [103]. Mice fed with an excess zinc diet showed decreased microbial diversity without affecting the bacterial burden. Recently, the association of a variant of zinc transporter *ZIP8* with CD was reported [104]. The healthy *ZIP8* variant carrier showed altered gut microflora partially overlapping with those in patients with CD. Thus, disturbed zinc homeostasis might be associated with dysbiosis.

5.3. Intestinal Zinc Absorption. ZIP4 plays an important role in zinc absorption from the small intestine [105]. ZIP4 localized on the apical membrane of intestinal epithelial cells controls the incorporation of luminal zinc into the host by multiple mechanisms [105–108]. The dysfunction of ZIP4-mediated zinc absorption due to *ZIP4* gene mutations causes acrodermatitis enteropathica [109–111]. ZnT1 is detected at the basolateral membrane [112]. Expression of ZnT1 is affected by dietary zinc supplementation. With zinc supplementation, ZnT1 expression is increased in the rat intestine [112, 113]. Since ZnT1 belongs to the SLC30A/ZnT family that mediates zinc export from the cytosol into the extracellular or intracellular compartment, ZnT1 may also mediate zinc transport from the intestinal epithelial cells to the extracellular region (i.e., the blood stream). Thus, ZIP4 and ZnT1 appear to have important roles in the intake of zinc. ZIP5 is localized on the basolateral side of the intestinal epithelial cells and participates in zinc excretion [106, 114]. Mice lacking ZIP5 in intestinal epithelial cells accumulate zinc in the pancreas [115]. Interestingly, expressions of ZIP4 and ZIP5 are oppositely regulated by zinc. In the zinc-deficient condition, expression of ZIP4 is increased and ZIP5 expression is decreased [108]. On the contrary, the ZIP4 level is suppressed in the zinc-adequate condition, whereas ZIP5 expression is induced [108]. Additionally, ZnT5B, which is a splicing variant of ZnT5, has been reported to be localized to the apical membrane of the epithelium in the small intestine [116]. Since ZnT5B is capable of bidirectional transport of zinc [116, 117], ZnT5B appears to mediate zinc influx as well as efflux from the epithelial cells. Thus, zinc absorption and excretion are likely determined by the regulation of specific zinc transporters.

5.4. Intestinal Epithelial Homeostasis. Zinc deficiency leads to alterations in the integrity and function of the intestinal epithelial cells, demonstrating a role for zinc in intestinal epithelial homeostasis [118]. However, exactly how zinc carries out this role is unclear. Recent studies have indicated that zinc transporters contribute to the maintenance of intestinal epithelial homeostasis by regulating cellular function.

ZIP4 has a role in the regulation of intestinal epithelial function (Figure 2(b)). Mice lacking ZIP4 in the intestinal epithelial cells have decreased expressions of Sox9, a marker for Paneth cells; induction of mucin, a marker of goblet cells; and loss of zinc in Paneth cells. These findings suggest that ZIP4-mediated zinc incorporation is necessary for the differentiation and maintenance of Paneth cells [119]. Moreover, ZIP4 also contributes to the proliferation of intestinal epithelial cells [119]. Mice lacking ZIP4 display disrupted villus integrity. Thus, ZIP4 appears to be important in preserving intestinal epithelial architecture.

ZnT2 is the zinc transporter expressed on the secretory granules in Paneth cells [120]. ZnT2 promotes the accumulation of zinc in granules in the Paneth cells [120]. Furthermore, ZnT2 regulates the secretion of antimicrobial molecules in Paneth cells (Figure 2(c)) [120]. The authors described degranulated Paneth cells in ZnT2-deficient mice and the unchanged overall structure of the villi-crypt axis. The findings indicate that ZnT2 are not involved in the continuous self-renewal of intestinal epithelial cells, but rather in Paneth cell function [120].

ZIP7 is an endoplasmic reticulum- (ER-) localized zinc transporter that is highly expressed in the crypts, rather than the villi, in the intestine [121]. In the crypts, intestinal stem cells, TA cells, and Paneth cells express ZIP7. A recent study described the essential role for ZIP7 in the maintenance of intestinal epithelial homeostasis (Figure 2(b)) [121]. The authors described that deletion of ZIP7 in the intestinal epithelial cells in mice caused an acute loss of proliferating cells by apoptosis, with the disappearance of intestinal stem cells [121]. Furthermore, ZIP7 deficiency elevated ER stress in TA cells and stem cells [121]. The villus-crypt structure was completely disrupted in the ZIP7-deficient intestine. Additionally, degenerated Paneth cells were found in the ZIP7-deficient crypts, suggesting the essential role of ZIP7 in Paneth cell maintenance [121]. These findings indicate that ZIP7 is indispensable for the rapid turnover of intestinal epithelial cells by preserving the survival of intestinal stem cells, proliferation of crypts cells, and resolving ER stress. Notch, EGF, and Wnt signaling critically contributes to the maintenance of intestinal stem cells as well as proliferation of intestinal epithelial cells [122]. Recent studies have implicated ZIP7 in these signaling events in other cell types. ZIP7 is involved in the activation of extracellular signal-regulated kinases, which is major downstream molecule of EGF in breast cancer cells [123]. ZIP7 participates in the regulation of the trafficking of Notch in T cell acute lymphoblastic leukemia cells [124]. Accordingly, the treatment of ZIP7-binding compound led to the alteration of the zinc level in the ER, disturbed Notch trafficking, and disrupted apoptosis via an ER stress mechanism. Although the relationship between ZIP7 and these signaling events in intestinal epithelial cells remains to be elucidated, ZIP7 may contribute to the maintenance of crypt homeostasis by multiple mechanisms.

6. Conclusion

Zinc plays a critical role in the maintenance of intestinal homeostasis by regulating intestinal epithelial cells, host immune cells, and intestinal commensal bacteria. Additionally, recent studies in mice revealed that zinc transporters contribute to regulation of epithelial functions and to the maintenance of intestinal epithelial homeostasis. These findings clarify the self-renewal mechanisms of intestinal epithelial cells as well as the pathogenesis of intestinal diseases. Due to various differences in the features of zinc transporters between mice and humans, the roles of zinc transporters in intestinal epithelial homeostasis and intestinal disease pathogenesis in humans are still undetermined. Further investigations of zinc transporters in humans may advance the understanding of the pathogenesis of gastrointestinal disorders.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported by grants from the Japan Society for Science (17K09373 and 17H04011) and the Vehicle Racing Commemorative Foundation.

References

[1] L. W. Peterson and D. Artis, "Intestinal epithelial cells: regulators of barrier function and immune homeostasis," *Nature Reviews Cancer*, vol. 14, no. 3, pp. 141–153, 2014.

[2] L. G. van der Flier and H. Clevers, "Stem cells, self-renewal, and differentiation in the intestinal epithelium," *Annual Review of Physiology*, vol. 71, no. 1, pp. 241–260, 2009.

[3] C. Andreini, L. Banci, I. Bertini, and A. Rosato, "Counting the zinc-proteins encoded in the human genome," *Journal of Proteome Research*, vol. 5, no. 1, pp. 196–201, 2006.

[4] C. Andreini and I. Bertini, "A bioinformatics view of zinc enzymes," *Journal of Inorganic Biochemistry*, vol. 111, pp. 150–156, 2012.

[5] K. J. Waldron, J. C. Rutherford, D. Ford, and N. J. Robinson, "Metalloproteins and metal sensing," *Nature*, vol. 460, no. 7257, pp. 823–830, 2009.

[6] C. J. Frederickson, J.-Y. Koh, and A. I. Bush, "The neurobiology of zinc in health and disease," *Nature Reviews Neuroscience*, vol. 6, no. 6, pp. 449–462, 2005.

[7] M. Murakami and T. Hirano, "Intracellular zinc homeostasis and zinc signaling," *Cancer Science*, vol. 99, no. 8, pp. 1515–1522, 2008.

[8] T. Hirano, M. Murakami, T. Fukada, K. Nishida, S. Yamasaki, and T. Suzuki, "Roles of zinc and zinc signaling in immunity: zinc as an intracellular signaling molecule," *Advances in Immunology*, vol. 97, pp. 149–176, 2008.

[9] S. L. Sensi, P. Paoletti, A. I. Bush, and I. Sekler, "Zinc in the physiology and pathology of the CNS," *Nature Reviews Neuroscience*, vol. 10, no. 11, pp. 780–791, 2009.

[10] T. Kambe, T. Tsuji, A. Hashimoto, and N. Itsumura, "The physiological, biochemical, and molecular roles of zinc transporters in zinc homeostasis and metabolism," *Physiological Reviews*, vol. 95, no. 3, pp. 749–784, 2015.

[11] T. Hara, T.-A. Takeda, T. Takagishi, K. Fukue, T. Kambe, and T. Fukada, "Physiological roles of zinc transporters: molecular and genetic importance in zinc homeostasis," *The Journal of Physiological Sciences.*, vol. 67, no. 2, pp. 283–301, 2017.

[12] T. Fukada and T. Kambe, "Molecular and genetic features of zinc transporters in physiology and pathogenesis," *Metallomics*, vol. 3, no. 7, pp. 662–674, 2011.

[13] S. Skrovanek, K. DiGuilio, R. Bailey et al., "Zinc and gastrointestinal disease," *World Journal of Gastrointestinal Pathophysiology*, vol. 5, no. 4, pp. 496–513, 2014.

[14] J. H. van Es, A. Haegebarth, P. Kujala et al., "A critical role for the Wnt effector Tcf4 in adult intestinal homeostatic self-renewal," *Molecular and Cellular Biology*, vol. 32, no. 10, pp. 1918–1927, 2012.

[15] K. A. Knoop and R. D. Newberry, "Goblet cells: multifaceted players in immunity at mucosal surfaces," *Mucosal Immunology*, vol. 11, no. 6, pp. 1551–1557, 2018.

[16] J. J. Worthington, F. Reimann, and F. M. Gribble, "Enteroendocrine cells-sensory sentinels of the intestinal environment and orchestrators of mucosal immunity," *Mucosal Immunology*, vol. 11, no. 1, pp. 3–20, 2018.

[17] H. C. Clevers and C. L. Bevins, "Paneth cells: maestros of the small intestinal crypts," *Annual Review of Physiology*, vol. 75, no. 1, pp. 289–311, 2013.

[18] A. Sbarbati and F. Osculati, "A new fate for old cells: brush cells and related elements," *Journal of Anatomy*, vol. 206, no. 4, pp. 349–358, 2005.

[19] H. Miller, J. Zhang, R. Kuolee, G. B. Patel, and W. Chen, "Intestinal M cells: the fallible sentinels?," *World Journal of Gastroenterology*, vol. 13, no. 10, pp. 1477–1486, 2007.

[20] N. Barker, J. H. van Es, J. Kuipers et al., "Identification of stem cells in small intestine and colon by marker gene Lgr5," *Nature*, vol. 449, no. 7165, pp. 1003–1007, 2007.

[21] N. Barker, "Adult intestinal stem cells: critical drivers of epithelial homeostasis and regeneration," *Nature Reviews Molecular Cell Biology*, vol. 15, no. 1, pp. 19–33, 2013.

[22] A. S. Darwich, U. Aslam, D. M. Ashcroft, and A. Rostami-Hodjegan, "Meta-analysis of the turnover of intestinal epithelia in preclinical animal species and humans," *Drug Metabolism and Disposition*, vol. 42, no. 12, pp. 2016–2022, 2014.

[23] Ö. H. Yilmaz, P. Katajisto, D. W. Lamming et al., "mTORC1 in the Paneth cell niche couples intestinal stem-cell function to calorie intake," *Nature*, vol. 486, no. 7404, pp. 490–495, 2012.

[24] W. de Lau, N. Barker, T. Y. Low et al., "Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling," *Nature*, vol. 476, no. 7360, pp. 293–297, 2012.

[25] K. S. Carmon, Q. Lin, X. Gong, A. Thomas, and Q. Liu, "LGR5 interacts and cointernalizes with Wnt receptors to modulate Wnt/β-catenin signaling," *Molecular and Cellular Biology*, vol. 32, no. 11, pp. 2054–2064, 2012.

[26] A. Glinka, C. Dolde, N. Kirsch et al., "LGR4 and LGR5 are R-spondin receptors mediating Wnt/β-catenin and Wnt/PCP signalling," *EMBO reports*, vol. 12, no. 10, pp. 1055–1061, 2011.

[27] N. Takeda, R. Jain, M. R. LeBoeuf, Q. Wang, M. M. Lu, and J. A. Epstein, "Interconversion between intestinal stem cell populations in distinct niches," *Science*, vol. 334, no. 6061, pp. 1420–1424, 2011.

[28] H. Tian, B. Biehs, S. Warming et al., "A reserve stem cell population in small intestine renders *Lgr5*-positive cells dispensable," *Nature*, vol. 478, no. 7368, pp. 255–259, 2012.

[29] G. C. Hansson, "Role of mucus layers in gut infection and inflammation," *Current Opinion in Microbiology*, vol. 15, no. 1, pp. 57–62, 2011.

[30] C. Gutzeit, G. Magri, and A. Cerutti, "Intestinal IgA production and its role in host-microbe interaction," *Immunological Reviews*, vol. 260, no. 1, pp. 76–85, 2014.

[31] M. S. Desai, A. M. Seekatz, N. M. Koropatkin et al., "A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility," *Cell*, vol. 167, no. 5, pp. 1339–1353.e21, 2016.

[32] T. Ayabe, D. P. Satchell, C. L. Wilson, W. C. Parks, M. E. Selsted, and A. J. Ouellette, "Secretion of microbicidal alpha-defensins by intestinal Paneth cells in response to bacteria," *Nature Immunology*, vol. 1, no. 2, pp. 113–118, 2000.

[33] S. Vaishnava, M. Yamamoto, K. M. Severson et al., "The antibacterial lectin RegIIIgamma promotes the spatial segregation of microbiota and host in the intestine," *Science*, vol. 334, no. 6053, pp. 255–258, 2011.

[34] S. Mukherjee and L. V. Hooper, "Antimicrobial defense of the intestine," *Immunity*, vol. 42, no. 1, pp. 28–39, 2015.

[35] D. Dinsdale, "Ultrastructural localization of zinc and calcium within the granules of rat Paneth cells," *Journal of Histochemistry & Cytochemistry*, vol. 32, no. 2, pp. 139–145, 1984.

[36] E. M. Porter, C. L. Bevins, D. Ghosh, and T. Ganz, "The multifaceted Paneth cell," *Cellular and Molecular Life Sciences CMLS*, vol. 59, no. 1, pp. 156–170, 2002.

[37] K. S. Kobayashi, M. Chamaillard, Y. Ogura et al., "Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract," *Science*, vol. 307, no. 5710, pp. 731–734, 2005.

[38] L. J. Giblin, C. J. Chang, A. F. Bentley, C. Frederickson, S. J. Lippard, and C. J. Frederickson, "Zinc-secreting Paneth cells studied by ZP fluorescence," *Journal of Histochemistry & Cytochemistry*, vol. 54, no. 3, pp. 311–316, 2006.

[39] T. Sato, J. H. van Es, H. J. Snippert et al., "Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts," *Nature*, vol. 469, no. 7330, pp. 415–418, 2011.

[40] E. Cario, G. Gerken, and D. K. Podolsky, "Toll-like receptor 2 enhances ZO-1-associated intestinal epithelial barrier integrity via protein kinase C," *Gastroenterology*, vol. 127, no. 1, pp. 224–238, 2004.

[41] M. H. Zaki, K. L. Boyd, P. Vogel, M. B. Kastan, M. Lamkanfi, and T.-D. Kanneganti, "The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis," *Immunity*, vol. 32, no. 3, pp. 379–391, 2010.

[42] S. Normand, A. Delanoye-Crespin, A. Bressenot et al., "Nod-like receptor pyrin domain-containing protein 6 (NLRP6) controls epithelial self-renewal and colorectal carcinogenesis upon injury," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 23, pp. 9601–9606, 2011.

[43] J. R. Turner, "Intestinal mucosal barrier function in health and disease," *Nature Reviews Immunology*, vol. 9, no. 11, pp. 799–809, 2009.

[44] K. L. Edelblum and J. R. Turner, "The tight junction in inflammatory disease: communication breakdown," *Current Opinion in Pharmacology*, vol. 9, no. 6, pp. 715–720, 2009.

[45] T. Suzuki, "Regulation of intestinal epithelial permeability by tight junctions," *Cellular and Molecular Life Sciences*, vol. 70, no. 4, pp. 631–659, 2013.

[46] S. Zeissig, N. Bürgel, D. Günzel et al., "Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease," *Gut*, vol. 56, no. 1, pp. 61–72, 2007.

[47] S. Vetrano, M. Rescigno, M. Rosaria Cera et al., "Unique role of junctional adhesion molecule-A in maintaining mucosal homeostasis in inflammatory bowel disease," *Gastroenterology*, vol. 135, no. 1, pp. 173–184, 2008.

[48] A. Michielan and R. D'Incà, "Intestinal permeability in inflammatory bowel disease: pathogenesis, clinical evaluation, and therapy of leaky gut," *Mediators of Inflammation*, vol. 2015, Article ID 628157, 10 pages, 2015.

[49] H. Tanaka, M. Takechi, H. Kiyonari, G. Shioi, A. Tamura, and S. Tsukita, "Intestinal deletion of claudin-7 enhances paracellular organic solute flux and initiates colonic inflammation in mice," *Gut*, vol. 64, no. 10, pp. 1529–1538, 2015.

[50] S. Jäger, E. F. Stange, and J. Wehkamp, "Inflammatory bowel disease: an impaired barrier disease," *Langenbeck's Archives of Surgery*, vol. 398, no. 1, pp. 1–12, 2013.

[51] J. Wehkamp, M. Koslowski, G. Wang, and E. F. Stange, "Barrier dysfunction due to distinct defensin deficiencies in small intestinal and colonic Crohn's disease," *Mucosal Immunology*, vol. 1, no. 1, pp. S67–S74, 2008.

[52] J. Wehkamp, N. H. Salzman, E. Porter et al., "Reduced Paneth cell α-defensins in ileal Crohn's disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 50, pp. 18129–18134, 2005.

[53] L. A. Simms, J. D. Doecke, M. D. Walsh, N. Huang, E. V. Fowler, and G. L. Radford-Smith, "Reduced alpha-defensin expression is associated with inflammation and not NOD2 mutation status in ileal Crohn's disease," *Gut*, vol. 57, no. 7, pp. 903–910, 2008.

[54] E. Cerrillo, I. Moret, M. Iborra et al., "Alpha-defensins (α-Defs) in Crohn's disease: decrease of ileal α-Def 5 via permanent methylation and increase in plasma α-Def 1-3 concentrations offering biomarker utility," *Clinical and Experimental Immunology*, vol. 192, no. 1, pp. 120–128, 2018.

[55] D. Takahashi, K. Hase, S. Kimura et al., "The epithelia-specific membrane trafficking factor AP-1B controls gut immune homeostasis in mice," *Gastroenterology*, vol. 141, no. 2, pp. 621–632, 2011.

[56] D. Boltin, T. T. Perets, A. Vilkin, and Y. Niv, "Mucin function in inflammatory bowel disease: an update," *Journal of Clinical Gastroenterology*, vol. 47, no. 2, pp. 106–111, 2013.

[57] M. E. V. Johansson, M. Phillipson, J. Petersson, A. Velcich, L. Holm, and G. C. Hansson, "The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 39, pp. 15064–15069, 2008.

[58] C. A. Anderson, G. Boucher, C. W. Lees et al., "Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47," *Nature Genetics*, vol. 43, no. 3, pp. 246–252, 2011.

[59] A. Franke, D. P. B. McGovern, J. C. Barrett et al., "Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci," *Nature Genetics*, vol. 42, no. 12, pp. 1118–1125, 2010.

[60] L. Jostins, S. Ripke, K. R. Weersma et al., "Host–microbe interactions have shaped the genetic architecture of inflammatory bowel disease," *Nature*, vol. 491, no. 7422, pp. 119–124, 2012.

[61] J. Z. Liu, S. van Sommeren, H. Huang et al., "Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations," *Nature Genetics*, vol. 47, no. 9, pp. 979–986, 2015.

[62] H. Clevers and R. Nusse, "Wnt/β-catenin signaling and disease," *Cell*, vol. 149, no. 6, pp. 1192–1205, 2012.

[63] B. Khor, A. Gardet, and R. J. Xavier, "Genetics and pathogenesis of inflammatory bowel disease," *Nature*, vol. 474, no. 7351, pp. 307–317, 2011.

[64] M. J. Koslowski, Z. Teltschik, J. Beisner et al., "Association of a functional variant in the Wnt co-receptor *LRP6* with early onset ileal Crohn's disease," *PLoS Genetics*, vol. 8, no. 2, article e1002523, 2012.

[65] K. J. Howell, J. Kraiczy, K. M. Nayak et al., "DNA methylation and transcription patterns in intestinal epithelial cells from pediatric patients with inflammatory bowel diseases differentiate disease subtypes and associate with outcome," *Gastroenterology*, vol. 154, no. 3, pp. 585–598, 2018.

[66] C. E. Semrad, "Zinc and intestinal function," *Current Gastroenterology Reports*, vol. 1, no. 5, pp. 398–403, 1999.

[67] A. S. Prasad, "Discovery of human zinc deficiency: its impact on human health and disease," *Advances in Nutrition*, vol. 4, no. 2, pp. 176–190, 2013.

[68] P. J. Winch, K. E. Gilroy, S. Doumbia et al., "Operational issues and trends associated with the pilot introduction of zinc for childhood diarrhoea in Bougouni district, Mali," *Journal of Health, Population and Nutrition*, vol. 26, no. 2, pp. 151–162, 2008.

[69] M. Lazzerini and H. Wanzira, "Oral zinc for treating diarrhoea in children," *Cochrane Systematic Review*, vol. 12, no. 1, article CD005436, 2016.

[70] C. L. F. Walker and R. E. Black, "Zinc for the treatment of diarrhoea: effect on diarrhoea morbidity, mortality and incidence of future episodes," *International Journal of Epidemiology*, vol. 39, Supplement 1, pp. i63–i69, 2010.

[71] A. H. Shankar and A. S. PRASAD, "Zinc and immune function: the biological basis of altered resistance to infection," *The American Journal of Clinical Nutrition*, vol. 68, no. 2, Supplement 2, pp. 447S–463S, 1998.

[72] J. Z. Liu, S. Jellbauer, A. J. Poe et al., "Zinc sequestration by the neutrophil protein calprotectin enhances *Salmonella* growth in the inflamed gut," *Cell Host & Microbe.*, vol. 11, no. 3, pp. 227–239, 2012.

[73] A. N. Alam, S. A. Sarker, M. A. Wahed, M. Khatun, and M. M. Rahaman, "Enteric protein loss and intestinal permeability changes in children during acute shigellosis and after recovery: effect of zinc supplementation," *Gut*, vol. 35, no. 12, pp. 1707–1711, 1994.

[74] S. K. Roy, R. Raqib, W. Khatun et al., "Zinc supplementation in the management of shigellosis in malnourished children in Bangladesh," *European Journal of Clinical Nutrition*, vol. 62, no. 7, pp. 849–855, 2008.

[75] R. Raqib, S. K. Roy, M. J. Rahman et al., "Effect of zinc supplementation on immune and inflammatory responses in pediatric patients with shigellosis," *The American Journal of Clinical Nutrition*, vol. 79, no. 3, pp. 444–450, 2004.

[76] M. J. Rahman, P. Sarker, S. K. Roy et al., "Effects of zinc supplementation as adjunct therapy on the systemic immune responses in shigellosis," *The American Journal of Clinical Nutrition*, vol. 81, no. 2, pp. 495–502, 2005.

[77] S. K. Sikora, D. Spady, C. Prosser, and W. El-Matary, "Trace elements and vitamins at diagnosis in pediatric-onset inflammatory bowel disease," *Clinical Pediatrics*, vol. 50, no. 6, pp. 488–492, 2011.

[78] D. Owczarek, "Diet and nutritional factors in inflammatory bowel diseases," *World Journal of Gastroenterology*, vol. 22, no. 3, pp. 895–905, 2016.

[79] G. C. Sturniolo, V. Di Leo, A. Ferronato, A. D'Odorico, and R. D'Incà, "Zinc supplementation tightens "leaky gut" in Crohn's disease," *Inflammatory Bowel Diseases*, vol. 7, no. 2, pp. 94–98, 2001.

[80] S. Siva, D. T. Rubin, G. Gulotta, K. Wroblewski, and J. Pekow, "Zinc deficiency is associated with poor clinical outcomes in patients with inflammatory bowel disease," *Inflammatory Bowel Diseases*, vol. 23, no. 1, pp. 152–157, 2017.

[81] A. N. Ananthakrishnan, H. Khalili, M. Song, L. M. Higuchi, J. M. Richter, and A. T. Chan, "Zinc intake and risk of Crohn's disease and ulcerative colitis: a prospective cohort study," *International Journal of Epidemiology*, vol. 44, no. 6, pp. 1995–2005, 2015.

[82] H. Iwaya, M. Kashiwaya, A. Shinoki et al., "Marginal zinc deficiency exacerbates experimental colitis induced by dextran sulfate sodium in rats," *The Journal of Nutrition*, vol. 141, no. 6, pp. 1077–1082, 2011.

[83] E. Suwendi, H. Iwaya, J.-S. Lee, H. Hara, and S. Ishizuka, "Zinc deficiency induces dysregulation of cytokine productions in an experimental colitis of rats," *Biomedical Research*, vol. 33, no. 6, pp. 329–336, 2012.

[84] J. Li, H. Chen, B. Wang et al., "ZnO nanoparticles act as supportive therapy in DSS-induced ulcerative colitis in mice by maintaining gut homeostasis and activating Nrf2 signaling," *Scientific Reports*, vol. 7, article 43126, no. 1, 2017.

[85] C. D. Tran, J. M. Ball, S. Sundar, P. Coyle, and G. S. Howarth, "The role of zinc and metallothionein in the dextran sulfate sodium-induced colitis mouse model," *Digestive Diseases and Sciences*, vol. 52, no. 9, pp. 2113–2121, 2007.

[86] G. C. Sturniolo, W. Fries, E. Mazzon, V. Di Leo, M. Barollo, and R. D'Incà, "Effect of zinc supplementation on intestinal permeability in experimental colitis," *The Journal of Laboratory and Clinical Medicine*, vol. 139, no. 5, pp. 311–315, 2002.

[87] J.-S. Park, J. Choi, J. Y. Kwon et al., "A probiotic complex, rosavin, zinc, and prebiotics ameliorate intestinal inflammation in an acute colitis mouse model," *Journal of Translational Medicine*, vol. 16, no. 1, p. 37, 2018.

[88] P. Popovics and A. J. Stewart, "GPR39: a Zn^{2+}-activated G protein-coupled receptor that regulates pancreatic, gastrointestinal and neuronal functions," *Cellular and Molecular Life Sciences*, vol. 68, no. 1, pp. 85–95, 2011.

[89] M. Hershfinkel, "The zinc sensing receptor, ZnR/GPR39, in health and disease," *International Journal of Molecular Sciences*, vol. 19, no. 2, p. 439, 2018.

[90] L. Sunuwar, M. Medini, L. Cohen, I. Sekler, and M. Hershfinkel, "The zinc sensing receptor, ZnR/GPR39, triggers metabotropic calcium signalling in colonocytes and regulates occludin recovery in experimental colitis," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 371, no. 1700, 2016.

[91] N. A. Hering, M. Fromm, and J.-D. Schulzke, "Determinants of colonic barrier function in inflammatory bowel disease and potential therapeutics," *Journal of Physiology*, vol. 590, no. 5, pp. 1035–1044, 2012.

[92] B. Zhang and Y. Guo, "Supplemental zinc reduced intestinal permeability by enhancing occludin and zonula occludens protein-1 (ZO-1) expression in weaning piglets," *British Journal of Nutrition*, vol. 102, no. 5, pp. 687–693, 2009.

[93] A. Finamore, M. Massimi, L. Conti Devirgiliis, and E. Mengheri, "Zinc deficiency induces membrane barrier damage and increases neutrophil transmigration in Caco-2 cells," *The Journal of Nutrition*, vol. 138, no. 9, pp. 1664–1670, 2008.

[94] Y. Miyoshi, S. Tanabe, and T. Suzuki, "Cellular zinc is required for intestinal epithelial barrier maintenance via the regulation of claudin-3 and occludin expression," *American Journal of Physiology-Gastrointestinal and Liver Physiology*, vol. 311, no. 1, pp. G105–G116, 2016.

[95] S. Wiegand, S. S. Zakrzewski, M. Eichner et al., "Zinc treatment is efficient against *Escherichia coli* α-haemolysin-induced intestinal leakage in mice," *Scientific Reports*, vol. 7, article 45649, no. 1, 2017.

[96] X. Wang, M. C. Valenzano, J. M. Mercado, E. P. Zurbach, and J. M. Mullin, "Zinc supplementation modifies tight junctions and alters barrier function of CACO-2 human intestinal epithelial layers," *Digestive Diseases and Sciences*, vol. 58, no. 1, pp. 77–87, 2013.

[97] Y. Shao, P. G. Wolf, S. Guo, Y. Guo, H. R. Gaskins, and B. Zhang, "Zinc enhances intestinal epithelial barrier function through the PI3K/AKT/mTOR signaling pathway in Caco-2 cells," *The Journal of Nutritional Biochemistry*, vol. 43, pp. 18–26, 2017.

[98] L. Cohen, I. Sekler, and M. Hershfinkel, "The zinc sensing receptor, ZnR/GPR39, controls proliferation and differentiation of colonocytes and thereby tight junction formation in the colon," *Cell Death and Differentiation*, vol. 5, no. 6, pp. e1307–e1312, 2014.

[99] G. J. Guthrie, T. B. Aydemir, C. Troche, A. B. Martin, S.-M. Chang, and R. J. Cousins, "Influence of ZIP14 (slc39A14) on intestinal zinc processing and barrier function," *American Journal of Physiology-Gastrointestinal and Liver Physiology*, vol. 308, no. 3, pp. G171–G178, 2015.

[100] T. Joeris, K. Müller-Luda, W. W. Agace, and A. M. Mowat, "Diversity and functions of intestinal mononuclear phagocytes," *Mucosal Immunology*, vol. 10, no. 4, pp. 845–864, 2017.

[101] A. Lahiri and C. Abraham, "Activation of pattern recognition receptors up-regulates metallothioneins, thereby increasing intracellular accumulation of zinc, autophagy, and bacterial clearance by macrophages," *Gastroenterology*, vol. 147, no. 4, pp. 835–846, 2014.

[102] C. Casén, H. C. Vebø, M. Sekelja et al., "Deviations in human gut microbiota: a novel diagnostic test for determining dysbiosis in patients with IBS or IBD," *Alimentary Pharmacology & Therapeutics*, vol. 42, no. 1, pp. 71–83, 2015.

[103] J. P. Zackular, J. L. Moore, A. T. Jordan et al., "Dietary zinc alters the microbiota and decreases resistance to Clostridium difficile infection," *Nature Medicine*, vol. 22, no. 11, pp. 1330–1334, 2016.

[104] D. Li, J.-P. Achkar, T. Haritunians et al., "A pleiotropic missense variant in SLC39A8 is associated with Crohn's disease and human gut microbiome composition," *Gastroenterology*, vol. 151, no. 4, pp. 724–732, 2016.

[105] J. Dufner-Beattie, F. Wang, Y. M. Kuo, J. Gitschier, D. Eide, and G. K. Andrews, "The acrodermatitis enteropathica gene ZIP4 encodes a tissue-specific, zinc-regulated zinc transporter in mice," *The Journal of Biological Chemistry*, vol. 278, no. 35, pp. 33474–33481, 2003.

[106] J. Dufner-Beattie, Y.-M. Kuo, J. Gitschier, and G. K. Andrews, "The adaptive response to dietary zinc in mice involves the differential cellular localization and zinc regulation of the zinc transporters ZIP4 and ZIP5," *The Journal of Biological Chemistry*, vol. 279, no. 47, pp. 49082–49090, 2004.

[107] T. Kambe and G. K. Andrews, "Novel proteolytic processing of the ectodomain of the zinc transporter ZIP4 (SLC39A4) during zinc deficiency is inhibited by acrodermatitis enteropathica mutations," *Molecular and Cellular Biology*, vol. 29, no. 1, pp. 129–139, 2009.

[108] B. P. Weaver, J. Dufner-Beattie, T. Kambe, and G. K. Andrews, "Novel zinc-responsive post-transcriptional mechanisms reciprocally regulate expression of the mouse Slc39a4 and Slc39a5 zinc transporters (Zip4 and Zip5)," *Biological Chemistry*, vol. 388, no. 12, pp. 1301–1312, 2007.

[109] K. Wang, B. Zhou, Y.-M. Kuo, J. Zemansky, and J. Gitschier, "A novel member of a zinc transporter family is defective in acrodermatitis enteropathica," *American Journal of Human Genetics*, vol. 71, no. 1, pp. 66–73, 2002.

[110] S. Schmitt, S. Küry, M. Giraud, B. Dréno, M. Kharfi, and S. Bézieau, "An update on mutations of the *SLC39A4* gene in acrodermatitis enteropathica," *Human Mutation*, vol. 30, no. 6, pp. 926–933, 2009.

[111] S. Küry, B. Dréno, S. Bézieau et al., "Identification of SLC39A4, a gene involved in acrodermatitis enteropathica," *Nature Genetics.*, vol. 31, no. 3, pp. 239-240, 2002.

[112] R. J. McMahon and R. J. Cousins, "Regulation of the zinc transporter ZnT-1 by dietary zinc," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 9, pp. 4841–4846, 1998.

[113] J. P. Liuzzi, R. K. Blanchard, and R. J. Cousins, "Differential regulation of zinc transporter 1, 2, and 4 mRNA expression by dietary zinc in rats," *The Journal of Nutrition*, vol. 131, no. 1, pp. 46–52, 2001.

[114] Z. L. Huang, J. Dufner-Beattie, and G. K. Andrews, "Expression and regulation of SLC39A family zinc transporters in the developing mouse intestine," *Developmental Biology*, vol. 295, no. 2, pp. 571–579, 2006.

[115] J. Geiser, R. C. De Lisle, and G. K. Andrews, "The zinc transporter *Zip5 (Slc39a5)* regulates intestinal zinc excretion and protects the pancreas against zinc toxicity," *PLoS One*, vol. 8, no. 11, article e82149, 2013.

[116] K. A. Jackson, R. M. Helston, J. A. McKay, E. D. O'Neill, J. C. Mathers, and D. Ford, "Splice variants of the human zinc transporter ZnT5 (*SLC30A5*) are differentially localized and regulated by zinc through transcription and mRNA stability," *Journal of Biological Chemistry*, vol. 282, no. 14, pp. 10423–10431, 2007.

[117] R. A. Valentine, K. A. Jackson, G. R. Christie, J. C. Mathers, P. M. Taylor, and D. Ford, "ZnT5 variant B is a bidirectional zinc transporter and mediates zinc uptake in human intestinal Caco-2 cells," *The Journal of Biological Chemistry*, vol. 282, no. 19, pp. 14389–14393, 2007.

[118] G. Vela, P. Stark, M. Socha, A. K. Sauer, S. Hagmeyer, and A. M. Grabrucker, "Zinc in gut-brain interaction in autism and neurological disorders," *Neural Plasticity*, vol. 2015, Article ID 972791, 15 pages, 2015.

[119] J. Geiser, K. J. T. Venken, R. C. De Lisle, and G. K. Andrews, "A mouse model of acrodermatitis enteropathica: loss of intestine zinc transporter ZIP4 (Slc39a4) disrupts the stem cell niche and intestine integrity," *PLoS Genetics*, vol. 8, no. 6, article e1002766, 2012.

[120] A. B. Podany, J. Wright, R. Lamendella, D. I. Soybel, and S. L. Kelleher, "ZnT2-mediated zinc import into Paneth cell granules is necessary for coordinated secretion and Paneth cell function in mice," *Cellular and Molecular Gastroenterology and Hepatology*, vol. 2, no. 3, pp. 369–383, 2016.

[121] W. Ohashi, S. Kimura, T. Iwanaga et al., "Zinc transporter SLC39A7/ZIP7 promotes intestinal epithelial self-renewal by resolving ER stress," *PLoS Genetics*, vol. 12, no. 10, article e1006349, 2016.

[122] H. Gehart and H. Clevers, "Tales from the crypt: new insights into intestinal stem cells," *Nature Reviews Gastroenterology & Hepatology*, vol. 16, no. 1, pp. 19–34, 2018.

[123] K. M. Taylor, S. Hiscox, R. I. Nicholson, C. Hogstrand, and P. Kille, "Protein kinase CK2 triggers cytosolic zinc signaling pathways by phosphorylation of zinc channel ZIP7," *Science Signaling*, vol. 5, no. 210, article ra11, 2012.

[124] E. Nolin, S. Gans, L. Llamas et al., "Discovery of a ZIP7 inhibitor from a Notch pathway screen," *Nature Chemical Biology*, vol. 15, no. 2, pp. 179–188, 2019.

Hindawi
Journal of Immunology Research
Volume 2018, Article ID 5404093, 11 pages
https://doi.org/10.1155/2018/5404093

Review Article

Zinc in Keratinocytes and Langerhans Cells: Relevance to the Epidermal Homeostasis

Youichi Ogawa, Manao Kinoshita, Shinji Shimada, and Tatsuyoshi Kawamura

Department of Dermatology, Faculty of Medicine, University of Yamanashi, Yamanashi 409-3898, Japan

Correspondence should be addressed to Youichi Ogawa; yogawa@yamanashi.ac.jp

Received 24 September 2018; Accepted 10 November 2018; Published 9 December 2018

Guest Editor: Toshiyuki Fukada

In the skin, the epidermis is continuously exposed to various kinds of external substances and stimuli. Therefore, epidermal barriers are crucial for providing protection, safeguarding health, and regulating water balance by maintaining skin homeostasis. Disruption of the epidermal barrier allows external substances and stimuli to invade or stimulate the epidermal cells, leading to the elicitation of skin inflammation. The major components of the epidermal barrier are the stratum corneum (SC) and tight junctions (TJs). The presence of zinc in the epidermis promotes epidermal homeostasis; hence, this study reviewed the role of zinc in the formation and function of the SC and TJs. Langerhans cells (LCs) are one of the antigen-presenting cells found in the epidermis. They form TJs with adjacent keratinocytes (KCs), capture external antigens, and induce antigen-specific immune reactions. Thus, the function of zinc in LCs was examined in this review. We also summarized the general knowledge of zinc and zinc transporters in the epidermis with updated findings.

1. Introduction

The epidermis is the outermost layer of the skin and is thus continuously exposed to various kinds of external substances and stimuli that can lead to potential harm. To counteract these risks and maintain homeostasis, the epidermis provides a barrier against the external environment. The importance of preserving epidermal homeostasis is evidenced by a reduction in the development of atopic dermatitis (AD) when moisturizer is applied to the skin during the neonatal period [1, 2]. Reports also suggest that skin barrier disruption leads not only to the development of AD but also to other allergic diseases such as asthma, food allergies, and allergic rhinitis [3, 4].

The epidermis is composed predominantly of keratinocytes (KCs) plus a small number of Langerhans cells (LCs), melanocytes, and epidermal-resident memory T cells, as well as others. The murine epidermis also contains unique dendritic epidermal T cells, a type of $\gamma\delta$T cells. The epidermal KCs are categorized into four layers, namely, the stratum basale, stratum spinosum, stratum granulosum (SG), and stratum corneum (SC). A functional epidermal barrier depends on the existence of the SC and tight junctions (TJs) formed in the SG (Figure 1) [5, 6]. LCs can link with KCs to form TJs (Figure 1) [7, 8].

The human body contains 2–3 g of zinc (Zn), with approximately 5% of the total Zn found in the skin [9]. The concentration of Zn is higher in the epidermis than in the dermis and subcutaneous tissue, which may be due to the Zn requirement for active proliferation and differentiation of KCs [10]. Zn facilitates over 1000 enzymatic reactions and is indispensable for over 2000 transcriptional activities [11–13]. Zn finger proteins are involved in various physiological reactions [14–16]. Moreover, approximately 10% of human proteins bind to Zn [17]. Therefore, the dysregulation of epidermal Zn levels due to nutritional deficiency or genetic abnormalities of Zn transporters affects various enzymatic reactions, transcriptional activities, and Zn finger protein functions in the epidermis, leading to the disruption of skin homeostasis [18–20]. This review is aimed at highlighting the association between Zn and epidermal barrier function to understand the importance of Zn in skin immunity. We also summarize the function of Zn and Zn transporters in the epidermis and epidermal cells.

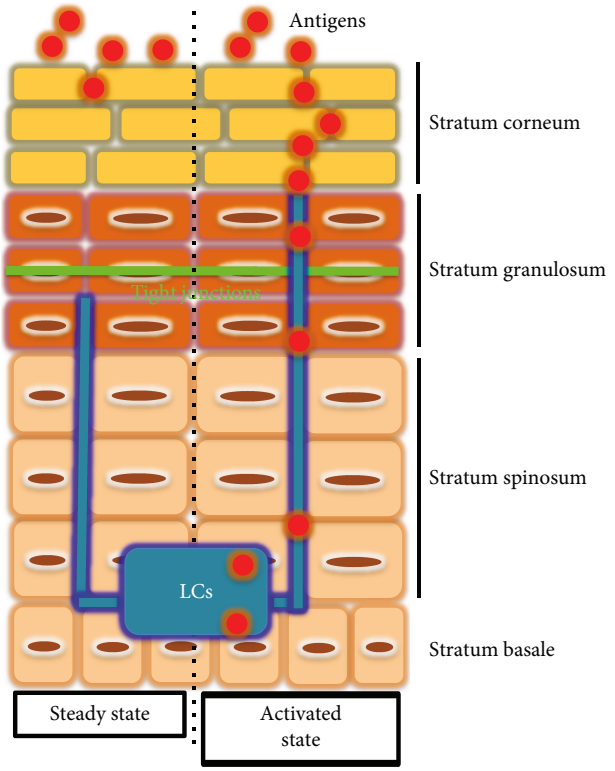


Figure 1: Structure of the epidermis. The epidermal KCs are categorized into four layers, namely, the stratum basale, stratum spinosum, stratum granulosum (SG), and stratum corneum (SC). A functional epidermal barrier depends on the existence of the SC and tight junctions (TJs) formed in the SG. LCs can link with KCs to form TJs. In steady state (left), LC dendrites lie beneath the TJs. Upon activation (right), LCs elongate their dendrites to penetrate the KC TJs by forming tricellulin-dependent TJs between KC-LC-KC. The elongated dendrites are able to reach beneath the SC and take up Ag from the extra-TJ environment without destroying barrier integrity. The original figure was published in [7].

2. Zinc and the Epidermal Barrier

The epidermal barrier function depends largely on the presence of SC and TJs formed in the SG [4, 21]. In this respect, homeostasis of the epidermis depends on normal KC differentiation (keratinization). LCs, a type of tissue-resident macrophages, also form TJs with KCs through linkages with KCs [7]. Most of the skin is covered with stratified epithelia that have both SC and TJs. However, skin appendages such as hair follicles and sweat glands lack SC, so TJs are the sole barrier structure in the skin appendages. The sections below summarized the involvement of Zn in the formation and function of SC and TJs.

2.1. Stratum Corneum. The outer layer of the SC consists of a cornified layer made of flattened and denucleated KCs (or corneocytes) and a SC-specific barrier structure called the cornified envelope that replaces the KC cell membranes. Through the process of terminal differentiation, SG KCs produce two membrane-circumscribed granules, keratohyalin granules and lamellar bodies. The former contains "intracellular" components of the SC such as filaggrin (FLG), loricrin, and keratin filaments, whereas the latter contains "extracellular" components such as lipids, corneodesmosin, and kallikreins. All of these intracellular and extracellular proteins are crucial for the formation and/or function of the epidermal barrier [21].

2.1.1. Zinc and Filaggrin and Its Metabolism. Among the components of keratohyalin granules, FLG has a crucial role in maintaining normal epidermal barrier function. FLG-deficient mice show an impaired SC barrier function and develop spontaneous dermatitis [22, 23]. Studies have shown that loss-of-function mutations in the FLG gene are strongly associated with the development of AD and ichthyosis vulgaris [24, 25]. These mutations were shown to range from 25 to 50% in the Northern European and Asian populations with these ailments [25, 26]. Moreover, FLG gene mutations were demonstrated as the strongest risk factor for AD in the genome-wide association studies (GWAS) [27]. These indicate the critical involvement of FLG in AD pathogenesis mediated by the disruption of the epidermal barrier.

FLG is produced in the SG as profilaggrin (FLG polymer) and is stored in keratohyalin granules. At the transition to the SC, the polymer is processed to the monomer by proteases such as Prss8 and SASPase [28, 29] and then binds to keratin and forms the fundamental structure of the corneocytes. At the outermost layer of the SC, FLG is citrullinated by peptidylarginine deiminase and then dissociated from keratin filaments [30]. These dissociated FLG are degraded to free amino acids, including glutamine, arginine, and histidine. The FLG-derived histidine-rich proteins are converted into urocanic acid (UCA) and pyrrolidine carboxylic acid (PCA) by proteases. UCA absorbs ultraviolet, maintains the acidic pH, and suppresses excess LC activation [31, 32]. PCA is a source of natural moisturizing factors. As a result, FLG is indispensable to the framework of SC and its metabolites are important for maintaining the epidermal barrier function (Figure 2).

Zn is involved in the regulation of FLG expression as well as its metabolism. It facilitates FLG production by increasing the activity of Prss8 [33]. Alternatively, Zn can also suppress FLG metabolism by decreasing PAD activity [34]. Moreover, Zn is required for histidine conversion to UCA [35] (Figure 2). *Propionibacterium acnes* (*P. acnes*) induce excess KC proliferation and FLG expression in the epidermis through the induction of IGF-1, which activates the IGF-1 receptor (IGF-1R) on KCs. This causes follicular plugging that is frequently observed in patients with acne vulgaris. In these cases, Zn helps to maintain homeostasis by directly suppressing the induction of IGF-1 and IGF-1R and the overexpression of FLG [36].

OVO-like proteins (OVOLs) are transcribed from ubiquitously conserved genes encoding a C2H2 zinc finger transcription factor in mammals [37]. Mutations in the OVOL1 gene, as well as the FLG gene, were demonstrated as a risk factor for AD in the GWAS [38–40]. Consistent with this finding, *Ovol1*-knockout mice show rapid disruption of the epidermal barrier [41]. OVOL1 regulates transcription

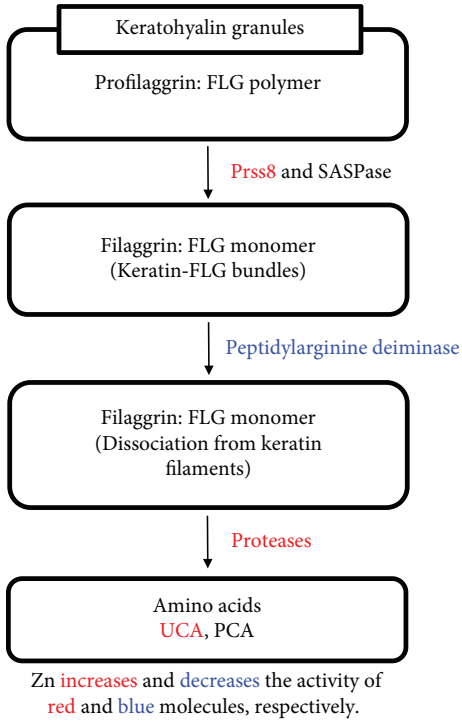


Figure 2: Filaggrin and its metabolism. FLG is produced in the SG as profilaggrin (FLG polymer) and is stored in keratohyalin granules. At the transition to the SC, the polymer is processed to the monomer by proteases such as Prss8 and SASPase and then binds to keratin and forms the fundamental structure of the corneocytes. At the outermost layer of the SC, FLG is citrullinated by peptidylarginine deiminase and then dissociated from keratin filaments. These dissociated FLG are degraded to free amino acids, including glutamine, arginine, and histidine. The FLG-derived histidine-rich proteins are converted into urocanic acid (UCA) and pyrrolidine carboxylic acid (PCA) by proteases. Zn facilitates FLG production by increasing the activity of Prss8. Alternatively, Zn can also suppress FLG metabolism by decreasing PAD activity. Moreover, Zn is required for histidine conversion to UCA.

in the nucleus by binding to p300, which is a member of the histone acetyltransferase (HAT) family and contains the Zn finger motif [42]. Additionally, HAT activity of p300 is negatively regulated by histone deacetylases (HDACs), which are Zn-dependent hydrolases [42]. Thus, OVOL1 expression and activity are regulated by Zn and Zn-related epigenetic enzymes. Aryl hydrocarbon receptor (AHR) is a ubiquitous ligand-activated transcription factor that is activated by both endogenous and exogenous ligands. AHR activation induces nuclear translocation of OVOL1, leading to the upregulation of FLG expression [43, 44]. Given this, it is evident that Zn and various Zn finger proteins are involved in the regulation of FLG expression.

2.1.2. Zinc and Cornified Envelope, Intercellular Lipid Lamellae, and Corneodesmosome. The cornified envelope (CE) is formed of keratins enclosed within an insoluble protein shell just beneath the corneocyte cell membrane [45]. It provides a solid physical barrier and consists of a 10 nm thick layer of highly cross-linked insoluble proteins, such as involucrin, envoplakin, periplakin, loricrin, and small proline-rich protein. A 5 nm thick layer of intercellular lipid lamellae composed of ceramides, free fatty acids, and cholesterol is covalently bound to these proteins. Adhesion between corneocytes is assumed to occur by the desmosome apparatus (corneodesmosome), composed of desmosomal cadherin, armadillo proteins, and plakins. The CE, intercellular lipid lamellae, and corneodesmosome are essential for effective physical and water barrier functions in the epidermis. The role of Zn in the formation and function of these proteins has not yet been elucidated.

2.1.3. Zinc and Corneocyte Desquamation. Shedding of corneocytes at the outermost layer of the SC is called desquamation and is an important step for maintaining epidermal homeostasis and preventing hyperkeratosis. This step is primarily assumed by a proteolytic cleavage of kallikrein- (KLK-) related peptidases. Out of 15 KLK proteins, human KCs express nearly all of them [46]. Among these KLKs, corneocyte desquamation is mainly conducted by KLK5, KLK7, and KLK14 [47]. The proteolytic activity is dependent on pH in the SC and is regulated by a cocktail of protease inhibitors, including lymphoepithelial Kazal-type 5 serine protease inhibitor (LEKTI) encoded by the serine protease inhibitor Kazal-type 5 (*SPINK5*) [48]. Importantly, the proteolytic activity of KLK5, KLK7, and KLK14 is impaired in the presence of Zn [49–51]. Furthermore, the expression of several KLK-related peptidases, including KLK5 and KLK7, is negatively regulated by transcriptional factor specificity protein 1 (Sp1), a C2H2-type Zn finger protein [52].

Several reports demonstrated the association between AD and Zn deficiency in humans and mice [53–55]. Consistent with this finding, the skin pH is increased in patients with AD [56], possibly due to the reduced UCA production caused by Zn deficiency (see Section 2.1.1). Additionally, Sp1 expression is decreased in the epidermis of patients with AD [52], and, as a consequence, KLK activity is often enhanced. Since KLKs process prointerleukin- (IL-) 1α and IL-1β that are abundantly stored in the corneocytes to active forms, this enhanced KLK activity leads to skin inflammation [57]. Additionally, KLK5 and KLK14 activate proteinase-activated receptor- (PAR-) 2, which is a G-protein-coupled receptor expressed on KCs, leading to the elicitation of itch common with AD [58]. Collectively, Zn suppresses excess inflammation and itching by inhibiting excess KLK activity. This action helps to maintain the epidermal barrier by suppressing IL-1-mediated FLG downregulation [57].

2.2. Zinc and Tight Junctions. TJs that are formed in the SG seal the intercellular spaces between SG KCs, thereby regulating the movement of water and inorganic ions via the paracellular pathway [59]. The major components of TJs are the transmembrane claudin proteins. Among the claudins expressed in the epidermis, claudin-1 is most critical for the formation and function of TJs, as confirmed in claudin-1-knockout mice, which showed severe dehydration and death soon after birth without modulating SC formation [60]. Providing further support to the importance of claudin-1, the expression is decreased in the epidermis of patients with

AD [61]. Other TJ components include transmembrane proteins such as occludin, JAM-A, tricellulin, and angulins and the intracellular scaffold proteins ZO-1, ZO-2, and ZO-3.

The role of Zn in the formation of TJs has been studied in the intestinal epithelium, but less so in the epidermis. For example, it has been reported that the chelation of intracellular Zn by TPEN (*N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)ethylenediamine) downregulates occludin and claudin-3, leading to TJ disruption in the intestinal epithelium [62]. It might be interesting to investigate the association between Zn deficiency in the epidermis and altered expression of TJ-associated proteins.

Zn finger E-box-binding homeobox- (ZEB-) 2 (also called SIP1) is a nuclear transcription factor that has C2H2-type Zn finger domains. Epidermal KC-specific ZEB-2 overexpression in mice showed epidermal barrier impairment along with the loss of expression of occludin and claudin-4 [63]. This suggests that the Zn finger protein, ZEB-2, negatively regulates the expression of occludin and claudin-4. The Zn endopeptidase meprinβ is expressed in the SG KCs just below the SC. Meprinβ is activated by KLK4 and facilitates proliferation and terminal differentiation of SG KCs, thereby contributing to the proper SC formation [64].

2.3. Epidermal Barrier Dysfunction and Th2 Response. Zn deficiency is known to drive Th2-type immune response and is associated with AD [65]. This suggests that Zn deficiency contributes to the disruption of the epidermal barrier through the downregulation of formation and/or function of SC and TJs. Epidermal barrier dysfunction allows external substances and stimuli to invade the epidermis and leads to the production of KC-derived Th2-inducing cytokines, including thymic stromal lymphoproteins (TSLP) and IL-33. TSLP downregulates FLG expression while IL-33 downregulates both FLG and claudin-1 expression [66–68]. Major Th2 cytokines, IL-4 and IL-13, downregulate the expression of FLG, the CE components (loricrin and involucrin), cell adhesion molecules (ZO-1), and ceramide lipids. IL-4 also inhibits the nuclear translocation of OVOL1, leading to the downregulation of FLG expression (see Section 2.1.1) [43]. IL-31, another Th2 cytokine, also downregulates FLG expression [69]. Taken together, epidermal barrier dysfunction, which is influenced by Zn deficiency, results in the disruption of skin homeostasis characterized by the Th2 immune response and vice versa.

3. Zinc and Langerhans Cells

LCs are antigen-presenting cells that occupy approximately 3% of the epidermis [70]. They were previously considered a subtype of dendritic cells (DCs) because of their ability to capture antigens (Ag), migrate to the draining lymph nodes (dLNs), and then present the Ag to T cells and initiate the immune response. However, recent evidence revealed that LCs originate from macrophage lineage of fetal liver progenitors and not from DC lineage [71–74]. Therefore, LCs are currently considered tissue-resident macrophages with the ability to migrate to the dLNs.

3.1. LCs and Tight Junctions. LCs express claudin-1 and form TJs with adjacent SG KCs [75]. As such, LCs are an important component of TJs to create an effective epidermal barrier. In steady state, LC dendrites lie beneath the TJs. Upon activation, LCs elongate their dendrites to penetrate the KC TJs by forming tricellulin-dependent TJs between KC-LC-KC. The elongated dendrites are able to reach beneath the SC and take up Ag from the extra-TJ environment without destroying barrier integrity (Figure 1) [7]. These LCs then induce Th2-type, but not Th1-type, humoral immune responses [8]. Furthermore, LCs take up KC-derived auto-Ag and present it on their MHC class II. These LCs expand polyclonal Ag-specific regulatory T cells and keep peripheral tolerance against auto-Ag [76]. Therefore, LCs assume the dual role of TJ component and surveillance agent of foreign and auto-Ag.

3.2. LCs and Zinc Deficiency. The association between Zn and LCs was revealed by analysis of skin specimen from patients with acrodermatitis enteropathica (AE; OMIM 201100) [77]. AE is an autosomal recessive disease caused by mutations in the *SLC39A4* gene that encodes ZRT/IRT-like protein 4 (ZIP4) [78]. ZIP4 is abundantly expressed in the apical side of the intestinal epithelium, thereby working as the primary gate absorbing Zn into the enterocytes. This absorbed Zn is subsequently transported to the bloodstream by Zn transporter 1 (ZnT1) [79, 80]. Therefore, ZIP4 dysfunction due to mutations results in decreased serum Zn levels. Interestingly, epidermal LCs were absent in AE skin lesions [77] (Figure 3). However, LCs were restored in the epidermis after patients were given Zn supplementation. These phenomena suggest that LCs disappeared from the epidermis when patients are deficient in Zn, but the effect can be easily reversed.

The association between LC loss and the development of characteristic AE skin lesion was investigated using dietary Zn-deficient (ZD) and Zn-adequate (ZA) mice [77]. Consistent with observations in patients with AE, epidermal LCs also disappeared in ZD mice (Figure 4).

"Allergic" contact dermatitis was significantly impaired in ZD mice compared with ZA mice. On the other hand, "irritant" contact dermatitis (ICD) was significantly enhanced in ZD mice compared to ZA mice. ICD is mediated by adenosine triphosphate (ATP), which is secreted by KCs in response to environmental irritants through lytic and nonlytic mechanisms [81–84]. As expected, ATP production from croton oil-applied skin was significantly increased in ZD mice compared with ZA mice. Additionally, ATP production from Pam212 KCs (murine immortalized KCs) was significantly increased by incubation with TPEN. These data suggest that KCs in ZD mice produce more ATP than KCs in ZA mice [77].

ATP is a potent inflammation inducer. Therefore, the epidermis is equipped with a mechanism to prevent ATP-mediated inflammation in the steady state. CD39 (ectonucleoside triphosphate diphosphohydrolase-1; ENTPD-1) plays a central role in ATP hydrolysis [83]. In the epidermis, CD39 is predominantly expressed in LCs, but not in KCs [83, 85, 86]. Thus, ATP is not hydrolyzed in the epidermis

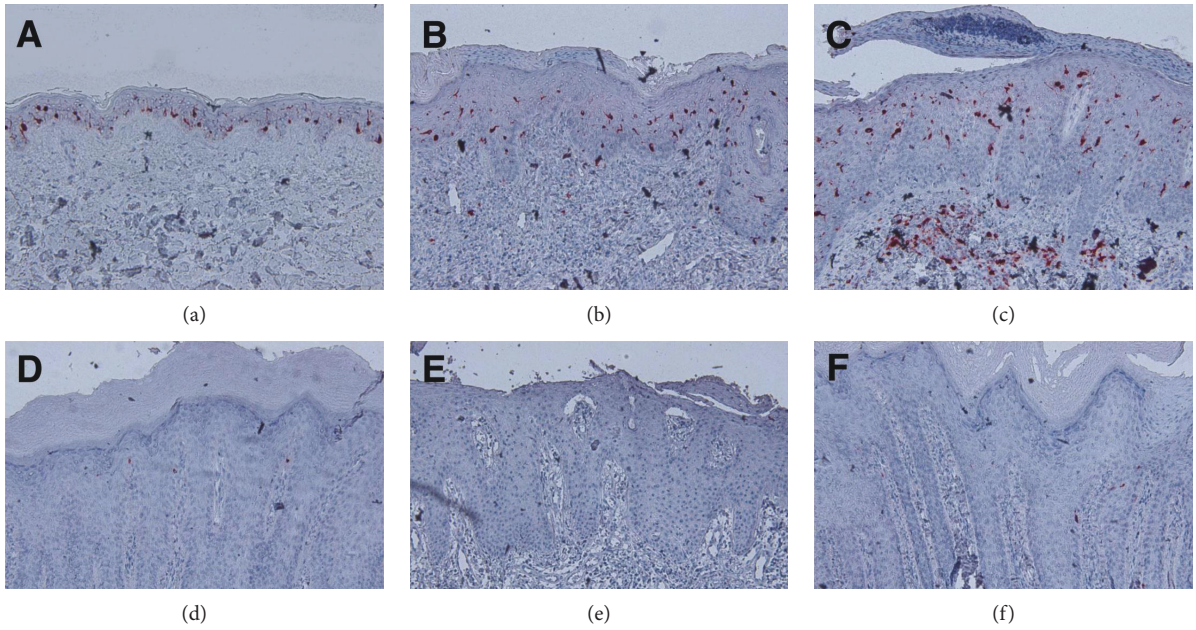


Figure 3: Loss of epidermal LCs in patients with AE. Immunohistochemical staining for langerin (red) in normal skin (a) and the erythematous lesions in atopic dermatitis (b), psoriasis vulgaris (c), or three AE (d–f) patients. Original magnification: 200x.

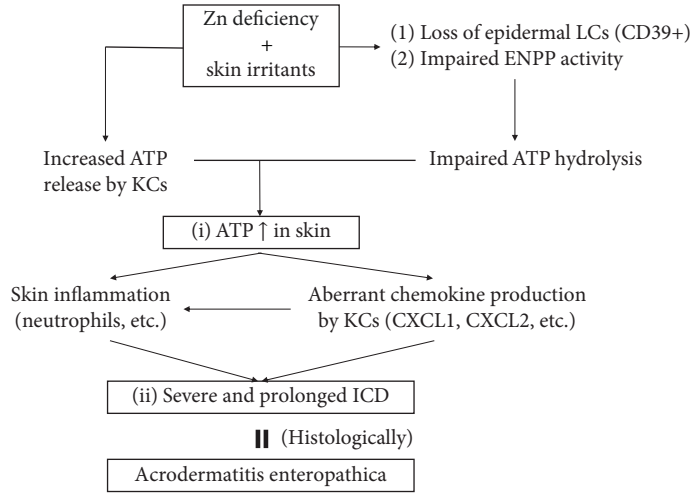


Figure 4: Model for the etiology of AE. (i) Skin irritants increase ATP release in the skin in Zn deficiency, due to increased ATP release by KCs, impaired ATP hydrolysis by LCs, and impaired ENPP activity. (ii) Increased ATP release induces severe and prolonged ICD via aberrant chemokine production by KCs and neutrophil-mediated skin inflammation. Histologically, cutaneous lesions in AE and ICD lesions in ZD mice demonstrate common histological features, such as subcorneal vacuolization and epidermal pallor.

of patients with AE and ZD mice, thereby eliciting ATP-mediated ICD in skin. In line with this concept, characteristic AE skin lesions develop in anogenital and periorificial areas and distal portions of the extremities, where frequent contact with external irritants including feces, urine, saliva, food, shoes, or excessive sweating is expected.

Taken together, the nature of AE skin lesions is ICD mediated by (1) increased ATP production by ZD KCs and (2) disabling of ATP hydrolysis due to the loss of CD39-expressing LCs.

As described, CD39 (ENTPD-1) potently hydrolyzes ATP. However, there are three groups of molecules that hydrolyze ATP, including ENTPDs, ectonucleotide pyrophosphatase/phosphodiesterases (ENPPs), and alkaline phosphatase (ALP) [87, 88]. The latter two molecules are Zn-dependent molecules. Among ENTPDs and ENPPs, ENTPD-1 (CD39), -2, -3, and -8 and ENPP-1, -2, and -3 aid in ATP hydrolysis. The expression of these molecules in LCs and KCs was not previously understood, with the exception of CD39. Thus, we determined that LCs strongly

expressed CD39 and weakly expressed ENTPD-2 and ENPP-1, -2, and -3. Normal human epidermal KCs weakly expressed CD39, ENTPD-2 and -3, and ENPP-1 and -2. Neither LCs nor KCs expressed ENTPD-8 or ALP. Therefore, although LCs strongly express CD39, other ATP-hydrolyzing molecules are weakly expressed in both LCs and KCs. KCs occupy approximately 97% of the epidermis, whereas LCs occupy approximately 3%. Therefore, we determined the degree of contribution of LCs and KCs to ATP hydrolysis. ATP hydrolysis was impaired by approximately 80% in LC-depleted epidermal suspension compared with sham-sorted epidermal suspension. This suggests that LCs assume approximately 80% of epidermal ATP hydrolysis, whereas KCs assume the remaining 20% [89].

Recently, it was demonstrated that Zn deficiency impairs the activity of ENPP-1 and ENPP-3, as well as ALP and CD73 [90]. As described, KCs weakly express ENPP-1 and ENPP-2. This explains one underlying mechanism by which ZD KCs increase ATP production.

3.3. LCs and Zinc Finger Proteins. In the steady state, LCs form the firm connections with adjacent KCs by claudin-1 and E-cadherin. Additionally, EpCAM (epithelial cell adhesion molecule) in mice and its human homolog, tumor-associated calcium signal transducer 2, also participate in this cell adhesion [91]. Claudin-1 and EpCAM colocalize in LCs [92]. Upon LC activation, LCs downregulate these adhesion molecules and then migrate to the dLNs. LCs upregulate ZEB-1 and ZEB-2 (see Section 2.2) during maturation, subsequently downregulating E-cadherin expression in LCs [93, 94]. Additionally, downregulation of EpCAM in LCs impairs claudin-1 expression in LCs [92]. This suggests that Zn deficiency leads to LC retention in the epidermis. Nevertheless, the presence of LCs is reduced with Zn deficiency [77]. For LC development and survival, LC-derived, but not KC-derived, autocrine transforming growth factor beta (TGF-β) is crucial [95–98]. This latent secreted protein is processed and activated by $\alpha v\beta 6$ and $\alpha v\beta 8$ integrins on KCs and then is recognized by LCs [99–101]. In patients with AE and ZD mice, the epidermal expression of TGF-β is strongly impaired, and thus, LCs are reduced [77]. However, the association between Zn deficiency and impaired epidermal TGF-β expression is not fully understood.

Collectively, Zn deficiency in the epidermis results in (1) disruption of epidermal barrier function (see Section 2), (2) LC disappearance due to impaired epidermal TGF-β expression, (3) impaired ATP hydrolysis due to the reduced number of CD39-expressing LCs and impaired ENPP activity, and (4) elicitation of ATP-mediated skin inflammation. Because of its importance, we are currently investigating the impact of LC loss on TJ development and function using AE skin specimens and ZD mice.

4. Zinc and Zinc Transporters in the Epidermis

The subject of Zn transporters in the epidermis has been widely reviewed by us and others [18–20]. Thus, here, we briefly summarized what is known and added recent findings. Zn is distributed to a higher degree in the epidermis than in the dermis. Within the epidermis, Zn is distributed primarily in the stratum spinosum [102]. LCs are present in the stratum spinosum, where Zn is found, and research suggests that LCs definitively require Zn for their survival and function [77].

The fundamental functions of Zn in the KCs are proliferation and anti-inflammation. KCs treated with nontoxic concentration of Zn increased their proliferation and survival [103]. Conversely, the chelation of intracellular Zn by TPEN facilitates KC apoptosis by activating caspase-3 and DNA fragmentation [104]. Zn has been reported to suppress the production of tumor necrosis factor-α, inducible nitric oxide synthase, and subsequent nitric oxide in KCs [65, 105, 106]. Zn also suppresses the expression of toll-like receptor 2 on KCs [107].

The rigorous Zn^{2+} regulation is conducted by Zn transporters (ZnTs and ZIPs) and metallothioneins (MTs) [108, 109]. So far, 10 ZnTs, 14 ZIPs, and 4 MTs were identified in humans [110, 111]. ZnTs and ZIPs mediate Zn efflux and uptake, respectively. MTs are ubiquitously expressed throughout various types of cells and are predominantly distributed in the cytoplasm and to a lesser extent in the nuclei and lysosomes. MTs can bind to metal ion including Zn via a unique cysteine-rich amino acid sequence and essentially control the availability of Zn [112]. Among 24 Zn transporters (10 ZnTs and 14 ZIPs), the function of only four Zn transporters (ZnT1, ZIP2, ZIP4, and ZIP10) in the epidermis or KCs has been elucidated so far.

ZnT1 in KCs is involved in the development of epidermodysplasia verruciformis (EV; OMIM 226400), which is a rare autosomal-recessive skin disease that can lead to nonmelanoma skin cancers resulting from selective susceptibility to oncogenic human papillomaviruses (HPVs) [113]. In KCs, EVER1 and EVER2 proteins form a complex with ZnT1 primarily in the ER and to a lesser extent in the nuclear membrane and Golgi apparatus. This complex maintains Zn homeostasis, which inhibits activator protein-1 (AP-1) activation that promotes HPV replication. Patients with EV have mutations in either the *EVER1* or *EVER2* genes [114, 115]. The complex of ZnT1 and mutated EVERs increases free Zn transport in the nucleus and subsequently enhances AP-1 activity, leading to aberrant replication of EV-related oncogenic HPVs and thereby developing skin cancers.

ZIP2 is expressed on the differentiating KCs of humans and mice. Since ZIP2 knockdown in KCs decreases intracellular Zn, suppresses KC differentiation, and downregulates involucrin expression, Zn taken up by KC ZIP2 is required for proper KC differentiation and CE formation (also see Section 2.1.2) [102]. ZIP4 is expressed on the undifferentiating KCs of humans. Since ZIP4 knockdown in KCs decreases intracellular Zn, suppresses KC differentiation, downregulates the expression of FLG and involucrin, and impairs the activity of p63 that is a critical regulator of epidermal formation, Zn taken up by KC ZIP4 is required for proper KC differentiation and SC formation (also see Section 2.1) [116]. Murine ZIP10 is expressed on the epidermal progenitor cells of the outer root sheath of hair follicles. Therefore, ZIP10 depletion in keratin 14-expressing cells leads to a thin epidermis and a hypoplasia of hair follicles via downregulation of

p63 activity [117]. The epidermal regulator p63 also controls the activity of ZNF750, a C2H2-type Zn finger protein. ZNF750 strongly regulates terminal epidermal differentiation. Thus, ZNF750 knockdown downregulates the expression of epidermal barrier-related proteins including FLG, loricrin, and SPINK5 [118]. In human skin, MT-1 and MT-2 are expressed in the actively proliferating KCs, such as the hair matrix, outer hair roots, and stratum basale [119]. Knockdown of both MT-1 and MT-2 in mice impairs KC proliferation [120]. MT expressions are upregulated in the hyperplastic KCs of inflamed skin lesions and skin cancers [121].

Collectively, Zn supports KC proliferation and survival. ZIP2 and ZIP4 are required for proper KC differentiation and subsequent epidermal barrier formation. ZIP10 is required for the successful epidermal formation. MTs are involved in KC proliferation.

5. Conclusions and Perspectives

Epidermal barrier homeostasis is the first line of defense for preventing the initiation of atopic march. SC and TJs are responsible for epidermal barrier function. Zn and Zn finger proteins regulate SC formation as well as its metabolism, while the contribution of Zn to TJ function is less well understood. However, when Zn is deficient, LCs are not present, and this important component of TJs is lacking. Therefore, Zn deficiency might lead to epidermal barrier dysfunction. Disruption of the epidermal barrier induces a Th2-type immune response by producing KC-derived Th2-promoting cytokines and T cell-derived Th2 cytokines. Meanwhile, these Th2-related cytokines impair the structure and function of the SC and TJs. In this way, epidermal barrier disruptions trigger a negative spiral of inflammation. Zn homeostasis in cells, including KCs, is maintained by ZnTs, ZIPs, and MTs. MT-mediated Zn in KCs facilitates its proliferation. On the other hand, the function of ZnTs and ZIPs in KC biology is less understood, because only the function of ZnT1, ZIP2, ZIP4, and ZIP10 has been elucidated from among 10 ZnTs and 14 ZIPs. The epidermis is composed of KCs, LCs, melanocytes, epidermal-resident memory T cells (TRMs), and others. The murine epidermis, but not the human epidermis, also contains dendritic epidermal T cells (DETCs), a type of $\gamma\delta$T cells. The role of Zn and Zn transporters in LCs, melanocytes, TRMs, and DETCs has not been analyzed to date. Additional research must be conducted to thoroughly understand the role of Zn and Zn transporters in maintaining healthy skin.

Conflicts of Interest

The authors declare no competing financial interest.

References

[1] K. Horimukai, K. Morita, M. Narita et al., "Application of moisturizer to neonates prevents development of atopic dermatitis," *The Journal of Allergy and Clinical Immunology*, vol. 134, no. 4, pp. 824–830.e6, 2014.

[2] E. L. Simpson, J. R. Chalmers, J. M. Hanifin et al., "Emollient enhancement of the skin barrier from birth offers effective atopic dermatitis prevention," *The Journal of Allergy and Clinical Immunology*, vol. 134, no. 4, pp. 818–823, 2014.

[3] J. M. Spergel and A. S. Paller, "Atopic dermatitis and the atopic march," *The Journal of Allergy and Clinical Immunology*, vol. 112, no. 6, pp. S118–S127, 2003.

[4] G. Egawa and K. Kabashima, "Multifactorial skin barrier deficiency and atopic dermatitis: essential topics to prevent the atopic march," *The Journal of Allergy and Clinical Immunology*, vol. 138, no. 2, pp. 350–358.e1, 2016, e351.

[5] M. J. Cork, S. G. Danby, Y. Vasilopoulos et al., "Epidermal barrier dysfunction in atopic dermatitis," *The Journal of Investigative Dermatology*, vol. 129, no. 8, pp. 1892–1908, 2009.

[6] A. De Benedetto, A. Kubo, and L. A. Beck, "Skin barrier disruption: a requirement for allergen sensitization?," *The Journal of Investigative Dermatology*, vol. 132, no. 3, pp. 949–963, 2012.

[7] A. Kubo, K. Nagao, M. Yokouchi, H. Sasaki, and M. Amagai, "External antigen uptake by Langerhans cells with reorganization of epidermal tight junction barriers," *The Journal of Experimental Medicine*, vol. 206, no. 13, pp. 2937–2946, 2009.

[8] T. Ouchi, A. Kubo, M. Yokouchi et al., "Langerhans cell antigen capture through tight junctions confers preemptive immunity in experimental staphylococcal scalded skin syndrome," *The Journal of Experimental Medicine*, vol. 208, no. 13, pp. 2607–2613, 2011.

[9] M. J. Jackson, "Physiology of zinc: general aspects," in *Zinc in Human Biology*, C. F. Mills, Ed., pp. 1–14, Springer, London, 1989.

[10] G. Michaelsson, K. Ljunghall, and B. G. Danielson, "Zinc in epidermis and dermis in healthy subjects," *Acta Dermato-Venereologica*, vol. 60, no. 4, pp. 295–299, 1980.

[11] C. Andreini, I. Bertini, and G. Cavallaro, "Minimal functional sites allow a classification of zinc sites in proteins," *PLoS One*, vol. 6, no. 10, article e26325, 2011.

[12] C. Andreini and I. Bertini, "A bioinformatics view of zinc enzymes," *Journal of Inorganic Biochemistry*, vol. 111, pp. 150–156, 2012.

[13] J. M. Berg and Y. Shi, "The galvanization of biology: a growing appreciation for the roles of zinc," *Science*, vol. 271, no. 5252, pp. 1081–1085, 1996.

[14] T. Fukada, S. Yamasaki, K. Nishida, M. Murakami, and T. Hirano, "Zinc homeostasis and signaling in health and diseases: zinc signaling," *Journal of Biological Inorganic Chemistry*, vol. 16, no. 7, pp. 1123–1134, 2011.

[15] A. Klug, "The discovery of zinc fingers and their development for practical applications in gene regulation and genome manipulation," *Quarterly Reviews of Biophysics*, vol. 43, no. 1, pp. 1–21, 2010.

[16] J. H. Laity, B. M. Lee, and P. E. Wright, "Zinc finger proteins: new insights into structural and functional diversity," *Current Opinion in Structural Biology*, vol. 11, no. 1, pp. 39–46, 2001.

[17] C. Andreini, L. Banci, I. Bertini, and A. Rosato, "Counting the zinc-proteins encoded in the human genome," *Journal of Proteome Research*, vol. 5, no. 1, pp. 196–201, 2006.

[18] Y. Ogawa, T. Kawamura, and S. Shimada, "Zinc and skin biology," *Archives of Biochemistry and Biophysics*, vol. 611, pp. 113–119, 2016.

[19] Y. Ogawa, M. Kinoshita, S. Shimada, and T. Kawamura, "Zinc and skin disorders," *Nutrients*, vol. 10, no. 2, 2018.

[20] B.-H. Bin, S. Hojyo, J. Seo et al., "The role of the Slc39a family of zinc transporters in zinc homeostasis in skin," *Nutrients*, vol. 10, no. 2, 2018.

[21] G. Egawa and K. Kabashima, "Barrier dysfunction in the skin allergy," *Allergology International*, vol. 67, no. 1, pp. 3–11, 2018.

[22] H. Kawasaki, K. Nagao, A. Kubo et al., "Altered stratum corneum barrier and enhanced percutaneous immune responses in filaggrin-null mice," *Journal of Allergy and Clinical Immunology*, vol. 129, no. 6, pp. 1538–1546.e6, 2012.

[23] S. P. Saunders, T. Moran, A. Floudas et al., "Spontaneous atopic dermatitis is mediated by innate immunity, with the secondary lung inflammation of the atopic march requiring adaptive immunity," *The Journal of Allergy and Clinical Immunology*, vol. 137, no. 2, pp. 482–491, 2016.

[24] C. N. A. Palmer, A. D. Irvine, A. Terron-Kwiatkowski et al., "Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis," *Nature Genetics*, vol. 38, no. 4, pp. 441–446, 2006.

[25] F. J. D. Smith, A. D. Irvine, A. Terron-Kwiatkowski et al., "Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris," *Nature Genetics*, vol. 38, no. 3, pp. 337–342, 2006.

[26] T. Nomura, A. Sandilands, M. Akiyama et al., "Unique mutations in the filaggrin gene in Japanese patients with ichthyosis vulgaris and atopic dermatitis," *The Journal of Allergy and Clinical Immunology*, vol. 119, no. 2, pp. 434–440, 2007.

[27] L. Paternoster, The EArly Genetics and Lifecourse Epidemiology (EAGLE) Eczema Consortium, M. Standl et al., "Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis," *Nature Genetics*, vol. 47, no. 12, pp. 1449–1456, 2015.

[28] C. Leyvraz, R. P. Charles, I. Rubera et al., "The epidermal barrier function is dependent on the serine protease CAP1/Prss 8," *The Journal of Cell Biology*, vol. 170, no. 3, pp. 487–496, 2005.

[29] T. Matsui, K. Miyamoto, A. Kubo et al., "SASPase regulates stratum corneum hydration through profilaggrin-to-filaggrin processing," *EMBO Molecular Medicine*, vol. 3, no. 6, pp. 320–333, 2011.

[30] R. Nachat, M.-C. Méchin, H. Takahara et al., "Peptidylarginine deiminase isoforms 1-3 are expressed in the epidermis and involved in the deimination of K1 and filaggrin," *Journal of Investigative Dermatology*, vol. 124, no. 2, pp. 384–393, 2005.

[31] N. K. Gibbs, J. Tye, and M. Norval, "Recent advances in urocanic acid photochemistry, photobiology and photoimmunology," *Photochemical & Photobiological Sciences*, vol. 7, no. 6, pp. 655–667, 2008.

[32] C. S. Leitch, E. Natafji, C. Yu et al., "Filaggrin-null mutations are associated with increased maturation markers on Langerhans cells," *The Journal of Allergy and Clinical Immunology*, vol. 138, no. 2, pp. 482–490.e7, 2016, e487.

[33] A. Shipway, H. Danahay, J. A. Williams, D. C. Tully, B. J. Backes, and J. L. Harris, "Biochemical characterization of prostasin, a channel activating protease," *Biochemical and Biophysical Research Communications*, vol. 324, no. 2, pp. 953–963, 2004.

[34] P. Stenberg and B. Roth, "Zinc is the modulator of the calcium-dependent activation of post-translationally acting thiol-enzymes in autoimmune diseases," *Medical Hypotheses*, vol. 84, no. 4, pp. 331–335, 2015.

[35] J. M. Hsu and B. Rubenstein, "Effect of zinc deficiency on histidine metabolism in rats," *The Journal of Nutrition*, vol. 112, no. 3, pp. 461–467, 1982.

[36] O. Isard, A. C. Knol, M. F. Ariès et al., "Propionibacterium acnes activates the IGF-1/IGF-1R system in the epidermis and induces keratinocyte proliferation," *The Journal of Investigative Dermatology*, vol. 131, no. 1, pp. 59–66, 2011.

[37] A. Kumar, A. Bhandari, R. Sinha et al., "Molecular phylogeny of OVOL genes illustrates a conserved C2H2 zinc finger domain coupled by hypervariable unstructured regions," *PLoS One*, vol. 7, no. 6, article e39399, 2012.

[38] T. Hirota, A. Takahashi, M. Kubo et al., "Genome-wide association study identifies eight new susceptibility loci for atopic dermatitis in the Japanese population," *Nature Genetics*, vol. 44, no. 11, pp. 1222–1226, 2012.

[39] L. Paternoster, M. Standl, C. M. Chen et al., "Meta-analysis of genome-wide association studies identifies three new risk loci for atopic dermatitis," *Nature Genetics*, vol. 44, no. 2, pp. 187–192, 2011.

[40] I. Marenholz, J. Esparza-Gordillo, F. Rüschendorf et al., "Meta-analysis identifies seven susceptibility loci involved in the atopic march," *Nature Communications*, vol. 6, no. 1, p. 8804, 2015.

[41] A. Teng, M. Nair, J. Wells, J. A. Segre, and X. Dai, "Strain-dependent perinatal lethality of Ovol1-deficient mice and identification of Ovol2 as a downstream target of Ovol1 in skin epidermis," *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, vol. 1772, no. 1, pp. 89–95, 2007.

[42] M. Nair, V. Bilanchone, K. Ortt, S. Sinha, and X. Dai, "Ovol 1 represses its own transcription by competing with transcription activator c-Myb and by recruiting histone deacetylase activity," *Nucleic Acids Research*, vol. 35, no. 5, pp. 1687–1697, 2007.

[43] G. Tsuji, A. Hashimoto-Hachiya, M. Kiyomatsu-Oda et al., "Aryl hydrocarbon receptor activation restores filaggrin expression via OVOL1 in atopic dermatitis," *Cell Death and Disease*, vol. 8, no. 7, article e2931, 2017.

[44] G. Tsuji, T. Ito, T. Chiba et al., "The role of the OVOL1-OVOL2 axis in normal and diseased human skin," *Journal of Dermatological Science*, vol. 90, no. 3, pp. 227–231, 2018.

[45] A. Kalinin, L. N. Marekov, and P. M. Steinert, "Assembly of the epidermal cornified cell envelope," *Journal of Cell Science*, vol. 114, Part 17, pp. 3069-3070, 2001.

[46] N. Komatsu, M. Takata, N. Otsuki et al., "Expression and localization of tissue kallikrein mRNAs in human epidermis and appendages," *The Journal of Investigative Dermatology*, vol. 121, no. 3, pp. 542–549, 2003.

[47] M. Brattsand, K. Stefansson, C. Lundh, Y. Haasum, and T. Egelrud, "A proteolytic cascade of kallikreins in the stratum corneum," *The Journal of Investigative Dermatology*, vol. 124, no. 1, pp. 198–203, 2005.

[48] C. Deraison, C. Bonnart, F. Lopez et al., "LEKTI fragments specifically inhibit KLK5, KLK7, and KLK14 and control desquamation through a pH-dependent interaction,"

Molecular Biology of the Cell, vol. 18, no. 9, pp. 3607–3619, 2007.

[49] M. Debela, P. Goettig, V. Magdolen, R. Huber, N. M. Schechter, and W. Bode, "Structural basis of the zinc inhibition of human tissue kallikrein 5," *Journal of Molecular Biology*, vol. 373, no. 4, pp. 1017–1031, 2007.

[50] M. Debela, P. Hess, V. Magdolen et al., "Chymotryptic specificity determinants in the 1.0 A structure of the zinc-inhibited human tissue kallikrein 7," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 41, pp. 16086–16091, 2007.

[51] C. A. Borgoño, I. P. Michael, J. L. V. Shaw et al., "Expression and functional characterization of the cancer-related serine protease, human tissue kallikrein 14," *Journal of Biological Chemistry*, vol. 282, no. 4, pp. 2405–2422, 2007.

[52] L. Bin, B. E. Kim, C. F. Hall, S. M. Leach, and D. Y. M. Leung, "Inhibition of transcription factor specificity protein 1 alters the gene expression profile of keratinocytes leading to upregulation of kallikrein-related peptidases and thymic stromal lymphopoietin," *The Journal of Investigative Dermatology*, vol. 131, no. 11, pp. 2213–2222, 2011.

[53] H. Takahashi, M. Nakazawa, K. Takahashi et al., "Effects of zinc deficient diet on development of atopic dermatitis-like eruptions in DS-Nh mice," *Journal of Dermatological Science*, vol. 50, no. 1, pp. 31–39, 2008.

[54] T. J. David, F. E. Wells, T. C. Sharpe, and A. C. C. Gibbs, "Low serum zinc in children with atopic eczema," *The British Journal of Dermatology*, vol. 111, no. 5, pp. 597–601, 1984.

[55] J. E. Kim, S. R. Yoo, M. G. Jeong, J. Y. Ko, and Y. S. Ro, "Hair zinc levels and the efficacy of oral zinc supplementation in patients with atopic dermatitis," *Acta Dermato-Venereologica*, vol. 94, no. 5, pp. 558–562, 2014.

[56] F. Rippke, V. Schreiner, T. Doering, and H. I. Maibach, "Stratum corneum pH in atopic dermatitis: impact on skin barrier function and colonization with *Staphylococcus aureus*," *American Journal of Clinical Dermatology*, vol. 5, no. 4, pp. 217–223, 2004.

[57] S. Kezic, G. M. O'Regan, R. Lutter et al., "Filaggrin loss-of-function mutations are associated with enhanced expression of IL-1 cytokines in the stratum corneum of patients with atopic dermatitis and in a murine model of filaggrin deficiency," *Journal of Allergy and Clinical Immunology*, vol. 129, no. 4, pp. 1031–1039.e1, 2012.

[58] K. Stefansson, M. Brattsand, D. Roosterman et al., "Activation of proteinase-activated receptor-2 by human kallikrein-related peptidases," *The Journal of Investigative Dermatology*, vol. 128, no. 1, pp. 18–25, 2008.

[59] N. Kirschner, P. Houdek, M. Fromm, I. Moll, and J. M. Brandner, "Tight junctions form a barrier in human epidermis," *European Journal of Cell Biology*, vol. 89, no. 11, pp. 839–842, 2010.

[60] M. Furuse, M. Hata, K. Furuse et al., "Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice," *The Journal of Cell Biology*, vol. 156, no. 6, pp. 1099–1111, 2002.

[61] A. De Benedetto, N. M. Rafaels, L. Y. McGirt et al., "Tight junction defects in patients with atopic dermatitis," *Journal of Allergy and Clinical Immunology*, vol. 127, no. 3, pp. 773–786.e7, 2011.

[62] Y. Miyoshi, S. Tanabe, and T. Suzuki, "Cellular zinc is required for intestinal epithelial barrier maintenance via the regulation of claudin-3 and occludin expression," *American Journal of Physiology. Gastrointestinal and Liver Physiology*, vol. 311, no. 1, pp. G105–G116, 2016.

[63] M. N. Tatari, B. De Craene, B. Soen et al., "ZEB2-transgene expression in the epidermis compromises the integrity of the epidermal barrier through the repression of different tight junction proteins," *Cellular and Molecular Life Sciences*, vol. 71, no. 18, pp. 3599–3609, 2014.

[64] C. Becker-Pauly, M. Höwel, T. Walker et al., "The alpha and beta subunits of the metalloprotease meprin are expressed in separate layers of human epidermis, revealing different functions in keratinocyte proliferation and differentiation," *The Journal of Investigative Dermatology*, vol. 127, no. 5, pp. 1115–1125, 2007.

[65] I. Wessels, M. Maywald, and L. Rink, "Zinc as a gatekeeper of immune function," *Nutrients*, vol. 9, no. 12, 2017.

[66] W. Amano, S. Nakajima, H. Kunugi et al., "The Janus kinase inhibitor JTE-052 improves skin barrier function through suppressing signal transducer and activator of transcription 3 signaling," *The Journal of Allergy and Clinical Immunology*, vol. 136, no. 3, pp. 667–677.e7, 2015.

[67] J. Seltmann, L. M. Roesner, F. W. von Hesler, M. Wittmann, and T. Werfel, "IL-33 impacts on the skin barrier by downregulating the expression of filaggrin," *Journal of Allergy and Clinical Immunology*, vol. 135, no. 6, pp. 1659–1661.e4, 2015.

[68] W. I. Ryu, H. Lee, H. C. Bae et al., "IL-33 down-regulates CLDN1 expression through the ERK/STAT3 pathway in keratinocytes," *Journal of Dermatological Science*, vol. 90, no. 3, pp. 313–322, 2018.

[69] C. Cornelissen, Y. Marquardt, K. Czaja et al., "IL-31 regulates differentiation and filaggrin expression in human organotypic skin models," *Journal of Allergy and Clinical Immunology*, vol. 129, no. 2, pp. 426–433.e8, 2012.

[70] M. Merad, F. Ginhoux, and M. Collin, "Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells," *Nature Reviews Immunology*, vol. 8, no. 12, pp. 935–947, 2008.

[71] L. Chorro, A. Sarde, M. Li et al., "Langerhans cell (LC) proliferation mediates neonatal development, homeostasis, and inflammation-associated expansion of the epidermal LC network," *The Journal of Experimental Medicine*, vol. 206, no. 13, pp. 3089–3100, 2009.

[72] G. Hoeffel, Y. Wang, M. Greter et al., "Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages," *The Journal of Experimental Medicine*, vol. 209, no. 6, pp. 1167–1181, 2012.

[73] C. Schulz, E. G. Perdiguero, L. Chorro et al., "A lineage of myeloid cells independent of Myb and hematopoietic stem cells," *Science*, vol. 336, no. 6077, pp. 86–90, 2012.

[74] L. C. Davies, S. J. Jenkins, J. E. Allen, and P. R. Taylor, "Tissue-resident macrophages," *Nature Immunology*, vol. 14, no. 10, pp. 986–995, 2013.

[75] S. C. Zimmerli and C. Hauser, "Langerhans cells and lymph node dendritic cells express the tight junction component claudin-1," *The Journal of Investigative Dermatology*, vol. 127, no. 10, pp. 2381–2390, 2007.

[76] D. Y. Kitashima, T. Kobayashi, T. Woodring et al., "Langerhans cells prevent autoimmunity via expansion of keratinocyte antigen-specific regulatory T cells," *eBioMedicine*, vol. 27, pp. 293–303, 2018.

[77] T. Kawamura, Y. Ogawa, Y. Nakamura et al., "Severe dermatitis with loss of epidermal Langerhans cells in human and mouse zinc deficiency," *The Journal of Clinical Investigation*, vol. 122, no. 2, pp. 722–732, 2012.

[78] E. Maverakis, M. A. Fung, P. J. Lynch et al., "Acrodermatitis enteropathica and an overview of zinc metabolism," *Journal of the American Academy of Dermatology*, vol. 56, no. 1, pp. 116–124, 2007.

[79] Y. Y. Yu, C. P. Kirschke, and L. Huang, "Immunohistochemical analysis of ZnT1, 4, 5, 6, and 7 in the mouse gastrointestinal tract," *The Journal of Histochemistry and Cytochemistry*, vol. 55, no. 3, pp. 223–234, 2006.

[80] X. Wang and B. Zhou, "Dietary zinc absorption: a play of Zips and ZnTs in the gut," *IUBMB Life*, vol. 62, no. 3, pp. 176–182, 2010.

[81] E. R. Lazarowski, R. C. Boucher, and T. K. Harden, "Mechanisms of release of nucleotides and integration of their action as P2X- and P2Y-receptor activating molecules," *Molecular Pharmacology*, vol. 64, no. 4, pp. 785–795, 2003.

[82] S. Koizumi, K. Fujishita, K. Inoue, Y. Shigemoto-Mogami, M. Tsuda, and K. Inoue, "Ca2+ waves in keratinocytes are transmitted to sensory neurons: the involvement of extracellular ATP and P2Y2 receptor activation," *The Biochemical Journal*, vol. 380, no. 2, pp. 329–338, 2004.

[83] N. Mizumoto, T. Kumamoto, S. C. Robson et al., "CD39 is the dominant Langerhans cell-associated ecto-NTPDase: modulatory roles in inflammation and immune responsiveness," *Nature Medicine*, vol. 8, no. 4, pp. 358–365, 2002.

[84] N. Mizumoto, M. E. Mummert, D. Shalhevet, and A. Takashima, "Keratinocyte ATP release assay for testing skin-irritating potentials of structurally diverse chemicals," *The Journal of Investigative Dermatology*, vol. 121, no. 5, pp. 1066–1072, 2003.

[85] J. G. Georgiou, K. K. Skarratt, S. J. Fuller et al., "Human epidermal and monocyte-derived Langerhans cells express functional P2X receptors," *The Journal of Investigative Dermatology*, vol. 125, no. 3, pp. 482–490, 2005.

[86] C. L. Ho, C. Y. Yang, W. J. Lin, and C. H. Lin, "Ecto-nucleoside triphosphate diphosphohydrolase 2 modulates local ATP-induced calcium signaling in human HaCaT keratinocytes," *PLoS One*, vol. 8, no. 3, article e57666, 2013.

[87] G. G. Yegutkin, "Nucleotide- and nucleoside-converting ectoenzymes: important modulators of purinergic signalling cascade," *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, vol. 1783, no. 5, pp. 673–694, 2008.

[88] H. Zimmermann, "Extracellular metabolism of ATP and other nucleotides," *Naunyn-Schmiedeberg's Archives of Pharmacology*, vol. 362, no. 4-5, pp. 299–309, 2000.

[89] Y. Ogawa, M. Kinoshita, N. Mizumura et al., "Purinergic molecules in the epidermis," *The Journal of Investigative Dermatology*, vol. 138, no. 11, pp. 2486–2488, 2018.

[90] T. Takeda, S. Miyazaki, M. Kobayashi et al., "Zinc deficiency causes delayed ATP clearance and adenosine generation in rats and cell culture models," *Communications Biology*, vol. 1, no. 1, p. 113, 2018.

[91] M. Ladwein, U. Pape, D. Schmidt et al., "The cell-cell adhesion molecule EpCAM interacts directly with the tight junction protein claudin-7," *Experimental Cell Research*, vol. 309, no. 2, pp. 345–357, 2005.

[92] T. Ouchi, G. Nakato, and M. C. Udey, "EpCAM expressed by murine epidermal Langerhans cells modulates immunization to an epicutaneously applied protein antigen," *The Journal of Investigative Dermatology*, vol. 136, no. 8, pp. 1627–1635, 2016.

[93] S. Konradi, N. Yasmin, D. Haslwanter et al., "Langerhans cell maturation is accompanied by induction of N-cadherin and the transcriptional regulators of epithelial-mesenchymal transition ZEB1/2," *European Journal of Immunology*, vol. 44, no. 2, pp. 553–560, 2014.

[94] J. Comijn, G. Berx, P. Vermassen et al., "The two-handed E box binding zinc finger protein SIP1 downregulates E-cadherin and induces invasion," *Molecular Cell*, vol. 7, no. 6, pp. 1267–1278, 2001.

[95] T. A. Borkowski, J. J. Letterio, A. G. Farr, and M. C. Udey, "A role for endogenous transforming growth factor beta 1 in Langerhans cell biology: the skin of transforming growth factor beta 1 null mice is devoid of epidermal Langerhans cells," *The Journal of Experimental Medicine*, vol. 184, no. 6, pp. 2417–2422, 1996.

[96] D. H. Kaplan, M. O. Li, M. C. Jenison, W. D. Shlomchik, R. A. Flavell, and M. J. Shlomchik, "Autocrine/paracrine TGFbeta1 is required for the development of epidermal Langerhans cells," *The Journal of Experimental Medicine*, vol. 204, no. 11, pp. 2545–2552, 2007.

[97] J. M. Kel, M. J. H. Girard-Madoux, B. Reizis, and B. E. Clausen, "TGF-beta is required to maintain the pool of immature Langerhans cells in the epidermis," *Journal of Immunology*, vol. 185, no. 6, pp. 3248–3255, 2010.

[98] A. Bobr, B. Z. Igyarto, K. M. Haley, M. O. Li, R. A. Flavell, and D. H. Kaplan, "Autocrine/paracrine TGF-β1 inhibits Langerhans cell migration," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 26, pp. 10492–10497, 2012.

[99] Z. Yang, Z. Mu, B. Dabovic et al., "Absence of integrin-mediated TGFbeta1 activation in vivo recapitulates the phenotype of TGFbeta1-null mice," *The Journal of Cell Biology*, vol. 176, no. 6, pp. 787–793, 2007.

[100] P. Aluwihare, Z. Mu, Z. Zhao et al., "Mice that lack activity of $\alpha v\beta 6$- and $\alpha v\beta 8$-integrins reproduce the abnormalities of Tgfb1- and Tgfb3-null mice," *Journal of Cell Science*, vol. 122, no. 2, pp. 227–232, 2008.

[101] J. Mohammed, L. K. Beura, A. Bobr et al., "Stromal cells control the epithelial residence of DCs and memory T cells by regulated activation of TGF-β," *Nature Immunology*, vol. 17, no. 4, pp. 414–421, 2016.

[102] Y. Inoue, S. Hasegawa, S. Ban et al., "ZIP2 protein, a zinc transporter, is associated with keratinocyte differentiation," *The Journal of Biological Chemistry*, vol. 289, no. 31, pp. 21451–21462, 2014.

[103] E. Emri, E. Miko, P. Bai et al., "Effects of non-toxic zinc exposure on human epidermal keratinocytes," *Metallomics*, vol. 7, no. 3, pp. 499–507, 2015.

[104] D. Wilson, G. Varigos, and M. L. Ackland, "Apoptosis may underlie the pathology of zinc-deficient skin," *Immunology and Cell Biology*, vol. 84, no. 1, pp. 28–37, 2006.

[105] A. Gueniche, J. Viac, G. Lizard, M. Charveron, and D. Schmitt, "Protective effect of zinc on keratinocyte activation markers induced by interferon or nickel," *Acta Dermato-Venereologica*, vol. 75, no. 1, pp. 19–23, 1995.

[106] J. Yamaoka, T. Kume, A. Akaike, and Y. Miyachi, "Suppressive effect of zinc ion on iNOS expression induced by interferon-gamma or tumor necrosis factor-alpha in murine

keratinocytes," *Journal of Dermatological Science*, vol. 23, no. 1, pp. 27–35, 2000.

[107] V. Jarrousse, N. Castex-Rizzi, A. Khammari, M. Charveron, and B. Dréno, "Zinc salts inhibit in vitro Toll-like receptor 2 surface expression by keratinocytes," *European Journal of Dermatology*, vol. 17, no. 6, pp. 492–496, 2007.

[108] T. Kambe, T. Tsuji, A. Hashimoto, and N. Itsumura, "The physiological, biochemical, and molecular roles of zinc transporters in zinc homeostasis and metabolism," *Physiological Reviews*, vol. 95, no. 3, pp. 749–784, 2015.

[109] M. A. Hediger, M. F. Romero, J. B. Peng, A. Rolfs, H. Takanaga, and E. A. Bruford, "The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteins," *Pflügers Archiv European Journal of Physiology*, vol. 447, no. 5, pp. 465–468, 2004.

[110] R. D. Palmiter and L. Huang, "Efflux and compartmentalization of zinc by members of the SLC30 family of solute carriers," *Pflügers Archiv European Journal of Physiology*, vol. 447, no. 5, pp. 744–751, 2004.

[111] D. J. Eide, "The SLC39 family of metal ion transporters," *Pflügers Archiv European Journal of Physiology*, vol. 447, no. 5, pp. 796–800, 2004.

[112] H. Tapiero and K. D. Tew, "Trace elements in human physiology and pathology: zinc and metallothioneins," *Biomedicine & Pharmacotherapy*, vol. 57, no. 9, pp. 399–411, 2003.

[113] M. Lazarczyk, C. Pons, J. A. Mendoza, P. Cassonnet, Y. Jacob, and M. Favre, "Regulation of cellular zinc balance as a potential mechanism of EVER-mediated protection against pathogenesis by cutaneous oncogenic human papillomaviruses," *The Journal of Experimental Medicine*, vol. 205, no. 1, pp. 35–42, 2008.

[114] N. Ramoz, M. Favre, G. Orth et al., "Evidence for a nonallelic heterogeneity of epidermodysplasia verruciformis with two susceptibility loci mapped to chromosome regions 2p21-p 24 and 17q25," *The Journal of Investigative Dermatology*, vol. 114, no. 6, pp. 1148–1153, 2000.

[115] N. Ramoz, L. A. Rueda, B. Bouadjar, L. S. Montoya, G. Orth, and M. Favre, "Mutations in two adjacent novel genes are associated with epidermodysplasia verruciformis," *Nature Genetics*, vol. 32, no. 4, pp. 579–581, 2002.

[116] B. H. Bin, J. Bhin, N. H. Kim et al., "An acrodermatitis enteropathica-associated Zn transporter, ZIP4, regulates human epidermal homeostasis," *The Journal of Investigative Dermatology*, vol. 137, no. 4, pp. 874–883, 2017.

[117] B. H. Bin, J. Bhin, M. Takaishi et al., "Requirement of zinc transporter ZIP10 for epidermal development: implication of the ZIP10-p 63 axis in epithelial homeostasis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 114, no. 46, pp. 12243–12248, 2017.

[118] G. L. Sen, L. D. Boxer, D. E. Webster et al., "ZNF750 is a p 63 target gene that induces KLF4 to drive terminal epidermal differentiation," *Developmental Cell*, vol. 22, no. 3, pp. 669–677, 2012.

[119] J. J. van den Oord and M. De Ley, "Distribution of metallothionein in normal and pathological human skin," *Archives of Dermatological Research*, vol. 286, no. 1, pp. 62–68, 1994.

[120] K. Hanada, D. Sawamura, I. Hashimoto, K. Kida, and A. Naganuma, "Epidermal proliferation of the skin in metallothionein-null mice," *The Journal of Investigative Dermatology*, vol. 110, no. 3, pp. 259–262, 1998.

[121] A. Zamirska, L. Matusiak, P. Dziegiel, G. Szybejko-Machaj, and J. C. Szepietowski, "Expression of metallothioneins in cutaneous squamous cell carcinoma and actinic keratosis," *Pathology Oncology Research*, vol. 18, no. 4, pp. 849–855, 2012.

Hindawi
Journal of Immunology Research
Volume 2018, Article ID 6872621, 11 pages
https://doi.org/10.1155/2018/6872621

Review Article

The Role of Zinc and Zinc Homeostasis in Macrophage Function

Hong Gao, Wei Dai, Lu Zhao, Junxia Min, and Fudi Wang

The First Affiliated Hospital, School of Public Health, Institute of Translational Medicine, Zhejiang University School of Medicine, Hangzhou 310058, China

Correspondence should be addressed to Lu Zhao; lzhao@zju.edu.cn, Junxia Min; junxiamin@zju.edu.cn, and Fudi Wang; fwang@zju.edu.cn

Received 1 June 2018; Revised 31 August 2018; Accepted 6 November 2018; Published 6 December 2018

Guest Editor: Ananda S. Prasad

Zinc has long been recognized as an essential trace element, playing roles in the growth and development of all living organisms. In recent decades, zinc homeostasis was also found to be important for the innate immune system, especially for maintaining the function of macrophages. It is now generally accepted that dysregulated zinc homeostasis in macrophages causes impaired phagocytosis and an abnormal inflammatory response. However, many questions remain with respect to the mechanisms that underlie these processes, particularly at the cellular and molecular levels. Here, we review our current understanding of the roles that zinc and zinc transporters play in regulating macrophage function.

1. Introduction

A healthy human body usually contains 2–4 grams of zinc [1]. Approximately 60% of the body's zinc is located in the skeletal muscle, 30% in the bone, 5% in the liver and the skin, and the remaining 2–3% in other tissues [2]. Internal zinc homeostasis is regulated by the cooperative activities of two metal transporter protein families. One family consists of ten solute-linked carrier 30 (SLC30 or ZnT) exporters, and the other family consists of fourteen solute-linked carrier 39 (SLC39, also known as Zrt- and Irt-like proteins, or ZIP) importers [3, 4]. The majority of labile zinc in the body is absorbed by intestinal epithelial cells via the metal transporter protein Slc39a4 [5], which is then transported into the plasma and utilized by nearly all cell types in the circulation. To maintain zinc homeostasis, excessive zinc is excreted through the kidneys [6] and the intestine [7] via Slc39a5.

Endogenous zinc is usually present in two forms in various organs and tissues. The majority of zinc is in a fixed pool in which zinc is tightly bound to metalloenzymes and zinc finger transcription factors; the remaining small amount of zinc is in a labile pool consisting of a variable amount of loosely bound zinc and free zinc ions [8]. In mammals, the plasma concentration of zinc ranges from 14 to 23 μmol/l under normal physiological conditions, and serum zinc accounts for only 0.1% of the body's total zinc pool, 80% loosely bound by albumin and 20% bound by macroglobulin [9, 10]. Thus, sufficient daily intake of zinc is required to achieve steady-state levels. In order to meet the daily requirement, the World Health Organization recommends a daily zinc intake of 9.4–10 mg and 6.5–7.1 mg for men and women, respectively [11].

Zinc plays an important role in the immune system and affects both innate and adaptive immune cells. Many studies found that zinc deficiency can lead to a reduced immune response and increased susceptibility to infection [12–16]. Moreover, endogenous zinc levels have been suggested to affect both the number and the function of various types of immune cells, including macrophages, neutrophils, dendritic cells, mast cells, T cells, and B cells [17–24]. The underlying molecular mechanisms have been discussed in previous studies [25, 26], and the importance of zinc as a signaling molecule has been suggested [17, 27].

Macrophages play a key role in innate immunity by regulating numerous homeostatic, developmental, and host defense responses. Moreover, macrophages also participate in a wide range of other biological activities, including modulating endogenous levels of reactive oxygen species [28, 29],

iron homeostasis [30], tissue repair, and metabolic processes [31]. Macrophages have three major functions—phagocytosis, antigen presentation, and immunomodulation—and are essential for maintaining normal immune status under a wide variety of pathophysiological conditions [32]. Many previous studies investigated the relationship between zinc and macrophages [33–37]; however, some studies yielded contradictory results, and the underlying mechanisms are poorly understood. Here, we provide an overview of the latest studies regarding the role of zinc in macrophages.

2. Zinc Homeostasis in Macrophages

The regulation of zinc homeostasis is a complicated process. As a divalent cation, zinc is hydrophilic and does not readily pass lipid-based cell membranes via passive diffusion; thus, specialized transporters are required in order to facilitate its transport in and out of the cytoplasm. In macrophages and many other immune cells, SLC39 and SLC30 family members have distinct expression patterns and have various functions in response to infectious stimuli (Table 1).

Multiple SLC30/SLC39 members are expressed in macrophages. In untreated mouse macrophages, *Slc39a1*, *Slc39a6*, and *Slc39a7* are the most robustly expressed genes in the Slc39a family, whereas the *Slc30a5*, *Slc30a6*, *Slc30a7*, and *Slc30a9* genes are the most robustly expressed genes in the Slc30a family [22], suggesting that these transporters play an important role in macrophages under physiological conditions. However, under pathological conditions, other key transporters are expressed. For example, upon stimulation with lipopolysaccharides (LPS), which are found in the outer membrane of gram-negative bacteria, *Slc39a10* expression is significantly downregulated, whereas *Slc39a14* expression is strongly upregulated [22, 38]. Moreover, Slc39a2, Slc30a4, and Slc30a7 are significantly upregulated in GM-CSF-activated peritoneal and bone marrow-derived macrophages [39].

Several SLC30/39 members have been found to participate in the function of macrophages by mediating zinc homeostasis. Our recent study using macrophage-specific *Slc39a10*-knockout mice revealed that Slc39a10 plays an essential role in p53-dependent macrophage survival following LPS stimulation [22]. Interestingly, the trans-fatty acid elaidate was found to increase the expression of *SLC39A10* and increase intracellular zinc levels in human macrophages [40], which also indicates the importance of Slc39a10 in zinc homeostasis in macrophages. In addition, several studies reported that SLC39A8 plays a role in inflammatory reactions [41, 42]. For example, LPS has been suggested to upregulate the expression of *SLC39A8* in human macrophages, thereby increasing zinc uptake and reducing proinflammatory pathways by inhibiting I$\kappa\beta$ kinase (IKK) [41] and IL-10 [42]. Furthermore, SLC39A14 was also found to be upregulated in response to LPS stimulation in macrophages, thereby regulating cytokine production [38]. Moreover, systemic inflammation in mice resulted in the IL-6-dependent upregulation of the zinc importer Slc39a14, which mediates zinc uptake by hepatocytes in the liver [43]. Although previous studies summarized above suggest functions of Slc39a8, Slc39a10, and Slc39a14 in macrophages, potential roles of other SLC39/30 transporters in macrophages [22, 44–48] remain to be explored.

Recently, a growing body of evidence supports the notion that zinc transporters transport not only zinc but also other divalent metals, including iron and manganese; for example, both SLC39A8 and SLC39A14 have been associated with iron and manganese transport [49–55]. These findings raise the question of whether other SLC30/39 family members are involved in the development and functions of macrophage through mediating the homeostasis of other metals, such as iron or manganese.

In addition to the two zinc transporter families, intracellular zinc levels are also regulated by metallothioneins (MTs). Because of its toxicity, intracellular labile zinc is generally present in extremely low levels. Laurin et al. reported that adding zinc to the culture medium increased the rate of MT degradation and decreased the rate of MT synthesis and accretion in a chicken macrophage cell line [56].

Several groups reported that MTs play a role in macrophage function. MT-I/II-knockout mice developed more severe brain injury accompanied by increased numbers of T cells in the injury site and circulating leukocytes and the decreased number of alternatively activated macrophages in the circulation after 7-day treatment with brain cryolesion. These observations indicate that MT-I/II may have a neuroprotective role via modulation of the immune response [57]. Besides, Zbinden et al. measured increased numbers of macrophages in the ischemic hind limb of MT-deficient mice 21 days after ischemia was induced; moreover, CD11b+ macrophages isolated from MT-deficient mice were more invasive, which indicates that MT plays an important role in the recovery of collateral flow and angiogenesis, an effect mediated partly by macrophages [58]. In addition, in *Salmonella typhimurium*-infected human monocyte-derived macrophages, NOD2 mediates the induction of MT via NF-κB- and caspase-1-mediated IL-1β secretion. Moreover, the elevated MT level was found to upregulate intracellular zinc in a MTF-1-dependent manner. However, the underlying mechanism remains unclear [59]. Furthermore, during alternative activation of macrophages, IL-4 increases intracellular zinc dependence on metallothionein-3 (MT-3) and Slc30a4 and weakens the antimicrobial defense against intracellular pathogens [60]. In addition, matrix metallothionein 7 (MMP7) cleaves the precursor forms of α-defensin and β-defensin to produce their respective active forms [61], and MMP12 destroys the pathogen's cell wall, leading to cell death [62]. In summary, a wide range of MTs are involved in maintaining macrophage function during the immune response.

3. Zinc and the Macrophage Cell Fate

Zinc homeostasis determines the cell fate of macrophages. In the innate immune system, monocytes migrate into the infected tissue and then differentiate into macrophages. Zinc supplementation increases the number of peritoneal macrophages in a *T. cruzi* infection model [63]. In addition, zinc-

Table 1: Summary of the immune cell expression and infection-related findings of SLC30 and SLC39 transporters based on previous literature.

(a)

Importer proteins	Expression in macrophages	Expression in other immune cells	Infection-related findings
Slc39a1	Strong expression in the plasma membrane and cytoplasm in THP1-derived macrophages [44]	Expressed in murine T cells [114]	HIV-1 stimulated Slc39a1 expression in alveolar macrophages [115]
Slc39a2	THP1 macrophages: weak expression mainly in nucleoli; TPEN significantly increases Slc39a2 expression Alveolar macrophages: strong expression in the plasma membrane and cytoplasm [44]	No expression in human monocytes or in granulocytes [46]; moderated expression in murine DCs [116]	Unknown
Slc39a3	Strong expression in human monocytes [46]	Expressed in human T cells and granulocytes [46]	Unknown
Slc39a4	Expressed in alveolar macrophages [117]	Uniform expression in human monocytes and in granulocytes [46]	Chronic alcohol exposure decreases Slc39a4 expression in alveolar macrophages [117]
Slc39a5	Unknown	No expression in human monocytes or in granulocytes [46]	Unknown
Slc39a6	Strong expression in murine macrophages [22]	Expressed in human DCs and T cells [20]	LPS decreases the expression of Slc39a6 in human DCs; Slc39a6-silenced macrophages have increased TNFα expression following LPS stimulation [20]
Slc39a7	Strong expression in murine macrophages [22], which can be inhibited by TPEN [45]	Expressed in murine T cells [114]	Unknown
Slc39a8	Strong expression in both human and murine macrophages	Strong expression in human T cells [21]	Both TNFα and LPS upregulate Slc39a8 expression in human macrophages, which increases zinc uptake and directly inhibits IKKβ [41] and IL-10 [42]
Slc39a9	Unknown	Expressed in murine T cells [114]	Unknown
Slc39a10	Strong expression in murine macrophages	Expressed in murine early B cells [23] and T cells [114]	Slc39a10$^{fl/fl}$; LysMCre+ mice have significantly decreased LPS-induced mortality due to increased macrophage apoptosis mediated by zinc-p53 signaling [22]
Slc39a11	Unknown	Expressed in murine T cells [114]	Unknown
Slc39a12	Unknown	Expressed in murine T cells, expression is increased by zinc deficiency [114]	Unknown
Slc39a13	Unknown	Unknown	Unknown
Slc39a14	Expressed in alveolar macrophages, expression is decreased by TPEN [44]	Expressed in leukocytes; Slc39a14-knockout mice have delayed leukocytosis [118]	LPS upregulates Slc39a14 expression and downregulates NF-κB in human macrophages [38]; Slc39a14-knockout mice have impaired zinc uptake and decreased plasma zinc and IL-6 levels following LPS stimulation [119]

(b)

Exporter proteins	Expression in macrophages	Expression in other immune cells	Infection-related findings
Slc30a1	Expressed in alveolar macrophages, expression is decreased by TPEN [44]	Expressed in murine DCs, expression is upregulated by LPS [18]	*M. tuberculosis* infection upregulates Slc30a1 expression in human macrophages [111]

Table 1: Continued.

Exporter proteins	Expression in macrophages	Expression in other immune cells	Infection-related findings
Slc30a2	Weak expression in macrophages in the nulliparous mammary gland [47]; increased expression in murine macrophages during infection [39]	No expression in human monocytes or granulocytes [46]	Unknown
Slc30a3	Expressed in alveolar macrophages, expression is decreased by TPEN [44]	Expressed at low levels in human peripheral blood lymphocytes [48]	Unknown
Slc30a4	Unknown	Expressed in murine DCs, expression is upregulated by LPS [18]; highly expressed in the human Molt-4 T cell line [48]	GM-CSF upregulate Slc30a4 expression to transport zinc into Golgi [39]
Slc30a5	Expressed in alveolar macrophages, expression is decreased by TPEN [44]	Expressed in murine mast cells and required for the mast cell-mediated delayed-type allergic response [19]	Unknown
Slc30a6	Expressed in THP-1 monocytes, expression is upregulated by zinc deficiency [48]	Expressed in murine DCs, expression is upregulated by LPS [18]	Unknown
Slc30a7	Expressed in THP-2 monocyte, expression is upregulated by zinc deficiency [48]	Expressed in human B lymphocytes with the target molecule CD40 [120]	GM-CSF upregulates Slc30a7 expression, leading to increased zinc transport into the Golgi apparatus [39]
Slc30a8	Unknown	Expressed in human peripheral blood lymphocytes [48]	May function as an autoantigen targeted by disease-associated autoreactive T cells in humans [121]
Slc30a9	Strong expression in murine macrophages [22]	Expressed at low levels in human circulating blood lymphocytes [48]; expressed in murine T cells, expression is decreased by zinc deficiency [114]	Unknown
Slc30a10	Unknown	Unknown	Unknown

DCs: dendritic cells; GM-CSF: granulocyte-macrophage colony-stimulating factor; IL: interleukin; LPS: lipopolysaccharides; TPEN: N,N,N′,N′-tetrakis(2-pyridylmethyl)-ethylenediamine (a membrane-permeable zinc chelator).

depleted monocytes have increased maturation, suggesting that low zinc status promotes their differentiation into macrophages [64]. High concentrations of zinc were found to decrease the viability of a human monocyte cell line and U-937 cells [65]. Moreover, another study confirmed that cell viability is significantly decreased in THP-1 monocytes/macrophages upon exposure to 100 μg/ml of ZnO (zinc oxide) particles. However, ZnO nanoparticles were found to induce the migration, adhesion, and cholesterol uptake of monocytes/macrophages, which may accelerate the formation of foam cells and lead to atherosclerosis [66]. Furthermore, a low-zinc environment can inhibit the differentiation of HL-60 cells into macrophages, and this inhibition can be partially prevented by the addition of exogenous zinc [67]. As in other cell types, both zinc deficiency and excessive zinc can induce apoptosis in macrophages. For example, using a genetic mouse model, we recently found that loss of Slc39a10 reduces zinc levels in macrophages, resulting in p53-dependent apoptosis, but not necroptosis, pyroptosis, ferroptosis, or autophagy [22]. On the other hand, zinc oxide nanoparticles have been shown to induce necrosis and apoptosis in RAW264.7 cells [68–70]. These results suggest that altered zinc homeostasis induces distinct forms of cell death under different circumstances.

4. Zinc and Macrophage Function

Innate immunity provides a rapid, nonspecific defense against pathogens and is activated by pathogen-associated molecular patterns (PAMPs). During this process, conserved structures in pathogens are recognized by their respective receptors, including Toll-like receptors (TLRs), which then trigger phagocytosis, cytokine secretion, the killing of target cells, and/or antigen presentation [71]. Monocytes/macrophages mediate host defense via phagocytosis and oxidative burst. In addition, these cells can serve as antigen-presenting cells (APCs) and can secrete proinflammatory cytokines in order to regulate the immune response [72, 73]. Zinc plays a critical role in the immune function of macrophages, and this function has been implicated in a variety of pathological processes, including decreased connective tissue contraction [34].

4.1. Zinc and Phagocytosis by Macrophages. The level of intracellular zinc influences the phagocytosis capacity of macrophages, and zinc was recently linked to the antimicrobial response in macrophages [33]. In chronic obstructive pulmonary disease (COPD), impaired efferocytosis (i.e., clearance) of apoptotic epithelial cells by alveolar

macrophages is mediated primarily by zinc restriction [44]. The transporters Slc39a1 and Slc39a2 respond differently to zinc deficiency and play important roles in macrophage-mediated efferocytosis [44]. On the other hand, zinc does not affect the phagocytic function of RAW264.7 cells [74] or bone marrow-derived macrophages [22] at nontoxic concentrations. Interestingly, a recent study by Mehta et al. found that alcohol abuse is associated with significant zinc deficiency in alveolar macrophages, which is accompanied by impaired immune function due to decreased phagocytosis-mediated bacterial clearance [75]. The authors also found that treating alveolar macrophages with zinc significantly improved their phagocytic capacity [75]. An earlier study by Wirth et al. found that zinc deficiency impairs the uptake and survival of protozoan parasites [76]. Zinc supplementation was also found to increase the phagocytosis of *E. coli* and *Staphylococcus aureus* by peritoneal macrophages in a mouse model of polymicrobial sepsis. Notably, Sheikh et al. reported that zinc deficiency decreases the phagocytic capacity of monocytes in children with enterotoxigenic *E. coli*-induced diarrhea, whereas treating patients with zinc (20 mg/day) or dietary zinc supplementation (10 mg/day) slightly improved the monocytes' phagocytic capacity and significantly decreased their cellular oxidative burst capacity [77]. From a clinical perspective, these effects of zinc supplementation with respect to alleviating symptoms in zinc-deficient children are highly encouraging.

4.2. Zinc and Oxidative Burst in Macrophages. The relationship between zinc and the level of oxidative burst in macrophages after bacterial infection is controversial. Mayer et al. reduced zinc concentrations in peripheral blood mononuclear cells—which include monocytes—either by treating the cells with TPEN (N,N,N′,N′-tetrakis(2-pyridylmethyl)-ethylenediamine) or by removing zinc from the culture medium using the chelator Chelex 100. They found that the level of oxidative burst was significantly increased in zinc-deficient macrophages following infection with gram-positive *S. aureus* [73]. In addition, zinc is an inhibitor of NADPH, which is the electron donor for catalyzing the production of O_2^- [78]. On the other hand, Srinivas et al. found that macrophages obtained from *E. coli*-infected rats released significantly higher amounts of superoxide and that *in vivo* superoxide production was increased by zinc supplementation; nevertheless, they also found that zinc supplementation *in vitro* inhibited the production of superoxide by macrophages harvested from septic rats [79].

4.3. Zinc and Inflammatory Signaling in Macrophages. Zinc also plays essential roles in the signaling and inflammatory output of monocytes and macrophages, including many upstream activators of the Toll-like receptor (TLR) family, including mitogen-activated protein kinase (MAPK), protein kinase C (PKC), phosphodiesterases, and NF-κB [36, 37]. Indeed, the relationship between zinc and inflammatory signaling in monocytes/macrophages relies primarily on TLR signaling (e.g., via TLR4), which is activated by the phosphorylation of interleukin-1 receptor-associated kinase 1 (IRAK1). Zinc is known to be required for the degradation of IRAK1 in LPS-stimulated TLR activation both *in vitro* and *in vivo*; however, zinc is not required for the phosphorylation or ubiquitylation of IRAK1 in macrophages [80]. Nevertheless, zinc has been found to mediate the degradation of procaspase-1 and the NLRP3 (NLR family, pyrin domain containing 3), as well as to inhibit the production of IL-1β in macrophages following LPS stimulation or *Salmonella* infection. This effect may compromise the cell's ability to clear microbial pathogens [45].

TLR4 signaling occurs via MyD88-dependent and TRIF-dependent pathways, and zinc has opposing effects on these two signaling pathways. Upon LPS stimulation, TLR4 first binds to the adapter proteins TIRAP and MyD88, which triggers the phosphorylation of MAP kinases and the early activation of NF-κB. Zinc signaling is required for preventing the dephosphorylation of the MAP kinases p38, MEK1/2, and ERK1/2, as well as the activation of NF-κB. Thus, zinc increases the release of inflammatory cytokines such as TNF-α, IL-1β, and IL-6 [81, 82]. Subsequently, the receptor complex is internalized and binds to TRAM and TRIF, inducing the delayed activation of NF-κB and the phosphorylation of IRF3. Phosphorylated IRF3 then translocated to the nucleus, where it induces the transcription of IFN-β [82, 83]. However, zinc can inhibit the phosphorylation of IRF3 and can prevent the secretion of IFN-β [82]. Moreover, zinc supplementation could downregulate inflammatory cytokines through upregulation of A20 to inhibit NF-κB activation [78, 84].

Zinc deficiency has diverse effects on inflammation. Zinc deficiency over the long term reduces the integrity of lysosomes, activates the NLRP3 inflammasome, and induces IL-1β secretion in macrophages [85], while in the short term, zinc depletion by TPEN inhibits inflammatory activation [86]. Moreover, without adequate zinc, an inflammatory response can also be elicited in cells, in part by causing the aberrant activation of immune cells and/or by altering promoter methylation [87]. In addition, a recent study found that zinc deficiency reduces the production of IL-6 and TNF-α in human monocytes [73]. Finally, zinc modulates LPS-induced inflammation in human macrophages by inducing SLC39A8 and by inhibiting C/EBPβ [42].

ZnO nanoparticles also affect the innate immune process. For example, ZnO nanoparticles have been shown to reduce bacterial skin infection by inducing oxidative stress and causing cell membrane breakdown in macrophages [88], as well as by reducing the innate immune response and attenuating the macrophage responses to bacterial infection [89]. In contrast, ZnO nanoparticles have been shown to induce a proinflammatory response in the RAW264.7 macrophage cell line [66, 90] and in peritoneal macrophages via TLR6-mediated MAPK signaling [91]. These seemingly contradictory results may be due—at least in part—to the different concentrations of nanoparticles and/or cell types used in the different studies.

Taken together, the evidence to date suggests that zinc regulates the function of macrophages in a variety of ways. For example, zinc deficiency induces the abnormal secretion of immune factors via distinct pathways in response to specific infections. In addition, oxidative stress caused by altered

levels of zinc can lead to dysfunction of the innate immune system during acute inflammation.

5. Zinc and Macrophage-Related Diseases

According to a 2002 report by the World Health Organization, zinc deficiency ranks fifth among the most important health risk factors in developing countries and eleventh worldwide [92]; moreover, abnormal zinc homeostasis causes a variety of health problems with various levels of severity. In addition to the immune system, other organs and systems can also be affected by changes in zinc.

5.1. Immunological Diseases. The relationship between zinc and rheumatoid arthritis (RA) has been studied for more than three decades. RA is a chronic systemic inflammatory disease characterized by inflammation of the synovial membrane and the progressive destruction of the articular cartilage and bone [93]. Importantly, the number and activation level of macrophages in the inflamed synovial membrane/pannus are correlated with the severity of RA. A recent meta-analysis of 1444 RA cases and 1241 healthy controls revealed that patients with RA often have decreased serum zinc levels [94]. Correspondingly, the mean level of zinc was significantly lower in hair samples of RA patients compared with healthy individuals [95]. These clinical observations are supported by *in vitro* studies. For example, zinc deficiency increases the levels of TNF-α, IL-1β, and IL-8 in a monocyte-macrophage cell line [96]. In contrast, zinc supplementation inhibits the LPS-induced release of TNF-α and IL-1β in monocytes [97].

Chronic alcoholism can increase the risk of pneumonia and the development of acute respiratory distress syndrome (ARDS) [98]. As the resident bona fide phagocytic cell type in the lungs, alveolar macrophages play a central role in maintaining alveolar homeostasis, lung host defense, and immune regulation [99]. Several groups have studied the relationship between zinc levels and macrophage function in the alveolar space. For example, Mehta et al. found that alcohol-fed rats have a 5-fold decrease in lung bacterial clearance compared to control-fed rats and providing dietary zinc supplementation to the alcohol-fed rats restored bacterial clearance and mitigated oxidative stress in the alveolar space, which was reflected by the relative balance between the thiol redox pair cysteine and cystine and by the increased nuclear binding of both PU.1 and Nrf2 in alveolar macrophages obtained from alcohol-fed rats [90, 100]. Similarly, Konomi et al. found that during pregnancy, intracellular zinc levels and the expression levels of the zinc transporters Zip1, ZnT1, and ZnT4 are decreased in alveolar macrophages after ethanol ingestion compared to control rats that did not ingest alcohol. In addition, bacterial clearance capacity was decreased in ethanol-treated alveolar macrophages, and the addition of zinc reversed these effects *in vitro* [101]. Furthermore, pulmonary zinc deficiency may be one of the mechanisms by which HIV-1 infection impairs alveolar macrophage immune function and renders infected individuals susceptible to severe pulmonary infection [102].

5.2. Nonimmunological Diseases. Evidence suggested that chronic inflammation that originated in the liver or adipose tissue plays an important role in the pathogenesis of obesity-related metabolic dysfunction [103]. In obese mice, zinc deficiency may increase leptin production and stimulate macrophage infiltration into the adipose tissue, suggesting that zinc is important in metabolic and macrophage-mediated inflammatory dysregulation in obesity [104]. Based on its anti-inflammatory and antioxidant functions, zinc also plays a protective role in atherosclerosis [105]. However, zinc deficiency does not appear to affect the uptake of low-density lipoprotein (LDL) by macrophages *in vitro* [106]. Interestingly, another study found that ZnO nanoparticles can induce the migration and adhesion of monocytes to endothelial cells and accelerate the formation of foam cells [107].

5.3. Pathogen Infection. A sufficient amount of zinc is essential for the host's defense against pathogenic organisms. For example, in both human monocyte-derived macrophages and mouse macrophages, increased intracellular zinc levels induced by the continuous stimulation of pattern recognition receptors (PRRs) can increase the clearance of bacteria via autophagy [59]. Moreover, treating mice with zinc and/or all-*trans* retinoic acid supplements helps protect against infection by the pathogen *Listeria monocytogenes* [108].

Interestingly, zinc is not only required by host cells but is also required for invading pathogens. According to the "nutritional immunity" theory, specific essential elements are sequestered from pathogens in order to restrict their growth [109, 110]. Zinc chelation was shown to restrict the growth of certain pathogens, for example, *Histoplasma capsulatum* [64]. A previous study found that zinc deprivation may be a defense mechanism utilized by the host's macrophages [35]. Moreover, when stimulated with granulocyte macrophage-colony stimulating factor (GM-CSF), macrophages infected with *Histoplasma capsulatum* sequester zinc by inducing zinc binding to metallothionein (MT) proteins [39]. In addition, human macrophages attack intracellular *Mycobacterium tuberculosis* pathogens by inducing a "burst of labile zinc" and by increasing the expression of the zinc-binding proteins MT1, MT2, and ZnT1 [111], as well as possibly releasing zinc stored in zincosomes [112]. Macrophages can also use a "zinc trap" [113] to kill pathogens; this mechanism may be impaired when intracellular zinc is either too high or too low.

6. Conclusions and Future Perspectives

The vital role that the micronutrient zinc plays in both health and disease has been known for many years. Regular intake of zinc and the coordinated function of zinc transporters are essential for maintaining zinc homeostasis and for maintaining health. With respect to innate immunity, the various functions of macrophages, which include phagocytosis and the secretion of immune-mediating factors, can be impaired by zinc imbalance, thereby inducing or exacerbating various inflammatory and/or disease processes, as illustrated in Figure 1.

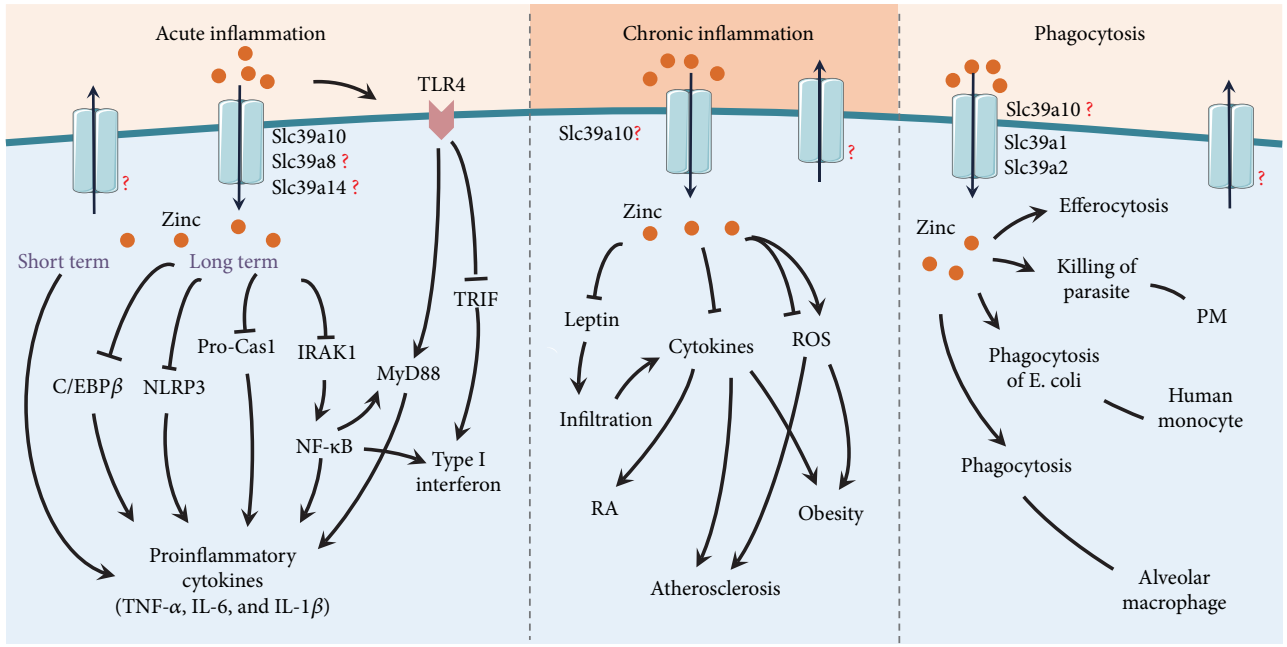


FIGURE 1: Schematic model depicting the putative roles that zinc plays in macrophages during acute inflammation, chronic inflammation, and phagocytosis. BMDM: bone marrow-derived macrophage; PM: peritoneal macrophage; RA: rheumatoid arthritis; ROS: reactive oxygen species; TRIF: Toll/IL-1R domain-containing adapter inducing IFN-β.

Despite extensive research, the molecular mechanisms by which zinc regulates the fate and function of macrophages remain poorly understood. Similarly, the function of zinc transporters is largely uninvestigated. In some cases, particularly when accompanied by a defect in a zinc transporter, oral zinc supplementation or restriction may not be sufficient for preventing diseases caused by cellular zinc imbalance; therefore, molecular approaches are needed in order to develop innovative new therapeutic approaches to correct the underlying defect. Given the development of powerful gene editing tools, the genetic manipulation of zinc transporters can be performed in various model systems, and research based on these models will likely shed light on the molecular function of these zinc transporters, as well as the mechanism of zinc in macrophages, ultimately guiding the treatment and prevention of zinc-related diseases.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Hong Gao and Wei Dai contributed equally to this work.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (31330036 and 31530034 to F. W.). We thank the members of the Wang and Min laboratories for helpful discussions.

References

[1] M. Maywald and L. Rink, "Zinc homeostasis and immunosenescence," *Journal of Trace Elements in Medicine and Biology*, vol. 29, pp. 24–30, 2015.

[2] T. Kambe, T. Tsuji, A. Hashimoto, and N. Itsumura, "The physiological, biochemical, and molecular roles of zinc transporters in zinc homeostasis and metabolism," *Physiological Reviews*, vol. 95, no. 3, pp. 749–784, 2015.

[3] L. Huang and S. Tepaamorndech, "The SLC30 family of zinc transporters - a review of current understanding of their biological and pathophysiological roles," *Molecular Aspects of Medicine*, vol. 34, no. 2-3, pp. 548–560, 2013.

[4] J. Jeong and D. J. Eide, "The SLC39 family of zinc transporters," *Molecular Aspects of Medicine*, vol. 34, no. 2-3, pp. 612–619, 2013.

[5] J. Geiser, K. J. T. Venken, R. C. de Lisle, and G. K. Andrews, "A mouse model of acrodermatitis enteropathica: loss of intestine zinc transporter ZIP4 (Slc39a4) disrupts the stem cell niche and intestine integrity," *PLoS Genetics*, vol. 8, no. 6, article e1002766, 2012.

[6] Q. Qin, X. Wang, and B. Zhou, "Functional studies of *Drosophila* zinc transporters reveal the mechanism for dietary zinc absorption and regulation," *BMC Biology*, vol. 11, no. 1, p. 101, 2013.

[7] J. Geiser, R. C. De Lisle, and G. K. Andrews, "The zinc transporter Zip5 (Slc39a5) regulates intestinal zinc excretion and protects the pancreas against zinc toxicity," *PLoS One*, vol. 8, no. 11, article e82149, 2013.

[8] C. J. Frederickson, "Neurobiology of zinc and zinc-containing neurons," *International Review of Neurobiology*, vol. 31, pp. 145–238, 1989.

[9] J. P. Barnett, C. A. Blindauer, O. Kassaar et al., "Allosteric modulation of zinc speciation by fatty acids," *Biochimica et*

Biophysica Acta (BBA) - General Subjects, vol. 1830, no. 12, pp. 5456–5464, 2013.

[10] J. G. Reyes, "Zinc transport in mammalian cells," *American Journal of Physiology*, vol. 270, no. 2, pp. C401–C410, 1996.

[11] W. Maret and H. H. Sandstead, "Zinc requirements and the risks and benefits of zinc supplementation," *Journal of Trace Elements in Medicine and Biology*, vol. 20, no. 1, pp. 3–18, 2006.

[12] A. H. Shankar and A. S. Prasad, "Zinc and immune function: the biological basis of altered resistance to infection," *American Journal of Clinical Nutrition*, vol. 68, no. 2, pp. 447s–463s, 1998.

[13] H. Haase and L. Rink, "The immune system and the impact of zinc during aging," *Immunity & Ageing*, vol. 6, no. 1, p. 9, 2009.

[14] P. J. Fraker and L. E. King, "Reprogramming of the immune system during zinc deficiency," *Annual Review of Nutrition*, vol. 24, no. 1, pp. 277–298, 2004.

[15] L. Rink, "Zinc and the immune system," *The Proceedings of the Nutrition Society*, vol. 59, no. 4, pp. 541–552, 2000.

[16] K. S. Vignesh, J. A. Landero Figueroa, A. Porollo, J. A. Caruso, and G. S. Deepe, "Zinc sequestration: arming phagocyte defense against fungal attack," *PLoS Pathogens*, vol. 9, no. 12, article e1003815, 2013.

[17] H. Haase and L. Rink, "Zinc signals and immune function," *BioFactors*, vol. 40, no. 1, pp. 27–40, 2014.

[18] H. Kitamura, H. Morikawa, H. Kamon et al., "Toll-like receptor-mediated regulation of zinc homeostasis influences dendritic cell function," *Nature Immunology*, vol. 7, no. 9, pp. 971–977, 2006.

[19] K. Nishida, A. Hasegawa, S. Nakae et al., "Zinc transporter Znt5/Slc30a5 is required for the mast cell-mediated delayed-type allergic reaction but not the immediate-type reaction," *The Journal of Experimental Medicine*, vol. 206, no. 6, pp. 1351–1364, 2009.

[20] M. Yu, W. W. Lee, D. Tomar et al., "Regulation of T cell receptor signaling by activation-induced zinc influx," *The Journal of Experimental Medicine*, vol. 208, no. 4, pp. 775–785, 2011.

[21] T. B. Aydemir, J. P. Liuzzi, S. McClellan, and R. J. Cousins, "Zinc transporter ZIP8 (SLC39A8) and zinc influence IFN-γ expression in activated human T cells," *Journal of Leukocyte Biology*, vol. 86, no. 2, pp. 337–348, 2009.

[22] H. Gao, L. Zhao, H. Wang et al., "Metal transporter Slc39a10 regulates susceptibility to inflammatory stimuli by controlling macrophage survival," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 114, no. 49, pp. 12940–12945, 2017.

[23] T. Miyai, S. Hojyo, T. Ikawa et al., "Zinc transporter SLC39A10/ZIP10 facilitates antiapoptotic signaling during early B-cell development," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 32, pp. 11780–11785, 2014.

[24] S. Hojyo, T. Miyai, H. Fujishiro et al., "Zinc transporter SLC39A10/ZIP10 controls humoral immunity by modulating B-cell receptor signal strength," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 32, pp. 11786–11791, 2014.

[25] V. von Bulow, S. Dubben, G. Engelhardt et al., "Zinc-dependent suppression of TNF-α production is mediated by protein kinase a-induced inhibition of Raf-1, IκB Kinase β, and NF-κB," *Journal of Immunology*, vol. 179, no. 6, pp. 4180–4186, 2007.

[26] A. S. Prasad, B. Bao, F. W. J. Beck, and F. H. Sarkar, "Zinc activates NF-κB in HUT-78 cells," *The Journal of Laboratory and Clinical Medicine*, vol. 138, no. 4, pp. 250–256, 2001.

[27] H. Haase, J. L. Ober-Blobaum, G. Engelhardt et al., "Zinc signals are essential for lipopolysaccharide-induced signal transduction in monocytes," *Journal of Immunology*, vol. 181, no. 9, pp. 6491–6502, 2008.

[28] Y. Zhang, S. Choksi, K. Chen, Y. Pobezinskaya, I. Linnoila, and Z. G. Liu, "ROS play a critical role in the differentiation of alternatively activated macrophages and the occurrence of tumor-associated macrophages," *Cell Research*, vol. 23, no. 7, pp. 898–914, 2013.

[29] A. P. West, I. E. Brodsky, C. Rahner et al., "TLR signalling augments macrophage bactericidal activity through mitochondrial ROS," *Nature*, vol. 472, no. 7344, pp. 476–480, 2011.

[30] M. Jung, C. Mertens, and B. Brune, "Macrophage iron homeostasis and polarization in the context of cancer," *Immunobiology*, vol. 220, no. 2, pp. 295–304, 2015.

[31] F. Ginhoux and S. Jung, "Monocytes and macrophages: developmental pathways and tissue homeostasis," *Nature Reviews Immunology*, vol. 14, no. 6, pp. 392–404, 2014.

[32] T. A. Wynn, A. Chawla, and J. W. Pollard, "Macrophage biology in development, homeostasis and disease," *Nature*, vol. 496, no. 7446, pp. 445–455, 2013.

[33] S. L. Stafford, N. J. Bokil, M. E. S. Achard et al., "Metal ions in macrophage antimicrobial pathways: emerging roles for zinc and copper," *Bioscience Reports*, vol. 33, no. 4, pp. 541–554, 2013.

[34] J. E. Nowak, K. Harmon, C. C. Caldwell, and H. R. Wong, "Prophylactic zinc supplementation reduces bacterial load and improves survival in a murine model of sepsis," *Pediatric Critical Care Medicine*, vol. 13, no. 5, pp. e323–e329, 2012.

[35] M. S. Winters, Q. Chan, J. A. Caruso, and G. S. Deepe, Jr, "Metallomic analysis of macrophages infected with Histoplasma capsulatum reveals a fundamental role for zinc in host defenses," *The Journal of Infectious Diseases*, vol. 202, no. 7, pp. 1136–1145, 2010.

[36] H. Haase and L. Rink, "Signal transduction in monocytes: the role of zinc ions," *Biometals*, vol. 20, no. 3-4, pp. 579–585, 2007.

[37] H. Haase and L. Rink, "Functional significance of zinc-related signaling pathways in immune cells," *Annual Review of Nutrition*, vol. 29, no. 1, pp. 133–152, 2009.

[38] A. Sayadi, A. T. Nguyen, F. A. Bard, and E. A. Bard-Chapeau, "Zip14 expression induced by lipopolysaccharides in macrophages attenuates inflammatory response," *Inflammation Research*, vol. 62, no. 2, pp. 133–143, 2013.

[39] K. Subramanian Vignesh, J. A. Landero Figueroa, A. Porollo, J. A. Caruso, and G. S. Deepe Jr., "Granulocyte macrophage-colony stimulating factor induced Zn sequestration enhances macrophage superoxide and limits intracellular pathogen survival," *Immunity*, vol. 39, no. 4, pp. 697–710, 2013.

[40] J. R. Zacherl, I. Tourkova, C. M. St. Croix et al., "Elaidate, an 18-carbon trans-monoenoic fatty acid, but not physiological fatty acids increases intracellular Zn(2+) in human macrophages," *Journal of Cellular Biochemistry*, vol. 116, no. 4, pp. 524–532, 2015.

[41] M. J. Liu, S. Bao, M. Gálvez-Peralta et al., "ZIP8 regulates host defense through zinc-Mediated inhibition of NF-κB," *Cell Reports*, vol. 3, no. 2, pp. 386–400, 2013.

[42] C. J. Pyle, S. Akhter, S. Bao, C. E. Dodd, L. S. Schlesinger, and D. L. Knoell, "Zinc modulates endotoxin-induced human macrophage inflammation through ZIP8 induction and C/EBPβ inhibition," *PLoS One*, vol. 12, no. 1, article e0169531, 2017.

[43] J. P. Liuzzi, L. A. Lichten, S. Rivera et al., "Interleukin-6 regulates the zinc transporter Zip14 in liver and contributes to the hypozincemia of the acute-phase response," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 19, pp. 6843–6848, 2005.

[44] R. Hamon, C. C. Homan, H. B. Tran et al., "Zinc and zinc transporters in macrophages and their roles in efferocytosis in COPD," *PLoS One*, vol. 9, no. 10, article e110056, 2014.

[45] M. Muroi and K. Tanamoto, "Zinc- and oxidative property-dependent degradation of pro-caspase-1 and NLRP3 by ziram in mouse macrophages," *Toxicology Letters*, vol. 235, no. 3, pp. 199–205, 2015.

[46] T. B. Aydemir, R. K. Blanchard, and R. J. Cousins, "Zinc supplementation of young men alters metallothionein, zinc transporter, and cytokine gene expression in leukocyte populations," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 6, pp. 1699–1704, 2006.

[47] S. Lee, S. R. Hennigar, S. Alam, K. Nishida, and S. L. Kelleher, "Essential role for zinc transporter 2 (ZnT2)-mediated zinc transport in mammary gland development and function during lactation," *The Journal of Biological Chemistry*, vol. 290, no. 21, pp. 13064–13078, 2015.

[48] S. Overbeck, P. Uciechowski, M. L. Ackland, D. Ford, and L. Rink, "Intracellular zinc homeostasis in leukocyte subsets is regulated by different expression of zinc exporters ZnT-1 to ZnT-9," *Journal of Leukocyte Biology*, vol. 83, no. 2, pp. 368–380, 2008.

[49] K. Tuschl, E. Meyer, L. E. Valdivia et al., "Mutations in *SLC39A14* disrupt manganese homeostasis and cause childhood-onset parkinsonism-dystonia," *Nature Communications*, vol. 7, article 11601, 2016.

[50] J. H. Park, M. Hogrebe, M. Grüneberg et al., "SLC39A8 deficiency: a disorder of manganese transport and glycosylation," *American Journal of Human Genetics*, vol. 97, no. 6, pp. 894–903, 2015.

[51] K. M. Boycott, C. L. Beaulieu, K. D. Kernohan et al., "Autosomal-recessive intellectual disability with cerebellar atrophy syndrome caused by mutation of the manganese and zinc transporter gene *SLC39A8*," *American Journal of Human Genetics*, vol. 97, no. 6, pp. 886–893, 2015.

[52] Y. Xin, H. Gao, J. Wang et al., "Manganese transporter Slc39a14 deficiency revealed its key role in maintaining manganese homeostasis in mice," *Cell Discovery*, vol. 3, article 17025, 2017.

[53] X. Huang, L. Li, W. Junhao, J. Min, and F. Wang, "Functional discoveries and mechanistic studies of manganese transporters," *Chinese Bulletin of Life Sciences*, vol. 30, pp. 603–614, 2018.

[54] C. Y. Wang, S. Jenkitkasemwong, S. Duarte et al., "ZIP8 is an iron and zinc transporter whose cell-surface expression is upregulated by cellular iron loading," *The Journal of Biological Chemistry*, vol. 287, no. 41, pp. 34032–34043, 2012.

[55] S. Jenkitkasemwong, C. Y. Wang, R. Coffey et al., "SLC39A14 is required for the development of hepatocellular iron overload in murine models of hereditary hemochromatosis," *Cell Metabolism*, vol. 22, no. 1, pp. 138–150, 2015.

[56] D. E. Laurin, D. M. Barnes, and K. C. Klasing, "Rates of metallothionein synthesis, degradation and accretion in a chicken macrophage cell line," *Proceedings of the Society for Experimental Biology and Medicine*, vol. 194, no. 2, pp. 157–164, 1990.

[57] M. W. Pankhurst, W. Bennett, M. T. K. Kirkcaldie, A. K. West, and R. S. Chung, "Increased circulating leukocyte numbers and altered macrophage phenotype correlate with the altered immune response to brain injury in metallothionein (MT)-I/II null mutant mice," *Journal of Neuroinflammation*, vol. 8, no. 1, p. 172, 2011.

[58] S. Zbinden, J. Wang, R. Adenika et al., "Metallothionein enhances angiogenesis and arteriogenesis by modulating smooth muscle cell and macrophage function," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 30, no. 3, pp. 477–482, 2010.

[59] A. Lahiri and C. Abraham, "Activation of pattern recognition receptors up-regulates metallothioneins, thereby increasing intracellular accumulation of zinc, autophagy, and bacterial clearance by macrophages," *Gastroenterology*, vol. 147, no. 4, pp. 835–846, 2014.

[60] K. Subramanian Vignesh, J. A. Landero Figueroa, A. Porollo, S. Divanovic, J. A. Caruso, and G. S. Deepe Jr., "IL-4 induces metallothionein 3- and SLC30A4-dependent increase in intracellular Zn^{2+} that promotes pathogen persistence in macrophages," *Cell Reports*, vol. 16, no. 12, pp. 3232–3246, 2016.

[61] C. L. Wilson, A. P. Schmidt, E. Pirila et al., "Differential processing of α- and β-defensin precursors by matrix metalloproteinase-7 (MMP-7)," *Journal of Biological Chemistry*, vol. 284, no. 13, pp. 8301–8311, 2009.

[62] A. M. G. Houghton, W. O. Hartzell, C. S. Robbins, F. X. Gomis-Rüth, and S. D. Shapiro, "Macrophage elastase kills bacteria within murine macrophages," *Nature*, vol. 460, no. 7255, pp. 637–641, 2009.

[63] V. Brazão, L. C. Caetano, M. del Vecchio Filipin, M. Paula Alonso Toldo, L. N. Caetano, and J. C. do Prado Jr, "Zinc supplementation increases resistance to experimental infection by *Trypanosoma cruzi*," *Veterinary Parasitology*, vol. 154, no. 1-2, pp. 32–37, 2008.

[64] S. Dubben, A. Honscheid, K. Winkler, L. Rink, and H. Haase, "Cellular zinc homeostasis is a regulator in monocyte differentiation of HL-60 cells by 1α, 25-dihydroxyvitamin D3," *Journal of Leukocyte Biology*, vol. 87, no. 5, pp. 833–844, 2010.

[65] G. B. Vega-Robledo, A. Polo-Jimenez, M. E. Morales-Martinez, S. Rojas-Dotor, and G. Rico-Rosillo, "Effect of zinc upon human and murine cell viability and differentiation," *Biological Trace Element Research*, vol. 120, no. 1-3, pp. 133–140, 2007.

[66] R. Roy, V. Parashar, L. K. S. Chauhan et al., "Mechanism of uptake of ZnO nanoparticles and inflammatory responses in macrophages require PI3K mediated MAPKs signaling," *Toxicology In Vitro*, vol. 28, no. 3, pp. 457–467, 2014.

[67] D. Glesne, S. Vogt, J. Maser, D. Legnini, and E. Huberman, "Regulatory properties and cellular redistribution of zinc during macrophage differentiation of human leukemia cells," *Journal of Structural Biology*, vol. 155, no. 1, pp. 2–11, 2006.

[68] V. Wilhelmi, U. Fischer, H. Weighardt et al., "Zinc oxide nanoparticles induce necrosis and apoptosis in macrophages in a p47phox- and Nrf2-independent manner," *PLoS One*, vol. 8, no. 6, article e65704, 2013.

[69] V. Wilhelmi, U. Fischer, D. van Berlo, K. Schulze-Osthoff, R. P. F. Schins, and C. Albrecht, "Evaluation of apoptosis induced by nanoparticles and fine particles in RAW 264.7 macrophages: facts and artefacts," *Toxicology In Vitro*, vol. 26, no. 2, pp. 323–334, 2012.

[70] R. Roy, S. K. Singh, L. K. S. Chauhan, M. Das, A. Tripathi, and P. D. Dwivedi, "Zinc oxide nanoparticles induce apoptosis by enhancement of autophagy via PI3K/Akt/mTOR inhibition," *Toxicology Letters*, vol. 227, no. 1, pp. 29–40, 2014.

[71] A. Brocard and B. Dreno, "Innate immunity: a crucial target for zinc in the treatment of inflammatory dermatosis," *Journal of the European Academy of Dermatology and Venereology*, vol. 25, no. 10, pp. 1146–1152, 2011.

[72] A. S. Prasad, "Zinc: role in immunity, oxidative stress and chronic inflammation," *Current Opinion in Clinical Nutrition and Metabolic Care*, vol. 12, no. 6, pp. 646–652, 2009.

[73] L. S. Mayer, P. Uciechowski, S. Meyer, T. Schwerdtle, L. Rink, and H. Haase, "Differential impact of zinc deficiency on phagocytosis, oxidative burst, and production of pro-inflammatory cytokines by human monocytes," *Metallomics*, vol. 6, no. 7, pp. 1288–1295, 2014.

[74] S. Triboulet, C. Aude-Garcia, L. Armand et al., "Analysis of cellular responses of macrophages to zinc ions and zinc oxide nanoparticles: a combined targeted and proteomic approach," *Nanoscale*, vol. 6, no. 11, pp. 6102–6114, 2014.

[75] A. J. Mehta, S. M. Yeligar, L. Elon, L. A. Brown, and D. M. Guidot, "Alcoholism causes alveolar macrophage zinc deficiency and immune dysfunction," *American Journal of Respiratory and Critical Care Medicine*, vol. 188, no. 6, pp. 716–723, 2013.

[76] J. J. Wirth, P. J. Fraker, and F. Kierszenbaum, "Zinc requirement for macrophage function: effect of zinc deficiency on uptake and killing of a protozoan parasite," *Immunology*, vol. 68, no. 1, pp. 114–119, 1989.

[77] A. Sheikh, S. Shamsuzzaman, S. M. Ahmad et al., "Zinc influences innate immune responses in children with enterotoxigenic *Escherichia coli*-induced diarrhea," *The Journal of Nutrition*, vol. 140, no. 5, pp. 1049–1056, 2010.

[78] A. S. Prasad, B. Bao, F. W. J. Beck, O. Kucuk, and F. H. Sarkar, "Antioxidant effect of zinc in humans," *Free Radical Biology & Medicine*, vol. 37, no. 8, pp. 1182–1190, 2004.

[79] U. Srinivas, B. Jeppsson, and J. H. Braconier, "Superoxide production of peritoneal macrophages in experimental gram-negative sepsis; influence of in vitro and in vivo supplements of zinc," *APMIS*, vol. 97, no. 7-12, pp. 682–688, 1989.

[80] Y. Wan, M. J. Petris, and S. C. Peck, "Separation of zinc-dependent and zinc-independent events during early LPS-stimulated TLR4 signaling in macrophage cells," *FEBS Letters*, vol. 588, no. 17, pp. 2928–2935, 2014.

[81] U. Siebenlist, G. Franzoso, and K. Brown, "Structure, regulation and function of NF-kappaB," *Annual Review of Cell Biology*, vol. 10, no. 1, pp. 405–455, 1994.

[82] A. Brieger, L. Rink, and H. Haase, "Differential regulation of TLR-dependent MyD88 and TRIF signaling pathways by free zinc ions," *Journal of Immunology*, vol. 191, no. 4, pp. 1808–1817, 2013.

[83] R. Ostuni, I. Zanoni, and F. Granucci, "Deciphering the complexity of toll-like receptor signaling," *Cellular and Molecular Life Sciences*, vol. 67, no. 24, pp. 4109–4134, 2010.

[84] A. S. Prasad, B. Bao, F. W. J. Beck, and F. H. Sarkar, "Zinc-suppressed inflammatory cytokines by induction of A20-mediated inhibition of nuclear factor-κB," *Nutrition*, vol. 27, no. 7-8, pp. 816–823, 2011.

[85] H. Summersgill, H. England, G. Lopez-Castejon et al., "Zinc depletion regulates the processing and secretion of IL-1β," *Cell Death & Disease*, vol. 5, no. 1, article e1040, 2014.

[86] D. Brough, P. Pelegrin, and N. J. Rothwell, "Pannexin-1-dependent caspase-1 activation and secretion of IL-1β is regulated by zinc," *European Journal of Immunology*, vol. 39, no. 2, pp. 352–358, 2009.

[87] C. P. Wong, N. A. Rinaldi, and E. Ho, "Zinc deficiency enhanced inflammatory response by increasing immune cell activation and inducing IL6 promoter demethylation," *Molecular Nutrition & Food Research*, vol. 59, no. 5, pp. 991–999, 2015.

[88] R. Pati, R. K. Mehta, S. Mohanty et al., "Topical application of zinc oxide nanoparticles reduces bacterial skin infection in mice and exhibits antibacterial activity by inducing oxidative stress response and cell membrane disintegration in macrophages," *Nanomedicine*, vol. 10, no. 6, pp. 1195–1208, 2014.

[89] C. D. Lin, Y. Y. Kou, C. Y. Liao et al., "Zinc oxide nanoparticles impair bacterial clearance by macrophages," *Nanomedicine (London, England)*, vol. 9, no. 9, pp. 1327–1339, 2014.

[90] M. Giovanni, J. Yue, L. Zhang, J. Xie, C. N. Ong, and D. T. Leong, "Pro-inflammatory responses of RAW264.7 macrophages when treated with ultralow concentrations of silver, titanium dioxide, and zinc oxide nanoparticles," *Journal of Hazardous Materials*, vol. 297, pp. 146–152, 2015.

[91] R. Roy, S. K. Singh, M. Das, A. Tripathi, and P. D. Dwivedi, "Toll-like receptor 6 mediated inflammatory and functional responses of zinc oxide nanoparticles primed macrophages," *Immunology*, vol. 142, no. 3, pp. 453–464, 2014.

[92] G. Chandel, K. Datta, and S. K. Datta, "Detection of genomic changes in transgenic Bt rice populations through genetic fingerprinting using amplified fragment length polymorphism (AFLP)," *GM Crops*, vol. 1, no. 5, pp. 327–336, 2010.

[93] E. Salgado and J. R. Maneiro, "New therapies for rheumatoid arthritis," *Medicina Clínica*, vol. 143, no. 10, pp. 461–466, 2014.

[94] L. Xin, X. Yang, G. Cai et al., "Serum levels of copper and zinc in patients with rheumatoid arthritis: a meta-analysis," *Biological Trace Element Research*, vol. 168, no. 1, pp. 1–10, 2015.

[95] A. Mierzecki, D. Strecker, and K. Radomska, "A pilot study on zinc levels in patients with rheumatoid arthritis," *Biological Trace Element Research*, vol. 143, no. 2, pp. 854–862, 2011.

[96] B. Bao, A. S. Prasad, F. W. J. Beck, and M. Godmere, "Zinc modulates mRNA levels of cytokines," *American Journal of Physiology. Endocrinology and Metabolism*, vol. 285, no. 5, pp. E1095–E1102, 2003.

[97] V. von Bulow, L. Rink, and H. Haase, "Zinc-mediated inhibition of cyclic nucleotide phosphodiesterase activity and expression suppresses TNF-α and IL-1β production in monocytes by elevation of guanosine 3′,5′-cyclic monophosphate," *Journal of Immunology*, vol. 175, no. 7, pp. 4697–4705, 2005.

[98] A. T. Jacobs and L. J. Ignarro, "Cell density-enhanced expression of inducible nitric oxide synthase in murine

macrophages mediated by interferon-beta," *Nitric Oxide*, vol. 8, no. 4, pp. 222–230, 2003.

[99] W. J. Janssen, "Alveolar macrophage dysfunction and chronic alcohol use. Time to think about zinc," *American Journal of Respiratory and Critical Care Medicine*, vol. 188, no. 6, pp. 635-636, 2013.

[100] A. J. Mehta, P. C. Joshi, X. Fan et al., "Zinc supplementation restores PU.1 and Nrf2 nuclear binding in alveolar macrophages and improves redox balance and bacterial clearance in the lungs of alcohol-fed rats," *Alcoholism, Clinical and Experimental Research*, vol. 35, pp. 1519–1528, 2011.

[101] J. V. Konomi, F. L. Harris, X. D. Ping, T. W. Gauthier, and L. A. S. Brown, "Zinc insufficiency mediates ethanol-induced alveolar macrophage dysfunction in the pregnant female mouse," *Alcohol and Alcoholism*, vol. 50, no. 1, pp. 30–38, 2015.

[102] P. C. Joshi, R. Raynor, X. Fan, and D. M. Guidot, "HIV-1-transgene expression in rats decreases alveolar macrophage zinc levels and phagocytosis," *American Journal of Respiratory Cell and Molecular Biology*, vol. 39, no. 2, pp. 218–226, 2008.

[103] G. S. Hotamisligil, "Inflammation and metabolic disorders," *Nature*, vol. 444, no. 7121, pp. 860–867, 2006.

[104] M. J. Liu, S. Bao, E. R. Bolin et al., "Zinc deficiency augments leptin production and exacerbates macrophage infiltration into adipose tissue in mice fed a high-fat diet," *The Journal of Nutrition*, vol. 143, no. 7, pp. 1036–1045, 2013.

[105] B. Bao, A. S. Prasad, F. W. J. Beck et al., "Zinc decreases C-reactive protein, lipid peroxidation, and inflammatory cytokines in elderly subjects: a potential implication of zinc as an atheroprotective agent," *The American Journal of Clinical Nutrition*, vol. 91, no. 6, pp. 1634–1641, 2010.

[106] A. Schmuck, F. Tricot, A. Hadjian, A. Favier, and A. M. Roussel, "Zinc deficiency does not enhance LDL uptake by P 388 D1 macrophages in vitro," *Biological Trace Element Research*, vol. 47, no. 1-3, pp. 75–80, 1995.

[107] Y. Suzuki, S. Tada-Oikawa, G. Ichihara et al., "Zinc oxide nanoparticles induce migration and adhesion of monocytes to endothelial cells and accelerate foam cell formation," *Toxicology and Applied Pharmacology*, vol. 278, no. 1, pp. 16–25, 2014.

[108] Y. Castillo, M. Tachibana, Y. Nakatsu, K. Watanabe, T. Shimizu, and M. Watarai, "Combination of zinc and all-trans retinoic acid promotes protection against Listeria monocytogenes infection," *PLoS One*, vol. 10, no. 9, article e0137463, 2015.

[109] P. G. Sohnle, M. J. Hunter, B. Hahn, and W. J. Chazin, "Zinc-reversible antimicrobial activity of recombinant calprotectin (migration inhibitory factor-related proteins 8 and 14)," *The Journal of Infectious Diseases*, vol. 182, no. 4, pp. 1272–1275, 2000.

[110] W. Alker and H. Haase, "Zinc and Sepsis," *Nutrients*, vol. 10, no. 8, 2018.

[111] H. Botella, P. Peyron, F. Levillain et al., "Mycobacterial p_1-type ATPases mediate resistance to zinc poisoning in human macrophages," *Cell Host & Microbe*, vol. 10, no. 3, pp. 248–259, 2011.

[112] G. Wellenreuther, M. Cianci, R. Tucoulou, W. Meyer-Klaucke, and H. Haase, "The ligand environment of zinc stored in vesicles," *Biochemical and Biophysical Research Communications*, vol. 380, no. 1, pp. 198–203, 2009.

[113] K. Subramanian Vignesh and G. S. Deepe Jr., "Immunological orchestration of zinc homeostasis: the battle between host mechanisms and pathogen defenses," *Archives of Biochemistry and Biophysics*, vol. 611, pp. 66–78, 2016.

[114] D. Daaboul, E. Rosenkranz, P. Uciechowski, and L. Rink, "Repletion of zinc in zinc-deficient cells strongly up-regulates IL-1β-induced IL-2 production in T-cells," *Metallomics*, vol. 4, no. 10, pp. 1088–1097, 2012.

[115] P. C. Joshi and D. M. Guidot, "HIV-1 transgene expression in rats induces differential expression of tumor necrosis factor alpha and zinc transporters in the liver and the lung," *AIDS Research and Therapy*, vol. 8, no. 1, p. 36, 2011.

[116] J. L. Peters, J. Dufner-Beattie, W. Xu et al., "Targeting of the mouse Slc39a2 (*Zip2*) gene reveals highly cell-specific patterns of expression, and unique functions in zinc, iron, and calcium homeostasis," *Genesis*, vol. 45, no. 6, pp. 339–352, 2007.

[117] T. V. Curry-McCoy, D. M. Guidot, and P. C. Joshi, "Chronic alcohol ingestion in rats decreases Krüppel-like factor 4 expression and intracellular zinc in the lung," *Alcoholism, Clinical and Experimental Research*, vol. 37, no. 3, pp. 361–371, 2013.

[118] I. Wessels and R. J. Cousins, "Zinc dyshomeostasis during polymicrobial sepsis in mice involves zinc transporter Zip14 and can be overcome by zinc supplementation," *American Journal of Physiology. Gastrointestinal and Liver Physiology*, vol. 309, no. 9, pp. G768–G778, 2015.

[119] T. B. Aydemir, S.-M. Chang, G. J. Guthrie et al., "Zinc transporter ZIP14 functions in hepatic zinc, iron and glucose homeostasis during the innate immune response (endotoxemia)," *PLoS One*, vol. 7, no. 10, article e48679, 2012.

[120] S. Tepaamorndech, P. Oort, C. P. Kirschke, Y. Cai, and L. Huang, "ZNT7 binds to CD40 and influences CD154-triggered p38 MAPK activity in B lymphocytes-a possible regulatory mechanism for zinc in immune function," *FEBS Open Bio*, vol. 7, no. 5, pp. 675–690, 2017.

[121] X. Xu, Y. Gu, L. Bian et al., "Characterization of immune response to novel HLA-A2-restricted epitopes from zinc transporter 8 in type 1 diabetes," *Vaccine*, vol. 34, no. 6, pp. 854–862, 2016.

Hindawi
Journal of Immunology Research
Volume 2018, Article ID 5749120, 9 pages
https://doi.org/10.1155/2018/5749120

Review Article

Role of Zinc Signaling in the Regulation of Mast Cell-, Basophil-, and T Cell-Mediated Allergic Responses

Keigo Nishida[1,2] **and Ryota Uchida**[1]

[1]*Laboratory of Immune Regulation, Graduate School of Pharmaceutical Sciences, Suzuka University of Medical Science, 3500-3 Minamitamagaki, Suzuka, Mie 513-8607, Japan*
[2]*Laboratory of Immune Regulation, Faculty of Pharmaceutical Sciences, Suzuka University of Medical Science, 3500-3 Minamitamagaki-cho, Suzuka, Mie 513-8670, Japan*

Correspondence should be addressed to Keigo Nishida; knishida@suzuka-u.ac.jp

Received 2 June 2018; Accepted 23 October 2018; Published 25 November 2018

Academic Editor: Alexandre Keller

Zinc is essential for maintaining normal structure and physiological function of cells. Its deficiency causes growth retardation, immunodeficiency, and neuronal degeneration. Zinc homeostasis is tightly regulated by zinc transporters and metallothioneins that control zinc concentration and its distribution in individual cells and contributes to zinc signaling. The intracellular zinc signaling regulates immune reactions. Although many molecules involved in these processes have zinc-binding motifs, the molecular mechanisms and the role of zinc in immune responses have not been elucidated. We and others have demonstrated that zinc signaling plays diverse and specific roles *in vivo* and *in vitro* in studies using knockout mice lacking zinc transporter function and metallothionein function. In this review, we discuss the impact of zinc signaling focusing particularly on mast cell-, basophil-, and T cell-mediated inflammatory and allergic responses. We also describe zinc signaling dysregulation as a leading health problem in inflammatory disease and allergy.

1. Introduction

This review discusses our current understanding of the roles of zinc transporters and zinc in inflammatory diseases and allergy. The essential trace element zinc [1] functions as a neurotransmitter [2] and an intracellular signaling molecule [3–6]. Effects of zinc on the immune and nervous systems have been demonstrated both in *in vivo* and *in vitro* studies, and zinc concentration is a key factor regulating these effects [7, 8]. A number of studies have shown that the depletion of zinc leads to impaired immune function. Decreased natural killer cell-mediated cytotoxic activity, antibody-mediated responses, and host defenses against pathogens and tumors have been observed in zinc-deficient mice [9–11].

Zinc plays an essential role in maintaining the conformation and the activity of many enzymes, transcription factors, signaling molecules, and other related factors. On the other hand, zinc can be toxic at a high concentration and can induce apoptosis in T and B cells [12, 13]. Intracellular concentration and distribution of zinc is controlled by zinc transporters including the *Slc39*/*Zrt- and Irt-like protein* (*ZIP*) and *Slc30*/*Zn transporter* (*ZnT*) families, which increase and decrease intracellular zinc levels, respectively [14–17], and this regulation is mediated by zinc-binding molecules such as metallothioneins [18, 19]. Zinc also acts as an intracellular signaling molecule. Extracellular stimuli can affect the intracellular zinc level either by modulating the transcription of zinc transporter genes and zinc-binding molecules or via transcription-independent routes such as zinc wave [20–22]. We categorize the former as late zinc signaling and the latter as early zinc signaling [3, 23]. While many studies have shown that zinc is important to the immune system and that imbalance in zinc homeostasis leads to various disorders, how zinc homeostasis and signaling are regulated in immune cells or whether zinc transporters and metallothioneins are involved in immune cell function is not fully understood.

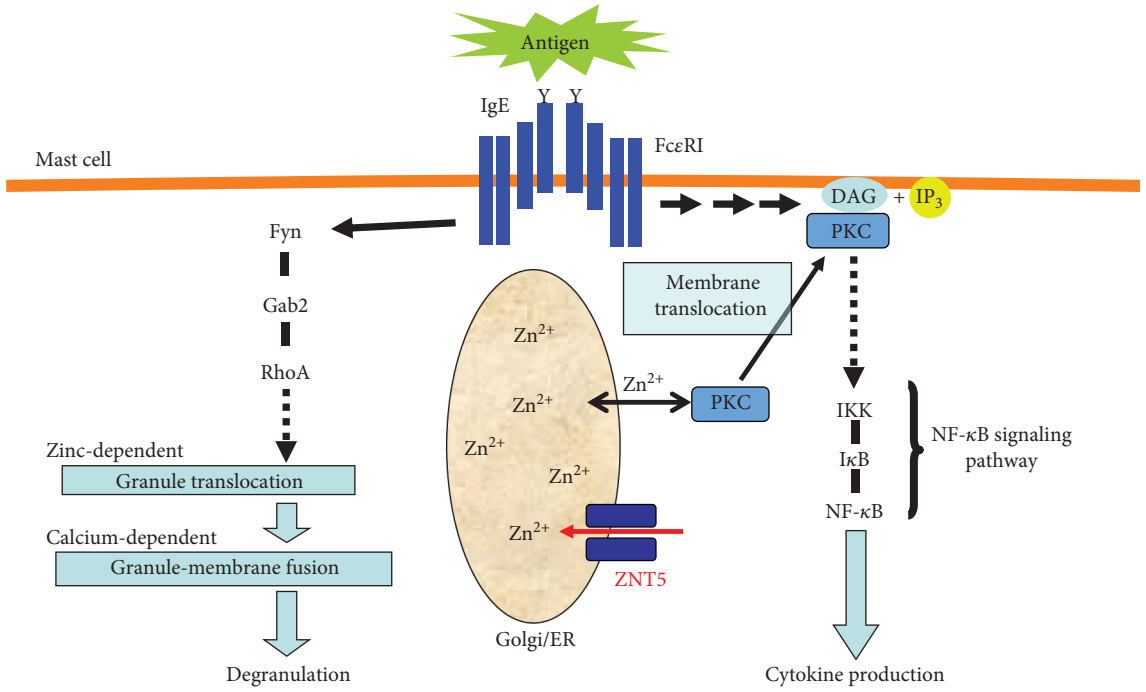


FIGURE 1: Zinc and zinc transporters are involved in FcεRI-mediated mast cell activation. Zinc is required in multiple steps of FcεRI-induced mast cell activation, including degranulation and cytokine production. Zinc levels depend on FcεRI-induced granule translocation, regulated by a Fyn/Gab2/RhoA-mediated signaling pathway. Zinc and ZNT5 are also required for PKC's translocation to the plasma membrane and NF-κB's subsequent nuclear translocation, leading to the production of cytokines such as IL-6 and TNFα. IgE: immunoglobulin E; FcεRI: high-affinity receptor for IgE; Gab2: Grb2-associated binding 2; Zn: zinc; ZNT5: Zn transporter 5; ER: endoplasmic reticulum; PKC: protein kinase C; DAG: diacylglycerol; IP_3: inositol-1,4,5-triphosphate; IKK: inhibitor of kappa B kinase; IκB: inhibitor of kappa B; NF-κB: nuclear factor-kappa B.

Here, we describe how zinc and its homeostasis and signaling affect biological events in inflammatory and allergic responses.

2. Role of Zinc in Mast Cell Function

Mast cells play important roles in allergic reactions such as anaphylaxis, asthma, and atopic dermatitis [24–26]. Activated mast cells secrete two classes of mediators. One class of mediators are preformed mediators stored in granules which are rapidly degranulated and secreted upon activation. The second class of mediators are cytokines and chemokines that are synthesized *de novo* and gradually secreted. These secreted molecules play leading roles in allergic inflammatory responses.

The zinc probe Zinquin has been used to determine the intracellular zinc level and distribution and to visualize distinct zinc pools in allergy-related cells. Mast cell granules intensely fluoresce with Zinquin [27]. Airway epithelial cells are also rich in zinc [28]. Zinc deficiency increases allergic eosinophilic inflammation, whereas dietary zinc supplementation alleviates the symptom [29]. Interestingly, zinc deficiency is a risk factor for the development of asthma [30, 31]. Also, high *SLC39A2*/*ZIP2* expression levels have been reported in the leukocytes of asthmatic infants [32]. These reports indicate that zinc is involved in the development of allergic diseases. However, the precise roles of zinc and zinc transporters in allergy-related cells have not been fully elucidated.

Zinc is required for both degranulation and cytokine production in mast cells [33] (Figure 1). The zinc chelator *N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) inhibits the release of histamine, the production of cytokines, and the secretion of lipid mediators in mast cells, and zinc supplementation rescues these inhibitory effects. Mast cell function is not affected by other metal chelators [33]. Similarly, zinc depletion caused by TPEN or the clinically used heavy metal chelator DMPS [34] inhibits the mRNA expression of chemokines such as eotaxin in human lung cell lines [35].

Mast cell degranulation begins when the stimulation of high-affinity receptor for IgE (FcεRI) triggers microtubule polymerization and granules are translocated to the plasma membrane. Fyn/Gab2/RhoA signaling, but not Lyn/SLP-76, plays a critical role in this calcium-independent and microtubule-dependent pathway [36]. However, in the second step of degranulation, which is calcium-dependent, the granules fuse with the plasma membrane. TPEN suppresses FcεRI-induced granule translocation, though it has little effect on calcium mobilization or other FcεRI functions such as the FcεRI-induced tyrosine phosphorylation of various signaling molecules. Since the translocation of granules depends on cytoskeletal proteins such as tubulin and actin [37] and microtubules are critical for both

granule translocation and vesicle transport [36, 38], it was hypothesized that TPEN affects the microtubule assembly. However, as shown by Kabu et al., TPEN does not suppress FcεRI-induced microtubule formation, suggesting that its target might be zinc-regulated molecules that directly link microtubules and granules. Kinesin receptors are linker-cargo proteins essential for microtubule-dependent vesicle trafficking [39], and therefore, the target(s) of TPEN might interact indirectly with granules via kinesin.

TPEN suppresses FcεRI-mediated cytokine production as well as interleukin- (IL-) 6 and tumor necrosis factor- (TNF-) α mRNA transcription. Its stimulation activates protein kinase C (PKC), which is involved in cytokine production through nuclear factor-kappa B (NF-κB) activation [40, 41]. Since TPEN inhibits the FcεRI-mediated translocation of PKC to the plasma membrane [33], PKC may be one of the targets of TPEN that affect cytokine production. In fact, PKC contains a zinc-binding motif and zinc is essential to maintain the structure of PKC [42]. Furthermore, its zinc-binding motif domain is required for the translocation of PKC to the plasma membrane after FcεRI stimulation [43].

ZnT5 is highly expressed in mast cells, and FcεRI stimulation enhances its transcription level. *ZnT5*-KO mice have defects in mast cell-mediated delayed-type allergic reactions such as contact hypersensitivity, but not in immediate-type reactions such as anaphylaxis [44]. Consistent with these *in vivo* findings, *ZnT5* is required for FcεRI-mediated cytokine production, but not for mast cell degranulation.

Mast cells lacking *ZnT5* exhibit reduced levels of FcεRI-induced IL-6 and TNF-α mRNA, and ZNT5 is required for FcεRI-induced plasma membrane translocation of PKC and the nuclear translocation of NF-κB [44]. Therefore, *ZnT5* is selectively required for mast cell-mediated delayed-type hypersensitivity reactions, and it is considered a novel component in PKC/NF-κB signaling (Figure 1). Furthermore, it was shown in experiments using ZnT5-deficient DT40 cells that ZNT5 expressed on the ER-Golgi interface is necessary for the enzymatic activity of zinc-dependent alkaline phosphatases (ALPs) that are processed to the holo form between the ER and the Golgi [45–47]. Thus, ZNT5 may act to supply zinc to the zinc finger-like domains in PKC and ALP.

These findings show that zinc and its transporters are involved in the regulation of degranulation and cytokine production in mast cell-mediated allergic responses and that zinc transporters modulate the PKC/NF-κB signaling pathway involved in the regulation of cytokine and chemokine gene expression.

3. Role of Zinc in Basophil-Mediated Cytokine Production

Basophils represent less than 1% of peripheral blood leukocytes. Like mast cells, they express FcεRI on the cell surface and release cytokines and chemical mediators in response to FcεRI activation [48, 49]. Under physiological conditions, basophils circulate in the blood while mast cells reside in peripheral tissues. Infiltration of basophils into peripheral tissues is often observed in allergic inflammatory diseases such as atopic dermatitis and bronchial asthma. Basophils have long been neglected in immunological studies due to their small numbers and morphological similarity to mast cells, but they are now recognized as a major source of cytokines such as IL-4 and TSLP that promote Th2-type allergic responses [50–53]. However, the molecular mechanisms of IL-4 production and the requirement of zinc in basophils have not been fully elucidated.

It has been reported that zinc-binding metallothionein (MT) proteins are required for FcεRI-induced IL-4 production in human and mouse basophils [54]. Transcription of *Mt-1* and *Mt-2* is significantly elevated after FcεRI stimulation in primary mouse basophils, while the expression of *Zips*, *ZnTs*, *Mt-3*, and *Mt-4* is not affected. Furthermore, FcεRI-induced IL-4 production in basophils was inhibited in the absence of *Mt-1* and *Mt-2*. It has also been shown that MTs are selectively required for FcεRI-induced calcineurin (CN)/nuclear factor of activated T cells (NFAT) signaling (Figure 2). Finally, Ugajin et al. indicated the requirement of MTs for IL-4 production by human basophils [55].

What is the role of free zinc in the regulation of IL-4 gene expression in basophils? CN consists of a catalytic subunit CN A and a regulatory subunit CN B, which form a heterodimer. The catalytic domain contains a Fe^{3+}-Zn^{2+} dinuclear center, and zinc is thought to act as a catalytic cofactor [56, 57]. On the other hand, it has been reported that zinc inhibits the activity of CN *in vitro* [58–60]. The intracellular concentration of free zinc was higher in *Mt-1/2*-deficient basophils due to the absence of MTs, which play an important role in cytosolic zinc storage. Experimentally increased zinc levels attenuated the CN activity in basophils. These findings suggest that the regulation of intracellular zinc levels mediated by MTs plays an important role in the modulation of CN/NFAT signaling in basophils. Consistent with our reports, some researchers indicated zinc-dependent inhibition of cytokine expression in T cells [61–63]. Thus, we and the other group suggested that intracellular free zinc acts as a suppressor of cytokine production by the stimulation of immune cells. In contrast, zinc positively regulates the expression of cytokines. Zinc supplementation enhances the cytokine production in immune cells [64–66]. These conflicting effects may be due to concentration-dependent effect of zinc on different signaling molecules involved in cytokine expression.

4. Role of Zinc in T Cell Receptor-Mediated Signaling

Zinc signaling in T cells has been first described by Yu et al. (Ref. [67]). They observed an increase in intracellular zinc concentration within 1 minute after stimulation of the T cell receptor (TCR) [67]. This increase is dependent on the extracellular zinc concentration, suggesting that TCR stimulation induces an influx of extracellular zinc into T cells. Moreover, this influx of zinc is inhibited by silencing ZIP6, a zinc transporter expressed on the cytoplasmic membrane.

Early events in TCR signaling include tyrosine phosphorylation of several signaling molecules. The Src protein kinase Lck is primarily responsible for the phosphorylation of tyrosine residues within the ITAM motifs of CD3ζ and ZAP70

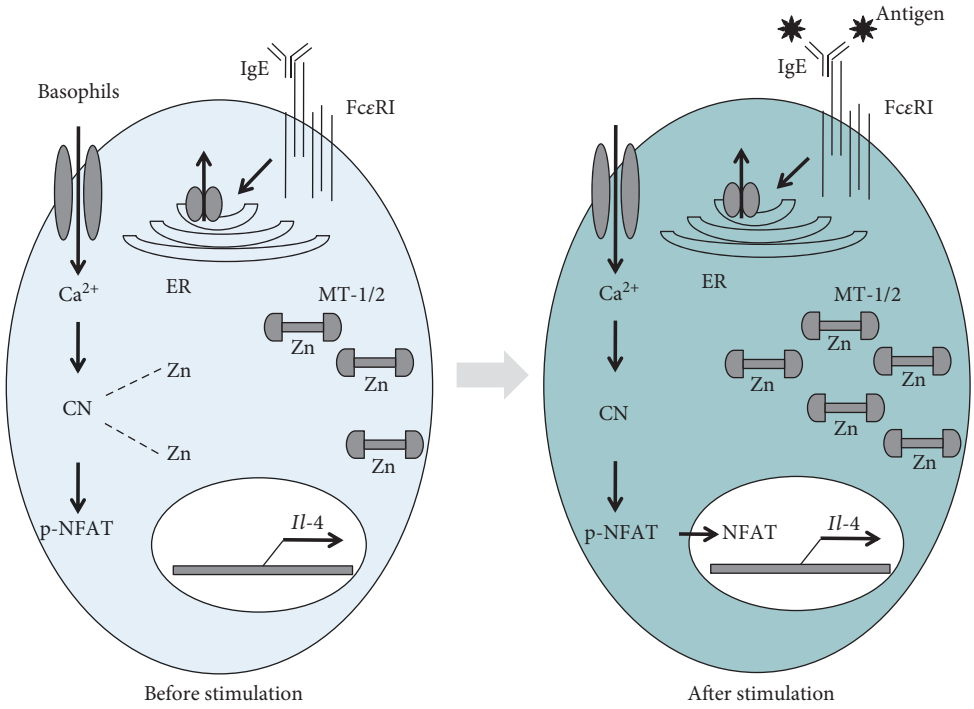


Figure 2: Metallothionein control in FcεRI-mediated IL-4 production in basophils. FcεRI stimulation activates basophils via signaling pathways that increase IL-4 production. Before stimulation, cytoplasmic free zinc ion inhibits CN. After FcεRI stimulation, cytoplasmic free zinc ion can induce the expression of metallothionein 1/2 and metallothioneins can bind to cytoplasmic free zinc ion. As a result, CN/NFAT signaling pathway can be activated and leads to increased IL-4 expression. IgE: immunoglobulin E; FcεRI: high-affinity receptor for IgE; ER: endoplasmic reticulum; Zn: zinc; MT-1/2: metallothionein 1/2; Ca: calcium; CN: calcineurin; NFAT: nuclear factor of activated T cells; IL-4: interleukin-4.

[68]. Extracellular zinc influences the phosphorylation of ZAP70 and inhibits negative regulatory feedback loops that at least partially accounts for the increase in ZAP70 phosphorylation. SHP-1, which dephosphorylates ZAP70 and other signaling molecules it is recruited to Lck [69], is a prime candidate target for ZIP6-mediated zinc signaling. In fact, the increase in zinc influx decreases the recruitment of SHP-1 to the TCR activation complex, augments ZAP70 phosphorylation, and sustains calcium influx. Yu et al. proposed that the influx of zinc following TCR stimulation leads to a local increase in cytoplasmic zinc that modifies early TCR signaling events. Thus, it is considered that ZIP6-mediated zinc signaling is dependent on extracellular zinc concentrations (Figure 3). In other words, extracellular zinc concentration controls the signal strength in TCR signaling. It has already been reported that the induction of IFN-γ production by zinc supplementation links the zinc status to Th1/Th2 polarization and corresponds to the observation that zinc deficiency leads to reduced Th1 immune response [70].

5. Role of Zinc in Inflammatory and Allergic Responses

The relationship between zinc homeostasis and immune function has been examined in a number of studies. The impact of single nutrient deficiency on immune response has been demonstrated by zinc homeostasis studies using experimental mouse and rat models. In addition, zinc deficiency is a frequent problem in humans and is associated with many chronic diseases. It is important to note that chronic diseases such as gastrointestinal disorders, chronic diarrhea, cirrhosis, renal disease, sickle cell anemia, some types of cancer, cystic fibrosis, pancreatic insufficiency, and autoimmune arthritis in humans can lead to a suboptimal zinc status [71–78]. Interestingly, zinc-containing compounds such as polaprezinc have been reported to improve the symptoms of autoimmune diseases in animal models [79, 80]. It has been suggested that the amelioration of autoimmune diseases by zinc occurs by inhibiting T cell activation, though detailed mechanisms involved in this process have not been elucidated.

Kitabayashi et al. hypothesized that zinc signaling might target proteins involved in inflammation and autoimmune diseases [81]. One such target protein is signal transducer and activator of transcription 3 (STAT3), which is a signaling molecule for the proinflammatory cytokine IL-6. The authors were particularly interested in how zinc affects the differentiation of Th17 cells that was known to be controlled by IL-6-induced STAT3 activation [82–85].

In addition, they used induced CIA (collagen-induced arthritis) and EAE (experimental autoimmune encephalomyelitis) mouse disease models, since both of these autoimmune diseases are mediated by antigen-specific Th17 cells and the development of these cells is controlled by STAT3

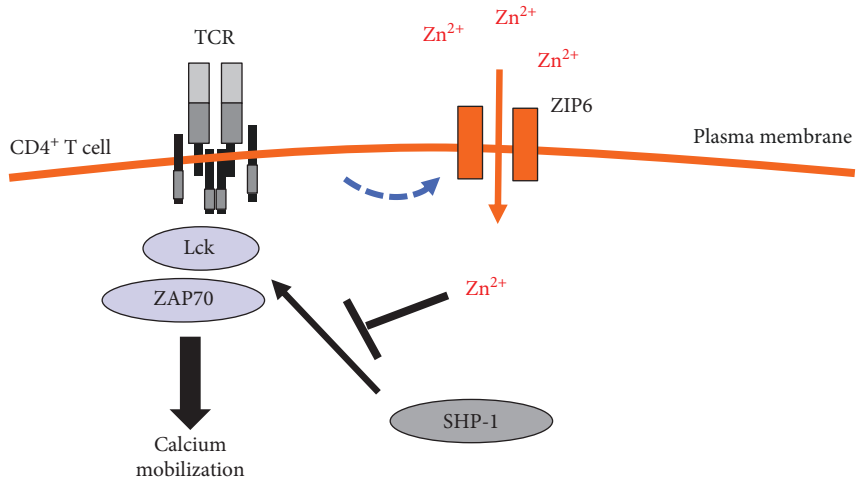


Figure 3: Zn functions as an ionic signaling molecule after T cell activation. Cytoplasmic zinc concentrations increased within 1 min after TCR triggering as the result of an influx via the zinc transporter *Zip6*. The increase was most pronounced in the immediate subsynaptic area and enhanced TCR signaling, at least in part as a result of the inhibition of SHP-1 recruitment. TCR: T cell receptor; Zn: zinc; SHP-1: Src homology region 2 (SH2) domain-containing phosphatase-1.

activation. Development of autoimmune diseases such as EAE was significantly suppressed by zinc supplementation. In one experiment, pathogenic Th17 cells were transferred into the mice and the mice were given zinc-supplemented drinking water. Development of EAE was similar in zinc-treated and control hosts, indicating that zinc supplementation did not alter the Th17 cell-mediated immune responses, including induced inflammation and Th17 cell activation. Thus, rather than affecting immune responses after pathogenic T cell development, zinc inhibits the development of pathogenic Th17 cells from naïve $CD4^+$ T cells, a process that depends on the IL-6-STAT3 signaling pathway. In addition to this, Kitabayashi et al. demonstrated that zinc directly binds STAT3 and inhibits its phosphorylation by Janus kinases (JAK) and does so without affecting the kinase activity of JAK proteins. Furthermore, the structure of STAT3 itself is altered by the zinc binding [81] (Figure 4).

While zinc supplementation can help restore normal immune functions as described above, there are cases where chelating zinc can be beneficial to suppress antigen-dependent allergic responses. Kabu et al. reported that TPEN inhibited antigen-induced anaphylaxis response [33]. In this case, TPEN attenuated the degranulation of mast cells *in vivo* and serum histamine level was decreased in TPEN-treated mice. Fukuyama et al. reported that TPEN suppressed asthmatic responses in mouse models of ovalbumin- (OVA-) induced airway hyperresponsiveness and allergic airway inflammation [86]. TPEN also attenuated the upregulation of cytokines such as TNFα, IL-13, and IL-4 in bronchoalveolar lavage fluids and goblet cell hyperplasia after OVA exposure. These observations suggest that zinc chelators and their derivatives can be potential antiallergic drugs that act differently from currently available drugs such as histamine antagonists.

Zinc deficiency may enhance some allergic diseases. In some clinical studies, serum zinc levels were lower in children with atopic dermatitis (AD) compared to the control group [87, 88]. Interestingly, Kim et al. reported a correlation between AD and hair zinc level and reported that AD patients with a low hair zinc level showed clinical improvement after oral zinc supplementation [89]. Another case of zinc deficiency was reported. In this report, serum zinc levels were significantly lower in atopic asthmatics than nonatopic asthmatics and healthy controls. In atopic asthmatics, highly significant correlations were found between zinc levels and total IgE levels [90]. A small number of studies have been conducted to evaluate the efficacy of zinc supplementation to improve clinical symptoms of asthma during nonexacerbation [91, 92]. Thus, zinc supplementation might help patients with a low serum zinc concentration to recover from allergic diseases, but it must be well adjusted to the actual requirement. However, if zinc concentration significantly exceeds the physiological level, zinc may also adversely affect the immune function. The opposing effects of varying zinc concentrations on distinct signaling pathways in different cell types such as mast cells, basophils, and T cells remain to be investigated.

6. Conclusion and Perspectives

We have briefly reviewed recent findings showing that zinc contributes to allergy and autoimmune disorders. Zinc chelators effectively inhibit anaphylaxis, and zinc supplements may effectively suppress rheumatoid arthritis and multiple sclerosis, as indicated by studies using mouse disease models. These findings may lead to future therapeutic applications for suppressing inflammatory or allergic responses.

Using strategic gene targeting, we have demonstrated the role of zinc transporters and metallothioneins in various signaling pathways. Zinc therefore functions as an intracellular signaling molecule; its intracellular status is affected by extracellular stimuli regulating changes in zinc transporter and metallothionein expression. Much about the mechanism of

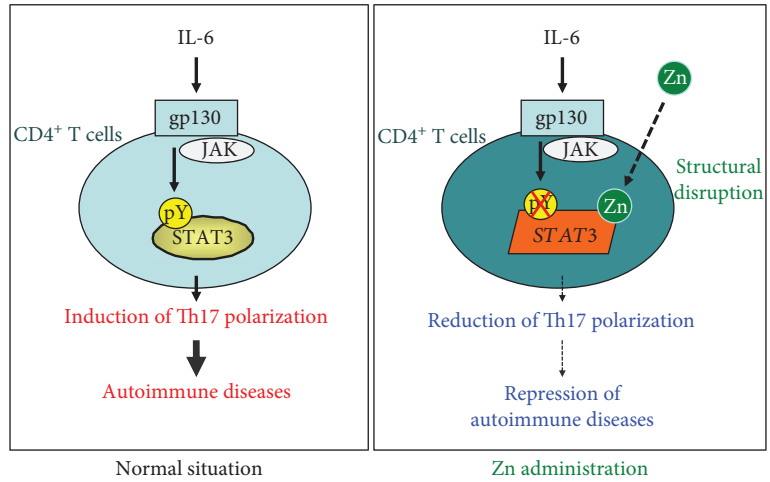


Figure 4: Zinc suppresses autoimmune diseases by inhibiting STAT3 activation. Zinc directly binds STAT3, altering its structure. The structurally altered STAT3 molecule cannot effectively transduce the IL-6 signaling pathway; this pathway is critical for autoimmune diseases involving Th17 cells. Zn: zinc; IL-6: interleukin-6; STAT3: signal transducer and activator of transcription 3; JAK: Janus kinases.

zinc signaling remains to be clarified, including the method by which zinc transporters transfer zinc to individual target proteins such as PKC. We can be certain that further research will advance our understanding of zinc signaling and the intracellular transport of zinc in the immune system.

Conflicts of Interest

The authors declare no competing financial interests.

Acknowledgments

We thank Dr. M. Kato for critical reading. This work was supported in part by the JSPS KAKENHI Grant numbers JP16K15152 (K. N.) and JP18H05299 (K. N.), the Takeda Science Foundation (K. N.), and SENSHIN Medical Research Foundation (K. N.). R. U. was supported by the Nagai Memorial Research Scholarship from the Pharmaceutical Society of Japan, and Public Interest Incorporated Foundation Tsukushi Scholarship Research Fund.

References

[1] A. S. Prasad, "Zinc: an overview," *Nutrition*, vol. 11, 1 Supplement, pp. 93–99, 1995.

[2] C. J. Frederickson and A. I. Bush, "Synaptically released zinc: physiological functions and pathological effects," *Biometals*, vol. 14, no. 3/4, pp. 353–366, 2001.

[3] T. Hirano, M. Murakami, T. Fukada, K. Nishida, S. Yamasaki, and T. Suzuki, "Roles of zinc and zinc signaling in immunity: zinc as an intracellular signaling molecule," *Advances in Immunology*, vol. 97, pp. 149–176, 2008.

[4] T. Fukada, S. Yamasaki, K. Nishida, M. Murakami, and T. Hirano, "Zinc homeostasis and signaling in health and diseases: zinc signaling," *Journal of Biological Inorganic Chemistry*, vol. 16, no. 7, pp. 1123–1134, 2011.

[5] I. Wessels, M. Maywald, and L. Rink, "Zinc as a gatekeeper of immune function," *Nutrients*, vol. 9, no. 12, article 1286, 2017.

[6] K. Nishida, T. Fukada, S. Yamasaki, M. Murakami, and T. Hirano, *13. Zinc in Allergy, Autoimmune, and Hard and Connective Tissue Diseases*, IOS Press, 2011.

[7] C. J. Frederickson, J. Y. Koh, and A. I. Bush, "The neurobiology of zinc in health and disease," *Nature Reviews Neuroscience*, vol. 6, no. 6, pp. 449–462, 2005.

[8] L. Rink, "Zinc and the immune system," *The Proceedings of the Nutrition Society*, vol. 59, no. 4, pp. 541–552, 2000.

[9] G. Fernandes, M. Nair, K. Onoe, T. Tanaka, R. Floyd, and R. A. Good, "Impairment of cell-mediated immunity functions by dietary zinc deficiency in mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 76, no. 1, pp. 457–461, 1979.

[10] P. J. Fraker, R. Caruso, and F. Kierszenbaum, "Alteration of the immune and nutritional status of mice by synergy between zinc deficiency and infection with Trypanosoma cruzi," *The Journal of Nutrition*, vol. 112, no. 6, pp. 1224–1229, 1982.

[11] C. L. Keen and M. E. Gershwin, "Zinc deficiency and immune function," *Annual Review of Nutrition*, vol. 10, no. 1, pp. 415–431, 1990.

[12] K. H. Ibs and L. Rink, "Zinc-altered immune function," *The Journal of Nutrition*, vol. 133, no. 5, pp. 1452S–1456S, 2003.

[13] W. G. Telford and P. J. Fraker, "Preferential induction of apoptosis in mouse CD4+CD8+ alpha beta TCRloCD3 epsilon lo thymocytes by zinc," *Journal of Cellular Physiology*, vol. 164, no. 2, pp. 259–270, 1995.

[14] T. Kambe, Y. Yamaguchi-Iwai, R. Sasaki, and M. Nagao, "Overview of mammalian zinc transporters," *Cellular and Molecular Life Sciences*, vol. 61, no. 1, pp. 49–68, 2004.

[15] R. J. Cousins, J. P. Liuzzi, and L. A. Lichten, "Mammalian zinc transport, trafficking, and signals," *The Journal of Biological Chemistry*, vol. 281, no. 34, pp. 24085–24089, 2006.

[16] T. Fukada and T. Kambe, "Molecular and genetic features of zinc transporters in physiology and pathogenesis," *Metallomics*, vol. 3, no. 7, pp. 662–674, 2011.

[17] T. Kimura and T. Kambe, "The functions of metallothionein and ZIP and ZnT transporters: an overview and perspective," *International Journal of Molecular Sciences*, vol. 17, no. 3, p. 336, 2016.

[18] G. K. Andrews, "Cellular zinc sensors: MTF-1 regulation of gene expression," *Biometals*, vol. 14, no. 3/4, pp. 223–237, 2001.

[19] R. D. Palmiter, "Protection against zinc toxicity by metallothionein and zinc transporter 1," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 14, pp. 4918–4923, 2004.

[20] S. Yamasaki, K. Sakata-Sogawa, A. Hasegawa et al., "Zinc is a novel intracellular second messenger," *The Journal of Cell Biology*, vol. 177, no. 4, pp. 637–645, 2007.

[21] S. Yamasaki, A. Hasegawa, S. Hojyo et al., "A novel role of the L-type calcium channel α_{1D} subunit as a gatekeeper for intracellular zinc signaling: zinc wave," *PLoS One*, vol. 7, no. 6, article e39654, 2012.

[22] K. Nishida and S. Yamasaki, *Zinc Signals in Cellular Functions and Disorders*, Springer, 2014.

[23] M. Murakami and T. Hirano, "Intracellular zinc homeostasis and zinc signaling," *Cancer Science*, vol. 99, no. 8, pp. 1515–1522, 2008.

[24] M. Metz, M. A. Grimbaldeston, S. Nakae, A. M. Piliponsky, M. Tsai, and S. J. Galli, "Mast cells in the promotion and limitation of chronic inflammation," *Immunological Reviews*, vol. 217, no. 1, pp. 304–328, 2007.

[25] T. Kawakami, T. Ando, M. Kimura, B. S. Wilson, and Y. Kawakami, "Mast cells in atopic dermatitis," *Current Opinion in Immunology*, vol. 21, no. 6, pp. 666–678, 2009.

[26] S. J. Galli and M. Tsai, "IgE and mast cells in allergic disease," *Nature Medicine*, vol. 18, no. 5, pp. 693–704, 2012.

[27] L. H. Ho, R. E. Ruffin, C. Murgia, L. Li, S. A. Krilis, and P. D. Zalewski, "Labile zinc and zinc transporter ZnT4 in mast cell granules: role in regulation of caspase activation and NF-kappaB translocation," *Journal of Immunology*, vol. 172, no. 12, pp. 7750–7760, 2004.

[28] A. Q. Truong-Tran, R. E. Ruffin, and P. D. Zalewski, "Visualization of labile zinc and its role in apoptosis of primary airway epithelial cells and cell lines," *American Journal of Physiology-Lung Cellular and Molecular Physiology*, vol. 279, no. 6, pp. L1172–L1183, 2000.

[29] M. Richter, R. Bonneau, M. A. Girard, C. Beaulieu, and P. Larivée, "Zinc status modulates bronchopulmonary eosinophil infiltration in a murine model of allergic inflammation," *Chest*, vol. 123, no. 3, article 446S, 2003Supplement, 2003.

[30] P. D. Zalewski, A. Q. Truong-Tran, D. Grosser, L. Jayaram, C. Murgia, and R. E. Ruffin, "Zinc metabolism in airway epithelium and airway inflammation: basic mechanisms and clinical targets. A review," *Pharmacology & Therapeutics*, vol. 105, no. 2, pp. 127–149, 2005.

[31] G. Riccioni and N. D'Orazio, "The role of selenium, zinc and antioxidant vitamin supplementation in the treatment of bronchial asthma: adjuvant therapy or not?," *Expert Opinion on Investigational Drugs*, vol. 14, no. 9, pp. 1145–1155, 2005.

[32] T. F. Xu, X. L. Wang, J. Z. Yang et al., "Overexpression of Zip-2 mRNA in the leukocytes of asthmatic infants," *Pediatric Pulmonology*, vol. 44, no. 8, pp. 763–767, 2009.

[33] K. Kabu, S. Yamasaki, D. Kamimura et al., "Zinc is required for FcεRI-mediated mast cell activation," *The Journal of Immunology*, vol. 177, no. 2, pp. 1296–1305, 2006.

[34] O. Torres-Alanís, L. Garza-Ocañas, and A. Pineyro-Lopez, "Evaluation of urinary mercury excretion after administration of 2,3-dimercapto-1-propane sulfonic acid to occupationally exposed men," *Journal of Toxicology: Clinical Toxicology*, vol. 33, no. 6, pp. 717–720, 1995.

[35] M. Richter, A. M. Cantin, C. Beaulieu, A. Cloutier, and P. Larivée, "Zinc chelators inhibit eotaxin, RANTES, and MCP-1 production in stimulated human airway epithelium and fibroblasts," *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 285, no. 3, pp. L719–L729, 2003.

[36] K. Nishida, S. Yamasaki, Y. Ito et al., "Fc {epsilon}RI-mediated mast cell degranulation requires calcium-independent microtubule-dependent translocation of granules to the plasma membrane," *The Journal of Cell Biology*, vol. 170, no. 1, pp. 115–126, 2005.

[37] B. L. Goode, D. G. Drubin, and G. Barnes, "Functional cooperation between the microtubule and actin cytoskeletons," *Current Opinion in Cell Biology*, vol. 12, no. 1, pp. 63–71, 2000.

[38] A. J. Smith, J. R. Pfeiffer, J. Zhang, A. M. Martinez, G. M. Griffiths, and B. S. Wilson, "Microtubule-dependent transport of secretory vesicles in RBL-2H3 cells," *Traffic*, vol. 4, no. 5, pp. 302–312, 2003.

[39] B. J. Schnapp, "Trafficking of signaling modules by kinesin motors," *Journal of Cell Science*, vol. 116, no. 11, pp. 2125–2135, 2003.

[40] H. Nechushtan, M. Leitges, C. Cohen, G. Kay, and E. Razin, "Inhibition of degranulation and interleukin-6 production in mast cells derived from mice deficient in protein kinase Cbeta," *Blood*, vol. 95, no. 5, pp. 1752–1757, 2000.

[41] S. Klemm, J. Gutermuth, L. Hültner et al., "The Bcl10–Malt1 complex segregates FcεRI-mediated nuclear factor κB activation and cytokine production from mast cell degranulation," *The Journal of Experimental Medicine*, vol. 203, no. 2, pp. 337–347, 2006.

[42] S. Corbalan-Garcia and J. C. Gomez-Fernandez, "Protein kinase C regulatory domains: the art of decoding many different signals in membranes," *Biochimica et Biophysica Acta*, vol. 1761, no. 7, pp. 633–654, 2006.

[43] E. Oancea, M. N. Teruel, A. F. G. Quest, and T. Meyer, "Green fluorescent protein (GFP)-tagged cysteine-rich domains from protein kinase C as fluorescent indicators for diacylglycerol signaling in living cells," *The Journal of Cell Biology*, vol. 140, no. 3, pp. 485–498, 1998.

[44] K. Nishida, A. Hasegawa, S. Nakae et al., "Zinc transporter Znt5/Slc30a5 is required for the mast cell-mediated delayed-type allergic reaction but not the immediate-type reaction," *The Journal of Experimental Medicine*, vol. 206, no. 6, pp. 1351–1364, 2009.

[45] T. Suzuki, K. Ishihara, H. Migaki et al., "Zinc transporters, ZnT5 and ZnT7, are required for the activation of alkaline phosphatases, zinc-requiring enzymes that are glycosylphosphatidylinositol-anchored to the cytoplasmic membrane," *Journal of Biological Chemistry*, vol. 280, no. 1, pp. 637–643, 2005.

[46] T. Suzuki, K. Ishihara, H. Migaki et al., "Two different zinc transport complexes of cation diffusion facilitator proteins localized in the secretory pathway operate to activate alkaline phosphatases in vertebrate cells," *The Journal of Biological Chemistry*, vol. 280, no. 35, pp. 30956–30962, 2005.

[47] T. Kambe, M. Matsunaga, and T. A. Takeda, "Understanding the contribution of zinc transporters in the function of the early secretory pathway," *International Journal of Molecular Sciences*, vol. 18, no. 10, article 2179, 2017.

[48] S. J. Galli, "Mast cells and basophils," *Current Opinion in Hematology*, vol. 7, no. 1, pp. 32–39, 2000.

[49] H. Karasuyama, K. Miyake, S. Yoshikawa, and Y. Yamanishi, "Multifaceted roles of basophils in health and disease," *Journal of Allergy and Clinical Immunology*, vol. 142, no. 2, pp. 370–380, 2018.

[50] J. T. Schroeder, D. W. MacGlashan jr., and L. M. Lichtenstein, "Human basophils: mediator release and cytokine production," *Advances in Immunology*, vol. 77, pp. 93–122, 2001.

[51] R. A. Seder, W. E. Paul, A. M. Dvorak et al., "Mouse splenic and bone marrow cell populations that express high-affinity Fc epsilon receptors and produce interleukin 4 are highly enriched in basophils," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 88, no. 7, pp. 2835–2839, 1991.

[52] C. L. Sokol, G. M. Barton, A. G. Farr, and R. Medzhitov, "A mechanism for the initiation of allergen-induced T helper type 2 responses," *Nature Immunology*, vol. 9, no. 3, pp. 310–318, 2008.

[53] D. Voehringer, K. Shinkai, and R. M. Locksley, "Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production," *Immunity*, vol. 20, no. 3, pp. 267–277, 2004.

[54] T. Ugajin, K. Nishida, S. Yamasaki et al., "Zinc-binding metallothioneins are key modulators of IL-4 production by basophils," *Molecular Immunology*, vol. 66, no. 2, pp. 180–188, 2015.

[55] T. Ugajin, S. Shibama, K. Nishida, and H. Yokozeki, "Metallothioneins are required for human basophil interleukin-4 gene induction via FcεRI stimulation," *Allergology International*, vol. 65, no. 4, pp. 466–468, 2016.

[56] M. M. King and C. Y. Huang, "The calmodulin-dependent activation and deactivation of the phosphoprotein phosphatase, calcineurin, and the effect of nucleotides, pyrophosphate, and divalent metal ions. Identification of calcineurin as a Zn and Fe metalloenzyme," *The Journal of Biological Chemistry*, vol. 259, no. 14, pp. 8847–8856, 1984.

[57] F. Rusnak and P. Mertz, "Calcineurin: form and function," *Physiological Reviews*, vol. 80, no. 4, pp. 1483–1521, 2000.

[58] T. B. Aydemir, J. P. Liuzzi, S. McClellan, and R. J. Cousins, "Zinc transporter ZIP8 (SLC39A8) and zinc influence IFN-gamma expression in activated human T cells," *Journal of Leukocyte Biology*, vol. 86, no. 2, pp. 337–348, 2009.

[59] J. Huang, D. Zhang, W. Xing et al., "An approach to assay calcineurin activity and the inhibitory effect of zinc ion," *Analytical Biochemistry*, vol. 375, no. 2, pp. 385–387, 2008.

[60] K. Takahashi, E. Akaishi, Y. Abe et al., "Zinc inhibits calcineurin activity in vitro by competing with nickel," *Biochemical and Biophysical Research Communications*, vol. 307, no. 1, pp. 64–68, 2003.

[61] K. Hayashi, S. Ishizuka, C. Yokoyama, and T. Hatae, "Attenuation of interferon-gamma mRNA expression in activated Jurkat T cells by exogenous zinc via down-regulation of the calcium-independent PKC-AP-1 signaling pathway," *Life Sciences*, vol. 83, no. 1-2, pp. 6–11, 2008.

[62] Z. Zhou, L. Wang, Z. Song, J. T. Saari, C. J. McClain, and Y. J. Kang, "Abrogation of nuclear factor-kappaB activation is involved in zinc inhibition of lipopolysaccharide-induced tumor necrosis factor-alpha production and liver injury," *The American Journal of Pathology*, vol. 164, no. 5, pp. 1547–1556, 2004.

[63] B. Bao, A. S. Prasad, F. W. J. Beck, and M. Godmere, "Zinc modulates mRNA levels of cytokines," *American Journal of Physiology-Endocrinology and Metabolism*, vol. 285, no. 5, pp. E1095–E1102, 2003.

[64] C. Driessen, K. Hirv, N. Wellinghausen, H. Kirchner, and L. Rink, "Influence of serum on zinc, toxic shock syndrome toxin-1, and lipopolysaccharide-induced production of IFN-gamma and IL-1 beta by human mononuclear cells," *Journal of Leukocyte Biology*, vol. 57, no. 6, pp. 904–908, 1995.

[65] L. Rink and H. Kirchner, "Zinc-altered immune function and cytokine production," *The Journal of Nutrition*, vol. 130, no. 5, pp. 1407S–1411S, 2000.

[66] N. Wellinghausen, M. Martin, and L. Rink, "Zinc inhibits interleukin-1-dependent T cell stimulation," *European Journal of Immunology*, vol. 27, no. 10, pp. 2529–2535, 1997.

[67] M. Yu, W. W. Lee, D. Tomar et al., "Regulation of T cell receptor signaling by activation-induced zinc influx," *The Journal of Experimental Medicine*, vol. 208, no. 4, pp. 775–785, 2011.

[68] E. H. Palacios and A. Weiss, "Function of the Src-family kinases, Lck and Fyn, in T-cell development and activation," *Oncogene*, vol. 23, no. 48, pp. 7990–8000, 2004.

[69] G. Altan-Bonnet and R. N. Germain, "Modeling T cell antigen discrimination based on feedback control of digital ERK responses," *PLoS Biology*, vol. 3, no. 11, article e356, 2005.

[70] A. S. Prasad, "Zinc and immunity," *Molecular and Cellular Biochemistry*, vol. 188, no. 1/2, pp. 63–69, 1998.

[71] Z. A. Bhutta, S. M. Bird, R. E. Black et al., "Therapeutic effects of oral zinc in acute and persistent diarrhea in children in developing countries: pooled analysis of randomized controlled trials," *The American Journal of Clinical Nutrition*, vol. 72, no. 6, pp. 1516–1522, 2000.

[72] S. K. Dutta, F. Procaccino, and R. Aamodt, "Zinc metabolism in patients with exocrine pancreatic insufficiency," *Journal of the American College of Nutrition*, vol. 17, no. 6, pp. 556–563, 1998.

[73] P. J. Fraker, L. E. King, T. Laakko, and T. L. Vollmer, "The dynamic link between the integrity of the immune system and zinc status," *The Journal of Nutrition*, vol. 130, no. 5, pp. 1399S–1406S, 2000.

[74] L. E. King, F. Osati-Ashtiani, and P. J. Fraker, "Depletion of cells of the B lineage in the bone marrow of zinc-deficient mice," *Immunology*, vol. 85, no. 1, pp. 69–73, 1995.

[75] N. F. Krebs, M. Sontag, F. J. Accurso, and K. M. Hambidge, "Low plasma zinc concentrations in young infants with cystic fibrosis," *The Journal of Pediatrics*, vol. 133, no. 6, pp. 761–764, 1998.

[76] A. S. Prasad, "Clinical and biochemical manifestation zinc deficiency in human subjects," *Journal de Pharmacologie*, vol. 16, no. 4, pp. 344–352, 1985.

[77] E. Tapazoglou, A. S. Prasad, G. Hill, G. J. Brewer, and J. Kaplan, "Decreased natural killer cell activity in patients with zinc deficiency with sickle cell disease," *The Journal of Laboratory and Clinical Medicine*, vol. 105, no. 1, pp. 19–22, 1985.

[78] B. S. Zemel, D. A. Kawchak, E. B. Fung, K. Ohene-Frempong, and V. A. Stallings, "Effect of zinc supplementation on growth and body composition in children with sickle cell disease," *The American Journal of Clinical Nutrition*, vol. 75, no. 2, pp. 300–307, 2002.

[79] T. Ohkawara, H. Takeda, K. Kato et al., "Polaprezinc (N-(3-aminopropionyl)-L-histidinato zinc) ameliorates

dextran sulfate sodium-induced colitis in mice," *Scandinavian Journal of Gastroenterology*, vol. 40, no. 11, pp. 1321–1327, 2005.

[80] C. D. Tran, J. M. Ball, S. Sundar, P. Coyle, and G. S. Howarth, "The role of zinc and metallothionein in the dextran sulfate sodium-induced colitis mouse model," *Digestive Diseases and Sciences*, vol. 52, no. 9, pp. 2113–2121, 2007.

[81] C. Kitabayashi, T. Fukada, M. Kanamoto et al., "Zinc suppresses Th17 development via inhibition of STAT3 activation," *International Immunology*, vol. 22, no. 5, pp. 375–386, 2010.

[82] E. Bettelli, M. Oukka, and V. K. Kuchroo, "T(H)-17 cells in the circle of immunity and autoimmunity," *Nature Immunology*, vol. 8, no. 4, pp. 345–350, 2007.

[83] M. Nishihara, H. Ogura, N. Ueda et al., "IL-6-gp130-STAT3 in T cells directs the development of IL-17+ Th with a minimum effect on that of Treg in the steady state," *International Immunology*, vol. 19, no. 6, pp. 695–702, 2007.

[84] I. I. Ivanov, B. S. McKenzie, L. Zhou et al., "The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17$^+$ T helper cells," *Cell*, vol. 126, no. 6, pp. 1121–1133, 2006.

[85] M. Veldhoen, R. J. Hocking, C. J. Atkins, R. M. Locksley, and B. Stockinger, "TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells," *Immunity*, vol. 24, no. 2, pp. 179–189, 2006.

[86] S. Fukuyama, Y. Matsunaga, W. Zhanghui et al., "A zinc chelator TPEN attenuates airway hyperresponsiveness and airway inflammation in mice in vivo," *Allergology International*, vol. 60, no. 3, pp. 259–266, 2011.

[87] M. Toyran, M. Kaymak, E. Vezir et al., "Trace element levels in children with atopic dermatitis," *Journal of Investigational Allergology & Clinical Immunology*, vol. 22, no. 5, pp. 341–344, 2012.

[88] T. J. David, F. E. Wells, T. C. Sharpe, A. C. C. Gibbs, and J. Devlin, "Serum levels of trace metals in children with atopic eczema," *British Journal of Dermatology*, vol. 122, no. 4, pp. 485–489, 1990.

[89] J. E. Kim, S. R. Yoo, M. G. Jeong, J. Y. Ko, and Y. S. Ro, "Hair zinc levels and the efficacy of oral zinc supplementation in patients with atopic dermatitis," *Acta Dermato-Venereologica*, vol. 94, no. 5, pp. 558–562, 2014.

[90] N. A. Mohamed, M. Rushdy, and A. S. M. Abdel-Rehim, "The immunomodulatory role of zinc in asthmatic patients," *Cytokine*, vol. 110, pp. 301–305, 2018.

[91] J. Ghaffari, A. Khalilian, E. Salehifar, E. Khorasani, and M. S. Rezaii, "Effect of zinc supplementation in children with asthma: a randomized, placebo-controlled trial in northern Islamic Republic of Iran," *Eastern Mediterranean Health Journal*, vol. 20, no. 6, pp. 391–396, 2014.

[92] S. Rerksuppaphol and L. Rerksuppaphol, "Zinc supplementation in children with asthma exacerbation," *Pediatric Reports*, vol. 8, no. 4, article 6685, 2016.

Hindawi
Journal of Immunology Research
Volume 2018, Article ID 4315140, 8 pages
https://doi.org/10.1155/2018/4315140

Review Article

Essential Role of Zinc and Zinc Transporters in Myeloid Cell Function and Host Defense against Infection

Muna Sapkota and Daren L. Knoell

Department of Pharmacy Practice, College of Pharmacy, 86120 Nebraska Medical Center Omaha, NE 68198-6120, USA

Correspondence should be addressed to Daren L. Knoell; daren.knoell@unmc.edu

Received 31 May 2018; Accepted 27 September 2018; Published 17 October 2018

Guest Editor: Toshiyuki Fukada

Zinc is an essential micronutrient known to play a vital role in host defense against pathogens. Diets that are deficient in zinc lead to impaired immunity and delayed recovery from and worse outcomes following infection. Sustained insufficient zinc intake leads to dysregulation of the innate immune response and increases susceptibility to infection whereas zinc supplementation in at-risk populations has been shown to restore host defense and reduce pathogen-related morbidity and mortality. Upon infection, zinc deficiency leads to increased pathology due to imbalance in key signaling networks that result in excessive inflammation and collateral tissue damage. In particular, zinc impacts macrophage function, a critical front-line cell in host defense, in addition to other immune cells. Deficits in zinc adversely impact macrophage function resulting in dysregulation of phagocytosis, intracellular killing, and cytokine production. An additional work in this field has revealed a vital role for several zinc transporter proteins that are required for proper bioredistribution of zinc within mononuclear cells to achieve an optimal immune response against invading microorganisms. In this review, we will discuss the most recent developments regarding zinc's role in innate immunity and protection against pathogen invasion.

1. Zinc and Human Health

Zinc (Zn) is an essential micronutrient in human health. According to the World Health Organization (WHO), an estimated 30% of the world's population is Zn deficient and inadequate intake contributes to 800,000 deaths worldwide [1]. Although severe Zn deficiency is rare, mild-to-moderate deficiency is prevalent throughout the world [2, 3]. It is the fifth leading risk factor for bacterial acquired diarrhea and pneumonia in developing countries [4]. These findings underscore the importance of Zn deficiency as a well-established nutritional problem in underdeveloped countries; however, it is important to recognize that Zn deficiency is also common in developed countries. According to the National Health and Nutrition Examination Survey (NHANES) that is conducted within the United States (US) population, the percentage of dietary intakes below the estimated average daily requirement is quite common in the elderly and up to 24% in 14- to 18-year-old females and 12% in adults over 19 years of age. Collectively, this indicates that inadequate Zn intake is quite common and may contribute substantially to comorbidity throughout the world across diverse populations.

Zinc is essential for the proper function of both eukaryotic and prokaryotic cells. It is required for normal growth, repair, and maintaining the structure and function of proteins and nucleic acids including enzymes and transcription factors. Despite the vital necessity of Zn, there exist no depots of Zn in the body that can be used to maintain metal levels for protracted periods of time [5]. This is further complicated in developing countries where other factors including limited protein-based food choices rich in bioavailable Zn significantly perpetuate Zn deficiency [6, 7]. A major contributing factor to this dilemma results from higher consumption of phytates, an inhibitor of Zn absorption in the gastrointestinal tract [8]. In addition, intestinal malabsorption, renal disease, alcoholism, cancer, aging, pharmacologic interactions, and alteration in Zn transporters can contribute to Zn deficiency [9, 10]. Further, the elderly, infants, and pregnant and lactating women are more susceptible to Zn deficiency and

corresponding infections that can be fatal if not corrected with dietary supplementation [11, 12].

This evidence substantiates that dietary Zn uptake is important in maintaining homeostasis, proper innate immune balance, and host defense [13]. Zn also possesses known anti-inflammatory and antioxidant effects, and nutritional supplementation has been reported to be beneficial in suppressing the duration and severity of infectious-based gastrointestinal and respiratory disease particularly in children less than 5 years of age [14]. Given the many roles of Zn in maintaining immune function, it is not surprising that Zn deficiency increases the risk of morbidity and mortality from infection.

2. Zinc and Infection in Mammals

Zn is essential for the proper function of the innate and adaptive immune systems; therefore, depletion of whole body Zn content adversely impacts the ability of the host to mount a balanced immune response against invading pathogens [15]. Multiple epidemiologic studies conducted over the past two decades demonstrate that Zn deficiency increases susceptibility to infections that the host would otherwise, under normal circumstances, be less vulnerable to. Most notably inadequate Zn intake is associated with new-onset upper respiratory tract and gastrointestinal tract infections in children less than 5 years old in developing countries [16, 17]. More recently, it has been shown that gastrointestinal giardiasis impairs intestinal mucosa function thereby lowering Zn absorption in young children resulting in Zn deficiency [18]. Given the importance of Zn relative to host defense, multiple studies have been conducted in humans and animals to determine whether Zn supplementation can restore immune function and prevent or reduce the severity of infection. Zn intake was originally shown to reduce the incidence of acute lower respiratory infection in preschool children [19]. Sánchez and colleagues more recently reported that the incidence of acute diarrhea and respiratory infection was reduced following the administration of a Zn amino acid chelate in children [20]. Sazawal and colleagues reported that Zn supplementation reduced duration and severity of diarrhea in infants and young children [21], and Prasad and colleagues reported that the incidence of bacterial infection in sickle cell disease was significantly reduced after Zn supplementation [22]. Bolick and colleagues reported that Zn-deficient diet mice had significantly greater weight loss and diarrhea compared to control mice when infected with enteroaggregative *Escherichia coli* (EAEC), a pathogen that is responsible for infectious diarrhea in children. In addition, Zn-deficient mice had reduced infiltration of leukocytes into the ileum in response to infection indicative of an impaired immune response [23]. Similarly, mice infected with HlyA-producing *E. coli* were able to better maintain epithelial function and reduce barrier disruption and fluid leakage with diminished bacterial translocation as a result of Zn supplementation [24]. More recently, administration of Zn oxide nanoparticles was shown to reduce fluid accumulation in the mouse ileum following the administration of cholera toxin protein [25]. Zinc supplementation has also been reported to be beneficial in animal models of severe infection. Sepsis mortality was significantly increased in Zn-deficient animals when compared to animals maintained on a Zn sufficient, control diet subject to cecal ligation puncture. Prior to death, Zn-deficient animals exhibited higher plasma cytokines, oxidative tissue damage, cell death, and more severe vital organ injury. However, when Zn supplementation was provided to Zn insufficient mice prior to the initiation of sepsis, it resulted in the normalization of the inflammatory response, diminished tissue damage, and significantly reduced mortality [26]. Zn supplementation has most often been reported to be beneficial for restoring immune function and downregulating conditions associated with chronic inflammatory responses [27]. Zn supplementation has also been shown to improve macrophage phagocytosis and oxidative burst function [28] and reduce C-reactive protein, IL-6, MMP2, and MMP9 production in a model of chronic liver inflammation [29]. Zn is also crucial for maintenance and function of the immune system during the process of aging [30]. Unfortunately, human studies where Zn supplementation has been provided to critically ill patients have not yet proven to be beneficial suggesting that critical illness is more complicated to treat [31]. Based on this, well-controlled, large, randomized control trials are required to determine whether optimal Zn dosing strategies exist. Notably, sepsis creates a significant challenge because it is difficult to anticipate the onset of sepsis thereby prohibiting preventive treatment strategies. Collectively, these studies strongly support the importance of Zn as an essential nutrient in host defense against pathogen invasion in the lung, gut, and systemic compartments and also highlight the need for more study aimed at determining how best to administer Zn to populations that are more susceptible to infection.

3. Zinc and Myeloid Cell Function against Pathogens

Invading pathogens are first encountered by cells that comprise the innate immune system. Myeloid lineage cells including polymorphonuclear neutrophils, monocytes, and macrophages are critical first responders engineered to recognize and eliminate pathogens. Micronutrient metabolism plays a critical role in innate immune defense against microbial infection. Macrophages exploit transition metals in part by manipulating their uptake and trafficking following pathogen recognition. Cation redistribution into the cytosol, in general, is designed to benefit the host in a number of important ways. It inhibits pathogen growth through deprivation of indispensable micronutrients, generates host protective Fenton reaction-dependent reactive oxygen species, and affords nonspecific inhibition of bacterial protein binding [32–34]. Importantly, internalized micronutrients also help orchestrate vital signaling pathways. Macrophages differ significantly from monocytes in their phenotype and function and the metabolic pathways responsible for zinc trafficking during macrophage host defense remain largely unexplored [33, 35]. In response to microbes, macrophages produce both proinflammatory cytokines and anti-inflammatory cytokines, like IL-10, in order to coordinate a

balanced response aimed at efficiently eliminating infection while minimizing damage to surrounding tissue [36]. Importantly, IL-10 stimulation of murine and human macrophages significantly reduces production of proinflammatory cytokines [37–40]. Zn has been shown to regulate endotoxin-mediated immune activation of human macrophages through a reduction in IL-10 production presumably leading to imbalance in immune regulation, although the latter was evaluated in this study [41]. In yet another mechanism of Zn sequestration, Histoplasma capsulatum induced mobilization of Zn within macrophages away from the phagosome and into the apparatus triggering superoxide formation and enhancing antimicrobial defense [42]. In the case of Salmonella infection, a bacteria-driven elevation of intracellular Zn levels occurs weakening antimicrobial defense and the ability of macrophages to eradicate the pathogen thereby demonstrating that certain bacteria can establish host subversion strategies that use Zn to their advantage [43, 44]. Based on these findings, it is clear that there exists a tug-of-war between macrophages and other myeloid-lineage cells and the pathogens that they encounter. In the case of bacteria that reside and thrive outside of macrophages, cellular activation most often leads to Zn-mediated changes that favor the host. In contrast, intracellular pathogens have the potential to subvert Zn metabolism and cellular activation in a manner that may favor pathogen survival and propagation within the macrophage.

Zinc is also important for monocyte and macrophage development and other vital functions including phagocytosis and cytokine production [45, 46]. Zinc is also necessary for adherence, migration, and differentiation of monocytes into tissue macrophages [47] and is crucial for the normal development and function of neutrophils. Zinc deficiency causes dysfunctional neutrophil phagocytosis, intracellular killing, and cytokine production [10]. In addition, neutrophil chemotaxis and the quantity of enzyme-containing cytosolic granulocytes decrease as a result of Zn deficiency [48]. A major role of neutrophils is to clear invading pathogens through formation of an extracellular network trap that is also disrupted under Zn-deficient conditions [49]. Therefore, recent studies have identified that intracellular Zn content within myeloid-lineage cells is absolutely critical in maintaining a defensive phenotype in a number of ways that influence organelle and protein function in order to enhance bacterial elimination.

Multiple cell signaling pathways are influenced by intracellular free Zn levels [50]. One of the most studied is the NF-$\kappa\beta$ signaling pathway. In particular, insufficient levels of intracellular Zn result in aberrant cell signaling causing an exaggerated inflammatory response as exhibited by the overproduction of cytokines and chemokines including TNFα, IL-6, IL-8, and IL-10. Zinc deficiency can also induce changes in the expression of other cytokines, DNA repair enzymes, zinc transporters, and signaling molecules that regulate immune function. As one example, macrophages have an armamentarium of recognition receptors, including Toll-like receptors (TLR), designed to recognize bacterial, viral, and fungal byproducts. Endotoxin is one of the most robustly studied ligands for TLR4 signaling. Transmission of cell signaling in monocytes is Zn dependent following LPS recognition and impact activation of the p38 MAPK, ERK1/2, and NF-$\kappa\beta$ pathways [51]. Depletion of Zn was also shown to inhibit endotoxin-induced activation of several MAPK kinase family members and IκBα in murine primary macrophages and corresponding cell lines [52]. In a polymicrobial sepsis mouse model, Zn deficiency was shown to enhance bacterial burden, NF-$\kappa\beta$ activity, and the corresponding IL1β, TNFα, and ICAM-1 gene expression and protein production in the lung when compared to Zn sufficient control animals [53]. Another study by this group revealed that Zn deficiency enhanced signaling through the JAK-STAT3 pathway and NF-$\kappa\beta$ pathway leading to increased inflammation and over activation of the inflammatory response in a polymicrobial sepsis mouse model [54]. Similarly, Zn has been shown to be critical in regulation of kinase activation and transcription factors that subsequently alter the expression of proteins [55]. Zinc has also been reported to alter the function of different immunologically relevant mitogens and bacterial stimulants. As one example, Zn was shown to enhance the immunogenicity of lipopolysaccharide with respect to cell recognition and cytokine induction in leukocytes by altering its structure into a more biologically active isoform [56].

Collectively, Zn-mediated regulation of innate immune host defense through myeloid lineage cells demonstrates that Zn is absolutely required to maintain proper function. In particular, deficits in Zn that typically occur across many populations as a result of insufficient dietary intake and or absorption result in dysregulated myeloid cell function in response to invading pathogens. The extent of immune dysfunction is context dependent in terms of host phenotype (age, gender, and health status), location of the infection (lung, gut, bloodstream), and cell type that pathogens encounter in these compartments (neutrophils, monocytes, macrophages, and other myeloid cell types not yet studied), as well as the pathogen (bacterial, viral, and fungal). A key aspect of Zn, beyond having sufficient amounts in our body, involves its rapid and controlled mobilization from the bloodstream into tissues and ultimately into cellular compartments and organelles where it has a direct impact on enzymatic function. In this regard, mammals have developed a sophisticated system to shuttle Zn in and out of cells and cellular organelles, and into specific locations within the cell allowing Zn to specifically interact with nucleic acids and proteins in a manner that positively impacts cellular function and bolsters host defense.

4. Zinc Transporters

The concentration of cellular Zn is rather high such that Zn can hardly be considered a trace element. Zinc is used as a cofactor in proteins much more frequently than most vitamins. Control over the fluctuating Zn pool occurs at remarkably low concentrations, often within the picomolar range, through the participation of many proteins [57]. Zinc homeostasis in mammals is primarily maintained through Zn transporters that are designed to regulate cellular uptake, efflux, and intracellular trafficking of Zn. The majority of

intracellular Zn exists in a tightly bound form most commonly associated with metalloproteins and Zn finger proteins. Approximately, ten percent of cellular Zn exists in a more loosely bound form commonly referred to as labile Zn. Whereas the tightly bound form of Zn is not subjected to rapid mobilization, the labile pool can be mobilized within and outside of cells very rapidly in response to cellular stress and infection. The labile pool therefore plays an important role in intracellular Zn fluxes and rapid alteration of cell function, but it is also more readily depleted in the setting of Zn deficiency [58]. There exist two major Zn transporter/carrier families known as (solute carrier family = SLC) SLC30 and SLC39 that have been extensively studied for their roles in maintaining cytosolic as well as cellular organelle Zn levels. There are 10 members of the human $SLC30^{1-10}$ or ZnT family that are primarily involved with transporting zinc from the cytosol to the extracellular space or into intracellular organelles and therefore function largely to efflux Zn out of the cytosol especially in conditions of excess [59]. The SLC39 family or ZIP (Zrt-, Irt-like proteins) is comprised of 14 members that are primarily involved in transporting Zn from the extracellular space or intracellular organelles into the cytosol and therefore function to increase cytosolic zinc concentrations [60]. ZIPs and ZnTs are involved in many cellular responses. Through the mobilization of Zn, they regulate enzymes, receptors, and transcription factors as well as cytokine and growth factor-mediated signaling pathways [61]. In collaboration with metallothioneins (MTs), these proteins collectively maintain cellular Zn content thereby countering rapid or prolonged changes in available Zn in a manner designed to maintain normal cellular function yet maintain cells in a vigilant state in advance of potentially harmful external challenges [62, 63].

Cytosolic labile Zn content can change rapidly in response to pathogens and plays an important role in Zn signaling that triggers immune activation [64]. Upon extracellular stimulation, Zn is rapidly released as a "zinc wave" into the cytosol from intracellular stores. This phenomenon activates two mitogen-activated protein kinase (MAPK) pathways, the extracellular signal-related kinase (ERK) and the c-Jun N-terminal kinase (JNK) signaling pathways [60] and the transcription factor NF-κB, which targets genes involved in cellular replication and apoptosis [65]. The expression and function of certain Zn transporter genes are responsive to physiologic stimuli and external challenges, whereas others appear to be constitutively expressed and vital for the day-to-day maintenance of body Zn composition. Moving forward, we will focus on Zn transporters that have been shown to be responsive to pathogen invasion and host defense. A variety of stimuli, including inflammatory cytokines and pathogens or bacterial byproducts, have been shown to induce the expression of MTs, ZnTs, and ZIP transporters [66]. Importantly, altered expression of Zn transporters during inflammatory conditions is part of the acute phase response that is designed to rapidly mobilize labile Zn in a manner that affords cell protection, maintains or increases vital functions, and bolsters host defense [63]. As a leading example, ZIP14 (slc39A14) was the first Zn transporter shown to be highly upregulated in the liver, white adipose tissue, and muscle in a mouse model after exposure to endotoxin LPS [67]. Studies conducted in a mouse model of endotoxemia first revealed that ZIP14 expression is upregulated through IL-6 and that this Zn transporter plays a major role in the mechanism responsible for hypozincemia, not to be mistaken with Zn deficiency as a result of insufficient dietary intake that accompanies the acute-phase response to inflammation and infection [68]. This group went on to reveal that ZIP14 plays an important role in LPS-mediated IL-6 release and that IL-6 synthesis after LPS stimulation is regulated by STAT3 and IκB phosphorylation. However, in LPS-injected Zip14 KO mice, mRNA expression of suppressor of cytokine signaling-3 (SOCS-3), a downstream target gene of the STAT3-IκB pathway, was markedly increased in LPS-injected null mice [67]. A related study showed that following intraperitoneal administration of lipopolysaccharide or the proinflammatory cytokines tumor necrosis factor (TNF) or interleukin-6 (IL6), mice exhibited quantitatively very different, highly tissue-specific, and markedly time-dependent up- and downregulation of ZIP8 and ZIP14 mRNA levels in vital organs. This revealed for the first time that most if not all tissues use ZIP8 and ZIP14 to some extent for Zn uptake. Some tissues are under basal conditions and others more so when inflammatory stressors are present that collectively lead to substantial alterations in plasma Zn levels due to Zn redistribution not just in the liver but across many vital organs [69]. More recently, using *Zip14* knockout (KO) mice, it was shown that ablation of *Zip14* delayed leukocytosis, prevented zinc accumulation in the liver, altered the kinetics of hypozincemia, and drastically increased serum IL-6, TNFα, and IL-10 concentrations following nonlethal sepsis further establishing that ZIP14 is vital to host defense in the setting of polymicrobial sepsis [70]. Consistent with these observations, the closest homologue to ZIP14, ZIP8, was shown to have induced expression in lung cells in response to LPS or TNF-α resulting in the expression of a heavily glycosylated, membrane-bound protein that lead to Zn import and cell survival [71]. A further work by this group revealed that ZIP8 plays a vital role in regulating the host response to sepsis, in part through monocyte and macrophage regulation, by downmodulating the NF-$\kappa\beta$ pathway. In particular, ZIP8 gene expression was induced by the NF-$\kappa\beta$ pathway following bacterial activation and the new pool of Zn transported into myeloid cells ultimately inhibited IKKβ activation [41]. Relative to myeloid-lineage cells, ZIP8 expression was observed to be elevated in macrophages following LPS exposure resulting in cellular accumulation of Zn and enhanced IL-10 release. Further, ZIP8 was vital to immune function because knockdown inhibited LPS-driven Zn accumulation and reduced Zn-dependent reduction of IL-10 release [41]. Similarly, *Mtb* infection of macrophages was shown to induce the expression of ZIP8 with a very little effect on other ZIPs although it remains to be determined whether ZIP8 enhances Zn uptake in favor of the host or pathogen [72]. Taken together, Zn transporter-mediated Zn redistribution via ZIP8 and ZIP14 upon infection likely serves multiple purposes [73]. In addition to these two closely related homologues, other Zn transporters have more recently been

shown to play a role in host defense as well. In particular, a recent study using macrophage-specific *Slc39a10*-knockout mice, we revealed that Slc39a10 plays an essential role in macrophage survival by mediating Zn homeostasis in response to LPS stimulation. Compared with *wild-type* mice, *Slc39a10*-knockout mice had significantly lower mortality following LPS stimulation as well as reduced liver damage and lower levels of circulating inflammatory cytokines [74]. Based on these findings, a picture is beginning to emerge that multiple Zn transporters play important roles in the strengthening innate immune function, in part by regulating macrophage behavior, in response to systemic bacterial invasion.

In the last decade, mutations in ZIP and ZnT transporter genes have been shown to be implicated in a number of inherited human diseases many of which lead to inflammation-based pathology and therefore may have relevance to infectious-based disease [75]. Moreover, dysregulation in the expression and activity of both transporter families has been suggested to be involved in the pathogenesis and progression of multiple diseases [76, 77]. As leading examples, mutations in ZIP4 (SLC39A4) and ZIP13 (SLC39A13) are responsible for the rare lethal autosomal-recessive inherited zinc deficiency disease, acrodermatitis enteropathica (AE) [78], and Ehlers-Danlos Syndrome (EDS) [79], respectively. Additionally, targeted ZnT3 disruption causes memory deficits with Alzheimer's disease-like abnormalities in mice [80]. Another study reported that a polymorphic genetic variant of ZnT10 was related to hypermagnesemia as well as a variety of neurological and hepatic disturbances [81]. Abnormal hematopoiesis and organ morphogenesis have been reported in ZIP8-hypomorphic mice [82]. Collectively, these studies strongly support the importance of the normal expression and function of Zn transporters in order to maintain Zn homeostasis in mammals but how this impacts the host response to pathogen invasion remains to be determined. In all likelihood, we predict that commonly occurring polymorphic variants of Zn transporters, including but not limited to ZIP8 and ZIP14, have the potential to increase susceptibility to infection by adversely altering Zn metabolism during the acute-phase response and thereby altering cellular defense mechanisms in myeloid and other parenchymal cell types.

5. Conclusion

Zinc is an essential metal that plays an important role in maintaining mammalian health and fending off invasive pathogens. It is fundamentally important in maintaining biochemical balance and influences multiple components of the innate immune system through modulation of protein function. In developing countries, limited uptake of Zn-containing foods, often a consequence of low economic status, has provided the best evidence that a lack of dietary Zn intake is a major contributing factor in susceptibility to bacterial infections. In recent years, using cell and animal models, it has become clear that the increased morbidity and mortality associated with Zn deficiency and bacterial infection are a consequence of impaired immune regulation. Zinc and a select number of Zn transporters have begun to emerge in recent years as a novel host defense strategy aimed at quickly mobilizing labile Zn within the body to, in most instances, bolster host defense and sequester Zn away from prokaryotic cells that also require Zn to maintain vital functions. Although exactly how Zn metabolism regulates, host defense will require much more study because there remain many unanswered questions. Additional studies will be required to better understand the multiple roles of Zn relative to innate immune signaling across different cell types, including but not limited to myeloid cells, through initiation and maintenance of an effective host response. What does seem clear is that a Zn deficit in most cases leads to exaggeration of the host defense response against pathogens. While at face value it would appear that amplification of host defense mechanisms would be advantageous, this is often not the case. In fact, exaggeration of the host response to infection has most commonly been shown to increase the risk of collateral tissue damage leading to poorer prognosis in terms of morbidity and mortality. To better understand the role of Zn transporters in pathogen-related pathological conditions, additional investigations of the molecular mechanisms of Zn-mediated regulation of immune function are warranted. In particular, some Zn transporters are known to transport other metal ions besides Zn; however, a number of these studies are currently limited and have primarily focused on the relative uptake affinity and biodistribution for other metal ions including iron, cadmium, and manganese. Whether these metals also play an important role in maintaining proper host defense against pathogens remains to be determined. In addition, this could lead to the design and synthesis of new compounds directed toward therapeutic targets that either modulate protein function or alter metal uptake and efflux via Zn transporters. Finally, a limited number of important studies have provided proof of principal that Zn supplementation can decrease the incidence and severity of bacterial infections in populations where the frequency of Zn-deficient intake is high. Most importantly, Zn has been shown to be the most beneficial when used as a preventive strategy prior to the development of established infection. In all likelihood, there does not exist a one size fits all therapeutic approach. Given the substantial biochemical footprint of Zn across the human proteome, it is also plausible that Zn supplementation could be disadvantageous under certain conditions against different pathogens. Perhaps most importantly, strategies that more accurately detect subacute Zn deficiency will expedite our ability to predict and prevent serious, life-threatening infection. Past studies have largely focused on the utility of blood and tissue Zn levels or protein biomarkers, with limited success. As an alternative approach, the genetic heterogeneity of Zn transporters relative to increased disease risk has begun to emerge and may provide a predictive tool to identify at-risk populations against different pathogens based on screening for polymorphic genetic variants that alter normal Zn metabolism and immune function. Scientific advances in the field of Zn biology coupled to advances in high throughput genetic and proteomic screening approaches will undoubtedly shed new light on this emerging field and hopefully provide translational

advances that improve our capacity to predict and prevent the extent of infectious disease in many different settings across vast populations.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Acknowledgments

This work was supported by the NHLBI grant # R01 HL118268.

References

[1] L. E. Caulfield and R. E. Black, "Zinc deficiency," in *Comparative Quantification of Health Risks: Global and Regional Burden of Disease Attributable to Selected Major Risk Factors*, vol. 1, pp. 257–280, WHO, Geneva, Switzerland, 2004.

[2] H. H. Sandstead, "Assessment of zinc nutriture," *The Journal of Laboratory and Clinical Medicine*, vol. 118, no. 4, pp. 299-300, 1991.

[3] H. H. Sandstead, "Zinc deficiency. A public health problem?," *American Journal of Diseases of Children*, vol. 145, no. 8, pp. 853–859, 1991.

[4] K. R. Wessells and K. H. Brown, "Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting," *PLoS One*, vol. 7, no. 11, article e50568, 2012.

[5] A. Sanna, D. Firinu, P. Zavattari, and P. Valera, "Zinc status and autoimmunity: a systematic review and meta-analysis," *Nutrients*, vol. 10, no. 1, 2018.

[6] A. S. Prasad, "Discovery of human zinc deficiency: 50 years later," *Journal of Trace Elements in Medicine and Biology*, vol. 26, no. 2-3, pp. 66–69, 2012.

[7] K. M. Hambidge, L. V. Miller, and N. F. Krebs, "Zinc requirements: assessment and population needs," *Nestlé Nutrition Institute Workshop Series*, vol. 70, pp. 27–35, 2012.

[8] J. R. Hunt, "Moving toward a plant-based diet: are iron and zinc at risk?," *Nutrition Reviews*, vol. 60, no. 5, pp. 127–134, 2002.

[9] A. J. R. Cabrera, "Zinc, aging, and immunosenescence: an overview," *Pathobiology of Aging & Age-related Diseases*, vol. 5, no. 1, article 25592, 2015.

[10] C. T. Chasapis, A. C. Loutsidou, C. A. Spiliopoulou, and M. E. Stefanidou, "Zinc and human health: an update," *Archives of Toxicology*, vol. 86, no. 4, pp. 521–534, 2012.

[11] R. S. Gibson, "Zinc deficiency and human health: etiology, health consequences, and future solutions," *Plant and Soil*, vol. 361, no. 1-2, pp. 291–299, 2012.

[12] H. Yasuda and T. Tsutsui, "Infants and elderlies are susceptible to zinc deficiency," *Scientific Reports*, vol. 6, no. 1, p. 21850, 2016.

[13] N. Roohani, R. Hurrell, R. Kelishadi, and R. Schulin, "Zinc and its importance for human health: an integrative review," *Journal of Research in Medical Sciences*, vol. 18, no. 2, pp. 144–157, 2013.

[14] M. Y. Yakoob, E. Theodoratou, A. Jabeen et al., "Preventive zinc supplementation in developing countries: impact on mortality and morbidity due to diarrhea, pneumonia and malaria," *BMC Public Health*, vol. 11, Supplement 3, p. S23, 2011.

[15] M. K. Ibrahim, M. Zambruni, C. L. Melby, and P. C. Melby, "Impact of childhood malnutrition on host defense and infection," *Clinical Microbiology Reviews*, vol. 30, no. 4, pp. 919–971, 2017.

[16] L. E. Caulfield, N. Zavaleta, A. H. Shankar, and M. Merialdi, "Potential contribution of maternal zinc supplementation during pregnancy to maternal and child survival," *The American Journal of Clinical Nutrition*, vol. 68, no. 2, pp. 499S–508S, 1998.

[17] R. E. Black, C. G. Victora, S. P. Walker et al., "Maternal and child undernutrition and overweight in low-income and middle-income countries," *The Lancet*, vol. 382, no. 9890, pp. 427–451, 2013.

[18] H. Astiazarán-García, G. Iñigo-Figueroa, L. Quihui-Cota, and I. Anduro-Corona, "Crosstalk between zinc status and Giardia infection: a new approach," *Nutrients*, vol. 7, no. 6, pp. 4438–4452, 2015.

[19] S. Sazawal, R. E. Black, S. Jalla, S. Mazumdar, A. Sinha, and M. K. Bhan, "Zinc supplementation reduces the incidence of acute lower respiratory infections in infants and preschool children: a double-blind, controlled trial," *Pediatrics*, vol. 102, no. 1, pp. 1–5, 1998.

[20] J. Sánchez, O. A. Villada, M. L. Rojas et al., "Effect of zinc amino acid chelate and zinc sulfate in the incidence of respiratory infection and diarrhea among preschool children in child daycare centers," *Biomédica*, vol. 34, no. 1, pp. 79–91, 2014.

[21] S. Sazawal, R. E. Black, M. K. Bhan, N. Bhandari, A. Sinha, and S. Jalla, "Zinc supplementation in young children with acute diarrhea in India," *New England Journal of Medicine*, vol. 333, no. 13, pp. 839–844, 1995.

[22] A. S. Prasad, F. W. J. Beck, J. Kaplan et al., "Effect of zinc supplementation on incidence of infections and hospital admissions in sickle cell disease (SCD)," *American Journal of Hematology*, vol. 61, no. 3, pp. 194–202, 1999.

[23] D. T. Bolick, G. L. Kolling, J. H. Moore II et al., "Zinc deficiency alters host response and pathogen virulence in a mouse model of enteroaggregative Escherichia coli-induced diarrhea," *Gut Microbes*, vol. 5, no. 5, pp. 618–627, 2014.

[24] S. Wiegand, S. S. Zakrzewski, M. Eichner et al., "Zinc treatment is efficient against Escherichia coli α-haemolysin-induced intestinal leakage in mice," *Scientific Reports*, vol. 7, no. 1, p. 45649, 2017.

[25] S. Sarwar, A. Ali, M. Pal, and P. Chakrabarti, "Zinc oxide nanoparticles provide anti-cholera activity by disrupting the interaction of cholera toxin with the human GM1 receptor," *The Journal of Biological Chemistry*, vol. 292, no. 44, pp. 18303–18311, 2017.

[26] D. L. Knoell, M. W. Julian, S. Bao et al., "Zinc deficiency increases organ damage and mortality in a murine model of polymicrobial sepsis," *Critical Care Medicine*, vol. 37, no. 4, pp. 1380–1388, 2009.

[27] E. John, T. C. Laskow, W. J. Buchser et al., "Zinc in innate and adaptive tumor immunity," *Journal of Translational Medicine*, vol. 8, no. 1, p. 118, 2010.

[28] V. Kloubert, K. Blaabjerg, T. S. Dalgaard, H. D. Poulsen, L. Rink, and I. Wessels, "Influence of zinc supplementation on immune parameters in weaned pigs," *Journal of Trace Elements in Medicine and Biology*, vol. 49, pp. 231–240, 2018.

[29] C. Xu, Z. Huang, L. Liu et al., "Zinc regulates lipid metabolism and MMPs expression in lipid disturbance rabbits," *Biological Trace Element Research*, vol. 168, no. 2, pp. 411–420, 2015.

[30] C. P. Wong and E. Ho, "Zinc and its role in age-related inflammation and immune dysfunction," *Molecular Nutrition & Food Research*, vol. 56, no. 1, pp. 77–87, 2012.

[31] D. K. Heyland, N. Jones, N. Z. Cvijanovich, and H. Wong, "Zinc supplementation in critically ill patients: a key pharmaconutrient?," *JPEN Journal of Parenteral and Enteral Nutrition*, vol. 32, no. 5, pp. 509–519, 2008.

[32] W. P. Lafuse, G. R. Alvarez, and B. S. Zwilling, "Regulation of Nramp1 mRNA stability by oxidants and protein kinase C in RAW264.7 macrophages expressing Nramp1Gly169," *Biochemical Journal*, vol. 351, no. 3, pp. 687–696, 2000.

[33] K. S. Vignesh, J. A. Landero Figueroa, A. Porollo, J. A. Caruso, and G. S. Deepe Jr., "Granulocyte macrophage-colony stimulating factor induced Zn sequestration enhances macrophage superoxide and limits intracellular pathogen survival," *Immunity*, vol. 39, no. 4, pp. 697–710, 2013.

[34] E. D. Weinberg, "Nutritional immunity, host's attempt to withhold iron from microbial invaders," *JAMA*, vol. 231, no. 1, pp. 39–41, 1975.

[35] S. L. Stafford, N. J. Bokil, M. E. S. Achard et al., "Metal ions in macrophage antimicrobial pathways: emerging roles for zinc and copper," *Bioscience Reports*, vol. 33, no. 4, pp. 541–554, 2013.

[36] M. Saraiva and A. O'garra, "The regulation of IL-10 production by immune cells," *Nature Reviews Immunology*, vol. 10, no. 3, pp. 170–181, 2010.

[37] L. Siewe, M. Bollati–Fogolin, C. Wickenhauser, T. Krieg, W. Müller, and A. Roers, "Interleukin-10 derived from macrophages and/or neutrophils regulates the inflammatory response to LPS but not the response to CpG DNA," *European Journal of Immunology*, vol. 36, no. 12, pp. 3248–3255, 2006.

[38] D. F. Fiorentino, A. Zlotnik, T. R. Mosmann, M. Howard, and A. O'Garra, "IL-10 inhibits cytokine production by activated macrophages," *The Journal of Immunology*, vol. 147, no. 11, pp. 3815–3822, 1991.

[39] C. Bogdan, Y. Vodovotz, and C. Nathan, "Macrophage deactivation by interleukin 10," *Journal of Experimental Medicine*, vol. 174, no. 6, pp. 1549–1555, 1991.

[40] M. J. Thomassen, L. T. Divis, and C. J. Fisher, "Regulation of human alveolar macrophage inflammatory cytokine production by interleukin-10," *Clinical Immunology and Immunopathology*, vol. 80, no. 3, pp. 321–324, 1996.

[41] C. J. Pyle, S. Akhter, S. Y. Bao, C. E. Dodd, L. S. Schlesinger, and D. L. Knoell, "Zinc modulates endotoxin-induced human macrophage inflammation through ZIP8 induction and C/EBPβ inhibition," *PLoS One*, vol. 12, no. 1, article e0169531, 2017.

[42] K. S. Vignesh, J. A. Landero Figueroa, A. Porollo, J. A. Caruso, and G. S. Deepe, "Zinc sequestration: arming phagocyte defense against fungal attack," *PLoS Pathogens*, vol. 9, no. 12, article e1003815, 2013.

[43] A. Wu, P. Tymoszuk, D. Haschka et al., "*Salmonella* utilizes zinc to subvert antimicrobial host defense of macrophages via modulation of NF-κB signaling," *Infection and Immunity*, vol. 85, no. 12, 2017.

[44] R. Kapetanovic, N. J. Bokil, M. E. S. Achard et al., "Salmonella employs multiple mechanisms to subvert the TLR-inducible zinc-mediated antimicrobial response of human macrophages," *The FASEB Journal*, vol. 30, no. 5, pp. 1901–1912, 2016.

[45] A. S. Prasad, "Discovery of human zinc deficiency: its impact on human health and disease," *Advances in Nutrition*, vol. 4, no. 2, pp. 176–190, 2013.

[46] G. Weiss and P. L. Carver, "Role of divalent metals in infectious disease susceptibility and outcome," *Clinical Microbiology and Infection*, vol. 24, no. 1, pp. 16–23, 2018.

[47] H. Haase and L. Rink, "Zinc signals and immune function," *BioFactors*, vol. 40, no. 1, pp. 27–40, 2014.

[48] K.-H. Ibs and L. Rink, "Zinc-altered immune function," *The Journal of Nutrition*, vol. 133, no. 5, pp. 1452S–1456S, 2003.

[49] L. S. Mayer, P. Uciechowski, S. Meyer, T. Schwerdtle, L. Rink, and H. Haase, "Differential impact of zinc deficiency on phagocytosis, oxidative burst, and production of pro-inflammatory cytokines by human monocytes," *Metallomics*, vol. 6, no. 7, pp. 1288–1295, 2014.

[50] A. S. Prasad, "Zinc in human health: an update," *The Journal of Trace Elements in Experimental Medicine*, vol. 11, no. 2-3, pp. 63–87, 1998.

[51] H. Haase, J. L. Ober-Blobaum, G. Engelhardt et al., "Zinc signals are essential for lipopolysaccharide-induced signal transduction in monocytes," *The Journal of Immunology*, vol. 181, no. 9, pp. 6491–6502, 2008.

[52] M. Maywald, I. Wessels, and L. Rink, "Zinc signals and immunity," *International Journal of Molecular Sciences*, vol. 18, no. 10, p. 2222, 2017.

[53] S. Bao, M. J. Liu, B. Lee et al., "Zinc modulates the innate immune response in vivo to polymicrobial sepsis through regulation of NF-κB," *American Journal of Physiology-Lung Cellular and Molecular Physiology*, vol. 298, no. 6, pp. L744–L754, 2010.

[54] M.-J. Liu, S. Bao, J. R. Napolitano et al., "Zinc regulates the acute phase response and serum amyloid A production in response to sepsis through JAK-STAT3 signaling," *PLoS One*, vol. 9, no. 4, article e94934, 2014.

[55] I. Wessels, M. Maywald, and L. Rink, "Zinc as a gatekeeper of immune function," *Nutrients*, vol. 9, no. 12, p. 1286, 2017.

[56] L. Rink and H. Kirchner, "Zinc-altered immune function and cytokine production," *The Journal of Nutrition*, vol. 130, no. 5, pp. 1407S–1411S, 2000.

[57] W. Maret, "Zinc biochemistry: from a single zinc enzyme to a key element of life," *Advances in Nutrition*, vol. 4, no. 1, pp. 82–91, 2013.

[58] A. Q. Truong-Tran, J. Carter, R. Ruffin, and P. D. Zalewski, "New insights into the role of zinc in the respiratory epithelium," *Immunology & Cell Biology*, vol. 79, no. 2, pp. 170–177, 2001.

[59] L. Huang and S. Tepaamorndech, "The SLC30 family of zinc transporters–a review of current understanding of their biological and pathophysiological roles," *Molecular Aspects of Medicine*, vol. 34, no. 2-3, pp. 548–560, 2013.

[60] E. Bafaro, Y. Liu, Y. Xu, and R. E. Dempski, "The emerging role of zinc transporters in cellular homeostasis and cancer," *Signal Transduction and Targeted Therapy*, vol. 2, article 17029, 2017.

[61] T. Takagishi, T. Hara, and T. Fukada, "Recent advances in the role of SLC39A/ZIP zinc transporters in vivo," *International Journal of Molecular Sciences*, vol. 18, no. 12, p. 2708, 2017.

[62] N. Gammoh and L. Rink, "Zinc in infection and inflammation," *Nutrients*, vol. 9, no. 6, p. 624, 2017.

[63] M. Foster and S. Samman, "Zinc and regulation of inflammatory cytokines: implications for cardiometabolic disease," *Nutrients*, vol. 4, no. 7, pp. 676–694, 2012.

[64] D. L. Knoell and M.-J. Liu, "Impact of zinc metabolism on innate immune function in the setting of sepsis," *International Journal for Vitamin and Nutrition Research*, vol. 80, no. 45, pp. 271–277, 2010.

[65] H. Haase and D. Beyersmann, "Intracellular zinc distribution and transport in C6 rat glioma cells," *Biochemical and Biophysical Research Communications*, vol. 296, no. 4, pp. 923–928, 2002.

[66] M. Yu, W. W. Lee, D. Tomar et al., "Regulation of T cell receptor signaling by activation-induced zinc influx," *The Journal of Experimental Medicine*, vol. 208, no. 4, pp. 775–785, 2011.

[67] T. B. Aydemir, S.-M. Chang, G. J. Guthrie et al., "Zinc transporter ZIP14 functions in hepatic zinc, iron and glucose homeostasis during the innate immune response (endotoxemia)," *PLoS One*, vol. 7, no. 10, article e48679, 2012.

[68] J. P. Liuzzi, L. A. Lichten, S. Rivera et al., "Interleukin-6 regulates the zinc transporter Zip14 in liver and contributes to the hypozincemia of the acute-phase response," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 19, pp. 6843–6848, 2005.

[69] M. Gálvez-Peralta, Z. Wang, S. Bao, D. L. Knoell, and D. W. Nebert, "Tissue-specific induction of mouse ZIP8 and ZIP14 divalent cation/bicarbonate symporters by, and cytokine response to, inflammatory signals," *International Journal of Toxicology*, vol. 33, no. 3, pp. 246–258, 2014.

[70] I. Wessels and R. J. Cousins, "Zinc dyshomeostasis during polymicrobial sepsis in mice involves zinc transporter Zip14 and can be overcome by zinc supplementation," *American Journal of Physiology-Gastrointestinal and Liver Physiology*, vol. 309, no. 9, pp. G768–G778, 2015.

[71] M.-J. Liu, S. Bao, M. Gálvez-Peralta et al., "ZIP8 regulates host defense through zinc-mediated inhibition of NF-κB," *Cell Reports*, vol. 3, no. 2, pp. 386–400, 2013.

[72] C. Pyle, A. Azad, A. Papp, W. Sadee, D. Knoell, and L. Schlesinger, "Elemental ingredients in the macrophage cocktail: role of ZIP8 in host response to *Mycobacterium tuberculosis*," *International Journal of Molecular Sciences*, vol. 18, no. 11, p. 2375, 2017.

[73] M. Jarosz, M. Olbert, G. Wyszogrodzka, K. Młyniec, and T. Librowski, "Antioxidant and anti-inflammatory effects of zinc. Zinc-dependent NF-κB signaling," *Inflammopharmacology*, vol. 25, no. 1, pp. 11–24, 2017.

[74] H. Gao, L. Zhao, H. Wang et al., "Metal transporter Slc39a10 regulates susceptibility to inflammatory stimuli by controlling macrophage survival," *Proceedings of the National Academy of Sciences*, vol. 114, no. 49, pp. 12940–12945, 2017.

[75] S. Hojyo and T. Fukada, "Roles of zinc signaling in the immune system," *Journal of Immunology Research*, vol. 2016, 21 pages, 2016.

[76] T. Kambe, A. Hashimoto, and S. Fujimoto, "Current understanding of ZIP and ZnT zinc transporters in human health and diseases," *Cellular and Molecular Life Sciences*, vol. 71, no. 17, pp. 3281–3295, 2014.

[77] T. Kimura and T. Kambe, "The functions of metallothionein and ZIP and ZnT transporters: an overview and perspective," *International Journal of Molecular Sciences*, vol. 17, no. 3, p. 336, 2016.

[78] K. Wang, B. Zhou, Y. M. Kuo, J. Zemansky, and J. Gitschier, "A novel member of a zinc transporter family is defective in acrodermatitis enteropathica," *The American Journal of Human Genetics*, vol. 71, no. 1, pp. 66–73, 2002.

[79] B.-H. Bin, T. Fukada, T. Hosaka et al., "Biochemical characterization of human ZIP13 protein a homo-dimerized zinc transporter involved in the spondylocheiro dysplastic ehlers-danlos syndrome," *Journal of Biological Chemistry*, vol. 286, no. 46, pp. 40255–40265, 2011.

[80] J.-Y. Lee, E. Cho, J. W. Seo, J. J. Hwang, and J. Y. Koh, "Alteration of the cerebral zinc pool in a mouse model of Alzheimer disease," *Journal of Neuropathology & Experimental Neurology*, vol. 71, no. 3, pp. 211–222, 2012.

[81] M. Quadri, A. Federico, T. Zhao et al., "Mutations in SLC30A10 cause parkinsonism and dystonia with hypermanganesemia, polycythemia, and chronic liver disease," *The American Journal of Human Genetics*, vol. 90, no. 3, pp. 467–477, 2012.

[82] M. Gálvez-Peralta, L. He, L. F. Jorge-Nebert et al., "ZIP8 zinc transporter: indispensable role for both multiple-organ organogenesis and hematopoiesis in utero," *PLoS One*, vol. 7, no. 5, article e36055, 2012.

Hindawi
Journal of Immunology Research
Volume 2018, Article ID 9365747, 9 pages
https://doi.org/10.1155/2018/9365747

Review Article

Function, Structure, and Transport Aspects of ZIP and ZnT Zinc Transporters in Immune Cells

Bum-Ho Bin,[1,2] **Juyeon Seo**,[3] **and Sung Tae Kim**[4]

[1]*Department of Molecular Science and Technology, College of Natural Sciences, Ajou University, Suwon 16499, Republic of Korea*
[2]*Division of Pathology, Department of Oral Diagnostic Sciences, School of Dentistry, Showa University, Tokyo 142-8555, Japan*
[3]*Basic Research & Innovation Division, AmorePacific R&D Unit, Yongin 17014, Republic of Korea*
[4]*Department of Pharmaceutical Engineering, Inje University, Gimhae 50834, Republic of Korea*

Correspondence should be addressed to Bum-Ho Bin; bbh82429@gmail.com and Sung Tae Kim; stkim@inje.ac.kr

Received 29 May 2018; Accepted 30 August 2018; Published 2 October 2018

Academic Editor: Ananda S. Prasad

Zinc is an important trace metal in immune systems, and zinc transporters are involved in many immune responses. Recent advances have revealed the structural and biochemical bases for zinc transport across the cell membrane, with clinical implications for the regulation of zinc homeostasis in immune cells like dendritic cells, T cells, B cells, and mast cells. In this review, we discuss the function, structure, and transport aspects of two major mammalian zinc transporter types, importers and exporters. First, Zrt-/Irt-like proteins (ZIPs) mediate the zinc influx from the extracellular or luminal side into the cytoplasm. There are 14 ZIP family members in humans. They form a homo- or heterodimer with 8 transmembrane domains and extra-/intracellular domains of various lengths. Several ZIP members show specific extracellular domains composed of two subdomains, a helix-rich domain and proline-alanine-leucine (PAL) motif-containing domain. Second, ZnT (zinc transporter) was initially identified in early studies of zinc biology; it mediates zinc efflux as a counterpart of ZIPs in zinc homeostasis. Ten family members have been identified. They show a unique architecture characterized by a Y-shaped conformation and a large cytoplasmic domain. A precise, comprehensive understanding of the structures and transport mechanisms of ZIP and ZnT in combination with mice experiments would provide promising drug targets as well as a basis for identifying other transporters with therapeutic potential.

1. The Role of Zinc Transporters in Immune Cells

Zinc plays critical roles in the immune system, and its transport proteins, called zinc transporters, are reportedly involved in many immune responses [1, 2]. Two types of zinc transporters are conserved in mammals: Zrt-/Irt-like protein (ZIP) zinc transporters, which increase cytoplasmic zinc level by zinc import, and ZnTs (zinc transporters), which decrease cytoplasmic zinc level by zinc export [1].

ZIP6 and ZIP10 were initially reported as the key mammalian zinc transporters in the regulation of immune cell function [3]. Dendritic cells (DCs) that are differentiated from hematopoietic bone marrow progenitor cells play important roles in presenting antigens to T cells as messengers between the innate and the adaptive immune systems [4]. When DCs are exposed to lipopolysaccharides (LPS), a ligand for toll-like receptor 4 (TLR4) is fragmented from the outer membrane of gram-negative bacteria, and intracellular zinc level is drastically decreased for maturation [3]. LPS stimulation activates the TLR4-TRIF axis, resulting in downregulation of zinc importers ZIP6 and ZIP10 and upregulation of zinc exporters Znt1 and Znt6. Similar to LPS, the zinc-chelating reagent, TPEN (*N*,*N*,*N*′,*N*′-tetrakis (2-pyridylmethyl)-ethylenediamine), induces a significant upregulation of the surface expression of the major histocompatibility complex (MHC) class II and CD86, leading to DC maturation. ZIP6 is also an important molecule in CD4 T cells [5]. CD4 T cells are white blood cells that mature in the thymus from thymocytes and play a central role in humoral immunity [6]. CD4 T cells express the T cell receptor (TCR) on their surface, which recognizes antigenic

peptides bound to MHC-α in DCs or other antigen presenting cells. TCR activation induces naïve CD4 T cells to differentiate into several T cell subsets including Th1, Th2, Th17, and Treg cells. When TCR signaling is activated by DCs, the ZIP6-mediated zinc level at spatially restricted regions near the immunological synapse between T cell and DC is increased [5]. This zinc accumulation inhibits tyrosine phosphatase SHP-1 recruitment for the negative regulation of TCR signaling, leading to the activation of the TCR-LCK-ZAP70 pathway for cell proliferation and cytokine production. TCR activation induces ZIP8 expression in human T cells [7]. ZIP8 is localized to the lysosomal membrane to transport zinc from the luminal side to the cytoplasm of T cells during TCR activation. An increased ZIP8-mediated zinc level blocks calcineurin activity, thereby mediating the phosphorylation of CREB for IFN-gamma transcription. This result supports several well-known reports that zinc deficiency reduces the production of cytokines such as IL-1, IL-2, and IFN-γ [7–10].

Recent advances have demonstrated that ZIP8 is important in various immune cells associated with innate immune function [11]. NF-κB, a central transcription factor that regulates innate immune responses including proinflammation pathways, regulates ZIP8 expression in monocytes, macrophages, DCs, and nonprofessional cells such as lung epithelia. When TNF-α and LPS bind to their receptors, IκB kinase (IKK) is activated, leading to IκBα protein phosphorylation. Phosphorylated IκBα is then dissociated from NF-κB, which activates NF-κB to translocate to the nucleus for the transcription of cytokines and ZIP8. ZIP8-mediated zinc upregulation inhibits IKK upon binding to a specific site within the kinase domain. Thus, ZIP8 negatively regulates the NF-κB pathway, indicating that the zinc-ZIP8-NF-κB axis plays crucial roles during host defense.

B cells play central roles in the adaptive immune system by producing antibodies. Though ZIP10 is enriched in the epidermis for epidermal progenitor survival [12, 13], ZIP10 is also essential for B cell survival and proper functioning [14, 15]. ZIP10-depletion during early B cell development induces a significant reduction in the B cell population by increasing apoptosis [15]. In the normal state, ZIP10-mediated intracellular zinc efficiently inhibits caspases. However, when ZIP10 is depleted, intracellular zinc level is decreased, leading to the activation of caspases for apoptosis. ZIP10 is also important for B cell antigen receptor (BCR) signaling [14]. ZIP10-depleted mature B cells proliferated poorly after BCR cross-linking due to the dysregulation of BCR signaling. In these cells, CD45R phosphatase activity for BCR signaling is reduced. Taken together, ZIP10 is indispensable for immature and mature B cell maintenance for humoral immune responses. Interestingly, in the chicken DT40 cells, ZIP9 is crucial for BCR signaling by regulating AKT and ERK [16].

The zinc transporter can indirectly modulate immune responses. Although ZIP4 is also expressed in the human epidermis [17], it is mainly expressed in the brush border of the small intestine for zinc uptake from diet [18]. Thus, mutations in ZIP4 lead to a zinc deficiency in the body. Though ZIP4 is expressed in intestinal enterocytes, mutations in ZIP4 leads to acrodermatitis enteropathica (AE), a type of skin disease involving blistering of the skin and enhancement of bacterial infection. In AE patients and mice with zinc-deficient diets (ZD), the skin pathogenic mechanism is associated with Langerhans cells (LCs) [19]. Zinc deficiency leads to an impaired production of TGF-β1, which is essential for LCs, leading to, at least in part, LC loss. Zinc deficiency itself could increase the apoptosis rate, further leading to LC loss in the skin of AE patients and ZD mice. The loss of LCs increases the accumulation of adenosine triphosphate (ATP), released from damaged keratinocytes, in the epidermis, leading to irritant contact dermatitis. Similarly, zinc transporters restricted to normal human cells, except immune cells, can mediate immune responses since normal human cells release many cytokines and compounds that can stimulate immune cells.

Many studies have highlighted the importance of the ZIP members in immune response; however, relatively little information about the ZnT members is available. ZnT5-mediated zinc homeostasis in allergic response is a well-known phenomenon. ZnT5-depleted mast cells show reduced Fc epsilon receptor I- (FcϵRI-) induced cytokine production [20]. ZnT5 regulates FcϵRI-induced translocation of protein kinase C (PKC) to the cell membrane for NF-κB-dependent cytokine production. In addition, ZnT8, which is expressed in β cells and associated with diabetes [21, 22], may modulate immune cells since diabetes disturbs the body's immune system.

Taken together, zinc transporters play indispensable roles in immune cells. Therefore, understanding the structure and transport mechanism of zinc transporters could offer new approaches to develop drugs to treat immune dysfunction-related diseases.

2. Structure of ZIPs

2.1. ZIP Family. Based on sequence analyses, ZIP family members contain 8 transmembrane domains (TMDs) as membrane transport proteins, with a cytoplasmic region between TM3 and TM4 [23, 24] (Figure 1(a)). The ZIP family is divided into four subfamilies according to sequence similarity: subfamilies I, II, gufA, and LIV-1. Subfamilies I, II, and gufA are phylogenetically closely related [24]. The LIV-1 subfamily is associated with the estrogen-regulated gene *LIV-1* and is mainly found in mammals [24]. Only LIV-1 subfamily members contain the HEXXH motif within TM5; they also exhibit diverse lengths of the N-terminal extracellular domain (ECD) [24] (Figure 1(a)). The ECD is very small or is not present in subfamilies I, II, and gufA, but is highly variable in LIV-1 [1, 25]. In mammals, 14 ZIP family members have been identified and are referred to as ZIP1–14. ZIP1, 2, and 3 are classified as the ZIP II subfamily. ZIP9 belongs to the ZIP I subfamily, and ZIP11 to the gufA subfamily. The remaining 9 ZIPs are classified as LIV-1 subfamily members, and the complicated roles of these ZIPs in mammalian cells may be associated with their ECDs. The largest cytoplasmic domain (CTD) of ZIP (ZIP-CTD) is located between TM3 and TM4. ZIP4-CTD is ubiquitinated

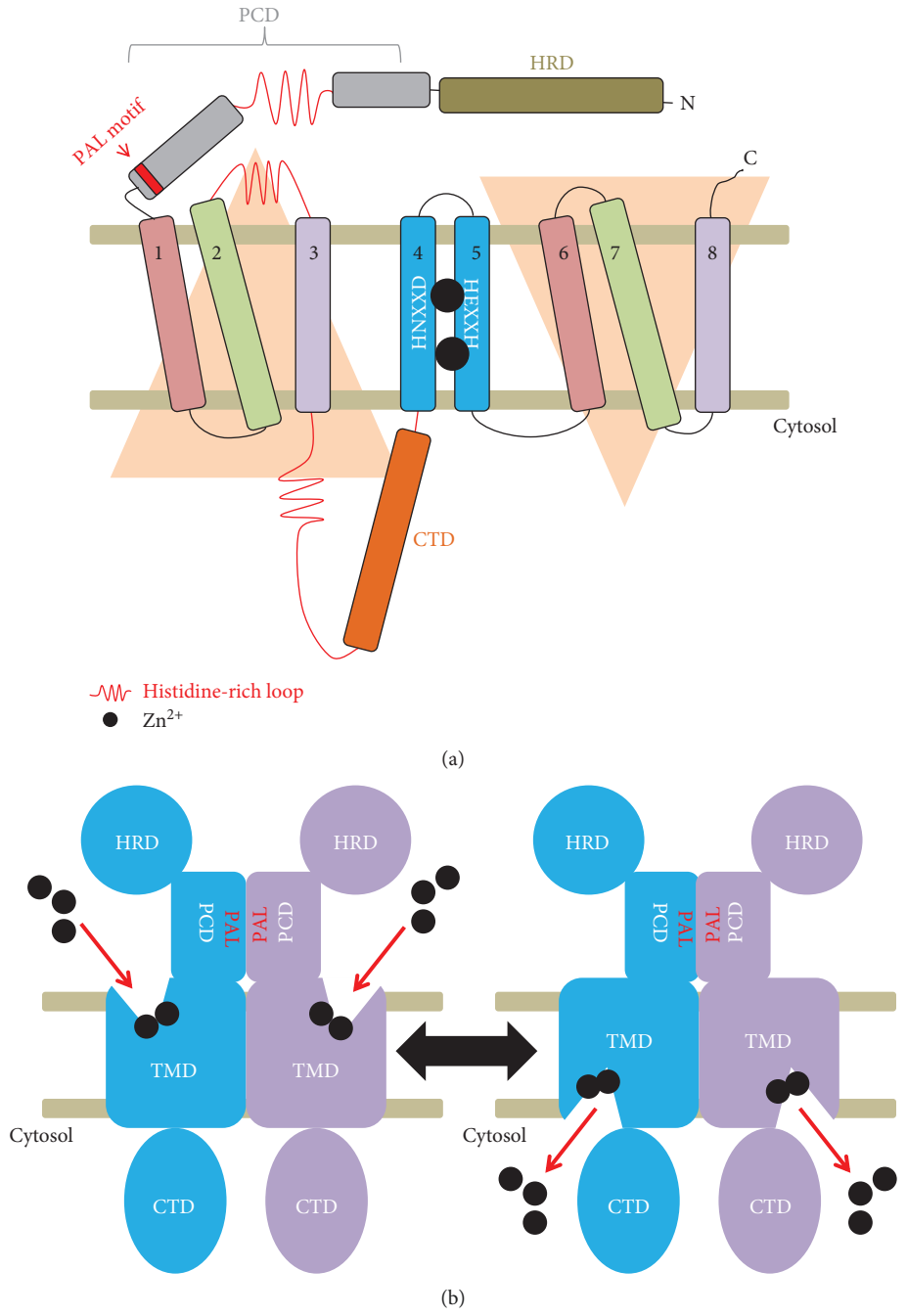


Figure 1: Overview of ZIP. (a) Topological model of ZIP. ZIP is composed of 8 TMDs with a large N-terminal domain including a PCD and HRD as well as a CTD. The pseudosymmetric TMs are shown in the same color. Zn^{2+} binds to the active site made up of TMD4 and 5 via the conserved HNXXD and HEXXH motifs. (b) Transport mechanism. ZIP has two major conformations, the inward-open and the outward-open conformations. The PAL motif and TMDs are involved in dimerization.

in response to metal cytotoxicity [26]. However, the roles of ZIP-CTD are unknown [23].

2.2. Structural Overview. Among 14 mammalian ZIP family members, only ZIP13, a LIV-1 subfamily member, has been successfully purified from an insect overexpression system owing to bottlenecks in the overexpression and purification procedures for other mammalian ZIPs [23]. For precise biochemical and structural analyses, prokaryotic ZIPs have been applied to mammalian ZIP research. ZIPB from *Bordetella bronchiseptica* was overexpressed, purified from *Escherichia coli*, and crystallized to determine the X-ray crystal structure [27]. The crystal structure of ZIPB revealed a novel 3 + 2 + 3-TM architecture; the first 3 TMs (TM1 to TM3) can be superimposed on the last three TMs (TM6–8) by rotating 180°, and TM4 and TM5 are symmetrically related and sandwiched by the two 3-TM repeats. This feature is unique among transporters.

2.3. Zinc Binding Site. The crystal structure of ZIPB demonstrated an inward-open conformation with a binuclear zinc center in the transport pathway, suggesting the existence of an outward-open conformation [27] (Figure 1(b)). The binuclear zinc center is formed by TM4 and TM5 with several conserved polar amino acid residues (Figure 1(a)). The HEXXHE motif, a LIV-1-specific motif, is found in TM5, and a comparative computational model of the ZIPB structure indicated that this motif serves as the binuclear zinc center. The ZIPB structure shows multiple conserved zinc binding sites near the metal exit cavity, indicating that these sites constitute a route of zinc release to the cytoplasm. Therefore, it is possible that the conserved histidine-rich motif found in mammalian ZIPs near TMs includes multiple zinc binding sites to transfer zinc efficiently from the binuclear zinc center.

2.4. Extracellular Domain. The crystal structure of only the ECD of ZIP4 (ZIP4-ECD) from *Pteropus alecto* has been revealed [25]. The ZIP4-ECD is stabilized by 4 conserved disulfide bonds. The ZIP4-ECD has two structurally independent subdomains with 14 α-helices, a helix-rich domain (HRD), and a PAL motif-containing domain (PCD) that is observed only in LIV-1 subfamily members. In particular, the HRD is conserved in ZIP4 and ZIP12 among 9 LIV-1 subfamily members, and others lack this domain. The HRD forms a novel protein fold from 156 amino acids from the N-terminus of ZIP4 and is composed of 9 α-helices, indicating a high α-helical content (73%) (Figure 1(a)). The PCD is conserved in ZIP4, 5, 6, 8, 10, and 12, but is lacking in ZIP7 and ZIP13. Interestingly, ZIP7 and ZIP13 are localized to intracellular compartments, like the ER and Golgi apparatus [28, 29], suggesting that the HRD and PCD are associated with their localization. The PCD, a C-terminal subdomain of ZIP4-ECD, forms from 130 amino acids; it is composed of 5 α-helices and exhibits a pair of helix-turn-helix folds. The longest α-helix of PCD possesses the PAL motif, which is structurally important for dimerization and stabilization. All of the helices in the PCD are involved in dimerization, and the PAL motif is at the center of the dimerization interface. However, ZIP13, which contains a short ECD with a degenerated PAL motif, also forms a dimer, suggesting that PCD may not be crucial for dimerization in ZIP13. PCD also contains an unstructured histidine-rich loop with potential roles in metal sensing, transport, or storage. According to a recent proposal, the cellular prion protein (PrP^C) evolved from ZIP approximately 500 million years ago, since the cysteine-flanked core (CFC) domain within PCD shows high structural similarity to PrP^C [30]. The PAL motif in PrP also represents a dimerization interface, similar to ZIP4-ECD [27, 30].

2.5. Oligomerization. ZIP13 has been purified as a homodimer [23] (Figure 1(b)). A purified prokaryotic ZIP, ZIPB in *n*-dodecyl-β-D-maltopyranoside (DDM) ran as a dimer on size exclusion columns based on their apparent detergent-solubilized molecular weights [27]. Interestingly, purified ZIPB shows two species, consistent with monomeric and dimeric states, and both species crystallized under identical conditions [27]. Moreover, purified ZIP13 sometimes ran as a monomer in blue native- (BN-) PAGE and Western blot analyses [23]. The ZIP monomer itself forms a perfect metal transport channel [27] and accordingly may act as a metal transporter. ZIP6 and ZIP10 reportedly form a heterodimer that is critical for the epithelial-to-mesenchymal transition [31]. Thus, the dimeric state might have further functions, for example, in signal transduction.

2.6. Transport Mechanism. The zinc transport mechanism is still controversial and depends on the type of ZIP. Early cell-based assays using isotopes have revealed that zinc flux via ZIP2 is time-, temperature-, and concentration-dependent and saturable with an apparent K_m of 3 μm in transfected K562 erythroleukemia cells [32]. ZIP2 activity was not affected by ATP, K^+, or Na^+ gradients, but was significantly stimulated by HCO_3^- treatment with high affinity to the first zinc and the next cadmium, suggesting a Zn^{2+}-HCO_3^- symporter mechanism [32]. ZIP8, which is involved in cadmium-induced toxicity in the testis [33], shows a Mn^{2+}-HCO_3^- symporter mechanism in ZIP8 cDNA-stable retroviral-infected mouse fetal fibroblasts in physiological conditions [34]. Previous reports have shown that the K_m for Cd^{2+} uptake by ZIP8 is 0.62 μM, and the K_m for Mn^{2+} uptake is 2.2 μM with a low affinity to Zn^{2+}. However, ZIP8 shows a Zn^{2+}-HCO_3^- symporter mechanism with an influx of two HCO_3^- anions per one Cd^{2+} (or one Zn^{2+}) cation, that is, electroneutral complexes, in an electrogenicity study of ZIP8 cRNA-injected *Xenopus* oocytes [35]. Cadmium (Cd^{2+}) and zinc (Zn^{2+}) uptake have V_{max} values of 1.8 ± 0.08 and 1.0 ± 0.08 pmol/oocyte/h, and K_m values of 0.48 ± 0.08 and 0.26 ± 0.09 μM. Many other metal transporters on the surface of mammalian cells might obscure these effects. *Xenopus* oocytes have negligible amounts of interfering transporters, but proper protein folding and distribution are sometimes a bottleneck in the determination of the precise transport mechanism. To resolve this issue, a proteoliposome-based kinetic analysis using purified ZIPB, a prokaryotic ZIP, has been applied to determine the transport mechanism. Similar to a zinc-selective channel, zinc flux via ZIPB was nonsaturable for a zinc concentration of up to 2 mM and was electrogenic [36], and this transport mechanism is consistent with ZIP2 [37]. However, this is inconsistent with the results of previous cell-based and electrogenic analyses, further emphasizing the lack of clarity regarding the zinc transport mechanism of mammalian ZIPs.

The crystal structure of ZIPB revealed that the eight TMs are grouped into two units, one including TM1, 4, 5, and 6 and another including the other four TMs as a traditional membrane carrier involving significant conformational changes [27] (Figure 1(b)). The solved crystal structure indicating an inward-open conformation of ZIPB without open entrances toward the extracellular space has a filter-like histidine 177 (H177) in the metal pathway below the metal binding sites toward the cytosol. H177 adopts two conformations for metal release from metal binding site 1. Below H177 toward the cytosol, where the exit cavity is located, a histidine-rich loop connecting TM3 and 4 probably facilitates zinc release as a "metal sink." Though a proteoliposome-

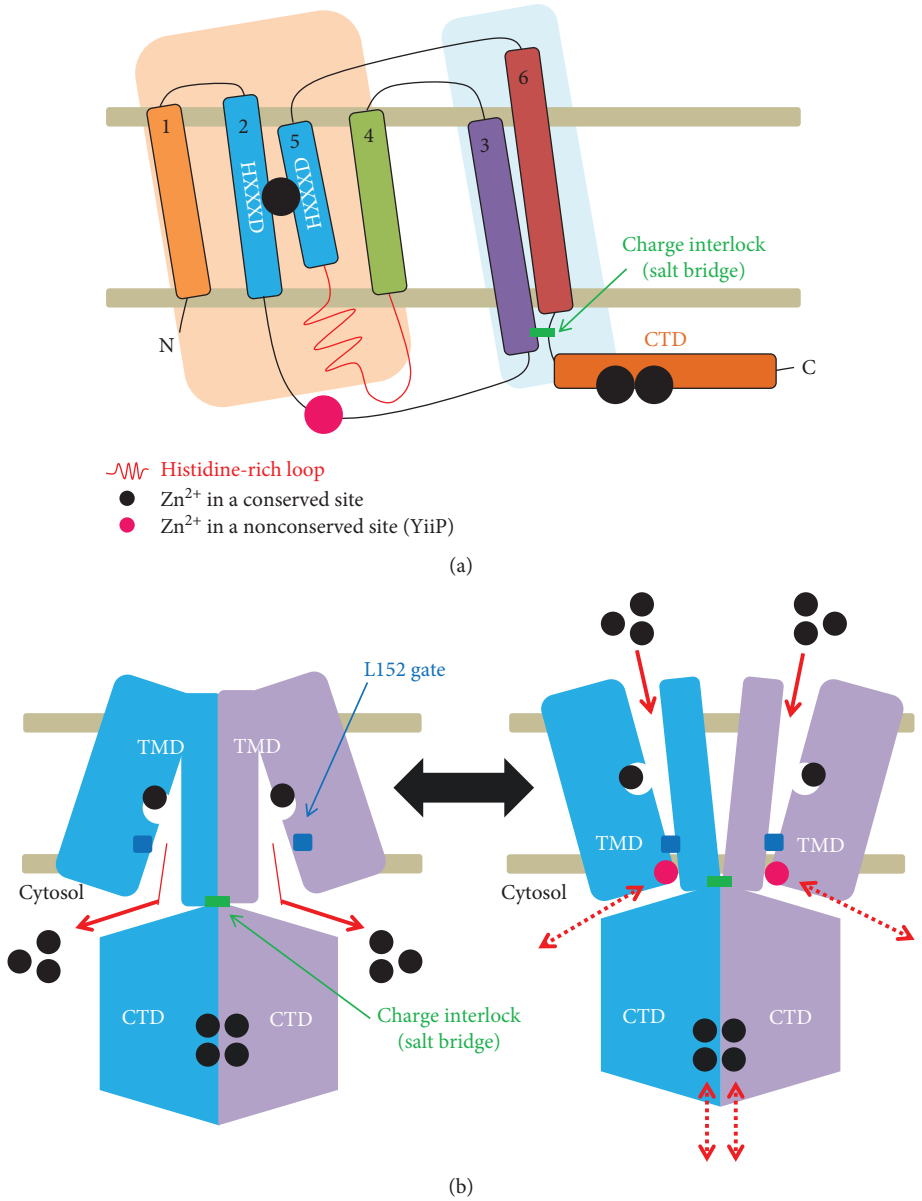


Figure 2: Overview of ZnT. (a) Topological model of ZnT. TMDs of ZnT are divided into two groups; TM1, 2, 4, and 5 form one bundle and TM3 and 6 form the other bundle. TM2 and TM4 share the Zn^{2+} binding site via HXXXD motifs. TM3 and 6 trigger the charge interlock that stabilizes the dimeric conformation. (b) Transport mechanism. ZnT has two major conformations, the inward-open and outward-open conformations, similar to ZIP. CTDs contain 4 Zn^{2+} that are not transported across the lipid bilayer but contribute to the stabilization of dimerization in a zinc-dependent manner. In the YiiP crystal structure, one Zn^{2+} is found in the link between the TMD and CTD (pink circle).

based kinetic analysis has revealed that ZIPB mediates zinc influx as a channel, the crystal structure of ZIPB implies that ZIPB may act as a transporter [27, 36]. The crystal structure for the outward-open conformation is needed to resolve this inconsistency. Zinc influx through ZIPB is extremely slow compared with typical ion channels; accordingly, ZIPB might adopt a unique mechanism that it uses to intermediate between typical transporters and channels.

3. Structure of ZnTs

3.1. ZnT Family. ZnTs belong to a superfamily of cation diffusion facilitators (CDF) observed in a wide range of taxa, including plants, bacteria, and fungi [38]. In mammals, at least 10 ZnT members have been identified [1, 38]. They are predicted to have six TMDs, except for ZnT5, which has additional TMDs, and their N- and C-termini face the cytoplasm, unlike those of ZIPs [1, 38] (Figure 2(a)). ZnTs show a histidine/serine-rich loop with diverse lengths between TM4 and TM5. Interestingly, ZnTs contain a large CTD at the C-terminus with a copper chaperon-like architecture, despite a lack of sequence homology [39]. This domain is important in diabetes research since variants of ZnT8 in the CTD are associated with diabetes risk [40]. Especially, variant W325R in the CTD increases the risk of developing diabetes by affecting thermostability.

Moreover, ZnT8 is a major autoantigen in type 1 diabetes, implying that the variant conformation of the CTD affects antigen recognition [41].

Though the first ZnTs were identified by selecting zinc-resistant baby hamster kidney cells [42], the overexpression and purification of mammalian ZnTs such as ZnT8 have become a success in recent years [43]. Instead, a prokaryotic CDF member has been well studied. YiiP from *E. coli* has been overexpressed, purified [44], and finally crystallized to determine its atomic structure [39].

3.2. Structural Overview. The crystal structure revealed that YiiP exhibits a unique Y-shaped structure formed by two monomers [39] (Figure 2(b)). Two TMDs of each monomer swing outward and plunge into the membrane at a 60° angle. The void space between two TMDs from each monomer is thought to be filled with phospholipids *in vivo*. In each monomer, helixes in the TMD could be divided into two groups; TM1, 2, 4, and 5 form a compact four-helix bundle and TM3 and 6 cross over in an antiparallel conformation outside the bundle to support the dimerization between monomers by conserved hydrophobic interactions at each TMD-TMD interface [39, 45]. At the dimer interface, Lys77 in TM3 and Asp207 from the intracellular loop that connects TM6 and the CTD stabilize the TM3-TM6 pairs by forming four interlocked salt bridges.

The crystal structure of YiiP reveals that the helix-breaking proline residue is lacking in TMDs of YiiP, indicating the limited conformational flexibility when zinc is transported [39]. However, TM5 is relatively short, with 2 residues among 4 coordinating residues in a tetrahedral arrangement for binding to one Zn^{2+} [45], suggesting that TM5 movement is a major event in zinc transport. The remaining 2 residues for completing the tetrahedral coordination for single Zn^{2+} binding are located on TM2. These evolutionarily conserved Zn^{2+} binding sites are located near the bottom of the extracellular cavity in the structure of YiiP, indicating that the solved crystal structure is the outward-open conformation (Figure 2(b)). Though the intracellular cavity is between the TMD and CTD, Zn^{2+} binding residues are not present, further suggesting that the solved crystal structure of YiiP is not the inward-open conformation.

Recent cryoelectron microscopic analysis using a YiiP homolog from *Shewanella oneidensis* shows the inward-open conformation for the cytoplasmic cavity [46]. The cryoelectron structure reveals that YiiP forms a still monodimer, without apparent conformational changes in CTDs. However, the cytoplasmic sides of TMDs involve significant conformational changes, producing dimeric interactions via TM3 and 6, resulting in interactions between the monomers in the whole regions of TM3 and TM6. Though the periplasm region of the compact four-helix bundle (TM1, 2, 4, and 5) moves closer to the two-helix bundle (TM3 and TM6) in the cryoelectron structure, the cytosol part of the four-helix bundle moves away from the two-helix bundle. Therefore, the cryoelectron structure of YiiP has a cytoplasmic cavity with an inward-open conformation (Figure 2(b)).

3.3. Zinc Binding Site and Oligomerization. Each YiiP monomer in the crystal structure has three Zn^{2+} binding sites [39, 45] (Figure 2). For transport across the lipid bilayer, Zn^{2+} is located in the center of the TMD by tetrahedral coordination via four residues from Asp45, Asp49 in TM2 and His153, and Asp157 in TM5. This coordination indicates that Zn^{2+} is completely surrounded by amino acid residues from TMs, and not by any other outer-shell constraints, like a water molecule. Thus, no breaking of the outer shell is needed to mediate Zn^{2+} release and accordingly little reorientation of TM2-TM5 could efficiently induce Zn^{2+} binding or release. The tetrahedral coordination prefers Zn^{2+} and Cd^{2+}, not Fe^{2+}, Ca^{2+}, Mn^{2+}, and so on, further suggesting that YiiP is a zinc transporter.

The intracellular loop (IL1) of YiiP that connects TM2 and TM3 includes a single Zn^{2+} binding site [39, 45] (Figure 2(a)). These sites are only observed in the YiiP sequences, indicating that they may not be conventionally important in ZnTs. However, several observations suggest that they may be functionally significant in some ZnT family members: (1) His71 that serves as a Zn^{2+} binding residue interacts with Gln203 in TM6 of the other monomer, (2) IL1 contains many zinc binding residues in some ZnT family members, and (3) this Zn^{2+} binding site is located near a cluster of highly conserved residues in the cytosolic region of TM3 at the dimer interface. Therefore, Zn^{2+} binding may be important for dimerization via direct or indirect interactions (hydrogen bonds or van der Waals forces) across the dimer interface.

Four bound Zn^{2+} are found at the CTD interface in the YiiP crystal structure [39, 45] (Figure 2(b)). These Zn^{2+} are coordinated by highly conserved residues, including extensive outer-shell constraints and water molecules. Some of the neighboring residues interact with these conserved residues, resulting in bidentate hydrogen bonds, thereby establishing a well-arranged network of outer-shell interactions. In addition, the bound Zn^{2+} shows resistance to EDTA chelation. As a result, Zn^{2+} binding at the CTD interface stabilizes the CTD-CTD interaction, thus strengthening the dimerization of YiiP monomers.

3.4. Cytoplasmic Domain and Oligomerization. The CTD exhibits a high structural similarity with that of the copper chaperone Hah1 [47, 48]. Despite low sequence similarity between the CTD and Hah1, they share an $\alpha\beta$-$\beta\alpha$ core structure. Since metallochaperones and their targets share a metallochaperone domain, a zinc chaperone that binds to YiiP and transfers zinc might exist. However, to date, a zinc chaperone has not been reported.

In the crystal structure of YiiP, the two positively charged protein surfaces of the CTD interface are held by four Zn^{2+} ions [39, 45]. If Zn^{2+} is absent from these sites, electrostatic repulsion pushes each CTD monomer away. The site-directed fluorescent resonance energy transfer (FRET) measurements using the CTD-labeled full-length purified YiiP protein shows that Zn^{2+} binding triggers hinge movement, pivoting around the $(Lys77\text{-}Asp207)_2$ charge interlock on the cytoplasmic membrane surface. However, the cryoelectron structure of the apo-zinc form of a YiiP homolog

based on 2D crystallization is identical to the crystal structure of the zinc-bound form of a CTD, without any apparent conformational changes [46]. These results imply that CTDs are important for the stabilization of the dimeric state, and thus TMD movement for zinc transport may occur without substantial CTD conformational changes.

The crystal structures of several bacterial CTD homologs have been solved and used for modeling analyses of mammalian ZnT [49, 50]. The apo and zinc forms of the CTD in CzrB from *Thermus thermophilus* reveal that only CTDs without TMDs could form a V-shaped dimer, and they show substantial zinc-dependent conformational changes [49]. Three Zn^{2+} ions are found in the monomer-monomer interface and their binding triggers a dramatic reorientation of the two monomers by a shift of about 40° through electrostatic repulsion. The crystal structure of the CTD from another CDF family member, MamM, in *Magnetospirillum gryphiswaldense* has also been solved [50]. However, only the apo form of the CTD has been solved, since the cation-bound form is limited by severe precipitation. The MamM-CTD also forms a V-shaped dimer, and metal binding triggers conformational changes. In general, CTD structures without TMDs are distinct from the full-length structure of YiiP. Therefore, the action/role of the CTD is still controversial. In the full-length structure of YiiP, CTDs are fastened by the charge interlock, which may prevent the dramatic movement of the CTD, as observed in the CTD-only structures of CzrB and MamM [39, 45, 49, 50].

3.5. Transport Mechanism. Using the purified YiiP protein, zinc efflux occurs via a Zn^{2+}/H^{+} antiporter [44]. YiiP mediates Zn^{2+} efflux to the outside of cells or to the luminal side of the intracellular compartment. At the same time, H^{+} ions are transported into the cytoplasm. Therefore, YiiP requires a proton motive force to mediate Zn^{2+} efflux. Indeed, isothermal titration calorimetric analyses have revealed that YiiP-mediated efflux is coupled with proton influx in a 1 : 1 zinc-for-proton exchange stoichiometry [44]. Zn^{2+} binding drives an all-or-nothing change in water accessibility to the transport site by the L152 gate in TM5 [51] (Figure 2(b)).

4. Conclusions

ZIPs and ZnTs play pivotal roles in the maintenance of cellular zinc homeostasis. Interestingly, both form dimers and share a traditional transport mechanism, including two major conformational states, that is, inward-open and outward-open. Their zinc binding sites are solved based on the crystal structures, providing insight into the zinc transport mechanism and providing a basis for drug development. Drugs that work as agonists are largely divided into two groups, "potentiators" and "correctors" [52]. Potentiators are compounds that stabilize transporters by binding to the specific site of transporters. Structure and biochemical data indicate that CTDs of ZnTs are involved in protein stability. Based on this, compounds that bind to CTDs of ZnTs can be classified as potentiators. Furthermore, drugs that directly bind near the active pore of transporters to stabilize the active conformation could increase the transport activity and may thus be classified as potentiators. In addition, compounds that regulate the degradation of transporters could be correctors. Many molecules involved in the degradation of zinc transporters are largely unknown. Thus, studies on the degradation and regulatory machinery for zinc transporter maintenance are strongly needed. Compounds that destabilize transporters could be useful as antagonists since the hyperactivation of zinc transporters may be related to immune dysfunction. Furthermore, ECDs of ZIP are very unique, but are similar to prions. Prion binding compounds could be screening targets to develop drugs.

Altogether, additional studies of the ECD and CTD may extend our knowledge of the diverse signaling mechanisms and their roles in disease pathogenesis. Therefore, a more precise understanding of the structures of ZIPs and ZnTs would be helpful to regulate immune cells, treat human diseases, and improve health life.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

This research was supported by the new faculty research fund of Ajou University and the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT, & Future Planning (2017R1C1B1004839).

References

[1] T. Fukada and T. Kambe, "Molecular and genetic features of zinc transporters in physiology and pathogenesis," *Metallomics*, vol. 3, no. 7, pp. 662–674, 2011.

[2] A. S. Prasad, "Zinc in human health: effect of zinc on immune cells," *Molecular Medicine*, vol. 14, no. 5-6, pp. 353–357, 2008.

[3] H. Kitamura, H. Morikawa, H. Kamon et al., "Toll-like receptor-mediated regulation of zinc homeostasis influences dendritic cell function," *Nature Immunology*, vol. 7, no. 9, pp. 971–977, 2006.

[4] I. Mellman and R. M. Steinman, "Dendritic cells: specialized and regulated antigen processing machines," *Cell*, vol. 106, no. 3, pp. 255–258, 2001.

[5] M. Yu, W. W. Lee, D. Tomar et al., "Regulation of T cell receptor signaling by activation-induced zinc influx," *The Journal of Experimental Medicine*, vol. 208, no. 4, pp. 775–785, 2011.

[6] J. Zhu, H. Yamane, and W. E. Paul, "Differentiation of effector CD4 T cell populations*," *Annual Review of Immunology*, vol. 28, no. 1, pp. 445–489, 2010.

[7] T. B. Aydemir, J. P. Liuzzi, S. McClellan, and R. J. Cousins, "Zinc transporter ZIP8 (SLC39A8) and zinc influence IFN-γ expression in activated human T cells," *Journal of Leukocyte Biology*, vol. 86, no. 2, pp. 337–348, 2009.

[8] F. W. Beck, A. S. Prasad, J. Kaplan, J. T. Fitzgerald, and G. J. Brewer, "Changes in cytokine production and T cell subpopulations in experimentally induced zinc-deficient humans," *The American Journal of Physiology*, vol. 272, no. 6, Part 1, pp. E1002–E1007, 1997.

[9] P. S. Dowd, J. Kelleher, and P. J. Guillou, "T-lymphocyte subsets and interleukin-2 production in zinc-deficient rats," *The British Journal of Nutrition*, vol. 55, no. 1, pp. 59–69, 1986.

[10] A. S. Prasad, F. W. Beck, S. M. Grabowski, J. Kaplan, and R. H. Mathog, "Zinc deficiency: changes in cytokine production and T-cell subpopulations in patients with head and neck cancer and in noncancer subjects," *Proceedings of the Association of American Physicians*, vol. 109, no. 1, pp. 68–77, 1997.

[11] M. J. Liu, S. Bao, M. Galvez-Peralta et al., "ZIP8 regulates host defense through zinc-mediated inhibition of NF-κB," *Cell Reports*, vol. 3, no. 2, pp. 386–400, 2013.

[12] B. H. Bin, J. Bhin, M. Takaishi et al., "Requirement of zinc transporter ZIP10 for epidermal development: implication of the ZIP10-p63 axis in epithelial homeostasis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 114, no. 46, pp. 12243–12248, 2017.

[13] B. H. Bin, S. Hojyo, J. Seo et al., "The role of the Slc39a family of zinc transporters in zinc homeostasis in skin," *Nutrients*, vol. 10, no. 2, 2018.

[14] S. Hojyo, T. Miyai, H. Fujishiro et al., "Zinc transporter SLC39A10/ZIP10 controls humoral immunity by modulating B-cell receptor signal strength," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 32, pp. 11786–11791, 2014.

[15] T. Miyai, S. Hojyo, T. Ikawa et al., "Zinc transporter SLC39A10/ZIP10 facilitates antiapoptotic signaling during early B-cell development," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 32, pp. 11780–11785, 2014.

[16] M. Taniguchi, A. Fukunaka, M. Hagihara et al., "Essential role of the zinc transporter ZIP9/SLC39A9 in regulating the activations of Akt and Erk in B-cell receptor signaling pathway in DT40 cells," *PLoS One*, vol. 8, no. 3, article e58022, 2013.

[17] B. H. Bin, J. Bhin, N. H. Kim et al., "An acrodermatitis enteropathica-associated Zn transporter, ZIP4, regulates human epidermal homeostasis," *The Journal of Investigative Dermatology*, vol. 137, no. 4, pp. 874–883, 2017.

[18] J. Geiser, K. J. T. Venken, R. C. De Lisle, and G. K. Andrews, "A mouse model of acrodermatitis enteropathica: loss of intestine zinc transporter ZIP4 (Slc39a4) disrupts the stem cell niche and intestine integrity," *PLoS Genetics*, vol. 8, no. 6, article e1002766, 2012.

[19] T. Kawamura, Y. Ogawa, Y. Nakamura et al., "Severe dermatitis with loss of epidermal Langerhans cells in human and mouse zinc deficiency," *The Journal of Clinical Investigation*, vol. 122, no. 2, pp. 722–732, 2012.

[20] K. Nishida, A. Hasegawa, S. Nakae et al., "Zinc transporter Znt5/Slc30a5 is required for the mast cell-mediated delayed-type allergic reaction but not the immediate-type reaction," *The Journal of Experimental Medicine*, vol. 206, no. 6, pp. 1351–1364, 2009.

[21] E. Kawasaki, "ZnT8 and type 1 diabetes," *Endocrine Journal*, vol. 59, no. 7, pp. 531–7, 2012.

[22] E. Kawasaki, M. Uga, K. Nakamura et al., "Association between anti-ZnT8 autoantibody specificities and SLC30A8 Arg325Trp variant in Japanese patients with type 1 diabetes," *Diabetologia*, vol. 51, no. 12, pp. 2299–2302, 2008.

[23] B. H. Bin, T. Fukada, T. Hosaka et al., "Biochemical characterization of human ZIP13 protein: a homo-dimerized zinc transporter involved in the spondylocheiro dysplastic Ehlers-Danlos syndrome," *The Journal of Biological Chemistry*, vol. 286, no. 46, pp. 40255–40265, 2011.

[24] K. M. Taylor and R. I. Nicholson, "The LZT proteins; the LIV-1 subfamily of zinc transporters," *Biochimica et Biophysica Acta (BBA) - Biomembranes*, vol. 1611, no. 1-2, pp. 16–30, 2003.

[25] T. Zhang, D. Sui, and J. Hu, "Structural insights of ZIP4 extracellular domain critical for optimal zinc transport," *Nature Communications*, vol. 7, article 11979, 2016.

[26] X. Mao, B. E. Kim, F. Wang, D. J. Eide, and M. J. Petris, "A histidine-rich cluster mediates the ubiquitination and degradation of the human zinc transporter, hZIP4, and protects against zinc cytotoxicity," *The Journal of Biological Chemistry*, vol. 282, no. 10, pp. 6992–7000, 2007.

[27] T. Zhang, J. Liu, M. Fellner, C. Zhang, D. Sui, and J. Hu, "Crystal structures of a ZIP zinc transporter reveal a binuclear metal center in the transport pathway," *Science Advances*, vol. 3, no. 8, article e1700344, 2017.

[28] B. H. Bin, J. Bhin, J. Seo et al., "Requirement of zinc transporter SLC39A7/ZIP7 for dermal development to fine-tune endoplasmic reticulum function by regulating protein disulfide isomerase," *The Journal of Investigative Dermatology*, vol. 137, no. 8, pp. 1682–1691, 2017.

[29] B. H. Bin, S. Hojyo, T. Hosaka et al., "Molecular pathogenesis of spondylocheirodysplastic Ehlers-Danlos syndrome caused by mutant ZIP13 proteins," *EMBO Molecular Medicine*, vol. 6, no. 8, pp. 1028–1042, 2014.

[30] G. Schmitt-Ulms, S. Ehsani, J. C. Watts, D. Westaway, and H. Wille, "Evolutionary descent of prion genes from the ZIP family of metal ion transporters," *PLoS One*, vol. 4, no. 9, article e7208, 2009.

[31] K. M. Taylor, I. A. Muraina, D. Brethour et al., "Zinc transporter ZIP10 forms a heteromer with ZIP6 which regulates embryonic development and cell migration," *The Biochemical Journal*, vol. 473, no. 16, pp. 2531–2544, 2016.

[32] L. A. Gaither and D. J. Eide, "Functional expression of the human hZIP2 zinc transporter," *The Journal of Biological Chemistry*, vol. 275, no. 8, pp. 5560–5564, 2000.

[33] T. P. Dalton, L. He, B. Wang et al., "Identification of mouse SLC39A8 as the transporter responsible for cadmium-induced toxicity in the testis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 9, pp. 3401–3406, 2005.

[34] L. He, K. Girijashanker, T. P. Dalton et al., "ZIP8, member of the solute-carrier-39 (SLC39) metal-transporter family: characterization of transporter properties," *Molecular Pharmacology*, vol. 70, no. 1, pp. 171–180, 2006.

[35] Z. Liu, H. Li, M. Soleimani et al., "Cd^{2+} versus Zn^{2+} uptake by the ZIP8 HCO_3^--dependent symporter: kinetics, electrogenicity and trafficking," *Biochemical and Biophysical Research Communications*, vol. 365, no. 4, pp. 814–820, 2008.

[36] W. Lin, J. Chai, J. Love, and D. Fu, "Selective electrodiffusion of zinc ions in a Zrt-, Irt-like protein, ZIPB," *The Journal of Biological Chemistry*, vol. 285, no. 50, pp. 39013–39020, 2010.

[37] M. C. Franz, J. Pujol-Gimenez, N. Montalbetti et al., "Reassessment of the transport mechanism of the human zinc transporter SLC39A2," *Biochemistry*, vol. 57, no. 26, pp. 3976–3986, 2018.

[38] T. Kimura and T. Kambe, "The functions of metallothionein and ZIP and ZnT transporters: an overview and perspective,"

International Journal of Molecular Sciences, vol. 17, no. 3, p. 336, 2016.

[39] M. Lu and D. Fu, "Structure of the zinc transporter YiiP," *Science*, vol. 317, no. 5845, pp. 1746–1748, 2007.

[40] D. S. Parsons, C. Hogstrand, and W. Maret, "The C-terminal cytosolic domain of the human zinc transporter ZnT8 and its diabetes risk variant," *The FEBS Journal*, vol. 285, no. 7, pp. 1237–1250, 2018.

[41] J. M. Wenzlau, K. Juhl, L. Yu et al., "The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 43, pp. 17040–17045, 2007.

[42] R. D. Palmiter and S. D. Findley, "Cloning and functional characterization of a mammalian zinc transporter that confers resistance to zinc," *The EMBO Journal*, vol. 14, no. 4, pp. 639–649, 1995.

[43] C. Merriman, Q. Huang, G. A. Rutter, and D. Fu, "Lipid-tuned zinc transport activity of human ZnT8 protein correlates with risk for type-2 diabetes," *The Journal of Biological Chemistry*, vol. 291, no. 53, pp. 26950–26957, 2016.

[44] Y. Chao and D. Fu, "Thermodynamic studies of the mechanism of metal binding to the *Escherichia coli* zinc transporter YiiP," *The Journal of Biological Chemistry*, vol. 279, no. 17, pp. 17173–17180, 2004.

[45] M. Lu, J. Chai, and D. Fu, "Structural basis for autoregulation of the zinc transporter YiiP," *Nature Structural & Molecular Biology*, vol. 16, no. 10, pp. 1063–1067, 2009.

[46] N. Coudray, S. Valvo, M. Hu et al., "Inward-facing conformation of the zinc transporter YiiP revealed by cryoelectron microscopy," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 6, pp. 2140–2145, 2013.

[47] A. L. Lamb, A. K. Wernimont, R. A. Pufahl, V. C. Culotta, T. V. O'Halloran, and A. C. Rosenzweig, "Crystal structure of the copper chaperone for superoxide dismutase," *Nature Structural Biology*, vol. 6, no. 8, pp. 724–729, 1999.

[48] A. L. Lamb, A. K. Wernimont, R. A. Pufahl, T. V. O'Halloran, and A. C. Rosenzweig, "Crystal structure of the second domain of the human copper chaperone for superoxide dismutase," *Biochemistry*, vol. 39, no. 7, pp. 1589–1595, 2000.

[49] V. Cherezov, N. Höfer, D. M. E. Szebenyi et al., "Insights into the mode of action of a putative zinc transporter CzrB in *Thermus thermophilus*," *Structure*, vol. 16, no. 9, pp. 1378–1388, 2008.

[50] N. Zeytuni, R. Uebe, M. Maes et al., "Cation diffusion facilitators transport initiation and regulation is mediated by cation induced conformational changes of the cytoplasmic domain," *PLoS One*, vol. 9, no. 3, article e92141, 2014.

[51] S. Gupta, J. Chai, J. Cheng, R. D'Mello, M. R. Chance, and D. Fu, "Visualizing the kinetic power stroke that drives proton-coupled zinc(II) transport," *Nature*, vol. 512, no. 7512, pp. 101–104, 2014.

[52] B. H. Bin, S. Hojyo, T. Ryong Lee, and T. Fukada, "Spondylocheirodysplastic Ehlers-Danlos syndrome (SCD-EDS) and the mutant zinc transporter ZIP13," *Rare Diseases*, vol. 2, no. 1, article e974982, 2014.

Hindawi
Journal of Immunology Research
Volume 2018, Article ID 5982169, 7 pages
https://doi.org/10.1155/2018/5982169

Research Article

Combined Treatment with Zinc Aspartate and Intravenous Immunoglobulins (IVIGs) Ameliorates Experimental Autoimmune Encephalomyelitis (EAE)

Diana Straubel,[1] Anja Thielitz,[2] Annegret Reinhold,[1] Kurt Grüngreiff,[3] and Dirk Reinhold[1]

[1]*Institute of Molecular and Clinical Immunology, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany*
[2]*University Clinic of Dermatology and Venereology, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany*
[3]*Clinics of Gastroenterology, City Hospital Magdeburg, Klinikum Magdeburg GmbH, Magdeburg, Germany*

Correspondence should be addressed to Dirk Reinhold; dirk.reinhold@med.ovgu.de

Received 1 June 2018; Revised 17 August 2018; Accepted 2 September 2018; Published 26 September 2018

Guest Editor: Shintaro Hojyo

Intravenous immunoglobulins (IVIGs) are widely used in replacement therapy of primary and secondary immunodeficiency disorders and in approved autoimmune indications. In addition, IVIG application is used off-label for treatment of other autoimmune diseases, e.g., multiple sclerosis (MS), an inflammatory autoimmune disorder with a clear T cell-mediated immune pathogenesis. The trace element zinc is shown to play a regulatory role in the maintenance of immune functions. Changes of zinc homeostasis affect both the innate and the adaptive immune system. On one hand, therapeutic zinc supplementation can normalize impaired immune functions due to zinc deficiency. On the other hand, therapeutic zinc supplementation is under consideration as a possible option to treat T cell-mediated autoimmune diseases. The aim of the present study was to investigate the influence of IVIG (Octagam®), zinc aspartate (Unizink®), and the combined application of both preparations in the experimental autoimmune encephalomyelitis (EAE), the animal model of MS. Therapeutic intraperitoneal application of zinc aspartate significantly diminished clinical signs during the relapsing-remitting phase of EAE in SJL/J mice. In contrast, IVIG given in a therapeutic manner did not influence the course of EAE. Interestingly, the combined application of both, IVIG and zinc aspartate, significantly reduced the severity of the disease during the acute and the relapsing-remitting phase of the EAE. Our data suggest that the combination of IVIG and zinc aspartate may have beneficial effects in autoimmune diseases, like MS. Further studies should verify the benefit of a controlled immunosuppressive therapy with IVIG and zinc for such diseases.

1. Introduction

The chronic autoimmune disease multiple sclerosis (MS) is the most frequent demyelinating disease of the central nervous system (CNS) with a prevalence of 0.1% in Northern America and Europe. MS can affect all functional systems of the CNS, leading to symptoms like weakness of one or several limbs, optic neuritis, cerebellar or brainstem dysfunction, sensory deficits, and cognitive impairment [1, 2]. The experimental autoimmune encephalomyelitis (EAE) is the accepted animal model of MS. EAE is characterized as a T cell-mediated autoimmune disease, driven by CNS inflammation, demyelination, and neuronal loss [3, 4].

The trace element zinc is shown to be essential for a wide range of physiological processes, including cell and tissue differentiation, proliferation, and apoptosis. Zinc is involved in the regulation of numerous structural and catalytic functions, in protein-protein interactions, and in signal transduction of several cell types [5–8]. An impairment of zinc homeostasis by genetic defects and/or zinc deficiency affects both the components of the innate and the adaptive immune system, whereas therapeutic zinc supplementation normalizes the diminished immune functions due to zinc deficiency [9]. In contrast, high dosages of zinc suppress functions of immune cells, particularly of T cells [6, 8, 10, 11].

Based on these observations, therapeutic zinc supplementation is considered for a long time as a possible option for T cell-mediated autoimmunity [6, 8]. To clarify, whether T cells could be potential targets of zinc supplementation in autoimmune diseases like MS, recently, we investigated the effect of zinc aspartate on T cell activation *in vitro* and on T cell-mediated autoimmunity *in vivo*. We have previously shown that zinc aspartate (Unizink®), a commercially available zinc supplement, is capable of suppressing the proliferation as well as Th1/Th2/Th17 cytokine production of human stimulated T cells and activated mouse splenocytes *in vitro* [10, 11]. Moreover, intraperitoneal (i.p.) administration of a medium-range dose of zinc aspartate in a therapeutic manner led to a significant reduction of the clinical severity of the EAE [10]. Thus, the trace element zinc is the only nontoxic metal which has the special capacity to suppress the proliferation as well as cytokine production of activated T cells and to be highly potent in the active EAE in mice.

Intravenous immunoglobulin (IVIG) preparations contain pooled immunoglobulin G (IgG) from the plasma of approximately thousand blood donors. IVIGs are used in a variety of conditions, especially in replacement therapy of primary and secondary immunodeficiency disorders, in approved autoimmune diseases, and in off-label indications for several autoimmune diseases [12]. IVIGs are currently being used as part of an "off-label" therapy in MS, particularly as a prophylactic approach in pregnant MS patients [13–15]. First studies have shown their efficacy in EAE [16, 17].

Concerning the combined application of IVIG and zinc preparations in EAE, no studies exist as yet. Thus, we wanted to answer the question whether the combination of IVIG and zinc aspartate is effective in EAE as an animal model of MS.

2. Materials and Methods

2.1. Materials. IVIG (Octagam®) was purchased from Octapharma GmbH (Langenfeld, Germany) and zinc aspartate (Unizink®) from Köhler Pharma GmbH (Alsbach-Hähnlein, Germany). Proteolipid protein (PLP) peptide (p)139–151, corresponding to the mouse sequence (HSLGKWLGHPDKF) was synthesized on a peptide synthesizer and purified by HPLC.

2.2. Mice. Female SJL/J mice, age 10–12 weeks, were purchased from JANVIER LABS (Le Genest-Saint-Isle, France) and housed in the animal facilities of the medical faculty of the Otto-von-Guericke-University, Magdeburg. All procedures were conducted according to protocols approved by the Institutional Animal Care and Use Committee (number 42502-2-781 UniMD).

2.3. Induction, Treatment, and Evaluation of Active EAE. Female SJL/J mice were immunized subcutaneously (s.c.) in depots distributed over 4 spots across the flanks with 200 μg PLP (p)139–151 in 0.2 ml emulsion consisting of equal volumes of PBS and complete Freund's adjuvant (CFA; Sigma, Taufkirchen, Germany), containing 4 mg/ml of *Mycobacterium tuberculosis* H37Ra (Difco, Detroit, MI). 200 ng pertussis toxin (PTX; List Biological Laboratories, Campbell, CA) was administered intraperitoneally (i.p.) at days 0 and 2 [18].

For therapeutic treatment, the mice received IVIG (10 mg/day), 30 μg/day zinc aspartate or both preparations intraperitoneally (i.p.) from day 11 to day 15 or from day 11 to day 19 after immunization. Equal volumes of phosphate-buffered saline (PBS) served as vehicle controls.

The mice were scored daily for clinical signs of EAE according to the following increasing severity scale: 0: no disease; 1: tail weakness (tail plegia); 2: hindlimb paraparesis and/or weak righting reflex; 3: hindlimb paraplegia; 4: paraplegia with forelimb weakness or paralysis; and 5: moribund animals. Mice with intermediate clinical signs were scored in 0.5 increments. For reasons of animal welfare, the mice were killed when reaching a score of 3 or above. Daily clinical scores were calculated as the average of all individual disease scores of each group [18].

2.4. Histological Analysis. For histological analysis, the mice were euthanized at day 20 postimmunization. Spinal cords were removed, fixed in 4% paraformaldehyde, embedded in paraffin, and stained with hematoxylin and eosin (H&E). Thoracic and lumbar spinal cord sections were evaluated, and total numbers of inflammatory foci were determined by an examiner blinded to the treatment status of the animal.

2.5. Statistical Analysis. Statistical comparison of EAE disease severity was accomplished by performing a Mann Whitney analysis as described previously [19]. Statistical analyses of the histological data were performed by the ANOVA test using the GraphPad Prism software (version 5.0).

3. Results

3.1. Influence of Preventive and Therapeutic Application of IVIG on the Clinical Course of EAE. Recently, we could show that i.p. administration of 30 μg/day zinc aspartate in a therapeutic manner led to a significant reduction of the clinical severity of the EAE in SJL/J mice [10].

In order to characterize the effect of IVIG application in active EAE, SJL/J mice were immunized with PLP (p)139–151. IVIGs (10 mg/day) were given i.p. in a preventive (treated from day 1 to day 10 after immunization) or in a therapeutic manner (treated from day 11 to day 19). PBS treatment served as vehicle control. We observed that the IVIG preparation was capable of diminishing the severity of EAE only in a preventive application (Figure 1(a)). In contrast, therapeutic administration of IVIG alone had no effect on the severity of EAE (Figure 1(b)).

3.2. Effects of Therapeutic Application of IVIG, Zinc Aspartate, and the Combination of Both on Clinical Signs of EAE. Next, we wanted to answer the question whether the therapeutic application of IVIG in combination with zinc aspartate can diminish the severity of the clinical course of EAE. The mice were treated i.p. with 10 mg/day IVIG, 30 μg/day zinc aspartate or with IVIG and zinc aspartate in combination from day 11 to day 15. As shown in Figure 2(c), the combination of IVIG and zinc aspartate

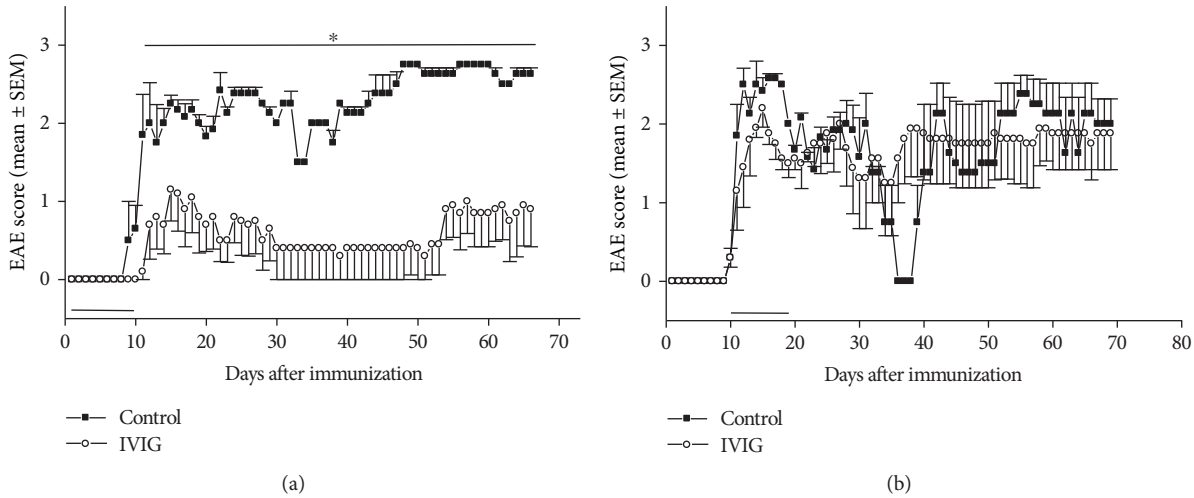


Figure 1: Effects of preventive and therapeutic application of IVIG on clinical signs of EAE. EAE was induced by immunization of SJL/J mice with PLP (p)139–151 as described in Materials and Methods. Mice were treated i.p. in a preventive manner from day 1 to day 10 (a) or in a therapeutic manner from day 11 to day 19 (b) with 10 mg/day IVIG. PBS treatment served as vehicle control. Treatment periods are indicated by the horizontal bar. Clinical disease scores were recorded daily. Data are presented as daily averages ± SEM of disease scores from 5 mice per treatment group. Significance is indicated by the horizontal bar above the curves. ∗ indicates a significance of $p < 0.05$ (Mann Whitney analysis).

caused a rapid remission of the acute phase of EAE and prevented further relapses. The mice treated with IVIG and zinc aspartate showed already in the acute phase of disease a significantly lower severity of the EAE (mean score of 0.5) than the mice of the PBS-treated control group (mean score 2.25). Moreover, the mean EAE score of the treated group was significantly lower than that of the control group in the first relapse of the disease.

As expected, therapeutic IVIG application alone from day 11 to day 15 after immunization showed no positive effect on the severity of the EAE (Figure 2(a)), whereas the therapeutic application of zinc aspartate led to an improvement in the clinical setting in the active phase and first relapse (Figure 2(b)). However, this effect was not as strong as the effect of the combination of IVIG and zinc aspartate.

This observation indicates a synergistic effect of IVIG and zinc in the treatment of relapsing-remitting EAE in mice.

Confirming the clinical data, histopathological analysis of EAE spinal cord tissues showed significantly decreased numbers of total inflammatory foci in EAE mice, which were treated i.p. with zinc aspartate or with the combination of zinc aspartate and IVIG from day 11 to day 15 (Figure 3).

Since therapy over a time period of 5 days already showed a significant improvement of EAE severity, we next investigated the combined effect of IVIG and zinc aspartate in a prolonged treatment protocol from day 11 to day 19 after immunization (Figure 4). In this experiment, the combination showed also a rapid improvement of the clinical picture in the acute phase of EAE (from day 12 to day 27) compared to the PBS control group. In addition, the first relapse of EAE in the group of treated animals was almost completely prevented (from day 47 to day 84). Only after a time period of 100 days after immunization the clinical course of both groups did not show any differences. Overall, these experiments establish the combination of IVIG and zinc as an effective treatment for relapsing-remitting EAE.

4. Discussion

In the past twenty years, it has been demonstrated that zinc is a key regulator of the immune system, capable of stimulating or suppressing different immune cells *in vitro* and *in vivo* in a dose- and cell type-dependent manner [6, 8]. Zinc has been reported to affect several cytokine-signalling cascades [20, 21]. We could show that zinc aspartate suppresses proliferation as well as interleukin- (IL-) 2, interferon- (IFN-) γ, IL-10, and IL-17 production of stimulated human and mouse T cells *in vitro* [10, 11].

Changes of zinc homeostasis affect both the innate and the adaptive immune system. On one site, impaired immune function due to zinc deficiency can be normalized by therapeutic zinc supplementation [9]. Several authors suggested therapeutic zinc supplementation as a possible option in T cell-mediated autoimmunity [6, 8]. Recently, we could show that i.p. administration of a medium-range dose of 30 μg/day zinc aspartate in a therapeutic manner reduced significantly the clinical severity of the EAE [10]. Moreover, oral administration of 12 μg/day zinc aspartate led to a significant reduction of the clinical severity of the EAE during the relapses of the disease [11].

In the absence of other therapeutic options, IVIG is used as part of an "off-label therapy" in several autoimmune diseases and, e.g., for the postpartum prophylaxis of MS in pregnant women [15, 22]. The work of several groups has well documented that IVIGs modulate the immune system, but the exact mechanism is still not clear. IVIG is shown to block Fc receptors in mononuclear phagocytes, to suppress MHC antigen presentation and antigen recognition by the T cell

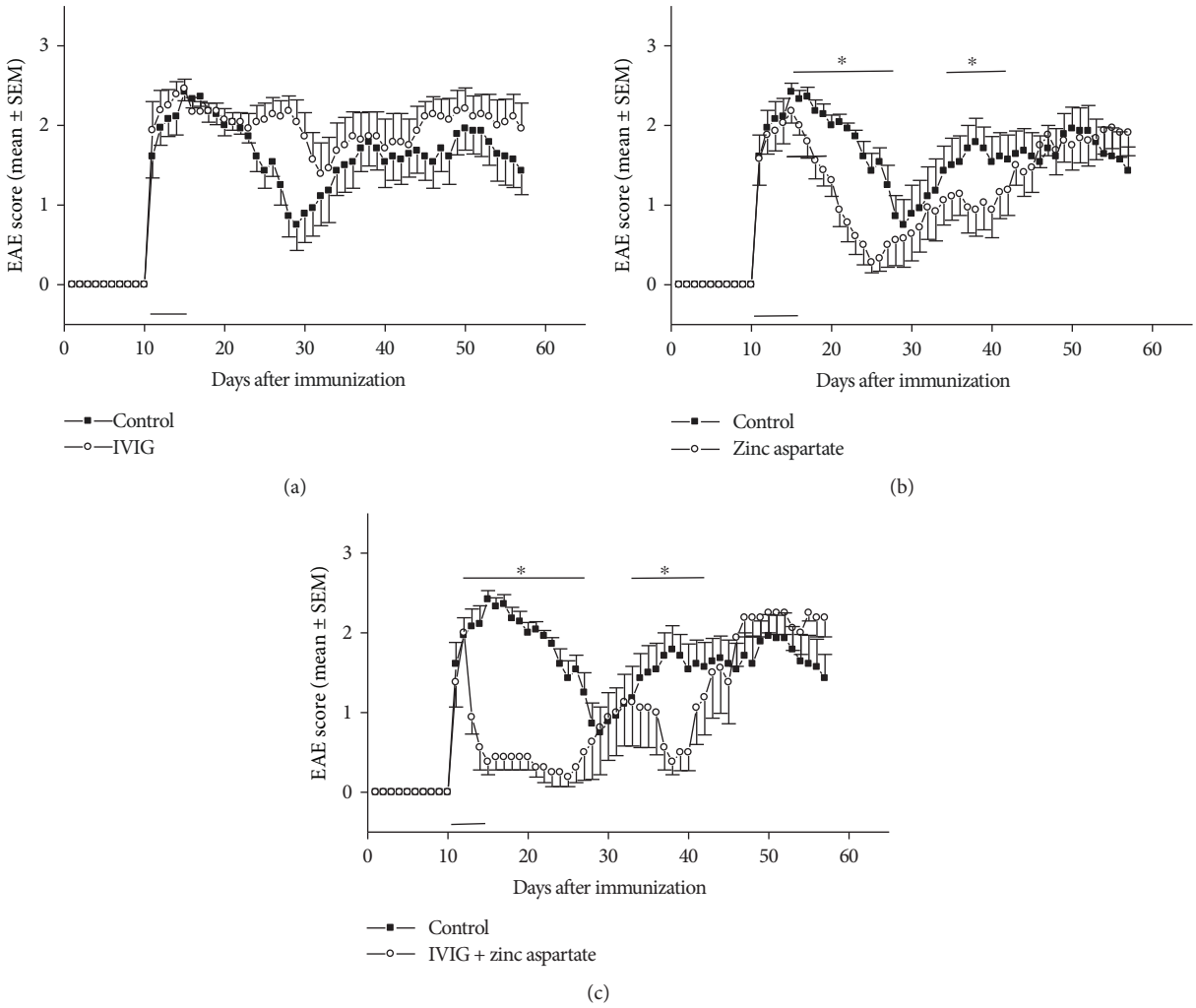


Figure 2: Effects of therapeutic application of IVIG, zinc aspartate, and the combination of both on clinical signs of EAE. EAE was induced by immunization of SJL/J mice with PLP (p)139–151. Mice were treated i.p. from day 11 to day 15 with 10 mg/day IVIG (a), 30 μg/day zinc aspartate (b), or combination of 10 mg/day IVIG and 30 μg/day zinc aspartate (c). PBS treatment served as vehicle control. Treatment periods are indicated by the horizontal bar. Clinical disease scores were recorded daily. Data are presented as daily averages ± SEM of disease scores from 10 mice per treatment group. Significance is indicated as horizontal bar above the curves. $*$ indicates a significance of $p < 0.05$ (Mann Whitney analysis).

receptor, to decrease the expression of proinflammatory cytokines, to block autoantibodies, and to induce regulatory T cells [23, 24]. Additionally, a functional role of the immunosuppressive cytokine transforming growth factor-β (TGF-β) was suggested for IVIG. Kekow et al. [25] reported that substantial amounts of latent TGF-β are present in commercially available IVIG preparations.

Comparing the effect of prophylactic and therapeutic IVIG administration on the course of active EAE in SJL/J mice, we found that IVIGs are capable of diminishing the severity of EAE in this animal model only after preventive application. This is in line with studies by other authors [16, 17]. The therapeutic administration of IVIG did not affect the severity of the EAE.

So far, no studies exist concerning the combined application of IVIG and zinc preparations in EAE. Thus, in the present study, we asked the question whether the combination of IVIG and zinc aspartate is effective in active EAE in SJL/J mice. Therefore, we combined the application of IVIG and zinc aspartate in a therapeutic manner and observed a significant reduction of the severity of the disease relapses. The therapeutic treatment with IVIG showed no effect on the acute phase of EAE or the further course. The therapeutic administration of zinc prevented the first relapse of the EAE but had no significant effect on the acute phase of the disease. Interestingly, the combined application of IVIG and zinc caused a rapid remission of the acute phase of EAE and prevented further relapses. Only about 100 days after the therapy, the course of disease of treated mice matched the course of the control group. This therapeutic effect not only was clinically evident from the EAE score but also could be demonstrated histologically. The number of inflammatory foci in the spinal cord was significantly reduced in animals treated with

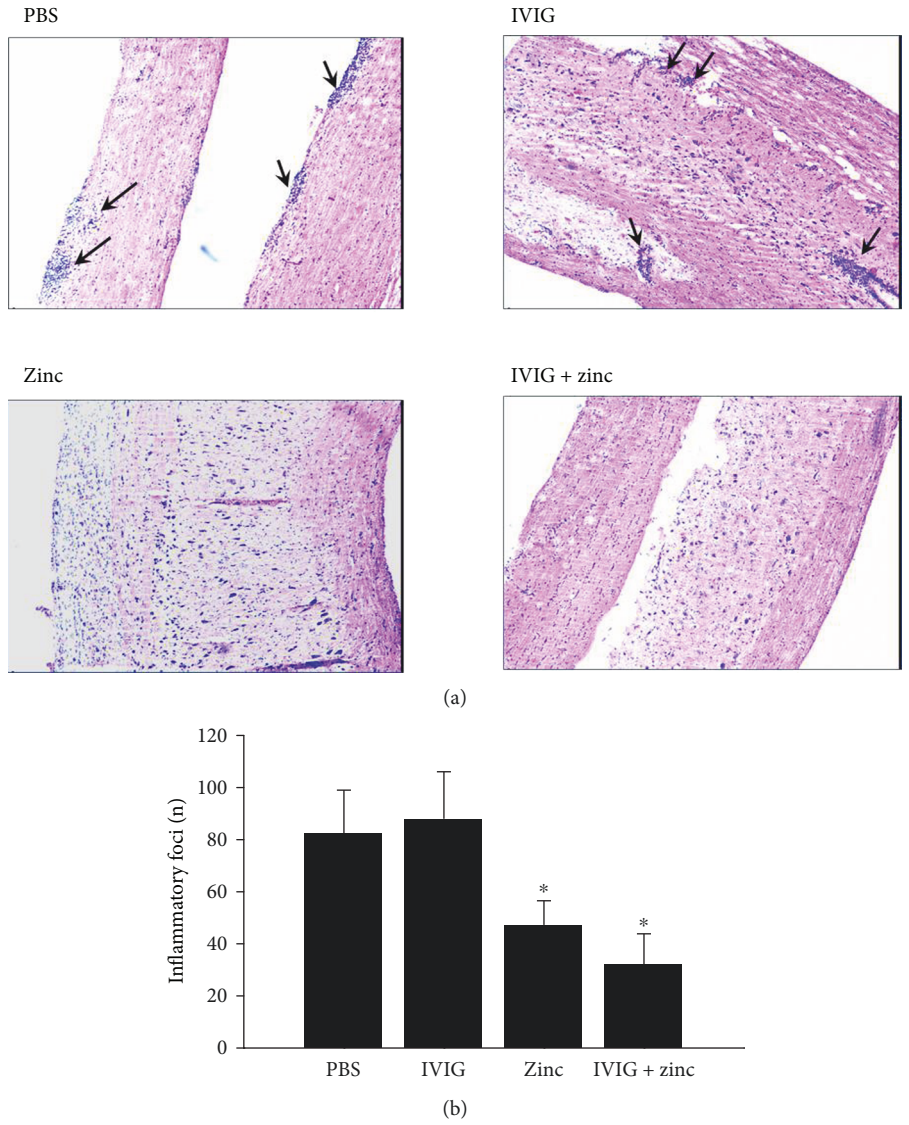


Figure 3: Effect of therapeutic application of IVIG, zinc aspartate, and the combination of both on formation of inflammatory lesions in the CNS of EAE mice. EAE was induced by immunization of SJL/J mice with $PLP_{139–151}$. Mice ($n = 4$ per group) were treated daily i.p. with 10 mg/day IVIG, 30 μg/day zinc aspartate, the combination of both preparations, or vehicle control from day 11 to day 15. Spinal cords were extracted on day 20, fixed in 4% paraformaldehyde, and embedded in paraffin. Sections were stained with H&E. (a) Representative histology of spinal cord longitudinal sections. Inflammatory infiltrations were visualized by H&E staining. The arrowheads show a typical heavy inflammatory cellular infiltration; original magnification ×200. (b) Inflammatory foci in H&E-stained spinal cord cross sections were quantified. Data represent the mean number of inflammatory foci + SEM; $^*p < 0.05$ (ANOVA).

zinc aspartate alone or with the combination of IVIG and zinc compared to the control group. Further studies should be performed to investigate the effect of the combined treatment of zinc aspartate and IVIG after the first and/or second relapse of EAE.

For both MS and EAE, a T cell-mediated pathogenesis, in which Th1 and Th17 cells play a crucial role, is discussed [26, 27]. Both IVIG and zinc preparations are capable of interfering with regulatory proteins of the immune cells. In $CD4^+$ T cells, the binding of zinc to STAT3 leads to a change in the α-helix structure of this protein, which subsequently prevents STAT3 activation and inhibits differentiation to Th17 cells [28]. IVIGs are shown to suppress Th17 differentiation by inhibiting STAT3 phosphorylation and ROR-γt expression [29–31].

Recently, it was demonstrated in the EAE animal model that IVIG can inhibit the initiation of pathogenic immune response by inhibiting the polarization of naïve T cells into Th17 and Th1 cells, simultaneously increasing regulatory T (Treg) cells [32].

Furthermore, it is known that the immunosuppressive cytokine TGF-β1 in the absence of proinflammatory cytokines like IL-6 inhibits the expression of ROR-γt in $CD4^+$ T cells and subsequently the differentiation to Th17 cells [33]. The IVIG preparation used in our study contained 11.10 ng/ml latent TGF-β1 as measured by a specific ELISA

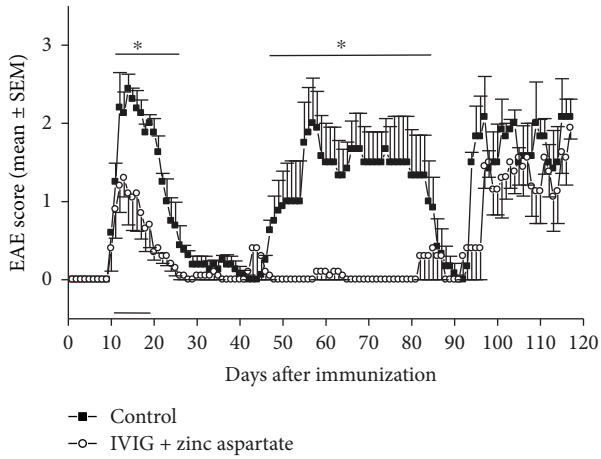


Figure 4: Effects of combined therapeutic application of IVIG and zinc aspartate on clinical signs of EAE. EAE was induced by immunization of SJL/J mice with PLP (p)139–151. Mice were treated i.p. from day 11 to day 19 with 10 mg/day IVIG and 30 μg/day zinc aspartate. PBS treatment served as vehicle control. Treatment periods are indicated by the horizontal bar. Clinical disease scores were recorded daily. Data are presented as daily averages ± SEM of disease scores from 5 mice per treatment group. Significance is indicated by the horizontal bar above the curves. $*$ indicates a significance of $p < 0.05$ (Mann Whitney analysis).

system (data not shown). This is in line with earlier observations [25].

In order to perform its function, latent TGF-β must be activated, which is realized *in vivo* especially by various proteases. These include endoproteases like matrix metalloproteases (MMP) [28, 34]. Interestingly, zinc is involved in the regulation of several of these enzymes [35].

The present work shows a significant improvement of the clinical course of EAE under zinc or zinc and IVIG administration. The results suggest that the combination of zinc and IVIG is capable of inhibiting the proliferation of Th1 and Th17 cells and thus suppressing the CNS inflammatory response during EAE. In addition, it was shown that the combination of IVIG and zinc significantly reduces the severity of the EAE in comparison to the administration of zinc aspartate alone.

In addition to Th1 and Th17 cells, $CD4^+CD25^+FoxP3^+$ Treg cells play also an important role in the pathogenesis of EAE and MS [28, 36, 37]. For this reason, it should be examined in future studies to what extent zinc and IVIG have an effect on the induction of regulatory T cells in the CNS. Moreover, further preclinical and clinical studies will help to clarify whether the combined application of IVIG and zinc can be beneficial for the treatment of T cell-mediated autoimmune diseases like MS.

Abbreviations

CFA: Complete Freund's adjuvant
CNS: Central nervous system
EAE: Experimental autoimmune encephalomyelitis
H&E: Hematoxylin and eosin
HPLC: High-performance liquid chromatography
IL: Interleukin
IFN: Interferon
IgG: Immunoglobulin G
i.p.: Intraperitoneal
IVIGs: Intravenous immunoglobulins
MS: Multiple sclerosis
PBS: Phosphate-buffered saline
PBMC: Peripheral blood mononuclear cells
PLP: Proteolipid protein
PTX: Pertussis toxin
TGF-β: Transforming growth factor-β
Treg: Regulatory T cells.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

There is no conflict of interest in this study.

Acknowledgments

We acknowledge grant support by the research alliance "Autonomy in Old Age" (EFRE and the State of Saxony-Anhalt). The authors thank Mrs. Marita Lotzing for the excellent technical assistance.

References

[1] E. Ellwardt and F. Zipp, "Molecular mechanisms linking neuroinflammation and neurodegeneration in MS," *Experimental Neurology*, vol. 262, Part A, pp. 8–17, 2014.

[2] B. Hemmer and R. Srivastava, "Hunting for autoantibodies in multiple sclerosis," *Neurology*, vol. 81, no. 11, pp. 944-945, 2013.

[3] C. S. Constantinescu, N. Farooqi, K. O'Brien, and B. Gran, "Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS)," *British Journal of Pharmacology*, vol. 164, no. 4, pp. 1079–1106, 2011.

[4] M. Rangachari and V. K. Kuchroo, "Using EAE to better understand principles of immune function and autoimmune pathology," *Journal of Autoimmunity*, vol. 45, pp. 31–39, 2013.

[5] A. S. Prasad, "Zinc and immunity," *Molecular and Cellular Biochemistry*, vol. 188, no. 1/2, pp. 63–69, 1998.

[6] N. Wellinghausen, H. Kirchner, and L. Rink, "The immunobiology of zinc," *Immunology Today*, vol. 18, no. 11, pp. 519–521, 1997.

[7] A. S. Prasad, B. Bao, F. W. J. Beck, O. Kucuk, and F. H. Sarkar, "Antioxidant effect of zinc in humans," *Free Radical Biology and Medicine*, vol. 37, no. 8, pp. 1182–1190, 2004.

[8] L. Rink and H. Haase, "Zinc homeostasis and immunity," *Trends in Immunology*, vol. 28, no. 1, pp. 1–4, 2007.

[9] A. S. Prasad, F. W. J. Beck, B. Bao et al., "Zinc supplementation decreases incidence of infections in the elderly: effect of zinc on generation of cytokines and oxidative stress," *The American Journal of Clinical Nutrition*, vol. 85, no. 3, pp. 837–844, 2007.

[10] D. Stoye, C. Schubert, A. Goihl et al., "Zinc aspartate suppresses T cell activation in vitro and relapsing experimental

autoimmune encephalomyelitis in SJL/J mice," *Biometals*, vol. 25, no. 3, pp. 529–539, 2012.

[11] C. Schubert, K. Guttek, K. Grüngreiff et al., "Oral zinc aspartate treats experimental autoimmune encephalomyelitis," *Biometals*, vol. 27, no. 6, pp. 1249–1262, 2014.

[12] N. Shehata, V. Palda, T. Bowen et al., "The use of immunoglobulin therapy for patients with primary immune deficiency: an evidence-based practice guideline," *Transfusion Medicine Reviews*, vol. 24, Supplement 1, pp. S28–S50, 2010.

[13] J. Yan, J. R. Richert, and M. D. Sirdofsky, "High-dose intravenous immunoglobulin for multiple sclerosis," *The Lancet*, vol. 336, no. 8716, p. 692, 1990.

[14] S. Schwarz, H. M. Meinck, and B. Storch-Hagenlocher, "Intravenöus immunoglobulins in multiple sclerosis," *Der Nervenarzt*, vol. 80, no. 8, pp. 918–928, 2009.

[15] A. Achiron, I. Kishner, M. Dolev et al., "Effect of intravenous immunoglobulin treatment on pregnancy and postpartum-related relapses in multiple sclerosis," *Journal of Neurology*, vol. 251, no. 9, pp. 1133–1137, 2004.

[16] A. Achiron, F. Mor, R. Margalit, I. R. Cohen, O. Lider, and S. Miron, "Suppression of experimental autoimmune encephalomyelitis by intravenously administered polyclonal immunoglobulins," *Journal of Autoimmunity*, vol. 15, no. 3, pp. 323–330, 2000.

[17] A. Ephrem, S. Chamat, C. Miquel et al., "Expansion of $CD4^{+-}$ $CD25^{+}$ regulatory T cells by intravenous immunoglobulin: a critical factor in controlling experimental autoimmune encephalomyelitis," *Blood*, vol. 111, no. 2, pp. 715–722, 2008.

[18] D. Reinhold, U. Bank, D. Entz et al., "PETIR-001, a dual inhibitor of dipeptidyl peptidase IV (DP IV) and aminopeptidase N (APN), ameliorates experimental autoimmune encephalomyelitis in SJL/J mice," *Biological Chemistry*, vol. 392, no. 3, pp. 233–237, 2011.

[19] K. K. Fleming, J. A. Bovaird, M. C. Mosier, M. R. Emerson, S. M. LeVine, and J. G. Marquis, "Statistical analysis of data from studies on experimental autoimmune encephalomyelitis," *Journal of Neuroimmunology*, vol. 170, no. 1-2, pp. 71–84, 2005.

[20] J. Kaltenberg, L. M. Plum, J. L. Ober-Blöbaum, A. Hönscheid, L. Rink, and H. Haase, "Zinc signals promote IL-2-dependent proliferation of T cells," *European Journal of Immunology*, vol. 40, no. 5, pp. 1496–1503, 2010.

[21] C. Kitabayashi, T. Fukada, M. Kanamoto et al., "Zinc suppresses Th17 development via inhibition of STAT3 activation," *International Immunology*, vol. 22, no. 5, pp. 375–386, 2010.

[22] I. Ringel and U. K. Zettl, "Intravenous immunoglobulin therapy in neurological diseases during pregnancy," *Journal of Neurology*, vol. 253, no. S5, pp. V70–V74, 2006.

[23] I. Schwab and F. Nimmerjahn, "Intravenous immunoglobulin therapy: how does IgG modulate the immune system?," *Nature Reviews Immunology*, vol. 13, no. 3, pp. 176–189, 2013.

[24] M. S. Maddur, S. Othy, P. Hegde et al., "Immunomodulation by intravenous immunoglobulin: role of regulatory T cells," *Journal of Clinical Immunology*, vol. 30, Supplement 1, pp. 4–S8, 2010.

[25] J. Kekow, D. Reinhold, T. Pap, and S. Ansorge, "Intravenous immunoglobulins and transforming growth factor β," *The Lancet*, vol. 351, no. 9097, pp. 184-185, 1998.

[26] J. Zepp, L. Wu, and X. Li, "IL-17 receptor signaling and T helper 17-mediated autoimmune demyelinating disease," *Trends in Immunology*, vol. 32, no. 5, pp. 232–239, 2011.

[27] D. Matusevicius, P. Kivisäkk, B. He et al., "Interleukin-17 mRNA expression in blood and CSF mononuclear cells is augmented in multiple sclerosis," *Multiple Sclerosis Journal*, vol. 5, no. 2, pp. 101–104, 1999.

[28] M. A. Travis and D. Sheppard, "TGF-β activation and function in immunity," *Annual Review of Immunology*, vol. 32, no. 1, pp. 51–82, 2014.

[29] M. S. Maddur, J. Vani, P. Hegde, S. Lacroix-Desmazes, S. V. Kaveri, and J. Bayry, "Inhibition of differentiation, amplification, and function of human T_H17 cells by intravenous immunoglobulin," *Journal of Allergy and Clinical Immunology*, vol. 127, no. 3, pp. 823–830.e7, 2011.

[30] M. S. Maddur, M. Sharma, P. Hegde, S. Lacroix-Desmazes, S. V. Kaveri, and J. Bayry, "Inhibitory effect of IVIG on IL-17 production by Th17 cells is independent of anti-IL-17 antibodies in the immunoglobulin preparations," *Journal of Clinical Immunology*, vol. 33, Supplement 1, pp. 62–S66, 2013.

[31] M. S. Maddur, S. V. Kaveri, and J. Bayry, "Comparison of different IVIG preparations on IL-17 production by human Th17 cells," *Autoimmunity Reviews*, vol. 10, no. 12, pp. 809-810, 2011.

[32] S. Othy, P. Hegde, S. Topcu et al., "Intravenous gammaglobulin inhibits encephalitogenic potential of pathogenic T cells and interferes with their trafficking to the central nervous system, implicating sphingosine-1 phosphate receptor 1-mammalian target of rapamycin axis," *The Journal of Immunology*, vol. 190, no. 9, pp. 4535–4541, 2013.

[33] L. Zhou and D. R. Littman, "Transcriptional regulatory networks in Th17 cell differentiation," *Current Opinion in Immunology*, vol. 21, no. 2, pp. 146–152, 2009.

[34] L. Kubiczkova, L. Sedlarikova, R. Hajek, and S. Sevcikova, "TGF-β - an excellent servant but a bad master," *Journal of Translational Medicine*, vol. 10, no. 1, p. 183, 2012.

[35] B. L. Vallee and K. H. Falchuk, "The biochemical basis of zinc physiology," *Physiological Reviews*, vol. 73, no. 1, pp. 79–118, 1993.

[36] M. Koutrolos, K. Berer, N. Kawakami, H. Wekerle, and G. Krishnamoorthy, "Treg cells mediate recovery from EAE by controlling effector T cell proliferation and motility in the CNS," *Acta Neuropathologica Communications*, vol. 2, no. 1, p. 163, 2014.

[37] J. Huan, N. Culbertson, L. Spencer et al., "Decreased FOXP3 levels in multiple sclerosis patients," *Journal of Neuroscience Research*, vol. 81, no. 1, pp. 45–52, 2005.