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ZINC DEFICIENCY AND IMMUNE FUNCTION

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INTRODUCTION

Generalized malnutrition and select nutrient deficiencies can result in a compromised immune response. Furthermore, immunocompetent defects associated with nutritional deficiencies can contribute to the establishment

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and severity of several disease states (22, 49). The influence of a nutrient on the immune response involves a complex interaction among at least three major effector mechanisms: antibody-mediated immunity, cell-mediated immunity, and phagocytosis. Although not considered a component of the immune response, mucosal and barrier immunity (i.e. skin acting as a barrier to prevent penetration of organisms) can be affected by the nutritional status of an individual. Defects in any of the above systems can result in ineffective host defenses.

During the last decade, the nutrient zinc (Zn), and its immunoregulatory properties, have been the focus of considerable interest. This interest has been fueled by observations concerning the influence of Zn nutriture on the functioning and ontogeny of the immune system in experimental animals and by numerous clinical reports suggesting an association between marginal Zn deficiency and impaired immune competence in several human populations. We discuss (*a*) the effects of Zn deficiency on the different components of the immune system, (*b*) potential mechanisms underlying these effects, and (*c*) evidence that Zn deficiency occurs in humans and is responsible for some immune defects.

The frequency and severity of Zn deficiency in human populations is an issue of considerable debate. However, based on recent detailed epidemiological investigations, as well as a number of dietary intervention studies, the argument can be made that marginal Zn deficiency may be a relatively common problem in select population subgroups. Individuals at high risk for Zn deficiency are thought to include infants, adolescents, women of reproductive age, and the aged (55, 76, 92). In addition, altered Zn metabolism may represent a serious complication of several diverse diseases including renal disease, alcoholism, diabetes, and sickle cell anemia. It is significant that all of the aforementioned diseases are also characterized by impaired immunocompetence (see section on Zn deficiency in humans).

The potentially serious consequences of consuming a diet inadequate in Zn are exacerbated by the apparent lack of readily mobilizable stores of Zn in the adult. The lack of these stores, coupled with a relatively high obligatory endogenous loss of the element, dictates a constant need for the adult organism to consume a diet adequate in Zn in order to maintain plasma Zn concentrations. For example, in rats plasma Zn concentrations can decrease by over 50% within 24 h after the introduction of a Zn-deficient diet (59). This rapid reduction in Zn can be functionally significant: In pregnant rats, embryotoxicity can occur when dams consume Zn-deficient diets for a period of time as short as three days (63). A number of hormonal signals can also trigger a rapid decrease in plasma Zn concentrations and a concomitant reduction in the transfer of plasma Zn to some extrahepatic tissues (26). Thus, depending on the tissue in question, "Zn deficiency" can arise either as a

function of low dietary Zn intake or as a result of a reduction in plasma Zn concentrations secondary to hormone-induced shifts in Zn metabolism.

COMPONENTS OF THE IMMUNE RESPONSE

Zn deficiency in adult animals can result in abnormal immune function. Zn-deficient animals can show an increased susceptibility to a number of pathogens including *Candida albicans, Francisella tularensis, Trypanosoma cruzi,* and *Trypanosoma musculi* (41, 67, 81, 91). In the following sections we discuss in detail some of the immune defects characteristic of Zn deficiency; for additional information on this subject a number of reviews can be consulted (24, 29, 44, 56). Below, a brief description of the components of the immune system is presented [for additional details see (49, 88, 93)].

Mucosal and Barrier Immunity

The immune system consists of innate and acquired mechanisms that protect the host from pathogens in the environment. Innate mechanisms function independent of previous exposure of the host to the infectious agent. Innate mechanisms include mechanical barriers, such as skin, and cellular components (macrophages, neutrophils and phagocytes). The influence of Zn deficiency on barrier immunity has received little attention, even though characteristics of Zn deficiency include skin lesions, gastrointestinal lesions including degenerative changes in the enterocytes and damage to the microvilli, and alterations in pulmonary function (55).

Humoral Immunity

Immunoglobulins bind specifically with antigen and facilitate its destruction. Immunoglobulins are heterogeneous and have different molecular weights and functional properties. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM. All of the antibodies are characterized by four polypeptide chains linked by disulfide bonds. Two of the chains, referred to as the light chains, have molecular weights of ~22,000 while the other two chains, referred to as heavy chains, have molecular weights of ~55,000. At the N-terminus portion of each of the polypeptide chains, a variable amino acid sequence dictates the antigen-binding properties of the immunoglobulin. The antibodies are produced by B lymphocytes that have differentiated into antibody-forming plasma cells. In mammals, the B-lymphocytes develop in the bone marrow and then seed secondary lymphoid tissue. The T-lymphocytes (thymus-derived), which are also derived from bone marrow stem cells, mature in the thymus and then, like B-cells, seed secondary lymphoid tissue.

The antigen-induced production of most antibodies involves a complex

interaction among a number of cells of the immune system. For example, T-dependent antigens initially associate with macrophages bearing class II major histocompatibility (MHC) antigens (Ia-positive macrophages). The antigen associates with an Ia molecule on the surface of the macrophage, and this complex can then associate with a helpter T-cell. Macrophages produce interleukin-1 (IL-1), a cytokine that acts in part to activate helper T-cells. IL-1 stimulation of the helper T-cell results in increased T-cell secretion of IL-2 and IL-3, increased expression of T-cell surface IL-2 receptors, and enhanced T-cell chemotaxis. The IL-1 signal also stimulates pre-B cells and the clonal expansion of mature B-cells, which produce antiody specific for the antigen. Following the association of the antigen-macrophage complex with the helper T-cell, the further stimulation of helper T-cell production of IL-2 as well as of B-cell growth factors occurs (IL-4 and IL-5). The IL-2 signal also stimulates the proliferation of activated T-cells (72).

In addition, the IL-1 signal effects a rapid redistribution of Zn from the plasma pool to the liver, bone marrow, and thymus (26). The reduction in plasma Zn may be critical for phagocytic cell activation (24), while the stimulation of Zn uptake by thymic and bone marrow tissues suggests a critical need for the element during T- and B-cell proliferation. The production and/or action of some cytokines is thought to be dependent on zinc. For example, the production and/or membrane binding of IL-1, IL-2, and interferon may be zinc dependent (33, 40, 43, 90, 101).

Cell-Mediated Immunity

Cell-mediated immunity is important in the killing of virus-, bacteria-, and protozoa-infected cells, and in the killing of tumor cells. One mechanism of destruction involves the binding of thymus-derived cytotoxic T-cells to the target cell. Theoretically, cytotoxic T-cells can kill any infected host cell because class I MHC antigens are present on all nucleated cells of the body. Following binding, cytotoxic T-cells kill their target cell through the release of cytotoxic proteins (perforins) that disrupt the integrity of the membrane of the target cell and result in ionic disequilibrium and subsequent osmotic rupture. Cell-mediated immunity is also provided by T-cells through the release of γ-interferon and lymphotoxin from activated T-cells. These products can activate macrophages and stimulate their ability to kill phagocytized organisms.

Macrophages and neutrophils (polymorphonuclear neutrophils, PMNs) are functionally similar cell types that can participate in the destruction of pathogens via enzymatic degradation. Macrophages can also be involved in the processing of antigens for regulatory T-cells, as well as in the killing of tumor cells and the regulation of the development and activation of other leukocytes. Natural killer cells (NK) a subset of lymphocytes that do not bear

either T- or B-cell markers, also have the capacity to kill tumor cells. NK cells do not have MHC restriction. Other white cells that are critical to the immune response include basophils, mast cells, and eosinophils.

Complement proteins are synthesized in various hepatic and extrahepatic sites and undergo well-defined, sequential reactions to produce products that are able to (*a*) perforate lipid bilayer membranes and induce cell lysis, and (*b*) attract and stimulate phagocytic cells. Complement proteins act via two major pathways. In the "classical pathway," after the binding of an antigen, sites on the constant region of certain immunoglobulin isotypes (IgM and IgE) are exposed; Clq, a complement component, may then attach to these sites. A series of interactions among other complement proteins then take place. In the "alternate" pathway, the complement cascade is initiated in the absence of antibody. A variety of substances associated with microorganisms (bacterial and fungal cell walls, parasite cuticles) and aggregated immunoglobulin can trigger this pathway. Zn deficiency has not been shown to negatively affect the complement system; indeed, Zn at the higher levels of the normal physiological range inhibits the complement cascade (74). Thus, one can speculate that the reduction in plasma Zn following infection may be a positive effector for this arm of the immune system.

INFLUENCE OF Zn DEFICIENCY ON THE IMMUNE RESPONSE IN EXPERIMENTAL ANIMALS

Zn Deficiency and Immune Function in Young and Adult Animals

Involution of the thymus and a small-sized spleen are characteristic signs of prolonged Zn deficiency in rodents, and a reduction in the mass of these tissues has been associated with a reduction in the number of white cells (7, 8, 33, 42, 49, 55, 71). Leukopenia is characteristic of Zn deficiency and is primarily the result of lymphopenia; neutrophil and monocyte counts are in the normal range (7, 71). The production of T-lymphocytes in particular has been shown to be impaired with Zn deficiency. Whether or not Zn deficiency has a differential effect on specific subsets of T-cells is currently the subject of debate. While some investigators have reported that the production of T-helper cells is particularly affected (43, 44), Dowd et al (33), using monoclonal antibodies specific for T-cell subset markers, were unable to detect a Zn deficiency–induced shift in the ratio of T-helper cells to T-suppressor and cytotoxic cells in Zn-deficient rats. Nash et al (78) reported that the percentage of autologous rosette-forming cells, a subset of immature T cells, increases over time in Zn-deficient mice.

Zn deficiency results in overall growth retardation and weight loss; however, the loss of lymphoid tissue mass exceeds that of other tissues (7, 8, 42).

While a chronic deficiency of Zn will result in atrophy of the entire organ, in the early stages a preferential loss in the cortex occurs in the region populated by glucocorticoid-sensitive immature thymocytes (31). One consequence of Zn deficiency is adrenal hypertrophy and an increase in circulating 11-hydroxysteroid concentrations (83); it has therefore been suggested that Zn deficiency–induced thymic atrophy could be due in part to the increase in circulating glucocorticoids. This idea was substantiated by the finding that adrenalectomy prevented much of the thymic atrophy associated with Zn deficiency (31). However, despite the prevention of the thymic atrophy, the adrenalectomized Zn-deficient mice still developed a functional T-cell loss that approached the loss occurring in nonadrenalectomized Zn-deficient mice (31). An effect of Zn deficiency on T-cell maturation has also been attributed to a possible reduction in the activity of deoxyribonucleotidyltransferase, a Zn-containing DNA polymerase found in high concentrations in the thymus of mature thymocytes (53). Possibly, Zn deficiency may also alter thymic epithelial function and impair thymic hormone production, which in turn would inhibit T-cell maturation in the thymus and in the periphery (5).

Some researchers believe that Zn deficiency may lead to a reduction in T-cell subsets (15, 32, 42, 49, 56). It is important to note that when data concerning the production of antibodies are evaluated on the basis of specific antibody-producing cells per 10^6 lymphocytes, rather than on a whole organ basis, the amount of antibody produced is similar in control and Zn-deficient animals (17). Thus, the overall reduction in antibody production in the deficient animal is primarily due to a reduction in total white cell number and is not due to a defect in lymphocyte function per se.

In addition to the above findings, overall responsiveness to T-cell independent antigens such as trinitrophenylated polysaccharide (TNP-LPS), as well as to the T-cell dependent mitogens, pokeweed mitogen (PWN), concanavalin A (Con-A), and phytohemagglutinin (PHA), can be markedly inhibited with Zn deficiency (15, 54, 102). Although the overall reduction in mitogen responsiveness can be impressive, when the number of cells producing specific antibody per 10^6 lymphocytes are considered, there is little evidence for specific lymphocyte defects. Indeed, the intrinsic ability of B- and T-cells isolated from Zn-deficient mice to produce IL-2 and antibody has been reported to be normal when tested in vitro (33).

The observation by DePasquale-Jardieu & Fraker (31) that adrenalectomy did not prevent a Zn deficiency–associated loss in lymphocytes supports the view that a deficit of Zn may directly influence lymphocyte proliferation. The stimulation of blast transformation in vitro to mitogens such as PHA can be inhibited by Zn-chelating agents, and Zn itself has been shown to act as a mitogen to both T-cells and B-cells (1, 23, 30, 66, 70, 85). Flynn (39) has suggested that Zn deficiency may affect T-cell proliferation (*a*) by depressing

the production of cytokines responsible for proliferation, (*b*) by interfering with the processing of antigen by accessory cells, or (*c*) by resulting in a loss of cell function or "activation state." Consistent with the above, Winchurch (100) has reported that Zn facilitates thymocyte proliferative response to IL-1, possibly by affecting the cell surface receptor for this cytokine. The binding of zinc to the plasma membranes of T-cells may also be crucial for antigen-induced activation of protein kinase C and its subsequent membrane binding (28, 29).

Similar to the Zn-deficient mouse and rat, thymic hypoplasia and lymphopenia is characteristic of Friesian cattle with lethal trait A46, a genetic defect that results in an impairment of Zn absorption (55). Zn supplementation of these animals results in an increase in thymic growth and white cell mass (55).

Delayed-type hypersensitivity, which is dependent on an interaction between effector T-cells (T_{dth}) and macrophages, is attenuated in Zn-deficient animals (45). Macrophage function can be inhibited in Zn-deficient animals. Defects in macrophage function include the reduced ability to take up the target as well as to kill it (41). Significantly, in contrast to lymphocytes, impaired macrophage function with Zn deficiency is evident even on a per cell basis. Thus the Zn-deficient environment directly affects the functioning of the cell independent of an indirect effect of the deficiency on proliferation of this cell line. However, if the macrophages isolated from Zn-deficient animals prior to pathogen challenge are incubated in the presence of supplemental Zn, the uptake of parasites, and their killing, approach control values (41). This observation suggests that the defect(s) in macrophage function is probably due to an actual limitation of Zn in a critical process such as maintaining membrane integrity or tubulin-mediated phagocytosis. Lastly, decreased NK cell activity and depressed cytotoxic responses to EL-4 tumor cells have been reported in Zn-deficient mice (38), although increased NK activity with Zn deficiency has also been reported (21). Finally, defective chemotaxis indicates that neutrophil function is also affected by Zn deficiency (57).

Zn-deficient animals show an increased susceptibility to pathogen challenge. Fraker et al (41) have reported that for mice a period of Zn deficiency lasting only eight days was sufficient to markedly enhance their susceptibility to the parasite *Trypanosoma cruzi*. Similar data obtained by others demonstrate an increased susceptibility of Zn-deficient animals to viral, parasitic, and bacterial challenges (41, 67, 81, 91). Significantly, virtually all of the immunological abnormalities discusssed above are rapidly corrected following Zn repletion. One significant exception should be noted: to some extent, immunological memory that is lost with postnatal Zn deficiency may not be recovered upon Zn repletion (44).

Zn Deficiency and the Development of the Immune System

Zn deficiency during prenatal development can have profound effects on the ontogeny of the immune system (11, 12, 14, 65, 95). Most studies concerning the effect of Zn deficiency on the development of the immune system differ from one another in the relative degree of Zn deficiency that is imposed. For example, studies investigating the function of Zn in early embryonic development and organogenesis generally use diets that are severely deficient in Zn and often contain less than 1 μg Zn/g (55, 63). In marked contrast, disturbances in the development of the immune system can be induced by using diets that are only marginally deficient in the element (<5 μg Zn/g). To illustrate the dramatic effects that marginal Zn deficiency can have on the ontogeny of the immune system in mice, we consider in some detail a series of studies conducted by Beach et al (11, 12, 14) on this subject.

The basic experimental design employed in these studies was as follows. During the first seven days of pregnancy, pregnant mice were fed Zn-adequate diets (100 μg Zn/g), after which time they were randomly assigned to one of three dietary groups: control Zn (100 μg Zn/g) ad libitum, marginal Zn (5 μg Zn/g) ad libitum, or control Zn at restricted intake levels. The third group of mice was included to separate the direct effects of Zn deficiency on the immune system from indirect effects that were due to Zn deficiency-induced reductions in maternal food intake. After the litters were born, all of the dams (and pups after weaning) were fed the control Zn diet ad libitum. Although dams fed the low Zn diets did not develop overt signs of Zn deficiency, their food intakes and weight gains were lower than those of controls during gestation. In addition, plasma Zn concentrations in the Zn-deprived dams were about 50% of control values on day 17 of gestation.

Litters born to the Zn-deprived dams had lower birth weights and a higher neonatal mortality rate than those of controls. Initial lymphoid organ weights (either absolute weight or tissue/body weight ratio) were markedly lower in pups born to the Zn-deprived dams; however, by postnatal week six, tissue weights were similar among the groups (11, 14). After weaning, all of the pups (F_1) were fed the Zn-adequate diet ad libitum. While whole body Zn concentrations were lower in the prenatally Zn-deprived pups than in the control groups, values were similar by day 10 postnatal (11).

The impact of prenatal dietary Zn deprivation on the immune system was evaluated at 6, 10, and 24 weeks of age. At 6 and 10 weeks of age the offspring of the prenatally Zn-deprived dams were characterized by a virtual absence of serum IgM; by 6 months of age IgM was detected in these pups but at levels that were only about half those of controls. Serum IgG_{2a} and IgA concentrations were significantly lower in the prenatally Zn-deprived pups than in the controls; however, IgG_{2a} and IgA levels were similar among the groups at 24 weeks of age. In contrast to the findings, serum levels of IgG_1

and IgG_{2b} were similar among the groups at all times tested. In addition to depressed IgM concentrations, the pups from the prenatally Zn-deprived dams were characterized by a markedly lower plaque-forming cell response (PFC) to sheep red blood cell (SRBC) immunization at 6 weeks of age; at 10 and 24 weeks of age, mice born to the Zn-deprived dams continued to show a lower PFC response to SRBC; the responses however, were no longer significantly lower than those found in controls. For both types of immunodeficiency, data from the food-restricted group clearly demonstrated that the immune defects occurred independent of the reduced maternal food intake in the Zn-deprived group. Furthermore, results from cross-fostering studies demonstrated that the impairment in the development of the immune system occurred prenatally, since control pups fostered onto dams given the low Zn diets during pregnancy developed normally while pups from these dams fostered onto control dams expressed immune defects similar to pups who were not cross fostered (11, 12, 14). Finally, immune defects caused by prenatal Zn deficiency can be persistent: thus, offspring of the prenatally Zn-deprived female mice (i.e. the F_2 generation) demonstrate low serum IgM levels and PFC response to SRBC (12, 14). The mechanism(s) underlying this observation has not been delineated. One explanation is that Zn deficiency during critical periods of ontogeny might result in changes in the methylation pattern(s) of immunoregulatory genes (95). Other epigenic defects have been ascribed to prenatally induced alterations in gene methylation patterns (58).

The observation that prenatal Zn deficiency can have a sustained effect on the development of the immune system is consistent with similar observations that intrauterine growth retardation (IUGR) is associated with depressed cell-mediated immunity and that this impairment can persist for years (35, 37). Persistent negative effects of prenatal methionein-choline deficiency, B12 deficiency, B6 deficiency, and protein-calorie deficiency on postnatal immune function have also been described (20, 79, 87, 99). Administration of the drug 6-mercaptopurine to pregnant mice also results in offspring with impaired immunocompetence (86). This drug has been shown to induce fetal zinc deficiency (3).

Although persistent immunological dysfunction that is due to prenatal Zn deficiency has not been demonstrated in humans, work with rhesus monkeys has shown that the young primate is very sensitive to the modulation of Zn in its diets. In a series of studies by Gershwin and co-workers (52, 57, 64), the influence of marginal Zn deprivation on the development of the immune system in primates was investigated by feeding rhesus monkeys either control (100 μg Zn/g) or marginal (4 μg Zn/g) Zn diets during pregnancy and lactation. The level of Zn used in the marginal diet does not produce overt signs of Zn deficiency in either the mother or infant. The Zn-deprived monkeys (both mothers and infants) exhibited significant reductions in im-

mune responsiveness that included depression in serum immunoglobulin levels, reduced proliferative responsiveness of peripheral blood lymphocytes to mitogens, and depressed neutrophil function, as assessed by chemotaxis to endotoxin-activated plasma and phagocytosis of *Candida albicans*.

Zn Deficiency and Autoimmune Disease

During the last decade scientists have learned that the restriction of several dietary components including select amino acids, calories, phosphorus, protein, and Zn can attenuate specific autoimmune diseases. Alternatively, dietary supplements of select nutrients, including iodine and W3 fatty acids, influence the course of autoimmune disease (62). Of critical importance is the observation that whereas in most cases the reported effect of dietary-induced modulations of autoimmune disease has been of benefit to the animal, reports also document negative effects of some dietary manipulations (62). The potential for negative effects secondary to some dietary manipulations must be kept in mind when considering extrapolation of the experimental animal results to possible diet therapies for humans.

A significant portion of research on Zn and autoimmune disease has been conducted using the New Zealand Black (NZB) mouse, which develops a form of lupus. The NZB strain spontaneously develops antinuclear antibodies associated with a progressive and lethal nephritis characterized by glomerular basement membrane thickening and cellular proliferation. Signs of the disease typically develop by 3–4 months of age, and most animals die between 8 and 15 months of age as a consequence of renal disease (49). Beach et al (9) reported that when young (6 week) NZB mice were fed low Zn diets (2.5 and 5 μg Zn/g), they experienced a significant delay in the onset of autoimmune hemolytic anemia, as reflected by higher hemoglobin levels, higher packed cell volumes, lower levels of serum immunoglobulins, and lower titers of antierythrocyte autoantibodies compared to levels in NZB mice fed control (100 μg Zn/g) or marginal (9 μg Zn/g) Zn diets. A reduced rate of weight gain and a prolonged life span was observed in mice fed the low Zn diets; over 80% of mice fed the low Zn diets lived beyond 10 months of age compared to only 30% of the mice in the control Zn diet groups. Young NZB mice that were fed the control Zn diets, at a caloric intake equivalent to that consumed by mice in the 5 μg Zn/g diet group, had a life span that was intermediate between the group fed an ad libitum Zn-adequate diet and the Zn-deficient group. Thus, the enhanced survival observed with the low Zn diets can only be partially attributed to a Zn deficiency–induced reduction in caloric intake.

It is important to note that mice fed the low Zn diets developed several signs of severe Zn deficiency including alopecia, dermatitis, corneal opacities, and edema of the feet and around the eyes and mouth. None of these

signs were noted in the mice fed the 9 or 100 μg Zn/g diets. Thus, it is possible that other factors secondary to the Zn deficiency syndrome (i.e. independent of Zn-dependent processes per se) and the accompanying caloric deprivation may be contributing to the salutory effect of Zn deficiency on autoimmune disease. Beach et al (9) also examined the effect of induced Zn deficiency in 6-month-old NZB mice. By this age, evidence of the disease is already present (i.e. presence of autoantibodies). Consistent with the effects of introducing the low Zn diets at an early age, induction of Zn deficiency in 6-month-old NZB mice retarded the further development of the autoimmune syndrome, although reversal of the disease did not occur (9). The induction of Zn deficiency in New Zealand Black/White (NZB/W) and MRL/1 mice also produced significant retardation in the development of autoimmune disease compared to development of the disease in Zn-adequate NZB/W mice (10, 13, 96).

Clearly, one mechanism by which the Zn-deficient diet exerts its effect on autoimmune disease is through the induction of anorexia, and the resultant caloric restriction. In addition to this effect, several immunological processes may be more directly affected by a Zn deficiency condition. For example, since Zn is required for normal T-lymphocyte activity, it may modify T-cell function in autoimmune diseases. Similarly, immunological abnormalities in murine lupus may involve hyperresponsive B-cell, which may be altered by Zn deficiency caused by inhibited autoantibody secretion, polyclonal B-cell activation, and/or B-stem cell development. Given that dietary Zn restriction does not affect disease progression in "old" MRL/1 and NZB mice, the salutory effect of Zn deficiency on the described autoimmune diseases may, in part, be a direct consequence of a lack of the metal at a critical step in one of the immune processes—most likely at an early stage of gene activation or expression rather than at the level of antibody-producing cells.

While there is little doubt that the correction of a Zn deficiency syndrome will benefit the average individual, the question arises whether or not the use of high levels of Zn supplements should be contraindicated in patients considered to be at risk for autoimmune disease. For example, patients with Down's syndrome exhibit an increased incidence of autoimmune disorders and low plasma Zn concentrations (69), and "improvements" in the immunological status of Down's syndrome patients have been reported following Zn supplementation (46). Similarly, type 1 diabetics are reportedly at risk for the development of autoimmune thyroid disease (80) and Zn deficiency (76); in human diabetics and in experimental diabetic animal models, diabetes-associated immune dysfunction has been shown to be responsive to Zn supplementation (75, 76). Consideration of the long-term consequences of Zn therapy for these patients should include the possibility that patients may develop autoimmune disease.

Zn DEFICIENCY IN HUMANS

Humans with low serum Zn levels tend to have an increased susceptibility to a variety of infectious diseases (49, 55). Some of the best evidence that Zn deficiency can result in impaired immune function has come from studies of patients with acrodermatitis enteropathica, an autosomally recessive disorder of Zn metabolism. Patients with this disorder exhibit marginal to severe Zn deficiency syndromes. These individuals are characterized by a high incidence of infections and by an immunological disorder that includes thymic atrophy and impaired cell-mediated immunity. Significantly, all of the above immune defects are corrected upon Zn supplementation (6, 77, 94).

A second group of subjects that have been reported to be characterized in part by Zn deficiency and by associated immunological abnormalities are children with severe protein calorie malnutrition (PCM). In a classic study (51), it was demonstrated that the thymic atrophy characteristic of children with PCM could be reversed with Zn supplementation. Similar improvements in impaired delayed hypersensitivity response in children with PCM have also been reported (50). Zn supplementation has also been proven to be of value in correcting some of the immunological defects characteristic of marasmic children (18). Immunological defects associated with Zn deficiency have also been described in patients receiving total parenteral nutrition (TPN) without adequate Zn supplementation. Allen et al (2) described the case of an adult male receiving long-term TPN who developed a condition of marginal Zn deficiency. Immunological defects noted for this subject included lymphopenia, depressed T-cell mitogenic response, increased numbers of circulating suppressor T-cells [OKT_8; decreased levels of helper T cell (OKT_4)], and decreased NK activity. All of the above abnormalities were corrected following Zn repletion.

Recently there has been considerable interest in the idea that some of the loss of immunological responsivity that is associated with aging may be corrected by Zn supplementation. While beneficial effects of Zn supplementation on cellular immunity in the elderly have been observed by some investigators (34, 98), others have not detected an effect of Zn supplementation on immune function in this population group (16). Kaplan et al (61) have suggested that lymphocyte IL-2 production is reduced in the "Zn-deficient" aged; Cossack (25) has reported that the activity of erythrocyte nucleoside phosphorylase is reduced in this subject group. In theory, alterations in either IL-2 production or cell nucleoside phosphorylase activity could affect T- and B-cell function. The frequency of Zn-responsive immune defects in the elderly clearly needs further clarification.

In addition to the above findings, a number of other diseases are reportedly characterized by secondary Zn deficiencies and attendant immunological

disorders in humans. This list of diseases includes diabetes, AIDS, Down's syndrome, alcoholism, uremia, and select cancers (4, 19, 36, 48, 68, 73, 84, 89). Given the diversity of the above list, it is imperative that we define the effects of Zn deficiency on the immune system as well as the frequency with which a deficit of this element results in immunological disorders. In this regard, several investigators have reported that one of the early effects of Zn deficiency in humans is a reduction in the concentration of active thymulin in the serum. Thymulin, which reportedly requires Zn to express its biological activity (82), is a thymic hormone with the amino acid sequence <Glu-Ala-Lys-Ser-Glyn-Gly-Gly-Ser-Asn-OH. Low concentrations of "active thymulin" have been reported in mildly Zn-deficient sickle cell patients, acrodermatitis enteropathica patients, nephrotic syndrome patients, type I diabetics, severely malnourished children, and "healthy" adult subjects given low Zn diets (29, 44, 49, 56, 60, 82, 97). Thymulin reportedly binds to high-affinity receptors, induces several T-cell markers, and promotes T-cell function including allogenic cytotoxicity, suppressor function, and IL-2 production (82).

SUMMARY

Zn deficiency can have marked effects on virtually all components of the immune system. That these effects can be functionally significant is demonstrated by the increased susceptibility of Zn-deficient animals to a number of bacterial, viral, and parasitic challenges. In addition, strong epidemiological data support the belief that Zn deficiency is a major factor underlying immune dysfunction in select human populations. Despite recognition of the importance of Zn in the ontogeny and functioning of the immune system, the biochemical lesions underlying the effects of Zn deficiency on immune responsivity have not been well characterized. Future efforts to delineate the effects of Zn on the production, release, and action of cytokines will likely produce significant advances in our understanding of the influence of this element on the immune system. The recent observation that Zn may be critical for the activity and binding of protein kinase C in lymphocyte membranes (27, 28) suggests that another fruitful area of research will involve examination of the influence of Zn deficiency on lymphocyte membrane structure and function. Finally, the recent recognition that Zn may be a critical factor in the activation/inactivation of immunoregulatory genes (47) provides us with yet another avenue of research.

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