Zinc and the immune system

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Zn is an essential trace element for all organisms. In human subjects body growth and development is strictly dependent on Zn. The nervous, reproductive and immune systems are particularly influenced by Zn deficiency, as well as by increased levels of Zn. The relationship between Zn and the immune system is complex, since there are four different types of influence associated with Zn. (1) The dietary intake and the resorption of Zn depends on the composition of the diet and also on age and disease status. (2) Zn is a cofactor in more than 300 enzymes influencing various organ functions having a secondary effect on the immune system. (3) Direct effects of Zn on the production, maturation and function of leucocytes. (4) Zn influences the function of immunostimulants used in the experimental systems. Here we summarize all four types of influence on the immune function. Nutritional aspects of Zn, the physiology of Zn, the influence of Zn on enzymes and cellular functions, direct effects of Zn on leucocytes at the cellular and molecular level, Zn-altered function of immunostimulants and the therapeutic use of Zn will be discussed in detail.

Zinc: Immune system

Raulin (1869) showed that Zn is essential for the growth of *Aspergillus niger*. Todd *et al*. (1934) were the first to show that Zn is necessary for the growth and development of rats. Approximately 100 years after the initial observation by Raulin, Prasad *et al*. (1963) described a Zn-deficiency syndrome in children from Persia practising geophagia. The syndrome was characterized by anaemia, hypogonadism, hepatosplenomegalie, skin alterations and growth and mental retardation. The discovery of acrodermatitis enteropathica (a rare autosomal recessive inheritable disease) as a Zn-specific malabsorption syndrome (Neldner & Hambidge, 1975) indicated clearly that these symptoms are related to Zn deficiency. This disease is also accompanied by thymic atrophy resulting in an immune defect and a high frequency of bacterial, viral and fungal infections. Without treatment this disease leads to death within a few years, but pharmacological Zn supplementation can reverse all symptoms (Neldner & Hambidge, 1975).

Nutritional aspects and physiology of zinc

The total body Zn content of human subjects is 2–4 g. However, Zn is referred to as a trace element, as its plasma concentration is only 12–16 μM. In the serum, Zn is predominantly bound to albumin (60 %, low-affinity), α_2-macroglobulin (30 %, high-affinity) and transferrin (10 %; Scott & Bradwell, 1983). The plasma Zn pool is a minor pool, but highly mobile and immunologically important. There is no specialized Zn storage system in the body, and therefore there must be a daily intake of Zn to achieve a steady-state. The distribution of Zn in the body is summarized in Table 1 (Mills, 1989; Favier & Favier, 1990).

The bioavailability of Zn depends on the composition of the diet. Zn is chelated by phytate and phosphate, resulting

Table 1. Zinc content of human organs

	Zn content	
Organ	μg/g organ dry wt	% whole-body Zn
Muscle	51	57·0
Bone	100	29·0
Skin	32	6·0
Liver	58	5·0
Brain	11	1·5
Kidneys	55	0·7
Heart	23	0·4
Hair	150	0·1
Plasma	1	0·1

Abbreviations: IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cells; PHA, phytohaemagglutinin; PKC, protein kinase C.

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in a poor resorption from vegetarian food in comparison with meat. Furthermore, the resorption is reduced by increased levels of other bivalent cations, such as Cu, Mg, Ca, Ni, Cd and Fe (Valberg *et al*. 1984; Favier & Favier, 1990). Fe is of special interest, as most pregnant women take Fe supplements, and some studies have shown a correlation between abortion or preterm delivery and Zn deficiency (Mills, 1989; Favier & Favier, 1990; Scholl *et al*. 1993). Thus, interaction between supplements must be taken into account when a single supplement is taken. The Zn content of food also shows a wide variation (Table 2; Favier & Favier, 1990). A balanced diet is therefore important for an adaquate Zn uptake. Variations in the bioavailability of Zn and Zn content make the Zn intake from food difficult to calculate. Health status and age are also important factors in the daily Zn intake. During the growth period, pregnancy and nursing there is an increased requirement for Zn, since Zn is essential for every proliferating cell, and the daily loss by excretion is approximately the same as the daily intake (Table 3; Ziegler *et al*. 1989; German Society of Nutrition, 1995). Zn absorption depends on the concentration in the diet and is about 20–40 % of the daily intake (Mills, 1989). However, there are a number of contradictory recommendations relating to the daily intake of Zn.

The daily intake must be adjusted according to health status, since Zn steady-state is regulated not only by uptake, but also by fluctuations in Zn excretion associated with a number of diseases and with inflammation (Klaiman *et al*. 1981; Yuzbasiyan-Gurkan *et al*. 1989; Weiss *et al*. 1998). Interestingly, healthy elderly subjects have decreased serum Zn levels, which may be due to decreased resorption or increased excretion (Cakman *et al*. 1996). Acute infections lead to a redistribution of Zn to the liver, decreasing the immunologically-important serum pool (Weiss *et al*. 1995).

All these factors may result in an immune deficiency, but there are no specific immunological defects, only inappropriate or inadequate nutrition. However, long-term Zn deficiency due to inappropriate nutrition may result in immunological or autoimmune diseases.

Table 2. Zinc content of some foods (mg/100g)

Meat and animal products		Vegetables and plant products	
Beef fillet	3·6	Wheat (white) flour	0·9
Liver	4–6	Wholemeal flour	3·0
Roast beef	2·5	Sugar	0·1
Pork fillet	3·6	Potato	0·2–0·3
Pork cutlet	1·3	Carrot	0·64
Pork shoulder	3·5	Radish	0·16
Calf fillet	4·3	Cauliflower	0·23
Poultry	2–3	Salad	0·22
Fish	1–2	Red cabbage	0·22
Oysters	20–150	Sauerkraut	0·32
Eggs	0·3–0·5	Fruit	0·1–0·3
Milk	0·2–0·4	Vegetable oil	0·1–0·2
Cheese	1–5	Sweetcorn	1·2
Butter	0·15	Coconut	0·5
		Rice	1·3

Table 3. Recommended daily intake of zinc (mg/d; German Society of Nutrition, 1995)

Infants and children:	0–< 12 months	5
	1–< 4 years	7
	4–< 7 years	10
	7–< 10 years	11
	10–< 13 years	12
Adolescents and adults:	Male	15
	Female	12
	Pregnant	15
	Nursing women	22

Cell biology of zinc

Exogenous Zn enters the cell within minutes (Wellinghausen *et al*. 1996*b*). However, it is not known how Zn enters the cell. Some specific Zn transporters have been reported in the nervous system, but these transporters inhibit the efflux of the intracellular Zn pool and some have been reported to be involved in intracellular redistribution, but there is no evidence that these transporters are involved in Zn uptake (Palmiter & Findley, 1995; Palmiter *et al*. 1996*a*,*b*; Tsuda *et al*. 1997). The transferrin receptor (CD71) has been reported to promote Zn influx, as for Fe, but this finding has not been confirmed by other workers (Wellinghausen *et al*. 1996*b*). Thus, there are no irrefutable data to indicate that CD71 is a Zn receptor. Different possible mechanisms for Zn uptake such as non-specific Ca channels, facilitated diffusion via amino acids and anionic exchange have been reported (Bentley, 1992; Hogstrand *et al*. 1996). In peripheral blood mononuclear cells (PBMC) exogenously-added Zn increases the free Zn content by about 70 % (Wellinghausen *et al*. 1996*b*), whereas the total Zn uptake is 300–600 %, indicating rapid binding of Zn to intracellular proteins (A Fischer, P Gabriel and L Rink, unpublished results).

Zn is a cofactor for more than 300 enzymes (Coleman, 1992*a*,*b*; Vallee & Falchuk, 1993). In some enzymes Zn is important for structural integrity, whereas in other enzymes it is the central ion for enzymic activity, but sometimes both these functions are involved, as in alcohol dehydrogenase. As a third possibility, Zn modulates the activity of a number of enzymes. Members of all six classes of enzymes use Zn as a cofactor (Table 4). A variety of general cell functions are therefore influenced by the Zn concentration. Factors interacting with DNA and RNA (e.g. transcription and replication factors), particularly, are Zn-dependent, because their structure shows a Zn-finger motif (Table 5). As a result cell proliferation does not occur in the absence of Zn,

Table 1. Enzymes with zinc as a co-factor (examples in each enzyme class)

Enzyme	No. of Zn ions and their function	Enzyme class
Alcohol dehydrogenase	One for stability and one for enzyme activity	Oxidoreductase
RNA polymerase	Two for catalytic activity	Transferase
Alkaline phosphatase	Two for catalytic activity and co-activity	Hydrolase
Carbonic anhydrase	One for catalytic activity	Lyase
Aldolase II (fungi)	One for catalytic activity	Isomerase
t-RNA synthetase	One or two for catalytic activity	Ligase

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Table 5. Some proteins containing zinc-finger motifs

Molecule containing Zn-fingers	Function	Reference
Oestradiol receptor	Hormone receptor	Humeny *et al.* (1999)
Glucocorticoid receptor	Hormone receptor	Schoenmakers *et al.* (1999)
AIRE-protein	Gene product, probably responsible for autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy	The Finnish-German APECED Consortium (1997)
MTB-Zf	MTB-Zf expression leads to the haem-oxygenase-1 gene product	Muraosa *et al.* (1996)
Myelin transcription factor 1	DNA-binding protein, important for oligodendrocyte development	Armstrong *et al.* (1995)
ZNF7	Lack of this protein expression in Burkitt lymphomas	Feduchi *et al.* (1994)
Z-225 (Egr-1)	Upregulation in RA synoviocytes	Aicher *et al.* (1994), Aicher *et al.* (1999)
WT-1	Expression in myeloid leucaemia and Wilms tumour	Deuel *et al.* (1999)
TFIIIA	Transcription factor	Pavletich & Pabo (1993)
TIEG	Transcription factor	Chalaux *et al.* (1999)
EGR3/Pilot	Transcription factor	Yamagata *et al.* (1994)
RFLAT-1	Transcription factor	Song *et al.* (1999)
mSNA	Transcription factor	Nakayama *et al.* (1998)
YY1	Transcription factor	De-Rinaldis *et al.* (1998)
REST	Transcription factor	Andres *et al.* (1999)
AREB6	Transcription factor	Turner & Crossley (1998)
Gli-1	Transcription factor	Hynes *et al.* (1997)
GATA-6	Transcription factor	Sakai *et al.* (1998)
WZF-1	Transcription factor	Skamoto *et al.* (1996)
RU 49	Transcription factor	Yang *et al.* (1996)
SP 1	Transcription factor	Philipsen & Suske (1999)
BCL 6-protein	Transcription repressor	Chang *et al.* (1996)
ZNF174	Transcription repressor	Williams *et al.* (1995)

RA, rheumatoid arthritis.

and highly-proliferating cell systems, such as the immune system, the skin and the reproductive system, are the most sensitive indicators of Zn deficiency. Furthermore, different factors important for signal transduction need Zn to maintain their normal function; thus, Zn can alter their function, but this change does not necessarily involve a loss of function (Maret *et al*. 1999). Enzymes inhibited by Zn include: caspase-3, fructose-1,6-bisphosphatase, glyceraldehyde-3-phosphase dehydrogenase, aldehyde dehydrogenase, tyrosine phosphatase, enolase. Also, the translocation of protein kinase C (PKC) to the cell membrane is Zn dependent.

More recently, Taylor & Blackshear (1995) reported that Zn influences the stability of mRNA by inhibiting its turnover. The resultant accumulation of mRNA is assumed to regulate gene expression, and might therefore also play a role in Zn-induced effects.

Apoptosis represents a physiological method of cell death. Without apoptosis development is not maintained, the exclusion of autoimmune T-cells and B-cells is lost and the killing activity of cytotoxic T-cells and NK cells decreases. Apoptosis is regulated by Zn and Zn chelation in the culture medium causes apoptosis (Jiang *et al*. 1995; Umezawa *et al*. 1999). On the other hand, the addition of Zn can protect cells against undergoing apoptosis. This protective action has been reported in relation to almost all apoptosis-inducing factors, including tumour necrosis factor-α, cytotoxic T-cells, dexamethasone, withdrawal of interleukin (IL) 2 (in IL-2-dependent T-cell lines), sporidesmin, cold shock, etoposide, hyperthermia, adenosine, and u.v. and γ-irradiation (Zalewski & Forbes, 1993). Interestingly, Zn can inhibit apoptosis even if added a short time after the apoptotic agent. Possible mechanisms for this anti-apoptotic effect are the inhibition of caspase-3, a direct interaction with bcl-2, or an induction of DNA synthesis by Zn (Fukamachi *et al*. 1998; Ishido *et al*. 1999; Maret *et al*. 1999). Other anti-apoptotic mechanisms suggested for Zn, are Ca antagonism, inhibition of the Ca^{2+}/Mg^{2+}-endonuclease and an interaction between Zn-finger proteins and the microtubuli system (Zalewski & Forbes, 1993). It is possible that Zn has various independent anti-apoptotic effects and that cell activation alone can prevent apoptosis. During the early stages of apoptosis there is a major redistribution of intracellular Zn. The increase in free Zn in the cytosol is a result of Zn release from intracellular pools and metalloenzymes (Maret, 1998).

All these effects of Zn are very important for the highly-proliferative and physiologically-active leucocytes, but they are not restricted to the cells of the immune system. However, a slightly decreased Zn status might be associated initially with immunological effects, in the form of an increased number of infections.

Specific interactions of zinc within the immune system

It has been established that Zn is an essential trace element for the immune system. However, the cellular and molecular mechanisms for the role of Zn within the immune system have been elucidated only during the last 10 years.

The innate as well as the specific parts of the immune system are influenced by Zn. The effects of Zn are multifaceted. Zn can induce adhesion of myelomonocytic cells to the endothelium, while Zn chelation diminishes cell recruitment (Chavakis *et al*. 1999). Thus, Zn is essential even in the earliest stages of an immune response. *In vivo*, Zn deficiency not only influences the recruitment of neutrophils but also decreases the chemotaxis of neutrophils. Under these conditions there is also impaired natural

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killer cell activity, phagocytosis of macrophages and neutrophils, and generation of the oxidative burst (Allen *et al*. 1983; Keen & Gershwin, 1990). At the molecular level, Zn is required for the interaction between the p58 killer cell inhibitory receptor on NK cells and major histocompatibility complex (MHC) class I molecules, mainly human leucocyte antigen C, on target cells (Rajagopalan *et al*. 1995), resulting in the inhibition of the killing activity. Interestingly, only the inhibitory signal is Zn-dependent, whereas the human leucocyte antigen C interaction and positive signals do not require Zn (Rajagopalan *et al*. 1995). Thus, Zn is needed to maintain the normal function of natural killer cells, and Zn deficiency may result in non-specific killing activity and functional loss. It is not only the proliferation of the immune system which depends on Zn; the proliferation of the pathogens is also Zn-dependent. Thus, decreasing Zn in the plasma is an acute-phase response to infection. Furthermore, the S-100 Ca^{2+}-binding protein calprotectin which is released by degradation of neutrophils inhibits reproduction of bacteria and *Candida albicans* by Zn chelation (Sohnle *et al*. 1991; Murthy *et al*. 1993; Clohessy & Golden, 1995).

Despite the fact that innate immunity is the first stage in the response of the immune system, initial observations relating to the influence of Zn were associated with the development of T-cells due to thymic atrophy (Fraker *et al*. 1995; Osati-Ashtiani *et al*. 1998). Zn is an essential cofactor for the thymic hormone thymulin (a nonapeptide) (Bach, 1981, 1983). Thymulin is secreted by thymic epithelial cells and induces markers of differentiation in immature T-cells (Saha *et al*. 1995). However, thymulin also modulates cytokine release by PBMC and induces proliferation of CD8 T-cells in combination with IL-2 (Coto *et al*. 1992; Safie-Garabedian *et al*. 1993). Zn supplementation can reverse the changes induced by Zn deficiency in the thymus and on peripheral cells (Mocchegiani *et al*. 1995*a*). The addition of Zn alone can induce the expression of the high-affinity receptor for IL-2 (Tanaka *et al*. 1989); a factor which can result in decreased proliferation of T-cells in Zn deficiency (Dowd *et al*. 1986; Crea *et al*. 1990).

Zn ions have been reported to induce blast transformation in human lymphocytes (Kirchner & Rühl, 1970; Rühl *et al*. 1971; Berger & Skinner, 1974; Sood *et al*. 1999). Zn addition induced the release of IL-1, IL-6, tumour necrosis factor α, soluble IL-2 receptor and interferon (IFN)-γ in human PBMC (Salas & Kirchner, 1987; Scuderi, 1990; Driessen *et al*. 1994). IL-1, IL-6, and tumour necrosis factor α are induced in monocytes in the absence of lymphocytes, whereas the induction of IFN-γ is dependent on the presence of monocytes (Rühl & Kirchner, 1978; Salas & Kirchner, 1987; Driessen *et al*. 1994; Wellinghausen *et al*. 1997*b*; Fig. 1). Tumour necrosis factor α release after Zn stimulation of PBMC is a result of *de novo* mRNA transcription (Wellinghausen *et al*. 1996*a*). However, how Zn activates an intracellular signal and which signal transduction pathways are involved are still unknown. Further investigations showed that only isolated monocytes respond to Zn stimulation, whereas isolated T-cells (Hadden, 1995; Wellinghausen *et al*. 1997*b*), B-cells (Crea *et al*. 1990), natural killer cells (Crea *et al*. 1990) or neutrophils (A Fischer, P Gabriel and L Rink, unpublished results; Fig. 1) do not produce cytokines after induction with Zn. The stimulation of monocytes and T-cells by Zn is dependent on the level of free Zn ions relative to the protein composition of the culture medium. Insulin and transferrin specifically enhance Zn-induced monocyte activation by a non-receptor-dependent mechanism (Phillips & Azari, 1974; Crea *et al*. 1990; Driessen *et al*. 1995*c*; Wellinghausen *et al*. 1996*b*), but high levels of serum proteins in the culture medium prevent monocyte stimulation. Zn concentrations > 100 μM in a serum-free culture medium stimulate monocytes, but actually inhibit T-cell functions, since T-cells have a lower intracellular Zn concentration and are more susceptible to increasing Zn levels than monocytes (Bulgarini *et al*. 1989; Goode *et al*. 1989; Wellinghausen *et al*. 1997*b*; Fig. 1). The increase in intracellular free Zn in monocytes and T-cells is the same (Wellinghausen *et al*. 1996*b*, 1997*b*). The molecular background for this effect is the inhibition of the IL-1 type I receptor-associated kinase by Zn, since T-cell stimulation by Zn is an indirect effect of IL-1 secreted by monocytes (Driessen *et al*. 1994; Wellinghausen *et al*. 1997*b*). Furthermore, Zn can alter the structure of ceramides (U Seydel, unpublished results) which are involved in the signal transduction of tumour necrosis factor and IL-1 (Wright & Kolesnick, 1995). This effect is comparable with the conformational alteration of lipopolysaccharide (LPS), described later (Table 5; Wellinghausen *et al*. 1996*c*). In contrast to the amplifying effect on LPS, the function of ceramides as second messengers may be reduced and could be responsible also for the inhibition of tumour necrosis factor-induced apoptosis (Flieger *et al*. 1990).

As *in vitro*, an excess of Zn *in vivo* inhibits T-cell function (Duchateau *et al*. 1981; Chandra, 1984). *In vitro* levels > 50 μM inhibit alloreactivity in the mixed lymphocyte reaction (aCampo *et al*. 2000). On the other hand, low serum Zn levels also alter normal T-cell functions. Some autoimmune diseases with a T-cell pathology, e.g. rheumatoid arthritis, are associated with moderate Zn deficiency (Simkin, 1976), and in some cases Zn supplementation was advantageous. Decreased plasma Zn levels in preganancy are associated with an increased

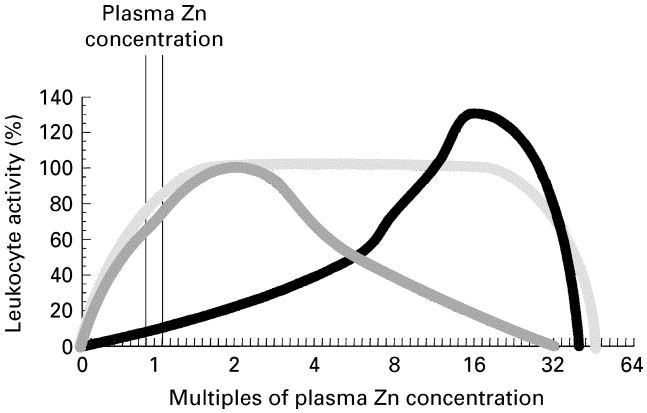

Fig. 1. Leucocyte activity and its dependence on plasma zinc concentrations. (▨), lymphocytes; (▬), monocytes; (▨), granulocytes. Note that zinc activates monocytes to a greater extent than either granulocytes or lymphocytes.

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risk of preterm delivery and abortion (Favier, 1992; Jameson, 1993; Bedwal & Bahuguna, 1994). This factor might be an effect of normally-suppressed alloreactive T-cells or non-specific natural killer cell activity. The physiological Zn concentrations, based on *in vitro* experiments, seem to be just below the optimal concentration for T-cell function (Fig. 1).

In contrast to the mechanisms involved in the inhibition of leucocytes, the direct activation of monocytes by Zn is still not understood. Tyrosine kinases as well as cAMP- and cGMP-dependent protein kinases are clearly involved (Wellinghausen *et al*. 1996*b*). Intracellular cAMP and calmodulin are therefore influenced in a different manner (Brewer *et al*. 1979; Heng *et al*. 1993). Inhibition of intra-erythrocytic calmodulin-mediated effects is possibly responsible for the benefits of Zn supplementation in sickle cell disease (Brewer & Bereza, 1982). In phospholipase C, Zn is integrated, in the active centre, but no stimulatory effects of Zn have been reported (Coleman, 1992*a*). Zn is necessary for the translocation of the PKC to the cell membrane, but an involvement of PKC in Zn-induced signal transduction in PBMC has not been confirmed (Csermely *et al*. 1988; Wellinghausen *et al*. 1996*b*).

Zn might simply bind to specific membrane receptors, triggering a signal transduction cascade. On the other hand, however, Zn could also exert its effects directly inside the cell. Perhaps more important than a specific interaction between Zn and certain molecules is a general influence of Zn on the fluidity of lipids, and thus also of biological membranes (Chvapil, 1976; Kruse-Jarres, 1989; Bettger & O'Dell, 1993). Membrane stabilization could have considerable consequences for the cell; for example, an activation or inhibition of ion channels and altered assembling of cell surface receptors, initiating or inhibiting signal transduction into the cell (Heldin, 1995).

In addition to its effects on upstream signalling molecules, Zn influences gene expression by structural stabilization and functional regulation of various immunologically-relevant transcription factors (Chesters, 1992; Coleman, 1992*a*; O'Halloran, 1993; Vallee & Falchuk, 1993) as summarized earlier. However, the relationship between activation of specific transcription factors and Zn-induced effects in PBMC still remains to be clarified.

Interaction of zinc with immunostimulants

The capacity of the immune system is measured using different outcome systems (e.g. proliferation, cytokine production) after stimulation of leucocytes *in vivo* or *in vitro*. Frequently used stimulants include lectins (e.g. phytohaemagglutinin; PHA), phorbol esters (e.g. phorbol 12-myristate 13-acetate), LPS, super-antigens (e.g. staphylococcal enterotoxins) or specific antigens inducing a recall response in most individuals (e.g. tetanus toxin or diphtheria toxin). Thus, to measure Zn-specific alterations of immune function it must be established that the immunostimulant itself is not affected by Zn. Indeed, the activity of many stimulants is influenced by Zn. Interestingly, one of the early reports regarding the immunobiology of Zn described the co-mitogenic effect of PHA (Duchateau *et al*. 1981; Fraker *et al*. 1986; Warner & Lawrence, 1986). However, PHA is frequently used *in vitro*, but does not appear in human blood *in vivo*. Thus, it is of greater relevance that Zn influences the immunostimulative effects of LPS from Gram-negative bacteria and some bacterial super-antigens (mainly produced by Gram-positive bacteria), as well as tetanus toxin and diphtheria toxin. Substimulatory concentrations of Zn enhanced the biological activity of LPS with respect to cytokine induction in leucocytes (Driessen *et al*. 1995*a*,*b*). This synergism depends on a Zn-induced structural alteration of LPS in its biologically-more-active less-fluid form (Wellinghausen *et al*. 1996*c*). LPS is an important pathogenic factor in sepsis, and Zn supplementation in patients with systemic Gram-negative infections or in intensive-care-unit patients may have devastating consequences if the activity of LPS is also enhanced *in vivo*. Recently, it was shown that parenteral Zn supplementation (30 mg/d) in septic patients exaggerated the acute-phase response, as indicated by a higher febrile response (Braunschweig *et al*. 1997). However, Klosterhalfen *et al*. (1996) reported that in a porcine sepsis model the induction of stress proteins by prophylactic administration of Zn can reduce the inflammatory response after LPS treatment. We recently found a time-dependent response in a mouse model (P Gabriel, J Schürman, G Tiegs and L Rink, unpublished results). If Zn is injected some minutes before LPS is administered it has a protective effect, whereas application simultaneously with or after LPS treatment resulted in a synergistic effect, as described earlier (Wellinghausen *et al*. 1996*c*). This finding may be explained by the influence of Zn on the IL-1 type 1 receptor-associated kinase as described earlier (p. 544), since the LPS signal transduction pathway via the toll-like receptors (members of the IL-1 receptor family) are also dependent on this kinase. Thus, Zn might be useful in the prophylactic treatment of patients at high-risk for sepsis, but unsuitable for already-septic patients.

The activity of some bacterial super-antigens is also influenced by Zn (Driessen *et al*. 1995*a*,*b*). There are different groups of super-antigens using binding sites on the MHC-II α-chain, β-chain, or both sites. All super-antigens binding to the MHC-II β-chain (e.g. the *Staphylococcus aureus* enterotoxins A, D and E and the *Mycoplasma arthritidis* super-antigen) do so by forming a Zn cluster involving histidine-81 of the MHC-II β-chain and three amino acids from the super-antigen (Fraser *et al*. 1992; Kim *et al*. 1994; Sundström *et al*. 1996; Bernatchez *et al*. 1997). Thus, binding to the MHC-II β-chain is Zn-dependent. The interaction between these super-antigens and the MHC-II β-chain can be diminished by the chelation of Zn (Bernatchez *et al*. 1997). On the other hand, cytokine induction by those super-antigens can also be inhibited by the addition of high Zn concentrations (Driessen *et al*. 1995*b*), whereas super-antigens binding to the Zn-independent MHC-II α-binding site (e.g. toxic shock syndrome toxin-1) are not influenced by Zn in any way. Without Zn the super-antigens cannot build up a Zn cluster with histidine-81 of the MHC-II β-chain, and it is likely that high Zn concentrations saturate both sites, thus preventing complex formation. However, Zn also mediates a super-antigen homodimerization, as recently described for *S. aureus* enterotoxins (Sundström *et al*. 1996). This homodimerization facilitates a T-cell-

independent interaction between the super-antigen and MHC-II molecules, resulting in direct activation of monocytes. The inhibitory effect of Zn on super-antigens could be an interesting therapeutic perspective for the treatment of food poisoning, Gram-positive sepsis or staphylococcal infections in which super-antigens play a causative role. Furthermore, the production of some super-antigens (e.g. toxic shock syndrome toxin-1) seems to be regulated by Zn repressor elements, since Zn chelation increases the super-antigen production (Tierno & Hanna, 1985; Balaban & Novick, 1995; Balaban *et al*. 1998). Interestingly, diphtheria-toxin production is also regulated by a Zn repressor (Groman & Judge, 1979; Pohl *et al*. 1997).

Phorbol 12-myristate 13-acetate, tetanus toxin and diphtheria toxin may also be influenced by Zn, but there are no conclusive data available. Phorbol 12-myristate 13-acetate directly activates PKC, and PKC is influenced by Zn as described earlier (p. 545). Tetanus toxin has a Zn-binding sequence in a region frequently used as a B- and T-cell epitope (Villiers *et al*. 1993). Zn saturation of this binding site leads to decreased recognition by antibodies and T-cells. Thus, Zn saturation of tetanus toxin may influence the outcome after tetanus toxin stimulation.

However, it is not only immunostimulants that are affected by Zn, since cytokine functions and detection are modulated by Zn-activated α_2-macroglobulin, which may also alter the outcome (James, 1990). In conclusion, it is difficult to find an immunostimulant and test system completely independent of Zn in its function *in vivo* or *in vitro*.

Zinc therapy and *in vitro* zinc supplementation

Zn has diverse effects on immune functions. During Zn deficiency different immune functions are decreased (Table 6; Wellinghausen *et al*. 1997*a*). All these impaired functions are completely restored by Zn supplementation. Since the defect in some immunological functions is related to a lack of the relevant leucocyte subset, only some of the functions were restored *in vitro* (Cakman *et al*. 1996, 1997).

Zn administration is the standard therapy for acrodermatitis enteropathica, as well as in non-specific malabsorption syndromes (Neldner & Hambidge, 1975; Cunningham-Rundles *et al*. 1980). The Zn supplement reversed immunological defects as well as other Zn-deficiency-associated syndromes. Furthermore, Zn supplementation is frequently used to compete with Cu for absorption in Wilson's diseases and to increase metallothionein.

Table 6. Immune functions which are decreased in zinc deficiency

Periperhal T-cell count
T-cell proliferation in response to PHA
Thymocyte count in thymus
Delayed-type hypersensitivity reaction
T-helper cell function
Cytotoxic T-cell activity
NK cell activity
Macrophage functions (phagocytosis, intracellular killing activity)
Neutrophil functions (chemotaxtis, oxidative burst)
Serum thymulin level

PHA, phytohaemagglutinin; NK, natural killer.

Various diseases are accompanied by altered Zn plasma levels. For some diseases the results of preliminary trials of Zn therapy have been published. Tables 7 and 8 summarize the diseases, the effects of Zn and the possible molecular mechanism associated with the beneficial effects of Zn therapy.

An interesting application of Zn is its use as an adjuvant in vaccination. Two groups of patients with reduced plasma Zn levels (the elderly, and haemodialysis patients) are known to have an impaired immune system and a poor response to vaccination (Sandstead *et al*. 1982; Lighart *et al*. 1984; Fraker *et al*. 1986; Bonomini *et al*. 1993; Cakman *et al*. 1996). The results of a number of vaccination trials which were accompanied by Zn supplementation have been published during the last two decades (Rawer *et al*. 1987; Grekas *et al*. 1992; Brodersen *et al*. 1995; Provinciali *et al*. 1998; Turk *et al*. 1998). The findings were extremely contradictory. This situation may be due to the lack of a standard level for Zn supplementation. In some studies extremely high (400 mg/d) levels of Zn were given, which have been shown in other trials to impair immune functions (reduced delayed-type hypersensitivity reaction occurs at a dose of 100 mg/d; Porter *et al*. 1977; Chandra, 1984; Patterson *et al*. 1985; Provinciali *et al*. 1998; Reinhold *et al*. 1999; Rink & Kirchner, 1999). We recently found that the response to diphtheria vaccination in haemodialysis patients is correlated with the actual plasma Zn level (Kreft *et al*. 2000). Possible mechanisms for this relationship are an increase in IFN-α production, as shown *in vitro*, or impaired T-cell functions (Sandstead *et al*. 1982; Cakman *et al*. 1996). However, high-dose Zn supplementation (seven to eight times the physiological level) *in vitro* inhibited T-cell functions and reduced IFN-α production. Thus, in order to reverse Zn deficiency, the pharmacological Zn dose should be adjusted in line with the actual requirements, and plasma Zn level should not exceed 30 μM.

The inhibitory effects of Zn may provide a new therapeutic tool for use in immunosuppressive therapy where a selective suppression of lymphocyte functions is desirable. Possible diseases are T-cell-mediated autoimmune diseases such as rheumatoid arthritis or graft *v*. host reactions following organ transplantation. We observed that Zn specifically inhibits the mixed lymphocyte culture (as an *in vitro* transplantation model) at concentrations of three to four times the physiological level.

Conclusion

It has been known for decades that Zn is essential for an intact immune system. However, experimental data and nutritional explanations for the precise function and daily intake requirements are few or contradictory. The problem is the differentiation of the effects of Zn on general cell growth and function, the specific effects on cells of the immune system or alterations of the outcome system via an effect on the immunostimulants. Furthermore, normal immune function is delicately regulated by the Zn level, and Zn deficiency, as well as Zn levels $>50\,\mu$M, alter normal immune functions. Fig. 1 shows that the function of leucocytes is dependent on the Zn concentration. Thus, therapeutic Zn administration must be adjusted according

Table 7. Zinc therapy studies

Disease or disorder	Symptoms	Effect of Zn supplementation	Possible mechanism	Reference
Cutaneous leishmaniasis	Ulcerative lesions susceptible to secondary infections	Decrease of disease activity	Leishmania is sensitive to zinc sulfate	Najim *et al.* (1998)
Growth retardation	Deficiency in the secretory activity of the testis, hypogonadism (male)	Recovery of testical functions, normalization of overall growth	Reactivation of enzymes and transcription factors	Prasad (1995, 1996)
Cystic fibrosis	Impaired Zn absorption	Improved Zn absorption by enzyme replacement	Re-activation of enzymes and transcription factors	Easley *et al.* (1998)
Parkinson's disease	Tremor	Reduction of Parkinson symptoms	Inhibition of β-carboline-2-N-methyltransferase	Gearhart *et al.* (1997)
Wilson's disease (WD)	Kayser-Fleischer-rings (cornea), muscle rigidity and dementia	Reduces Cu absorption and Kayser-Fleischer rings in size. Increases metallothionein in presymptomatic WD patient	Inhibits Cu resorption Induced metallothionein synthesis which also prevents Cu accumulation	Brewer & Yuzbasiyan-Gurkan (1992), Brewer *et al.* (1994), Esmaeli *et al.* (1996), Shimizu *et al.* (1999), Sturniolo *et al.* (1999)
Sickle cell disease	Hyperzincuria causes an impaired cell-mediated immunity	Increase in lymphocyte function and IL-2 production. Decreased hospitalizations and vaso-occlusive pain	Reconstitution of thymocyte functions	Prasad *et al.* (1999)
Common cold	Rhinitis, tearing, low-grade fever and malaise	Zinc gluconate reduces the duration of symptoms	Cell membrane stabilization to reduce viral penetration. Altered viral capsid formation. Increased IFN-α production	Mossad *et al.* (1996), Cakman *et al.* (1997)
Hypozincaemia	Increased incidence of concomitant systematic bacterial infections	Zn supplementation can reduce bacterial infections	Reconstitution of thymocyte functions, increased NK and phagocytotic activity	Worwag *et al.* (1999)
Acrodermatitis enteropathica	Vesicles and bullae of the skin and mucous membranes, alopecia, diarrhoea and failure to thrive. Autosomal recessive disorder of Zn resorption	Zn therapy reverses all symptoms	Reactivation of enzymes and transcription factors, reconstitution of thymocyte functions, increased NK and phagocytotic activity	Prasad (1995)
AIDS	Increased apoptosis of immune cells. Hypozincaemia associated with increased opportunistic infections	Decreased apoptosis and Zn is an adjunct to AZT therapy in AIDS pathology	Zn inhibits T-cell apoptosis and increases thymocyte proliferation	Mocchegiani *et al.* (1995*b*), Neves *et al.* (1998), Wellinghausen *et al.* (2000)
Rheumatoid arthritis (RA)	Lower level of Zn may be due to an accumulation of Zn-containing proteins in the liver and in the inflamed joints in RA	Decreased symptoms	Impairment of PMN phagocytosis, T-cell suppression and blocking IL-1 signal transduction	Zoli *et al.* (1998), Wellinghausen *et al.* (1997*b*)
Herpes simplex virus	Small, transient, irritating and sometimes painful fluid-filled blisters on the skin and mucous membranes	Shortening infection	Inhibition of virion functions by binding to sulfhydryl groups of glycoprotein B and increased IFN-α production	Varadinova *et al.* (1993), Cakman *et al.* (1996)
Down syndrome	Immature myeloid cells in the peripheral blood	Haematological symptoms reversed	Direct effects on leucocytes and on thymus hormones	Trubiani *et al.* (1996), Antonucci *et al.* (1997)
Crohn's disease	Decreased Zn levels lead to acrodermatitis enteropathica and decreased visual acuity	Alleviation of skin lesions and improvement of visual acuity. Low plasma concentrations of thymulin restored	T-cell suppression and thymus reconstitution	Brignola *et al.* (1993), Krasovec & Frenk (1996), Myung *et al.* (1998)

AIDS, acquired immune deficiency syndrome; AZT, zidovudine; IL, interleukin; IFN, interferon; NK, natural killer; PMN, polymorphonuclear leucocyte.

to the plasma Zn level. On the other hand, Zn can be used as an immunosuppressant with low toxicity. Last, but not least, both *in vivo* and *in vitro* the adequacy of the Zn concentration must be taken into account whenever abnormal cellular functions are observed. This approach is not restricted to the immune system, but the immune system is the first system to be affected by changing Zn levels, due to its high cell turnover.

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Table 8. Diseases associated with altered zinc levels

Disease	Plasma Zn level	Type of disorder	Reference
Alzheimer's disease	elevated	Zn-mediated NF-kB activation resulting in the activation of the amyloid precursor protein (APP) promoter region. APP forms amyloid-β and Zn inhibits the modulation of APP into non-amyloidogenic peptides. Amyloid-β-protein forms Ca-permeable and Zn-sensitive channels resulting in cellular toxicity	Kawahara *et al.* (1997), Lin *et al.* (1999)
Hyperzincaemia	elevated	Plasma levels > 200 μM cause symptoms consistent with Zn deficiency, probably an inborn defect of Zn metabolism	Sampson *et al.* (1997)
Depression	decreased	Zn deficiency impairs neuro- and immunoactivity of the mammalian organisms. Hypozincaemia in severe depression may be related to activation of cell-mediated immunity in that illness. T-cell suppression and modulation of neurotransmitter systems	Nowak (1998), Nowak & Schlegel-Zawadzka (1999)
Preterm delivery	decreased	Decreased plasma Zn levels are associated with preterm delivery and abortion	Scholl *et al.* (1993)

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