Zinc and immune function: the biological basis of altered resistance to infection[1–3]

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ABSTRACT   Zinc is known to play a central role in the immune system, and zinc-deficient persons experience increased susceptibility to a variety of pathogens. The immunologic mechanisms whereby zinc modulates increased susceptibility to infection have been studied for several decades. It is clear that zinc affects multiple aspects of the immune system, from the barrier of the skin to gene regulation within lymphocytes. Zinc is crucial for normal development and function of cells mediating nonspecific immunity such as neutrophils and natural killer cells. Zinc deficiency also affects development of acquired immunity by preventing both the outgrowth and certain functions of T lymphocytes such as activation, T_h1 cytokine production, and B lymphocyte help. Likewise, B lymphocyte development and antibody production, particularly immunoglobulin G, is compromised. The macrophage, a pivotal cell in many immunologic functions, is adversely affected by zinc deficiency, which can dysregulate intracellular killing, cytokine production, and phagocytosis. The effects of zinc on these key immunologic mediators is rooted in the myriad roles for zinc in basic cellular functions such as DNA replication, RNA transcription, cell division, and cell activation. Apoptosis is potentiated by zinc deficiency. Zinc also functions as an antioxidant and can stabilize membranes. This review explores these aspects of zinc biology of the immune system and attempts to provide a biological basis for the altered host resistance to infections observed during zinc deficiency and supplementation.   *Am J Clin Nutr* 1998; 68(suppl):447S–63S.

KEY WORDS   Zinc, zinc deficiency, immunity, infection, micronutrients, ontogeny, apoptosis

INTRODUCTION

Animal studies in the 1930s first documented the essentiality of zinc for growth and survival of animals (1). Unfortunately, it was not until the 1960s and the seminal work of Prasad (2) that the importance of zinc deficiency in human populations was appreciated. Later, it became clear that zinc was also crucial for patients maintained on parenteral nutrition (3). A central clinical feature of zinc deficiency in these latter 2 cases was the increased susceptibility to infectious diseases. This led researchers to speculate that zinc must be important for host immunity. Indeed, the past 2 decades have witnessed a rapid growth in knowledge of the underlying mechanisms whereby zinc exerts its ubiquitous effects on immune function, disease resistance, and general health. Analysis of animal and human studies examining the in vivo effects of nutritional zinc deficiency and supplementation on immune cells and their function underscores the essential role of zinc in normal development and function of many key tissues, cells, and effectors of immunity. In vitro studies have elucidated the role of zinc at the cellular level, and recent advances in molecular biology and cell biology have begun to clarify the role of zinc in gene expression, mitosis, and apoptosis of lymphoid cells. From these studies, many of which are reviewed herein, it is clear that even mild zinc deficiency can impair multiple mediators of host immunity ranging from the physical barrier of the skin to acquired cellular and humoral immunity (4–10). This article represents a synthesis and expansion of 2 previous reviews examining the role of zinc on health and immune function (9, 10). Two figures are presented that integrate the concepts relating zinc to development and function of immune cells (**Figure 1**) and the role of zinc in the cellular processes of activation and proliferation (**Figure 2**).

ZINC DEFICIENCY

In persons suffering from marginal zinc deficiency, clinical signs are depressed immunity, impaired taste and smell, onset of night blindness, impairment of memory, and decreased spermatogenesis in males (9, 10). Severe zinc deficiency is characterized by severely depressed immune function, frequent infections, bullous pustular dermatitis, diarrhea, alopecia, and mental disturbances (3, 9). Similar effects of mild and severe zinc deficiency

arise in zinc-deficient laboratory animals (9–11). A rare genetic disorder, known as acrodermatitis enteropathica, occurs in cattle and humans, resulting in decreased zinc absorption accompanied by characteristic hyperpigmented skin lesions, poor growth, and low plasma zinc concentrations (12, 13).

ZINC STATUS AND SUSCEPTIBILITY TO INFECTIOUS DISEASES

Influence of zinc on experimental infections in animals

Numerous animal and human studies indicate that zinc deficiency decreases resistance to infectious diseases. Zinc-deficient animals have suppressed immune responses and are more susceptible to a diverse range of infectious agents including *Herpes simplex* virus (14) and Semliki forest virus (15); bacteria such as *Francisella tularensis* (16), *Listeria monocytogenes* (17, 18), *Salmonella enteritidis* (19), and *Mycobacterium tuberculosis* (20); the protozoan parasites *Trypanosoma cruzi* (21), *Trypanosoma musculi* (22), *Toxoplasma gondii* (23), and *Plasmodium yoelii* (24); eukaryotes such as *Candida albicans* (15, 25); and the helminths *Heligmosomoides polygyrus* (26, 27), *Strongyloides ratti* (28), *Trichinella spiralis* (29), *Fasciola hepatica* (30), and *Schistosoma mansoni* (31).

A selective overview of these studies illustrates the broad role of zinc during infection. Zinc-deficient mice infected with *T. musculi* harbored >3 times as many parasites as control mice, and this was associated with delay in production of protective antibodies (22). Likewise, 40% of moderately zinc-deficient mice succumbed to *P. yoelii* 17XNL malaria, a normally nonlethal infection (24). Again, this was accompanied by delayed appearance of protective immunoglobulin (Ig) G antibodies. With respect to *C. albicans*, genetically susceptible strains of mice were rendered resistant when fed zinc-enriched diets or when zinc was administered intraperitoneally (15, 25), and normally resistant mice became susceptible when maintained on a low-zinc diet (25). For the helminth infections, zinc-deficient animals either harbored more worms or had prolonged expulsion times. For *H. polygyrus*, marginal zinc deficiency had little effect (27), although severe zinc deficiency did increase worm burdens (26). In murine *S. mansoni*, zinc deficiency resulted in decreased pathology with no change in worm burdens (31). For *Listeria*, delayed hypersensitivity response, a measure of T lymphocyte activation, was reduced but actual bacterial clearance did not change substantially (17, 18). In naturally occurring bovine acrodermatitis enteropathica, calves had impaired resistance to viruses, fungi, and bacteria. This condition was corrected by increased dietary zinc intakes (32).

Influence of zinc on infectious diseases in human populations

Several studies have demonstrated the benefits of zinc supplementation on infectious diseases in human populations. Double-blind, placebo-controlled trials of daily zinc supplementation showed that zinc reduces the incidence and duration of acute and chronic diarrhea by 25–30% (33–35) and can reduce the incidence of acute lower respiratory infections by 45% (36). Some studies implied that zinc may reduce clinical disease caused by *Plasmodium falciparum* (37, 38), and it was recently shown in a controlled trial carried out in Papua New Guinea that zinc supplements could reduce malaria attributable to health center atten-

dance by >35% (39). Zinc supplementation also reduced the frequency of recurrent boils in hospital patients (40). Furthermore, decreased *S. mansoni* egg counts were observed in children given zinc supplements compared with those given a placebo (41). Humans suffering from acrodermatitis enteropathica also had fewer infections when given supranormal concentrations of zinc (42), and plasma zinc concentrations were substantially lower in patients with diffuse lepromatous leprosy compared with those with the more limited bacillary form (43).

Zinc supplements also have beneficial effects when administered during infection. Zinc lozenges were shown to decrease the duration of the common cold (44, 45), and studies in preschool children showed that zinc supplementation during a diarrheal episode reduced duration of sickness up to 30% (33).

In patients with HIV, zinc deficiency is frequently seen and disease progression is accompanied by decreased serum zinc concentrations and depressed phytohemagglutinin (PHA) mitogenic responses (46, 47). These changes are partially reversible by zinc supplementation (48). Likewise, in the murine model of AIDS the amount of zinc in multiple tissues, including the spleen, was depressed (49). In contrast, recent dietary analysis of a large cohort of AIDS patients indicated that high zinc intake was associated with more rapid progression of the disease (50). The actual effect of zinc supplementation on the progression of AIDS or the general health of AIDS patients remains to be evaluated.

Direct effects of zinc on infectious agents

In general, it is not clear to what extent the availability of zinc affects in vivo growth of infectious agents themselves. Most microorganisms require zinc in some amount for basic cellular processes. For example, yeast (51), *P. falciparum* (52), and HIV-1 (53) require zinc for replication and other functions. During the acute phase response zinc is redistributed from plasma to the liver and to lymphocytes (54). It has been suggested that this is an adaptive response intended to deprive invading pathogens of zinc (54–56). However, lowered plasma zinc concentrations resulting from the acute phase response are generally well above the concentrations at which the growth of most pathogens is affected (51, 52, 55). Still, highly localized suppression of extracellular zinc to microbiostatic concentrations may be created by the acute phase response in conjunction with calprotectin, a zinc binding protein produced by polymorphonuclear leukocytes (51, 57). In contrast, it is also true that very high zinc concentrations can be microbicidal. For example, the relatively low frequency of urinary tract infections in men may be due, in part, to the very high microbicidal zinc concentration in semen (58). Moreover, the effect of zinc on the common cold may be due to the increased zinc concentrations in the nasal mucosa, which may alter the conformation of the binding site between the virus and ICAM-1 (59).

The balance between zinc availability or deficiency for host immunity and the invading pathogen is affected by multiple factors. It appears, however, that in most cases any benefit to the pathogen of zinc availability is well compensated for by the concomitant improvement in host immune function.

EFFECTS OF ZINC ON OVERALL IMMUNE FUNCTION

Zinc is a potent mediator of host resistance to infection. Because susceptibility to a broad range of pathogenic agents is affected by zinc, it is likely that multiple immunologic effectors

are involved. The general effects of zinc on innate or nonspecific and adaptive or specific immunity are presented below as a framework for the more detailed discussions of zinc and specific immunologic effectors presented later.

Effects of zinc on barrier and nonspecific immunity

Evaluation of the effects of zinc on host immunity must begin with the effect of zinc on components of innate immunity. Zinc deficiency damages epidermal cells, resulting in the characteristic skin lesions of acrodermatitis enteropathica or severe zinc deficiency (42, 60). Damage to the linings of the gastrointestinal and pulmonary tracts is also observed during zinc deficiency (9, 61). As detailed below, zinc deficiency affects other mediators of nonspecific immunity, such as polymorphonuclear leukocyte function (6, 42, 62–64), natural killer cell function (6, 65–67), and complement activity (68).

Overview of effects of zinc on specific immunity

Lymphopenia is common in zinc-deficient humans and animals and occurs in both the central and peripheral lymphoid tissues (9, 10, 69). Adult mice fed zinc-deficient diets for 2 wk had reduced numbers of T and B lymphocytes in peripheral blood and spleen tissue (69). Peripheral blood lymphocyte and macrophage concentrations were eventually reduced by >50% (6, 69). Importantly, even marginal zinc deficiency substantially suppressed peripheral blood lymphoid cell concentrations in mice and humans (61, 69–71).

Zinc deficiency results not only in decreased lymphocyte concentrations, but in depressed T and B lymphocyte function (69, 72–76). In general, murine T and B lymphocyte proliferative responses to mitogens were greatly depressed in zinc-deficient compared with pair-fed animals (77–79). Suppression ranged from 5% to 50% with a tendency for greater suppression in T lymphocytes.

Some reports in rat and murine systems, however, documented no effect of zinc deficiency on mitogenic responses of lymphocytes (80, 81). Indeed, some data suggest that the primary effect of zinc deficiency is deletion of T and B lymphocytes with little loss of function in the surviving cells. This is based on plaque-forming cell (PFC) assays measuring antibody production in vitro in which data were expressed as PFC per viable splenocytes rather than PFC per whole spleen (81). These findings notwithstanding, it is difficult to explain the effects of zinc on immunity solely on the grounds of changing cell numbers. Given the ubiquitous involvement of zinc in cellular events, ranging from gene expression to membrane stability (9, 10, 82), one would expect multiple metabolic and structural defects in the surviving lymphocytes in zinc-deficient hosts.

Effects of high-dose zinc supplementation on immune function

Elevated zinc intake has been shown in some cases to potentiate immune function above basal levels. In rats, PHA-induced mitogen responses were increased in animals fed a rich 1000-ppm Zn diet for 14 d compared with a 40-ppm control diet (83). Likewise, mice fed a high-zinc diet had increased numbers of splenic PFC in response to T lymphocyte–dependent antigens (25). Additional murine studies confirmed increased T lymphocyte and macrophage function with supplemental doses of zinc (15). Interestingly, the mice were more also more resistant to endotoxin shock (15). A high-zinc diet also diminished the amount of lipid peroxidation in the livers of mice infected with *Plasmodium berghei* (84).

Studies in humans concerning excess zinc intake on the immune system are few. One study reported that 11 men receiving 300 mg Zn/d, 20 times the recommended dietary allowance (RDA) (9), for 6 wk experienced decreased proliferative responses of lymphocytes to PHA and reductions in chemotaxis and phagocytosis of circulating polymorphonuclear leukocytes (85). Additional studies suggest that very high zinc intakes in adults and children can result in copper deficiency, anemia, growth retardation, and immunodepression (86–88). One recent study of nutritional rehabilitation in marasmic children suggested that zinc down-regulated certain monocyte functions (89). Importantly, other larger and longer-term controlled trials of high-dose zinc supplementation in adults did not induce immunosuppression (90–92). Moreover, deleterious immunologic effects were not observed in trials in which clinically healthy and otherwise normal children received daily zinc supplementation up to 2 times the RDA. Therefore, short-term intake of zinc several-fold above the RDA is generally considered safe in preschool children and adults (82, 93, 94). It should, however, be mentioned that high-dose zinc supplements given to mouse pups during the perinatal period reduced PFC responses (95), and high doses may also have adverse health effects in human neonates (94). As for any nutritional supplement, caution must be exercised in taking excessive doses of zinc for prolonged periods of time.

EFFECTS OF FETAL ZINC DEFICIENCY ON IMMUNOLOGIC DEVELOPMENT

Gestational zinc deficiency in mice and nonhuman primates has short- and long-term deleterious effects on the offspring (96–100). Substantial reductions are seen in lymphoid organ size and gamma globulin concentrations in pups born to marginally zinc-deficient mice. In rhesus monkeys, prenatal zinc deficiency led to hypogammaglobulinemia, reductions in mitogenesis of peripheral blood lymphocytes, and reduced neutrophil function (101).

Additional murine studies showed that many of the immunodeficiencies observed at birth persisted into adulthood even though the pups were fed a diet containing normal amounts of zinc after weaning. At 6 wk of age, serum IgM concentrations were only 1% of normal and IgG_{2a} and IgA concentrations remained 40–60% below normal (96–98). As expected, the PFC response to T-dependent and T-independent antigens was markedly reduced (96–98). Indirect evidence for such effects in humans is also available. Intrauterine growth retardation, which has been linked to maternal zinc deficiency (102), results in depressed cellular immunity that can persist for years (103, 104). Furthermore, nutritional deficiencies occurring up to 1 y of age have resulted in permanent reductions in thymus size (105).

The observation that prenatal zinc deficiency in mice results in long-term suppression of IgM, IgA, and IgG_{2a} concentrations suggests that a transient prenatal window may exist for the development of cells producing these antibodies. Indeed, during immunologic ontogeny, groups of certain B lymphocytes emerge in sequential waves, with each wave being idiotypically distinct, thereby recognizing specific predefined antigenic determinants (106, 107). Many of these cells produce antibodies of the IgM and IgA isotypes that recognize capsular polysaccharides of bacteria and other common pathogens (106). These so-called natural

antibodies are believed to provide an early line of defense for the immunologically naive neonate against invasive pathogens. Natural antibodies also influence the developing antibody repertoire of the immune system. Postnatally, the bone marrow undergoes developmental maturation and loses the potential to generate cells secreting these antibodies (108). Thus, if gestational zinc deficiency influences the development of cells producing such antibodies, the antibody repertoire to bacterial antigens would be permanently altered in adults having experienced transient gestational zinc deprivation.

To test this hypothesis, we examined the antibody responses to the common bacterial antigens α-1,3-dextran and phosphorylcholine in adult mice whose mothers had been moderately deprived of zinc during late gestation. Adult mice who had been subjected to transient fetal zinc deficiency in utero had similar concentrations of total serum IgM, but more than 2-fold greater IgM responses to phosphorylcholine and up to 50% lower responses to α-1,3 dextran compared with control animals (AH Shankar, unpublished observations, 1998). The data suggest that transient zinc deficiency in utero permanently altered the response to certain bacterial antigens. Moreover, whereas total IgM concentrations were similar in both sets of animals, the findings indicate that antibody repertoire is more sensitive to mild or transient zinc deficiency in utero.

The potential consequences of altered antibody diversity can be seen in immune responses to 2 common childhood respiratory pathogens, *Streptococcus pneumoniae* and *Haemophilus influenza*. During infection with *S. pneumoniae*, children often mount a potent antibody response. However, only certain antibodies are protective, and their absence can result in severe infection or death (107, 109). Likewise, in children it has been shown that among the antibodies produced against *H. influenza*, those of the Hib-id1 idiotype have the greatest antibacterial activity (110).

Zinc status may also affect placental transport of antibodies from the mother to the fetus during the last trimester of pregnancy. In contrast with natural antibodies, these maternal antibodies recognize a greater diversity of antigens with high specificity. However, they are not replenished after birth and gradually decay to low values by 6 mo of age. In addition to providing early protection, maternal antibodies also influence qualities of the emerging immune system in the child (108). Because zinc is important for normal placental development (111, 112), deficiency may result in impaired in utero acquisition of maternal antibodies by the child as well. The actual effect of gestational or perinatal zinc deficiency on transfer of maternal antibodies to the child in utero or through breast milk in unknown.

Perhaps the most remarkable effects of gestational zinc deficiency is the demonstration in mice that some immunodeficiencies, particularly suppressed IgM concentrations, persisted to the second- and third-generation offspring (96). This suggests that gestational zinc deficiency may have epigenetic effects (113). Given the potential short- and long-term immunologic consequences of even moderate gestational zinc deficiency, a better understanding of its influence on public health is clearly needed.

INFLUENCE OF ZINC ON IMMUNOSUPPRESSIVE CONDITIONS

Persons suffering from sickle cell anemia have depressed peripheral T lymphocyte numbers, decreased CD4$^+$-CD8$^+$ T lymphocyte ratios, loss of delayed hypersensitivity, decreased natural killer cell activity, decreased production of the T lympho-

cyte cytokine interleukin (IL)-2, and suppressed activity of thymulin, a thymic hormone (4, 70, 114, 115). Zinc supplementation restored these immunologic indexes to near normal (69, 116). Likewise, in patients with Down syndrome, zinc supplements restored immediate hypersensitivity, normalized in vitro assays of lymphocyte function and neutrophil chemotaxis (117), and increased resistance to infection (118). Zinc supplements also restored delayed hypersensitivity in alcoholics (119) and stimulated cell-mediated and humoral immunity in humans and mice with hypogammaglobulinemia (25, 120).

In elderly humans and animals, impairments in wound healing and resistance to infection were corrected by zinc supplementation (121–124), suggesting that immunodeficiency in the elderly is due in part to zinc deficiency. Low plasma zinc concentrations in elderly patients were associated with peripheral blood lymphopenia, although not with depressed serum IgA concentrations or delayed hypersensitivity responses (125). Zinc supplementation restored normal lymphocyte and neutrophil zinc concentrations, increased concentrations of circulating T lymphocytes, and improved delayed hypersensitivity and IgG antibody responses to tetanus toxoid. As might be expected, increased resistance to infection was also observed (122, 126–130).

EFFECTS OF ZINC ON SPECIFIC CELLS OF THE IMMUNE SYSTEM

As discussed above, zinc deficiency has multiple effects on the immune system. The reader is referred to Figure 1 as a guide to the various effects of zinc deficiency in the context of overall immunologic development and function.

T lymphocytes

Development of T lymphocytes

Studies of zinc deficiency in bovine (131, 132), porcine (133, 134), rat (135), and murine (5, 6, 73, 77, 136) systems, and in severely zinc-deficient children (7, 137–140), describe substantial reductions in the size of the thymus, the central organ for T lymphocyte development. Mice maintained on a zinc-deficient diet for as little as 2 wk showed moderate thymic involution. After 4 wk the thymus retained only 25% of its original size, and at 6 wk only a few thymocytes remained in the thymic capsule (6). The percentage of thymic atrophy exceeded that of other organs and the percentage of overall weight loss. The reduction in thymic size and cellularity was observed mostly in the thymic cortex, where immature thymocytes develop. Such changes were not observed in pair-fed animals, confirming that zinc was responsible for the effect (6, 73, 77). After only 1 wk of normal zinc intake, thymic size increased and the cortex was repopulated with cells (73). In contrast with the effects of severe zinc restriction on thymic size in adult mice, moderate zinc restriction had minimal effects on thymic size (5). However, marginal zinc deficiency in the early postnatal period did result in substantial reductions in thymic size (77).

In the peripheral lymphoid organs, T lymphocytes were progressively depleted from the spleen, lymph nodes, and peripheral blood in zinc-deficient animals (6, 69, 73). Zinc-deficient children with acrodermatitis enteropathica or patients receiving total parenteral nutrition have reduced numbers of lymphocytes, particularly T lymphocytes in the blood and peripheral lymphoid tis-

sues. Decreased CD4^{+}:CD8^{+} cell ratios are also seen. Recent studies in an experimental human model showed that the percentage of CD8^{+}CD73^{+} T lymphocytes, precursors to cytotoxic T lymphocytes, was decreased in zinc deficiency (141). The presence of the CD73 molecule on cytotoxic T lymphocytes is required for antigen recognition, proliferation, and cytolysis (141). These and the previously mentioned changes are reversed with zinc supplementation (7, 32, 65, 138, 141–144).

T lymphocyte function

T lymphocyte responses such as delayed hypersensitivity and cytotoxic activity are suppressed during zinc deficiency and reversed by zinc supplementation (6, 7, 32, 137, 142, 144, 145). Suppressed delayed hypersensitivity responses in malnourished children are also restored after zinc supplementation (139, 140, 146, 147). In one study in children, synergism between zinc and vitamin A tended to potentiate the T-lymphocyte proliferative response to tuberculin (145). Patients receiving total parenteral nutrition devoid of zinc had reduced T lymphocyte PHA responses (65, 142, 143), which returned to normal after 20 d of zinc supplementation. The influence of zinc on T-dependent antibody production and T lymphocyte cytokine production patterns are discussed below in the sections titled B lymphocytes and cytokines, respectively.

In vitro effects of zinc on T lymphocytes

Several in vitro studies showed that zinc is required for T lymphocyte proliferation in response to IL-1 (148), PHA, concanavalin A, or IL-2 (149–152). Proliferation of human T lymphocytes was enhanced by zinc when stimulated with mitogenic concentrations of PHA, concanavalin A, or phorbol ester (153). Moreover, the mitogen response of cells from zinc-deficient patients could be improved by addition of 10 μmol $ZnCl_2$/L in vitro. Zinc also restored proliferative T lymphocyte responses in the presence of high concanavalin A concentrations, 20 μg/mL, which are known to be suppressive. One murine study found no effect when zinc chloride was added to spleen cell cultures containing subactivating concentrations of PHA or concanavalin A (154). In contrast, zinc potentiated the suppression of T lymphocyte mitogenesis caused by phorbol ester pretreatment (155). In another experimental system, zinc suppressed the allogeneic proliferative response of human T lymphocytes to HeLa cells (153).

It was also shown that zinc can potentiate the response to some bacterial superantigens by facilitating binding between the superantigen and class II molecules on antigen-presenting cells (156, 157). The clinical significance of this phenomenon, including the role of zinc in the development of toxic shock during sepsis, requires further study.

Lastly, there is also evidence that zinc addition in vitro alters the expression, function, or both, of lymphocyte surface molecules governing cell–cell interactions. The addition of zinc chloride at μmol/L levels enhanced the rosetting of peripheral blood T lymphocytes with sheep erythrocytes. This effect was mediated at the T lymphocyte level because pretreatment of the lymphocytes themselves, but not the sheep erythrocytes, had the same effect (158). It was also reported that zinc enhanced the transcription and expression of the adhesion molecule ICAM-1 on the surface of lymphoid cells, but not on fibroblasts (159).

The in vitro studies make apparent the many roles played by zinc in T lymphocyte activation and proliferation. The effects of zinc on T lymphocytes are modulated by factors such as the antigen presenting cell type, adhesion molecules, co-stimulatory molecules, antigen type, and the overall milieu in which T lymphocyte activation occurs.

B lymphocytes

Development of B lymphocytes

B lymphocyte development in bone marrow is adversely affected by zinc deficiency (160–162). When mice were fed a marginally zinc-deficient diet for just 30 d, nucleated bone marrow cells were reduced by one third with preferential reduction in small nongranular cells. Total B lymphocytes and their precursors were reduced nearly 75%. Losses were predominantly in pre-B and immature B lymphocytes, which declined by $\approx$50% and 25%, respectively. Mature B lymphocytes were less affected. This finding is consistent with the previously mentioned study wherein spleen cells remaining in zinc-deficient mice appeared functionally normal (81) with typical markers of T and B lymphocyte maturation (163). Thus, zinc deficiency blocks development of B lymphocytes in the marrow, resulting in fewer B lymphocytes in the spleen.

B lymphocyte function

B lymphocyte antibody responses are inhibited by zinc deficiency (5, 73, 164, 165). As previously mentioned, gross effects of zinc deficiency on B lymphocyte mitogen and plaque-forming responses have been observed. Zinc is required for the B lymphocyte mitogenic and cytokine response to lipopolysaccharide (156, 166). In vitro antibody production, determined by PFC assay, was strongly inhibited in splenic B lymphocytes from zinc-deficient mice (72).

Interestingly, T-dependent antibody responses are more affected by zinc deficiency than are T-independent ones. The PFC responses to the T-dependent antigen sheep red blood cells and T-independent antigen dextran were reduced by 90% (72, 73) and 50% (69, 79, 167), respectively, in zinc-deficient mice. Similar differences in effects were seen in moderately zinc-deficient animals (72, 73). In 1 study, mild zinc deficiency affected antibody responses to T-dependent antigens but not T-independent antigens (71). When zinc-deficient mice were returned to a normal diet, IgM PFC activity recovered within 1 wk, although restoration of IgG PFC activity required 1 mo, suggesting that greater perturbations in B or T lymphocyte functions are required for isotype switching (73, 164). T lymphocyte–mediated effects of zinc deficiency on B lymphocyte activity were also shown by restoration of PFC responses after infusion of normal thymocytes into zinc-deficient mice (72). In zinc-deficient mice infected with P. yoelii, antiparasite IgM concentrations were not affected, although IgG concentrations were suppressed nearly 10-fold (AH Shankar, unpublished observations, 1998). These findings are consistent with the idea that T-dependent B lymphocyte functions are more affected than T-independent ones. This is further supported by the observation that B lymphocytes are less dependent on zinc for proliferation than are T lymphocytes (168, 169).

It is also important to note that zinc-deficient mice had reduced antibody recall responses to T-dependent and T-independent antigens for which they had previously been immunized (69, 164, 165, 170), implying that immunologic memory is affected by zinc status. The potentially important effect of this for maintenance of vaccine efficacy in humans remains unexplored.
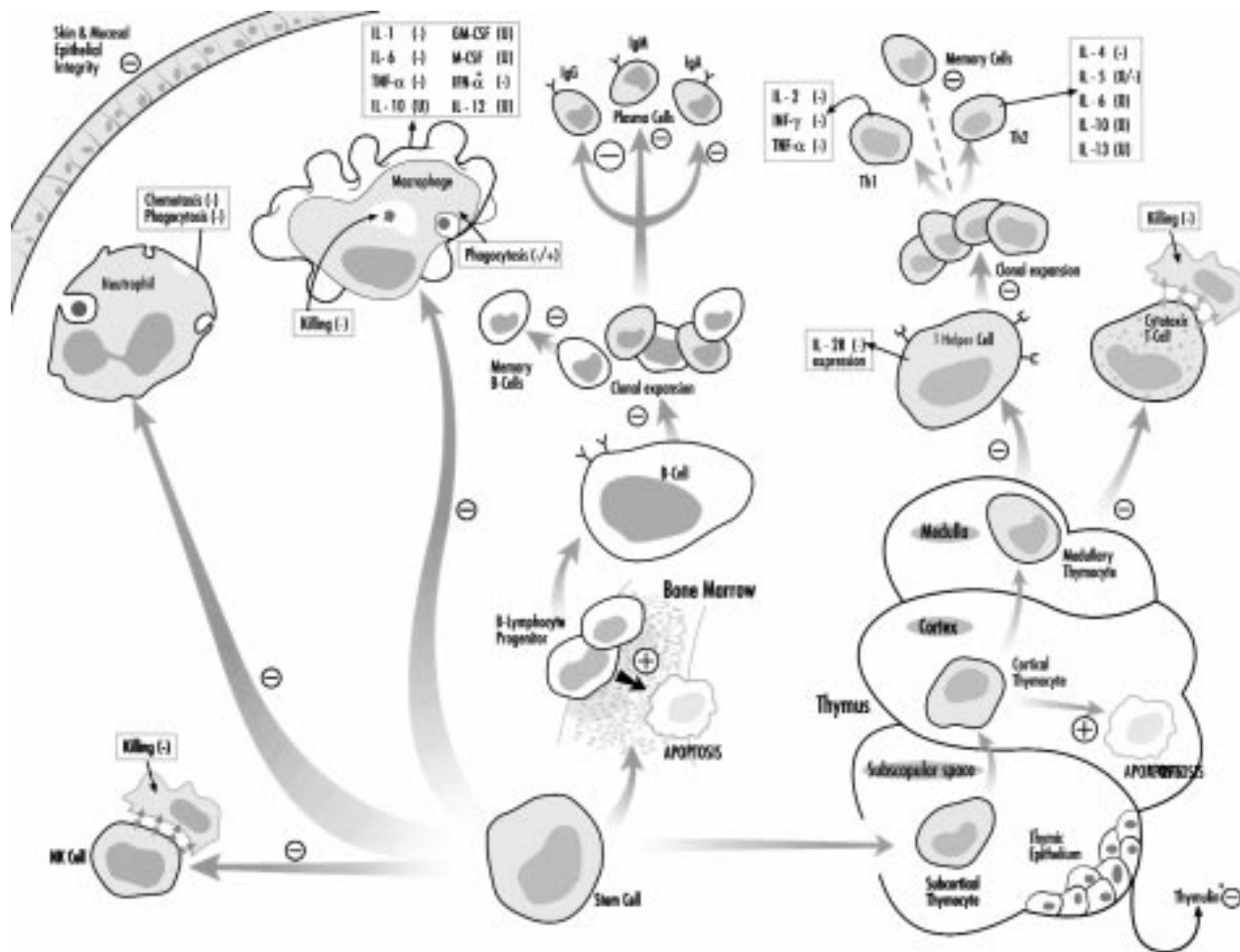
FIGURE 1. The effects of zinc on the development and function of certain immunologic cells and cytokines. GM-CSF, granulocyte macrophage colony stimulating factor; IG, immunoglobulin; IFN, interferon; IL, interleukin; IL-2R, interleukin-2 receptor; M-CSF, monocyte colony stimulating factor; NK, natural killer; 0, zinc deficiency has little or no effect on the process or activity; TNF, tumor necrosis factor; U, the effect of zinc deficiency on the particular process or activity is unknown; ⊖, zinc deficiency down-regulates or inhibits the process or activity; ⊕, zinc deficiency enhances the process or activity; *, zinc is needed for the structural integrity of the molecule.

In vitro effects of zinc on B lymphocytes

As for T lymphocytes, in vitro zinc-induced polyclonal activation of human B lymphocytes from blood, spleen, and lymph nodes was reported (171). These effects may, however, be secondary to zinc activation of helper T lymphocytes. In contrast with the effects on T lymphocytes, rosette formation between murine B lymphocytes and erythrocytes was inhibited when B lymphocytes were precultured with zinc in the presence of zinc ionophore, indicating that receptor down-regulation had occurred (172).

Natural killer cells

Studies in humans and animals describe decreased natural killer cell activity in zinc deficient states (6, 65), although 1 study reported increased natural killer cell activity (66). Natural killer cell function was depressed after in vivo treatment of cells with 1,10-phenanthroline, a zinc chelator, and the depression was reversed by the readdition of zinc, but not of calcium or magnesium (65). Exogenous zinc also stimulated production of interferon γ by human peripheral blood natural killer cells (67, 173).

However, exposure of natural killer cells to high concentrations of zinc in vitro inhibited cytotoxicity by rendering target cells more resistant to damage (66, 174). This and other reports of zinc-mediated inhibition (66) of natural killer cell activity may be partially explained by the recent demonstration that the killer cell inhibitory receptor requires zinc (175). The zinc-induced down-regulation of CD16, one of the Fcγ antibody receptor subtypes on natural killer cells, may also be due to a zinc-dependent metalloprotease (176). Overall, the in vitro data are consistent with the decreased natural killer cell activity seen in zinc-deficient animals and humans (6, 61, 65).

Neutrophils

Polymorphonuclear leukocyte function is altered in zinc-deficient animals and patients with acrodermatitis enteropathica and other types of zinc deficiency. In most cases, absolute numbers of peripheral polymorphonuclear leukocytes were not affected, but chemotactic responses were impaired and were reversible by in vitro addition of zinc (42, 62, 63). In addition, in vitro addi-

tion of zinc potentiated the polymorphonuclear leukocyte response against staphylococcal bacteria (177). One study observed that exercise-induced potentiation of polymorphonuclear leukocyte microbicidal superoxide formation was attenuated by zinc supplementation (64). Unfortunately, the effects of zinc deficiency on microbicidal functions such as total oxygen production, phagocytosis, and extravasation remain to be adequately evaluated.

Monocytes and macrophages

Effects on monocyte and macrophage function have been observed during zinc deficiency (178–181). In humans, the chemotactic response of monocytes from acrodermatitis enteropathica patients is suppressed and can be restored after addition of zinc in vitro (42, 62). Monocytes from zinc-deficient mice have impaired killing of intracellular parasites, which is rapidly corrected in vitro by addition of zinc (178). Reduced macrophage phagocytosis of *Candida* has also been observed in zinc-deficient animals (15, 25). In other studies, however, the ability of macrophages from zinc-deficient rodents to phagocytose particles was either enhanced (179, 182) and accompanied by greater numbers of cells bearing Fcγ and complement receptors (179), or remained unchanged (183). High concentrations of zinc in vitro inhibited macrophage activation, mobility, phagocytosis, and oxygen consumption (65, 184, 185). When marasmic children were rehabilitated with a zinc-containing regimen, monocyte phagocytic and fungicidal activity was suppressed (89). Because elevated zinc concentrations can inhibit complement activation (68), complement-mediated phagocytosis may be adversely affected by high zinc concentrations. Additional studies are clearly needed for a better understanding of the conditions under which zinc affects monocyte and macrophage phagocytosis.

There is much speculation regarding the role of zinc in the killing of pathogens by oxygen radicals produced by macrophages. With the exception of 1 report (186), available data do not provide evidence linking zinc status with macrophage production of oxygen radicals (21, 165, 180). Unfortunately, data regarding the effects of zinc on the production of other microbicidal radicals, ie, nitric oxide, are lacking. It was proposed that the product of the *Nramp* gene in mice, responsible for resistance to mycobacteria, *Leishmania donovani*, and *Salmonella typhimurium* (187), may be a zinc transporter involved in nitric oxide–mediated microbicidal activity of macrophages. In humans, monocyte production of cytokines IL-1β, IL-6, interferon α (IFN-α) and tumor necrosis factor α (TNF-α), were stimulated by addition of zinc in vitro (188–190). However, zinc deficiency enhanced human monocyte-mediated cytotoxicity against target cells, which returned to normal after zinc supplementation (65).

Zinc deficiency suppressed macrophage support of mitogen-induced T lymphocyte proliferation (181). Although inhibition of mitogenesis was due to effects on both macrophages and T lymphocytes, suppression of macrophage function occurred earlier in the onset of zinc deficiency. Moreover, macrophage activity was corrected within 30 min of incubation with zinc salts in vitro. The rapid restoration of certain macrophage functions after zinc addition suggests that rapid therapeutic effects of zinc supplementation on diarrhea or the common cold may involve some aspects of macrophage function. Moreover, there may be greater benefit of zinc supplementation for infections in which specific macrophage functions are central to resistance. Unfortunately, there is limited information concerning the effects of zinc on microbicidal or immunologic functions of macrophages in humans, including phagocyto-

sis, free radical production, antigen processing, antigen presentation, and cytokine production.

EFFECTS OF ZINC STATUS ON SOLUBLE MEDIATORS OF IMMUNITY

Glucocorticoids

The release of glucocorticoid hormones from the adrenal glands can result in certain physiologic changes, such as thymic atrophy, that are reminiscent of zinc-deficiency (191–193). Because zinc deficiency raises blood glucocorticoid concentrations (194–196), thymic atrophy may be mediated, in part, by glucocorticoids. Indeed, when adrenalectomized mice were fed a zinc-deficient diet for up to 6 wk, changes in thymic weight were small or absent (196). In addition, when adult mice were given a slow-release corticosteroid implant, thymus size was reduced >than 80% (161). Steroid-implanted mice also showed large reductions in pre-B and immature B lymphocytes in bone marrow (161, 197), suggesting that the effects of zinc deficiency on early B lymphocyte development may also involve glucocorticoids.

The contribution of glucocorticoids to the effects of zinc deficiency must, however, be interpreted with caution. Zinc deficiency has profound effects on human thymocytes, which are relatively resistant to glucocorticoids (196). In addition, although the thymus of zinc-deficient adrenalectomized mice remained normal in size, zinc-dependent decreases in the cortical to medullary area still occurred (196). Likewise, in adrenalectomized mice, zinc deficiency reduced IgM and IgG PFC responses to sheep red blood cells, 50% of the loss in T lymphocyte helper function in control animals occurred before detectable increases in plasma corticosterone (196). Last, in marginally zinc-deficient mice, loss of lymphocytes in the spleen, depressed immunity, and decreased IL-2 production were observed despite the absence of thymic shrinkage or increased glucocorticoid concentrations (69, 72).

Thymulin

Thymulin is a 9-peptide hormone (Glu-Ala-Lys-Ser-Gln-Gly-Ser-Asn) secreted by thymic epithelial cells. Thymulin binds to high-affinity receptors on T lymphocytes and promotes T lymphocyte maturation, cytotoxicity, and IL-2 production (198). Zinc is bound to thymulin in a 1:1 stoichiometry via the side chains of asparagine and the hydroxyl groups of the 2 serines (199). Thymulin activity, in vitro and in vivo in both animals and humans, is dependent on plasma zinc concentrations such that marginal changes in zinc intake or availability affect thymulin activity (32, 70, 120, 200–203). Thymulin is readily detectable in the serum of zinc-deficient patients, but is not active (70). The binding of zinc results in a conformational change that produces the active form of thymulin (200). The overall role of thymulin on the immunologic lesions caused by zinc deficiency has not been well studied. The use of thymulin as an indicator of zinc deficiency has been suggested, although thymulin concentration is also modulated by zinc-independent factors. Assays regarding zinc status and thymulin zinc saturation may prove more useful, much as the index of transferrin saturation provides useful information regarding iron status.

Cytokines

Cytokines, also known as interleukins, are key messengers of immunologic cells that regulate multiple aspects of leukocyte

biology. Their effects are mediated through corresponding receptors on target cells. Production or biological activity of multiple cytokines (IL-1, IL-2, IL-3, IL-4, IL-6, IFN-γ, IFN-α, TNF-α, and migration inhibitory factor) influencing the development and function of T lymphocytes, B lymphocytes, macrophages, and natural killer cells is affected by zinc deficiency. The cytokines IL-1 (204), IL-2 (71, 76, 204), IL-4 (26, 188), and IFN-γ (67) have been reported to be suppressed during zinc deficiency. A decline in IL-2 receptor expression has been observed in some (76, 152), but not all (71), studies. The amino acid sequence of IL-3 contains an active zinc binding site that is important for activity (205). Further studies are needed to determine the in vivo role of zinc in modulating IL-3 activity. Zinc also plays a critical role in the dimerization of IFN-α necessary for its activity (206). Confirming the general inhibitory effects of zinc deficiency on cytokine production in vivo, in vitro addition of 1,10-phenanthroline reversibly inhibited antigen-stimulated production of migration inhibitory factor from lymphocytes (20, 25, 207). As previously mentioned, monocyte production of cytokines IL-1β, IL-6, IFN-α, and TNF-α was stimulated by the addition of zinc in vitro (188–190, 204).

T helper cells can be categorized as Th1 and Th2 cells, according to their functions in cell-mediated (Th1) and antibody-mediated (Th2) immunity. These subsets are well characterized in the murine immune system, where IL-2, IFN-γ, and TNF-α are considered products of Th1 cells, and IL-4, IL-5, IL-6, IL-10, and IL-13 are products of Th2 cells (208). Although not as clear cut in humans, segregated T lymphocyte production of cytokines has been observed (208). Th1 cell–associated cytokines are known to promote macrophage activation and production of cytophilic IgG isotypes. Th2 cell–associated cytokines tend to suppress macrophage function and cell-mediated immunity while promoting production of the noncytophilic IgG isotypes and IgE (208).

In an experimental human model, we showed recently that the production of IFN-γ was decreased whereas the production of IL-4, IL-6, and IL-10 was not affected during zinc deficiency (209). Earlier studies in experimental human model subjects and in patients with sickle cell disease and head and neck cancer showed a significant effect of zinc deficiency on IL-2 activity (70, 210). In summary, the studies showed that even mild zinc deficiency in humans may be accompanied by an imbalance of Th1 cell and Th2 cell function, resulting in dysregulated resistance to infection.

CELL BIOLOGY OF ZINC IN THE IMMUNE SYSTEM

General cell biology of zinc

Zinc is present in the body almost exclusively as Zn^{2+} bound to cellular proteins. Zinc has a high affinity for electrons, enabling interactions with several amino acid side chains. Interactions, especially with sulfur and nitrogen atoms in the amino acids cysteine and histidine, respectively, enable zinc to crosslink remote regions within and between polypeptides to modify tertiary protein structure and function. Given this ability, its relative nontoxicity, and the fact that it does not engage in damaging redox reactions, zinc is ideally suited to play a central role in intracellular metabolism.

The zinc content within lymphocytes, 150 pmol/10^6 peripheral blood lymphocytes (211), is typical of most cells in the body. It is thought that activated lymphocytes take up zinc via multiple mechanisms, including receptors for zinc-transferrin, albumin, α2-macroglobulin, and metallothionein, the major zinc binding protein of the body (9, 149, 212–214)

Zinc uptake also involves other less well characterized mechanisms such as anionic channels or transporters (215). Recently, zinc transporters and channels mediating both the inward and outward movement of zinc were isolated from mammalian cells (216, 217). The continued study of these transporters and channels will help elucidate the mechanisms of zinc homeostasis within cells. In rats, a system for transport of zinc into the nucleus is suggested by the rapid appearance of ^{65}Zn in the nucleus of spleen cells within 2 h of intragastric feeding with ^{65}Zn (218).

Role of zinc in the cell cycle of lymphocytes

When lymphocytes were treated with PHA or concanavalin A, within several hours there was a rapid increase in cellular zinc (10, 219–221). These findings are consistent with studies indicating a requirement for zinc during mid to late G1 phase in promotion of thymidine kinase expression (222), and in another less well defined step involved in cell transition to S phase (223). The zinc-dependent activity of DNA polymerase may account in part for the influence of zinc during S phase (224). Zinc may also play a role in transition to the G2 and M phases. A greater proportion of S compared with G2 phase cells were observed in PHA-stimulated lymphocytes from mildly zinc-deficient patients suffering from sickle cell anemia (116). The ratio returned to normal after a period of zinc supplementation (116). The M phase of the cell cycle may also be affected by zinc deficiency because defective tubulin polymerization is seen in tissues from zinc-deficient animals (225, 226), and zinc is known to bind the amino-terminal of tubulin, thereby stabilizing microtubule formation (225, 226).

Mechanisms underlying the effect of zinc on cell replication

Zinc influences the activity of multiple enzymes at the basic levels of replication and transcription, as shown in Figure 2. These include DNA polymerase, thymidine kinase, DNA-dependent RNA polymerase, terminal deoxyribonucleotidyl transferase and aminoacyl transfer RNA synthetase (220, 222, 227–231), and the family of transcriptional regulators known as zinc finger DNA binding proteins (232, 233). In addition, zinc forms the active enzymatic sites of many metalloproteases (232, 233).

The activity of the major enzyme regulating DNA replication, DNA polymerase, is zinc dependent. It is inhibited by zinc deficiency and zinc chelators and is enhanced by addition of low concentrations of zinc in vitro (229). Thymidine kinase, crucial for phosphorylated pyrimidines, is also very sensitive to dietary zinc depletion (219, 229). Zinc is, in fact, required for expression of multiple genes regulating mitosis, including thymidine kinase, ornithine decarboxylase, and *CMYC* (219, 234, 235). As mentioned, several transcription factors, such as NFκ-B (236), metallothionein transcription factor 1 (237), and RING (238), contain zinc finger–like domains that are influenced by changes in intracellular pools of zinc. In addition, zinc deprivation affects the activity of RNA polymerase (230) needed for transcription.

Role of zinc in lymphocyte activation

Zinc also plays a role in multiple aspects of T lymphocyte activation and signal transduction (239) as detailed in Figure 2. Zinc is implicated in the noncovalent interaction of the cytoplas-
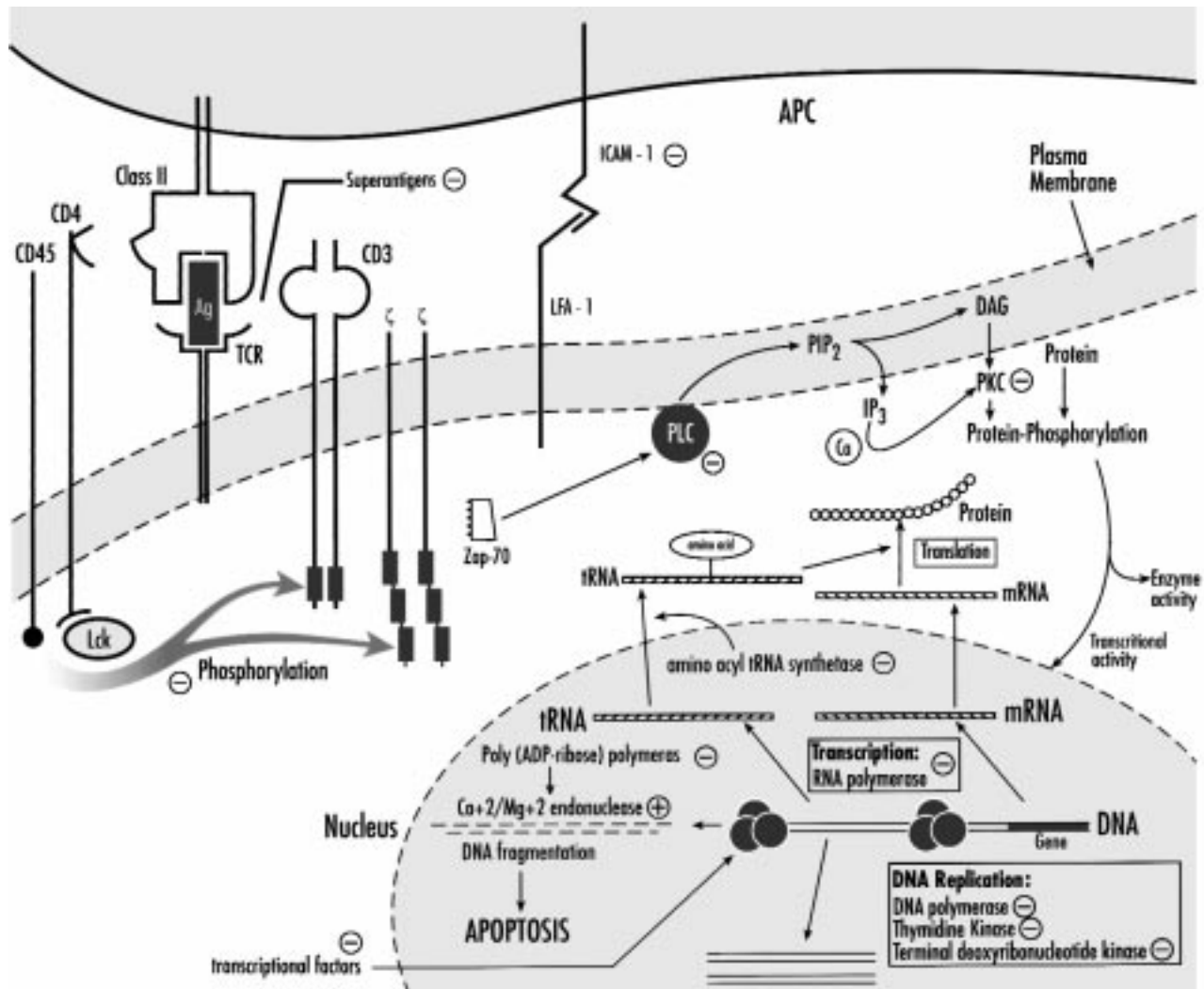
FIGURE 2. The influence of zinc deficiency on activation of lymphocytes. T lymphocyte activation via antigen presentation by class II or via super-antigen binding directly to the T lymphocyte receptor (TCR). Ag, antigenic peptide; APC, antigen presenting cell; Lck, p56^{lck}; PLC, phospholipase C; PIP$_2$, phosphatidyl inositol; IP$_3$, inositol triphosphate; DAG, diacylglycerol, PKC, protein kinase C; ⊖indicates that zinc deficiency down-regulates or inhibits the process or activity; ζ, ζ-chains of the CD3 T lymphocyte receptor complex; Zap-70, ζ-associated protein-70; ⊕ indicates that zinc deficiency enhances the process or activity. Cell activation pathways shown are limited to those where zinc may play a role.

mic tails of CD4 and CD8 with the tyrosine kinase p56^{lck}, an essential protein in the early steps of T lymphocyte activation. Through this and possibly other pathways, zinc stimulates autophosphorylation of tyrosine residues by p56^{lck} and subsequent phosphorylation of the T lymphocyte receptor complex involving CD45 (240, 241). Zinc is then involved in the activity of phospholipase C to give rise to inositol triphosphate and diacylglycerol (242). In addition, zinc affects the phosphorylation of proteins mediated by protein kinase C (243). Subsequent changes through protein phosphorylation regulate activation and cell proliferation.

Influence of zinc on apoptosis

The major mechanism of cell death in the body and in culture is apoptosis, a form of cell suicide characterized by a decrease in cell volume, dramatic condensation of the chromatin and cyto-

plasm, and fragmentation of nuclear DNA (244). Apoptosis is a normal physiologic process enabling a variety of important processes from epithelial turnover to T and B lymphocyte development (245). The dysregulation of such a basic process would, therefore, have important health consequences.

Zinc-deficient animals exhibit enhanced spontaneous and toxin-induced apoptosis in multiple cell types (243, 246–249). As previously discussed, zinc thymic atrophy is a central feature of zinc deficiency. It is now known that this atrophy is accompanied by apoptotic cell death of thymocytes (160). Several studies showed that zinc is a regulator of lymphocyte apoptosis in vivo. Zinc supplementation decreased mycotoxin-induced apoptosis of macrophages and T lymphocytes in mice (250). In addition, zinc administration to mice 48 h before intraperitoneal injection of lipopolysaccharide greatly abrogated subsequent apoptotic

DNA cleavage in thymocytes and loss in thymic weight (251). In vitro, greater numbers of lymphocytes and thymocytes undergo apoptosis when cultured with a zinc-free medium (252) or with zinc chelators (253–255). Conversely, apoptosis of T lymphocytes induced by in vitro exposure to toxins (250) and other agents (243) is prevented by the addition of high concentrations of zinc salts. Cells can also be rescued from apoptosis with physiologic concentrations of zinc salts (5–25 μmol/L) if uptake is facilitated by the zinc ionophore pyrithione (253). It has been suggested that zinc is a major intracellular regulator of apoptosis because lymphocytes maintain intracellular zinc at concentrations slightly above those needed to suppress apoptosis (256). In addition, a dose-responsive relation is seen between intracellular zinc concentrations and the degree of susceptibility to apoptosis (10, 256).

The subcellular mechanisms whereby zinc affects apoptosis are not well understood, but it is likely that zinc acts at multiple levels. There was a good correlation between inhibition of Ca^{2+}/Mg^{2+} DNA endonuclease activity and inhibition of apoptotic DNA fragmentation (257). Although in vivo data are lacking, the Ca/Zn balance may regulate endonuclease activity (258). Nucleoside phosphorylase, another zinc-dependent enzyme, may inhibit apoptosis by preventing the accumulation of toxic nucleotides (126, 259, 260). Likewise, poly (ADP-ribose) polymerase, the zinc-dependent nuclear enzyme, interacts with and inhibits the Ca^{2+}/Mg^{2+} DNA endonuclease (261, 262). In lymphocytes undergoing apoptosis, cytoplasmic zinc increases significantly, possibly originating from release of zinc from nuclear proteins (263).

Role of zinc as an antioxidant

Zinc has other properties that could contribute to its role in lymphocyte function. It is an antioxidant, protecting cells from the damaging effects of oxygen radicals generated during immune activation (264, 265). Zinc also regulates the expression in lymphocytes of metallothionein and metallothionein-like proteins with antioxidant activity (213, 266). Membrane zinc concentrations are strongly influenced by dietary zinc deficiency and supplementation (267, 268). Zinc concentrations in cell membranes appear to be important in preserving their integrity through poorly defined mechanisms involving binding to thiolate groups (265). It is noteworthy that zinc release from thiolate bonds can prevent lipid peroxidation (269). In addition, nitric oxide induces zinc release from metallothionein, the primary zinc binding and transport protein in the body, which may limit free radical membrane damage during inflammation. Indeed, zinc supplementation has been shown to prevent pulmonary pathology due to hyperoxia in rats (270).

DISCUSSION

The studies presented herein represent the scope of zinc-mediated effects on infectious disease and immune function. Although the effects of zinc deficiency on immunity are profound and ubiquitous, it is remarkable that greater effects on health are not observed in marginally zinc-deficient populations. This may be partially explained by the redundancy of immunologic effectors compensating for suboptimal function of any one effector. However, it is also clear that not all aspects of immunity or infections are affected equally by zinc deficiency. For example, in vitro intracellular killing by macrophages is very sensitive

to zinc deficiency and is restored rapidly after supplementation. T lymphocyte–macrophage interactions are less sensitive to zinc deficiency and macrophage phagocytosis is the least sensitive. Thus, infections in which macrophage killing is a central effector, such as malaria or tuberculosis, may be more sensitive to zinc deficiency. Likewise, zinc deficiency may have greater effects on infections requiring T lymphocyte–dependent antibody responses and those in which IgG is crucial for protection. Infections for which T lymphocyte–independent responses are sufficient may be less affected. In the case of T lymphocytes, infections for which Th1 cytokines, eg, IFN-γ, are needed for resistance, may be more affected by zinc deficiency. It is also clear that development of immunologic cells is most sensitive to zinc deficiency during the perinatal period, indicating that this is a crucial window of intervention.

Future directions

Continued understanding of how zinc influences the immune system and alters host resistance to infection will depend on greater integration of modern immunology, molecular biology, and cellular biochemistry into laboratory and field-based studies. A few specific areas are detailed below that represent only a small fraction of the immunologic processes for which major gaps remain in the knowledge of zinc immunobiology.

Although adhesion molecules play a major role in cell-to-cell interaction, the influence of zinc on their function remains mostly unexplored. Given the role of zinc in the tertiary structure of proteins, it is likely that zinc deficiency may affect adhesion molecule function. Likewise, we know very little about how zinc might affect lymphocyte trafficking in the body.

With regard to macrophage function, it is still not clear how zinc influences the ability of the cell to engulf and kill organisms. Additional studies examining the effects of zinc on nitric oxide and oxygen radical production would, therefore, be useful. The role of zinc in antigen digestion and presentation to T lymphocytes is unknown.

With respect to B lymphocyte function, the processes of isotype switching and affinity maturation are crucial to optimal antibody-mediated resistance to infection. Knowledge of the influence of zinc on antibody affinity would also provide information relevant to vaccine efficacy. For both T and B lymphocytes, the role of zinc in the development of memory cells is potentially important for vaccine efficacy and needs to be further explored. As indicated for macrophages, the role of zinc in the antigen processing and presentation functions of B lymphocytes is completely unknown.

It is clear that zinc plays a major role in immunologic ontogeny and development of the antigen-recognition repertoire. In addition, subsets of T and B lymphocytes known as γδ-T lymphocytes and CD5 B lymphocytes provide pseudospecific or oligoclonal immunity. Although more limited in antigenic reactivity than their conventional polyclonal T and B lymphocyte counterparts, their localization to the pleural and peritoneal cavities and intestinal epithelium provides a rapid response against invading organisms. The effects of zinc on development of these cells and their function remains unknown. As detailed elsewhere in this supplement (271), the health effects of gestational zinc deprivation are potentially far-reaching and need to be fully explored given the possible long term or even generational effects on populations.

Concerning cytokines and T lymphocyte regulation, the seminal work by Prasad et al (209, 210) concerning the influence of

zinc on Th1 and Th2 regulation provides a foundation on which to build. Little is known regarding effects of zinc on cytokine activation of cells because most studies have focused on cytokine secretion. Moreover, most of the studies of T and B lymphocytes have used mitogens for cell activation. Studies are needed that examine lymphocyte activation in response to the more physiologic antigen driven responses involving all aspects of cell adhesion, co-stimulation, and cytokine activation.

It will be important to apply knowledge of the immunologic mechanisms modulated by zinc deficiency to the design of therapies and public health interventions involving targeted zinc supplementation for certain diseases or high-risk groups. By extension, it would be useful to identify the specific immunologic lesions caused by zinc deficiency responsible for altered host resistance in natural infections and in experimental infectious disease models. The role of zinc in immunity and the biological basis of altered resistance to infection will no doubt be fruitfully explored as researchers continue to integrate knowledge and techniques from diverse disciplines to examine the effects on zinc on immunity, health, and disease.

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