Biogerontology **1:** 133–143, 2000.

Review article

Zinc, metallothioneins, immune responses, survival and ageing

Eugenio Mocchegiani*, Mario Muzzioli & Robertina Giacconi
*Immunology Centre, Geriatric and Gerontological Research Department 'Nino Masera', Italian National Research Centres on Ageing (I.N.R.C.A.), via Birarelli 8, 60121 Ancona, Italy; *Author for correspondence (e-mail: e.mocchegiani@inrca.it; fax: +39-071-206791)*

Received 30 September 1999; accepted in revised form 12 November 1999

Key words: ageing, immune responses, metallothioneins, NK activity, survival, zinc

Abstract

Zinc is required as a catalytic, structural (zinc fingers) and regulatory ion. In this capacity, it is involved in many homeostatic mechanisms, including immune responses. Metallothioneins (MTs) may play key roles because of their preferential binding to zinc especially in ageing. MTs protect from oxidative damage during transient stress conditions at young-adult age. This protection no longer exists in ageing and in age-related diseases (cancer and infections) because the stress condition is constant. As such, MTs may constantly deplete zinc from plasma and tissues. This phenomenon causes increased MTs levels on the one hand, but on the other hand induces low zinc ion bioavailability for normal immune responses. This may be particularly relevant for thymic functions and natural killer activity. Therefore MTs which are protective in young-adults may become dangerous in immune responses during ageing. Physiological supplementation of zinc in ageing corrects central and peripheral immune defects, resulting prolonged survival and decreased mortality (50%) from infections and tumours, especially during middle age. Because of increased MT gene expression and protein levels in the liver and atrophic thymus of old mice, MTs are proposed as genetic markers of immunosenescence.

Introduction

Many studies show zinc to be the catalytic component of more than 300 enzymes, a structural constituent of many proteins and important in preventing free radical formation. Therefore zinc is a pivotal element in correct the functioning of various tissues, organs and systems (Mills 1989), including immune responses (Wellinghausen et al. 1997a). In particular, zinc is essential for thymic functions by means of a zinc-dependent thymic hormone called thymulin (ZnFTS) (Dardenne et al. 1982) required for T-cell maturation and differentiation (Goldstein 1984). Zinc is also important for liver extrathymic T-cell functions (Mocchegiani et al. 1997) prominent in ageing (Abo 1993) and, in turn, zinc, thymulin and peripheral immune responses show age-related declines (Mocchegiani and Fabris 1995; Mocchegiani et al. 1995). Zinc-binding proteins, such as Metallothioneins (MTs), may play key roles in the zinc effect upon the immune system. MTs are protective against stresses (Kagi 1993) and bind zinc with higher binding affinity (kd) than thymulin (Mocchegiani et al. 1998a). MTs increase in ageing (Mocchegiani et al. 1997) and show preferential binding with zinc in the aged, rather than with copper in the young (Hamer 1986). Thus, a shift of zinc towards binding MTs in the aged with subsequent low zinc ion bioavailability for thymulin activation has been recently proposed to play a role in thymic involution (Mocchegiani et al. 1998a). Such a shift towards zinc sequestration might also be involved in depressed peripheral immune responses in ageing (Mocchegiani et al. 1997), despite MTs being protective in zinc deficiency (Kelly et al. 1996) and functioning as donors of zinc for thymulin reactivation in thymic epithelial cells (TEC) (Savino et al. 1984; Coto et al. 1992).

Here we review the role of zinc and MTs in immune responses, including Natural Killer (NK) activity, during ageing with a discussion of some age-related diseases (cancer and infections). Because NK activity is decreased in ageing (Muzzioli et al. 1992), this is relevant for host defence (Herberman and Ortaldo 1981) where high MTs levels are an index for unfavourable prognosis in cancer and infections (Abdel-Maaged and Agrawal 1997). The efficacy of supplementing zinc in central and peripheral immune responses and in survival during ageing is examined.

Biology of zinc

Zinc is one of the most important trace elements in the body, but its presence in nature does not exceed 0.02% (Mills 1989). The major characteristics of zinc include a highly concentrated charge, a small radius (0.65 Å), no variable valence (low risk of free radical production), ready passage from one symmetry in its surroundings to another without exchange, rapid exchange of ligands (on and off reactions), and binding mostly to S- and N-donors in biological systems. These properties enable zinc to play three major biological roles: as catalyst, structural and regulatory ion (Vallee and Falchuk 1993a).

Catalytic action

Zinc is required for the biological function of more than 300 enzymes. In particular, zinc is essential and directly involved in catalysis by the enzyme. The removal of the catalytic zinc results in an active apoenzyme which usually retains the native tertiary structure. The catalytic zinc is usually bound by three-protein ligands and a water molecule, signifying an open co-ordination site which is considered essential for the function of zinc in catalysis (Vallee and Auld 1989). Indeed, the function of zinc is to both polarize the substrate and to activate the H_2O molecule, which then acts as a nucleophile. Because of its flexible co-ordinator geometry, the zinc ions act as a template to bring together the substrate and the nucleophile (Matthews 1988). The lowering of the energy barrier for the transition state and hence the acceleration of the conversion of substrate to products is the basis to catalysis by zinc metalloenzymes.

Structural action

Zinc is involved in gene expression, including MTs gene expression (Dunn et al. 1987). Zinc plays structural and functional roles in several proteins involved in DNA replication and reverse transcription, as well as in a number of eukaryotic transcription factors where the potential binding domains are referred to as zinc fingers (Coleman 1992). In this context a bulk of data shows zinc fingers in DNA replication of many proteins involved in cell proliferation, in cell differentiation, in cell growth arrest, in cell division, in signal transmission, in growth factors production, in protoncogenes activation, in chemokine production, and in codifying hormone nuclear receptor superfamily and in nuclear transcription factor activation (Table 1) (Coleman 1992; Mackay and Crosseley 1998; Berg and Godwin 1997; Hooper 1996), in mRNA stability (Taylor and Blackshear 1995), and in maintaining the extracellular matrix (Vallee and Auld 1990). In addition, zinc is bound to enzymes, proteins and peptides with different binding affinity (kd) ranging from 10^{-2} to 10^{-14} mol/l, including thymulin (kd = $5.0 \pm 2 \times 10^{-7}$ M) (see review Mocchegiani et al. 1998a). The compound is inactive or of low biological activity in the absence of zinc-binding (Hooper 1996). Thereby zinc, other than in physiological condition, is also crucial in pathologies because the proteins, peptides, protoncogenes and transcription factors cited above are also involved in tumour growth and the progress of infections (Nagase 1996; Zornig et al. 1996; Tachibana et al. 1997; Anzai et al. 1998).

Regulatory role

Zinc regulates both enzymatic activity and the stability of the protein as an activator or as an inhibitor ion. In this context, gene overexpressions of metalloproteinases (MMPs) are under the control of the gene expression of some tissue inhibitors of matrix metalloproteinases (TIMPs) (Kolkenbrock et al. 1991), of α-2 macroglobulin (Souttrp-Jensen 1989) and of β-amyloid precursor protein (Miyazaki et al. 1993), which are, in turn, codified by zinc-finger motifs (Nagase 1996). Thereby a good balance of the gene expression metalloproteinases (either as activator or as inhibitor) is necessary for an optimal functioning of many body homeostatic mechanisms. Indeed, an imbalance between MMPs and TIMPs or α-2 macroglobulin is characteristic of cancer (Stettler-Stevenson et al. 1993) and inflammation (Nagase 1996), as well as during ageing (Ashcroft et al. 1997; Li et al. 1999).

Table 1. Structural action of zinc as 'zinc fingers' for protein or peptide DNA replication in various biological functions.

Compounds (proteins or peptides)	Biological function
SP1, A-20, ALR, MMPs, BP10, BP1, Staf 50 TIMPs, α-2 macroglobulin	Cell proliferation
GATA family, mp1, glycoprotein IIb/IIIa	Cell differentiation
Nil-2-a, TGF-β	Cell growth arrest
Cyclin T1	Cell division
Protein kinase-C (PKC), ZNF 162	Signal transmission
EGF, IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, IFN-α, TNF-α	Cell growth
p53, p21, c-myc, c-fos, GFI-1, HIC-1, c-jun, bcl-2	Protoncogene for prevention or activation apoptosis
RANTES	Chemokine production
NF-kB, AP-1, EGR-1, EGR-2, BTE, KS-1, WT-1, TF III A, Finb, TRAF-2, ZEB	Nuclear transcription factor activation
Melatonin, growth hormone, IGF-1, glucocorticoids, prostaglandins	Hormone nuclear receptor superfamily codification

Since zinc ion bioavailability is essential for immune functions (Wellinghausen et al. 1997a), attention is addressed to the role of MTs in immune responses, including NK activity, because of MT increments in ageing (Mocchegiani et al. 1997). Moreover, the kd of MTs is higher (1.4×10^{-13} M) (Kagi 1993) than thymulin ($5.0 \pm 2 \times 10^{-7}$ M) (Dardenne et al. 1982), and thymulin, NK activity and zinc ion bioavailability decrease in ageing (Muzzioli et al. 1992; Mocchegiani and Fabris 1995; Mocchegiani et al. 1995; Pawelec and Solana 1997). Moreover, low zinc ion bioavailability is a risk factor for infection relapses in the elderly (Mocchegiani et al. 1999b), who, in turn, display high MT levels (Sobocinki et al. 1978) and decreased immune functions (Fabris and Mocchegiani 1995).

Biology of metallothioneins and ageing

Metallothioneins (MTs) are low-molecular-weight metal-binding proteins with 61 aminoacids; among them 20 are cysteines. MTs play pivotal roles in metal-related cell homeostasis because of their high affinity for metals, in particular zinc and copper (Bremner and Beattie 1990). Twenty cysteine aminoacids are in reduced form and bind seven zinc atoms through mercaptide bonds forming metal thiolate clusters (Kagi 1988; Valle and Maret 1993b; Maret and Vallee 1998). The zinc/cysteine interactions form two clusters of two different types, either as bridging or as terminal cysteine thiolate. Three bridging and six terminal cysteine thiolates provide an identical co-ordination environment for each of the three zinc atoms in a β-domain cluster. Two different zinc sites are present in α-domain clusters; two of them have one terminal ligand and three bridging ligands, while the other two have two terminal and two bridging ligands (Kagi 1988; Maret and Vallee 1998). Four different isoforms of MTs exist, characterized by the length of the aminoacid chain. Isoforms I, II, III and IV are mapped on chromosome 16 in man and on chromosome 8 in mice, with complex polymorphisms (West et al. 1990). Isoforms I and II are present in many organs, while isoform III, called GIF, is exclusively present in the brain and isoform IV is still not well defined (Palmiter et al. 1992). MTs bind zinc with high binding affinity (kd = 1.4×10^{-13} M) (Kagi 1993). MTs distributes cellular zinc because zinc undergoes rapid inter- and intracluster exchange (Otvos et al. 1993). Moreover, MTs are antioxidant agents because the zinc-sulphur cluster is sensitive to changes of cellular redox state and oxidizing sites induce the transfer of zinc from its binding sites in MTs to those of lower affinity in other proteins (Maret and Vallee 1998), as it occurs for superoxide dismutase activation (Suzuki et al. 1995; Lazo et al. 1998). Thereby the redox properties of MTs are crucial for their role of protection against the cytotoxic effect of reactive oxygen species, ionizing radiations, electrophilic anti-cancer drugs and mutagens, and metals (Kagi 1993). Consistent with this role, MT gene expression (MT I, II isoforms) induced by means of transcription factor MTF-1 (Palmiter 1994), is present from molluscs to humans (Kagi 1993).

This role is peculiar to young-adult age but it may be questioned in ageing. Indeed, this protection occurs in young-adult age during transient stress conditions (Kagi 1993). By contrast, stress condition is constant in ageing, not transient, as documented by high levels of glucocorticoids during the whole circadian cycle (Mocchegiani et al. 1998b). MT protection may

become, at least, dangerous. Indeed, despite the fact that MTs are increased in the liver (Mocchegiani et al. 1997) and brain (Suzuki et al. 1992) of old mice, the cellular oxidative damage is constant in ageing (free radical theory of ageing) (Ashok and Ali 1999). Moreover, high MT levels are observed in cancer, infections and dementia (Ebadi and Swanson 1988; Sobocinski et al. 1995; Zambenedetti et al. 1998) with a diagnostic role of unfavourable prognosis (Abdel-Mageed and Agrawal 1997). Because of the low zinc ion bioavailability, both in ageing and in age-related diseases (Fabris and Mocchegiani 1995), MTs do not transfer zinc in ageing, cancer and infections. By contrast, MTs constantly sequester zinc to maintain their original role of protection. This phenomenon induces low zinc ion bioavailability for immune responses with possible different roles for MTs: from protective in young-adult age to dangerous in old age. Indeed, MTs preferentially bind zinc rather than copper (Orlowski and Piotrowski 1998), especially in ageing (Hamer 1986) as was recently also confirmed in old liver by an HPLC procedure (E. Mocchegiani 1999: unpublished observation), and low zinc ion bioavailability is present in ageing, but not low copper (Jacob et al. 1985). On the other hand, high MT levels obstruct antioxidative responses after exposure to H_2O_2 in the brain of trisomy 16 mouse (experimental model of Down's syndrome) (Scortengangna et al. 1998). Oxidative damage and impaired immune responses in Down's syndrome subjects (accelerated ageing) are restored by supplementing zinc (Chiricolo et al. 1993, Fabris et al. 1993). Thereby, the biological role of MTs is crucial in antioxidative and immune responses during ageing and age-related diseases.

Zinc, immune responses and ageing

Zinc is relevant for immunocompetence. Decreased chemotaxis by neutrophils and monocytes and impairment of cell-mediated immune responses, including thymic endocrine activity, NK activity, and cytokine production are observed in zinc deprived diets (Wellinghausen et al. 1997a). In this context, zinc affects more Th1 cells, believed to be involved in IL-2 release, rather than Th2 cells, suggesting an imbalance of Th1/Th2 paradigm in zinc deficiency because of the zinc involvement in the synthesis and release of IL-2 and IFN-α by Th1 (Prasad 1998). Moreover, zinc recognizes superantigens by means of MHC class II molecules (Papagerogiou et al. 1999). Although the zinc effect may be related to possible phenomena involving competition with copper, progressive depletion as well as excessive intake of copper and zinc result in impaired immunocompetence (Chandra 1984). Because of zinc deficiency in ageing, but not copper (Jacob et al. 1985), possible competition phenomena may be avoided in ageing. Indeed the crude zinc balance (difference between zinc intake and zinc excretion), is negative in old mice (Mocchegiani et al. 1995) and old humans (Turnlund et al. 1986) as compared to positive values in young-adult age. This negativity is associated with reduced thymic endocrine activity, decreased NK activity (Mocchegiani et al. 1995), abnormalities in cytokine production (Fagiolo et al. 1993), an imbalance of the Th1/Th2 paradigm towards Th2 cells (Cakman et al. 1996), increments of memory T-cells (CD45RO) and decrements of naive T-cells (CD45RA) (Franceschi et al. 1995; Rink and Seyfath 1997; Pawelec and Solana 1997). Because of the involvement of zinc in the gene expression of anti-inflammatory cytokines (IL-2, IL-12, IFN-α) (Driessen et al. 1994) and increments of proinflammatory cytokines (IL-4) are associated with an imbalance of the Th1/Th2 paradigm in old mice (Frasca et al. 1997), it is evident that zinc ion bioavailability is important for anti-inflammatory cytokine production with subsequent normal Th1 cell development. When zinc ion bioavailability decreases, a loss of T-cells coming from the bone marrow occurs, with constant impairment of immune responses in old people (Fabris et al. 1997). The reduced thymic endocrine activity is of interest because of its influence in T-cell maturation and differentiation, in NK activity, and in cytokine production (Goldstein 1984). Indeed, a thymic hormone, called thymulin (ZnFTS), essential for T-cell maturation and differentiation, requires zinc for its activation (Dardenne et al. 1982). The zinc unbound form (FTS) is inactive with an inhibitory action on the active form (ZnFTS). FTS increases in ageing, whereas ZnFTS is reduced. The *in vitro* addition of $zinc^{++}$ (200 nM) to old plasma samples containing FTS restores circulating active thymulin ZnFTS (AT) plasma levels, also showing the total amount of thymulin (TT) produced (active ZnFTS + inactive FTS) (Fabris et al. 1984). Since the TT/AT ratio represents the unsaturable fraction of FTS by zinc, it is a good marker to test real zinc ion bioavailability because of the strict inverse correlation between zinc plasma levels and the ratio itself [Ratio = 1 ($\log_{-2}$) = no zinc deficiency, ratio $\geq$ 2 ($\log_{-2}$) = low zinc ion bioavailability]. This ratio in ageing is always

Table 2. In vitro effect of inactive zinc-unbound FTS (FTS), of inactive FTS + $Zinc^{++}$ and active zinc-bound FTS (ZnFTS) on NK activity in spleen cells from old inbreed Balb/c mice.

Spleen cells of mice	NK activity (L.U. $20/10^7$)
Young control	73.2 ± 14.1
Old control	25.2 ± 7.5*
Old + inactive FTS (10^{-6} M)	24.5 ± 6.8*
Old + inactive FTS (10^{-6} M) + Zn^{++} (10^{-6} M)	71.2 ± 13.7
Old + active ZnFTS (10^{-6} M)	71.4 ± 13.5

* $P < 0.01$ when compared to young controls (paired Student's *t*-test). *In vitro* experiments were performed in zinc-chelating medium in order to avoid zinc interference in the medium (Mocchegiani and Fabris 1995). Preincubation time 4 h. Data of NK assay are expressed in Lytic Unit (LU) against tumoral target YAC-1. ZnFTS (10^{-6} M) is the minimum to obtain thymulin biological activity in thymulin bioassay (Mocchegiani et al. 1995). $Zinc^{++}$ 10^{-6} M because of zinc bound in thymulin molecule in equimolar ratio (Dardenne et al. 1982).

> 2 despite the fact that plasma zinc levels are in the normal range (Fabris et al. 1984). Thus low zinc ion bioavailability is present in ageing, causing impaired immune responses, including NK activity. Indeed, NK activity is controlled by active thymulin (ZnFTS) (Muzzioli et al. 1992). *In vitro* experiments in splenocytes from old inbred Balb/c mice (low responder to YAC-1) (Muzzioli et al. 1986) show that zinc addition to inactive FTS restores NK activity as obtained with active ZnFTS (Table 2).

These findings, which one the hand demonstrate NK activity controlled by active thymulin (ZnFTS), on the other hand pinpoint the relevance of zinc ion bioavailability for NK activity which, in turn, is controlled by IL-2 (Henney et al. 1981). Zinc affects IL-2 synthesis (Tanaka et al. 1990) and thymulin influences IL-2 receptor expression on NK cells (Saha et al. 1995). Thereby thymulin and zinc decrements may be both involved in impaired IL-2 production and NK activity in ageing.

Thus zinc, via thymulin, is one of the mechanisms by which zinc may impact upon the immune responses. Many other mechanisms have been proposed. Direct mechanisms activating DNA- and RNA-polymerases, nucleoside phosphorylase, ecto-5 nucleotidase and protein-kinase C (PKC) or indirect mechanisms involving transcription factors (NF-kB and AP-1) and hormones (melatonin, growth hormone, thyroid hormones and IGF-1) have been documented (Mocchegiani et al. 1998a).

Whatever the mechanisms involved, the relevance of zinc ion bioavailability in immune responses, in particular in NK activity for host defence (Herberman and Ortaldo 1981), is evident. In addition, the liver extrathymic NK activity is also reduced in ageing because of the major quota of zinc ions bound with liver MTs (Mocchegiani et al. 1997). Liver NK cells (NK 1.1+) from old mice (Tsukahara et al. 1997), from irradiated mice (Halder et al. 1998) and from G-CSF transgenic mice (Kawamura et al. 1999) have TCR intermediate intensity as compared to the TCR high intensity of spleen NK cells. These findings suggest liver NK activity impairments, also in ageing and stress (Mocchegiani et al. 1997) despite liver NK cell major abundance and more expression in IL-2 β-chain receptor than spleen NK cells (Tsukahara et al. 1997). Since low zinc ion bioavailability and high MTs levels are present in ageing and stress (Mocchegiani et al. 1997), zinc and MT homeostasis is crucial for NK activity of thymic (Muzzioli et al. 1992) or liver (Tsukahara et al. 1997) origin during ageing.

Metallothioneins, immune responses and ageing

MTs protect the cells by stresses inducing the secretion by macrophages for a prompt immune response of IL-1, IL-6, IFN-α, TNF-α, which are, in turn, involved in new synthesis of MTs in the liver (Cousins and Leinart 1988; Cui et al. 1998). In turn, IL-1 induces alterations in zinc status and in liver MT concentrations (Bui et al. 1994). These findings suggest the existence of an interplay between zinc, MTs and the immune system. On the other hand, IL-1 induces MTmRNA gene expression in thymic epithelial cells (TEC) of young humans (Coto et al. 1992) by means of PKC, which, in turn, is zinc dependent (Coto et al. 1992) and participates in the process of metal-induced MT gene expression (Yu et al. 1997). Moreover, MTs are donors of zinc for thymulin reactivation in TEC (Savino et al. 1984). Experiments in MT-null mice or in mice exposed to endotoxins or radiation support the existence of links between MTs and the immune system. GM-CSF and GM-CSF receptor decrements are present in MT-null mice, inducing glial cell death (Penkova et al. 1999). MTmRNA, proinflammatory cytokines and chemokines increased in the lung of mice exposed to endotoxins (LPS) (Johnston et al. 1998). Moreover, thymocytes apoptosis is augmented in irradiated MT-null mice (Kondo et al. 1997; Deng et al. 1999). Such links attain relevance in condi-

Table 3. Tissue zinc and MT concentrations in the thymus and liver of young and old inbreed Balb/c mice.

	Young mice	Old mice
Zinc into the thymus (μg/gr)	62.3 ± 11.2	107.4 ± 27.5*
Zinc into the liver (μg/gr)	110.6 ± 23.4	125.6 ± 21.4
MTs into the thymus (μg/gr w.w.)	3.5 ± 0.4	8.6 ± 0.7*
MTs into the liver (μg/gr w.w.)	5.3 ± 1.0	12.5 ± 4.7*

* $P < 0.01$ as compared to young mice (paired Student's *t*-test). Liver and thymus MTs concentrations were tested with silver saturation method (Mocchegiani et al. 1997). Tissue zinc content was tested in AAS with the method of Leblondel et al. (1986).

tions characterized by high MT and pro-inflammatory cytokine levels, impaired immune responses and low zinc ion bioavailability, such as in cancer and inflammation, as well as in ageing because of the presence of the same altered immune and nutritional characteristics of cancer and inflammation which are, in turn, diseases related to the ageing process (Fabris and Mocchegiani 1995). MTs protect against stresses (Kagi 1993), including tumour growth (Kloth et al. 1995), and during zinc deficiency and zinc toxicity or as a type of zinc reservoir or as sequestering excess zinc, respectively (Kelly et al. 1996). However, high MTs and zinc content in the liver and atrophic thymus from old mice (Table 3), suggest that MTs play different roles in immunosenescence: turning from protective to dangerous (Mocchegiani et al. 1998a).

Increments of MTmRNA gene expression that were more pronounced in old liver, than atrophic thymus, have been recently found by means of RT-PCR. Significant inverse correlations between increased MTmRNA and decreased liver NK cells and activity are also observed (E. Mocchegiani 1999: unpublished results). The MT increments in lymphocytes of adult people (Yukow and Makhijani 1998) are further significant support for this possibly different role of MTs in ageing. Indeed, the findings of Kelly et al. (1996) and Kloth et al. (1995) were obtained in young age, which may mimic transient abnormal conditions in young-adult age. By contrast, zinc deficiency, impaired immune responses and stress condition are constant in ageing (Fabris and Mocchegiani 1995, Mocchegiani et al. 1998b). Thereby MTs may constantly retrieve zinc from plasma and tissues. This, at least, might induce low zinc ion bioavailability and, consequently, impair immune responses (Mocchegiani et al. 1998a). Such an assumption is supported by the following findings: (i) the necessity for the *in vitro* zinc addition to old plasma samples for thymulin reactivation (Mocchegiani and Fabris 1995; Mocchegiani et al. 1995); (ii) the presence of atrophic thymus in MT transgenic mice showing melanoma development (Iiai et al. 1994); (iii) the demand for endogenous zinc for T-cell growth in adult-old individuals (Miller and Strittmatter 1992), for TEC restoration number in old thymus (Mocchegiani and Fabris 1995), and for NK activity restoration, via active thymulin, in old mice (Muzzioli et al. 1992). Consistent with these findings, MTs may not be donors of zinc in ageing but rather sequesters of zinc. This phenomenon has been proposed for thymic involution in ageing (Mocchegiani et al. 1997, 1998a). On the other hand, increased MT levels induce down-regulation of many other biological functions related to zinc, such as metabolism, gene expression, and signal transduction (Kagi 1993). Since MTs have higher kd for zinc than thymulin (Mocchegiani et al. 1998a) and Atomic Absorption Spectrophotometry (AAS) detects both bound and unbound zinc, this way on the justify high the thymus and liver zinc content in old mice (Table 3), on the other hand, it suggests low zinc ion bioavailability as being crucial for entire immune efficiency in ageing. Moreover, because of the preferential binding of MTs with zinc rather than copper in ageing (Hamer 1986; E. Mocchegiani 1999: unpublished results), different roles of MTs (from protective to dangerous) may be further supported because of zinc deficiency, not copper, in ageing (Jacob et al. 1985) and zinc ion bioavailability is essential for immunity (Wellinghausen et al. 1997a).

Supplementing zinc in ageing

Supplementing zinc in rodents from birth prevents age-related immune modifications (Iwata et al. 1979). Because of the constant presence of low zinc ion bioavailability in ageing (Fabris et al. 1984), supplementing zinc in old mice induces: (i) thymus regrowth, as recently shown by Magnetic Resonance Imaging (MRI) (Sbarbati et al. 1998), (ii) TEC and thymocyte T-subsets restoration numbers (Dardenne et al. 1993; Mocchegiani et al. 1995), (iii) both mitogen T-cell responsiveness (PHA and ConA) and NK activity recovery (Mocchegiani et al. 1995). Moreover, *in vitro* zinc prevents thymocytes apoptosis in old mice (Provinciali et al. 1998) by means of endonuclease enzyme activation (Monti et al. 1992). Thus, zinc is crucial

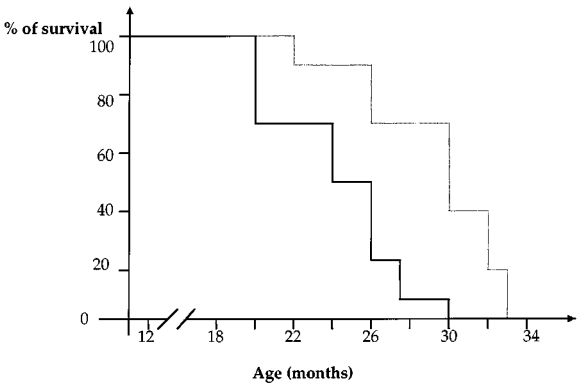

Figure 1. Survival curves (Kaplan–Meier) from the age of 12 months in 50 male zinc treated inbreed Balb/c mice (hatched line) and in 50 male mice of controls (full line) in our (INRCA, Ancona, Italy) housing conditions (5 mice per cage; 12-h light/12-h dark cycle from 7:00 a.m. to 7:00 p.m. at constant temperature 20 $\pm$ 1 °C and humidity 50 $\pm$ 5%). 50 mice are sufficient for survival analysis (Piantanelli et al. 1994).

to maintaining immune responses in ageing with consequent possible prevention of age-related diseases. Indeed, supplementing zinc (22 mg/l of zinc sulphate = 18 μg Zn^{++}/day) (Mocchegiani et al. 1995) in drinking water in mice from the age of 12 months (adult age) prolongs their survival with an emphasis in the middle age (22–28 months) (Figure 1) because of mortality reduction (more than 50%) by infections and tumours as compared to controls (Mocchegiani et al. 1998b).

Modest immune modifications are observed in old people treated with high doses of zinc for short periods (Prasad et al. 1993; Boukaiba et al. 1993) as well as with physiological zinc doses (RDA) (USDA 1980) for long periods (one year) (Bodgen et al. 1990). Zinc accumulation might exist in both conditions, causing toxic effects (Fosmire 1990). Indeed, high zinc is toxic in IFN-α (Cakman et al. 1997), IL-1β (Wellinghausen et al. 1997b) and IL-2 productions (Mocchegiani et al. 1999a). By contrast, supplementing physiological zinc (15 mg Zn^{++}/day) (RDA) for two months in Down's syndrome restores cell-mediated immune responses, including NK activity, with decreased morbidity by infections and better cognitive performances also after four months of follow-up (Fabris et al. 1993). Old infected humans treated with zinc sulphate (12 mg Zn^{++}/day) for one month show immune restorations with infection relapse reduction also after four months of follow-up (Mocchegiani et al. 1999b). Similar immune recovery after zinc physiological treatment has also been foound by others in the elderly (Fortes et al. 1998), in cancer (Ames 1998) and in infections (Shankar and Prasad 1998).

However, because whether zinc affects MTmRNA gene expression (Cousins and Lee-Ambrose 1992; Hernandez et al. 1996; Sullivan et al. 1998), the relevant question rises of supplementing zinc may further affect the high MT levels. This phenomenon may be limited or avoided in ageing. Indeed, *in vitro* physiological zinc does not further increase MTmRNA induced by IL-1 in the young human thymus (Coto et al. 1992), and IL-1 increases in ageing (Fagiolo et al. 1993). Although further studies are required, MT molecules in ageing may be fully saturated by pre-existing zinc ions. Such an interpretation is supported by recent findings in cancer. Despite MT increments in tumour (Ebadi and Swanson 1988), zinc (100 μg/dl = normal zinc circulating levels, Fabris and Mocchegiani 1995) arrests human prostatic carcinoma cell growth in the G2/M phase of cell cycle (Liang et al. 1999). Thus supplementing zinc might induce MTs to regain their role of protection; on the other hand, it may arrest tumour growth and lead cancer cells to cell death by means of p21 overexpression (Liang et al. 1999). This phenomenon has been recently suggested for increased α-2 macroglobulin concentrations in cervical carcinoma (Mocchegiani et al. 1999a).

Conclusions

Zinc is crucial for immune responses. Free zinc ion bioavailability is relevant because zinc is bound to enzymes, proteins and peptides with different binding affinity. Thymulin and MTs are of interest because they bind zinc with different binding affinity; higher for MTs. The shift of zinc towards MTs rather than thymulin may occur. MTs protect cells under stress by binding zinc in thiolate groups forming clusters. As such, MTs prevent cell destruction or cell death. This occurs in young-adult age during transient exposure to reactive oxygen species. Because stresses are not transient in ageing (Mocchegiani et al. 1998b), MTs may constantly sequester zinc ion inducing low zinc ions bioavailability for thymulin activation and subsequent impaired peripheral immune responses, including NK activity. Thereby, despite increased MTs in ageing, MTs may have different roles: protective in young-adult age and dangerous for peripheral immune responses in ageing, as recently proposed for thymic involution, as MTs increase in the atrophic thymus of old mice (Mocchegiani et al.

1998a). Physiological supplementation of zinc. performed with caution to avoid possible competition phenomena with copper, corrects central and peripheral immune defects in ageing with subsequent prolonged survival. The benefit may be also extended to infections because of infection relapse reduction in the elderly by supplementing zinc. Major zinc ion bioavailability may not affect the high MTs levels in ageing, as supported by *in vitro* thymic experiments after IL-1 stimulation (Coto et al. 1992) and by an *in vitro* cancer model (Liang et al. 1999). Thus supplementing zinc is of benefit and MTs may regain their original task of protection in ageing, as recently proposed for increased α-2 macroglobulin in cancer (Mocchegiani et al. 1999a). Augmented MT levels (Table 3) and increased MTmRNA gene expression (E. Mocchegiani 1999: unpublished results) are present in the liver and atrophic thymus of old mice. Thus MTs may be possible genetic markers of immunosenescence. Further studies are in progress in our laboratory.

Acknowledgement

This paper was supported by INRCA and Italian Health Ministry.

References

Abo T (1993) Extrathymic pathway of T-cell differentiation: a primitive and fundamental immune system. Microbiol Immunol 37: 247–250

Adbel-Magged A and Agrawal KC (1997) Antisense downregulation of metallothionein induces growth arrest and apoptosis in human breast carcinoma cells. Cancer Gene Ther 4: 199–207

Ames BN (1998) Micronutrients prevent cancer and delay aging. Toxicol Lett 102–103: 5–18

Anzai H, Kitadai Y and Bucana CD et al. (1998) Expression of metastasis-related genes in surgical specimens of human gastric cancer can predict disease recurrence. Eur J Cancer 34: 558–565

Ashcroft GS, Herrick SE and Tarnuzzer RW et al. (1997) Human ageing impairs injury-induced *in vivo* expression of tissue inhibitor of matrix metalloproteinases (TIMP)-1 and -2 proteins and mRNA. J Pathol 183: 169–176

Ashok BT and Ali R (1999) The aging paradox: free radical theory of aging. Exp Gerontol 34: 293–303

Berg JM and Godwin HA (1997) Lessons from zinc-binding peptides. Ann Rev Biophys Biomol Struct 26: 357–371

Bodgen JD, Oleske JM and Lavenhar MA et al. (1990) Effects of one year of supplementation with zinc and other micronutrients on cellular immunity in the elderly. J Am Coll Nutr 9: 214–225

Boukiba N, Flament C and Archer F et al. (1993) A physiological amount of zinc supplementation: effects on nutritional, lipid and thymic status in an elderly population. Am J Clin Nutr 57: 566–572

Bremner I and Beattie JH (1990) Metallothionein and trace minerals. Ann Rev Nutr 10: 63–70

Bui LM, Dressendorfer RH and Keen CL et al. (1994) Zinc status and interleukin 1-beta induced alterations in mineral metabolism in rats. Proc Soc Exp Biol Med 206: 438–444

Cakman I, Rohwer J and Schuz RM et al. (1996) Dysregulation of Th1 and Th2 amd subpopulation in elderly. Mech Ageing Dev 87: 197–209

Cakman C, Kirchner H and Rink L (1997) Zinc supplementation reconstitutes the production of interferon-alpha leukocytes from elderly persons. J Interferon Cytokines Res 17: 469–472

Chandra RK (1984) Excessive intake of zinc impairs immune responses. J Am Med Assoc 252: 1443–1446

Chandra RK (1992) Nutrition and immunoregulation. Significance for host resistance to tumours and infections in human and rodents. J Nutr 122: 754–757

Cherian MG and Chun HM (1993) Biological function of metallothioneins. In: Suzuki KT, Imura N and Kimura M (eds) Metallothionein III, pp 87–110. Birkhauser Verlag, Basel

Chiricolo M, Musa AR and Monti D et al. (1993) Enhanced DNA repair in lymphocytes of Down's syndrome patients: the influence of zinc nutritional supplementation. Mutat Res 295: 105–111

Chung J, Nartey NO and Cherian MG (1996) Metallothionein levels in liver and kidney of Canadians, a potent indicator of enviromental exposure to cadmium. Arch Environ Health 41: 319–323

Coleman JE (1992) Zinc proteins, enzymes, storage proteins transcription factors and replication proteins. Ann Rev Biochem 61: 897–946

Coto JA, Hadden EM and Sauro M et al. (1992) Intereleukin-1 regulates secretion of zinc-thymulin by human thymic epithelial cells and its action on T-lymphocyte proliferation and nuclear protein kinase C. Proc Natl Acad Sci USA 89: 7752–7756

Cousins RJ and Leinart AS (1988) Tissue-specific regulation of zinc metabolism and metallothionein genes by interleukin 1. FASEB J 2: 2844–2890

Cousins RJ and Lee-Ambose LM (1992) Nuclear zinc uptake and interactions and metallothionein gene expression are influenced by dietary zinc in rats. J Nutr 122: 56–64

Cui L, Takagi Y and Wasa M et al. (1998) Zinc deficiency enhances interleukin-1 alpha-induced metallothionein-1 expression in rats. J Nutr 128: 1092–1098

Dardenne M, Pleau JM and Nabama B et al. (1982) Contribution of zinc and other metals to the biological activity of the serum thymic factor. Proc Natl Acad Sci USA 79: 5370–5373

Dardenne M, Boukaiba N and Gagernault MC et al. (1993) Restoration of the thymus in aging mice by *in vivo* zinc supplementattion. Clin Immunol Immunopathol 66: 127–135

Deng DX, Cai L and Chakrabarti S et al. (1999) Increased radiation-induced apoptosis in mouse thymus in the absence of metallothionein. Toxicology 134: 39–49

Driessen C, Hirv, K and Rink L et al. (1994) Induction of cytokines by zinc ions in human peripheral blood mononuclear cells and separated monocytes. Lymphokine Cytokine Res 13: 15–20

Dunn MA, Blalock TL and Cousins RJ (1987) Metallothionein review. Proc Soc Exp Res Biol Med 185: 107–119

Ebadi E and Swanson S (1988) Zinc and metallothioneins in cancer. In: Ebadi M (ed) Nutrition, Growth and Cancer, pp 161–175. Alan R Liss, New York

Fabris N and Mocchegiani E (1995) Zinc, human diseases and aging. A review. Aging Clin Exp Res 7: 77–93

Fabris N, Mocchegiani E and Albertini G (1993) Psycoendocrine-immune interactions in Down's syndrome: role of zinc. In: Castells S and Wisnieski KE (eds) Growth Hormone Treatment in Down's Syndrome, pp 203–218. John Wiley and Sons, New York

Fabris N, Mocchegiani E and Amadio L et al. (1984) Thymic hormone deficiency in normal ageing and Down's syndrome: is there a primary failure of the thymus? Lancet 1: 983–986

Fabris N, Mocchegiani E and Provinciali M (1997) Plasticity of neuroendocrine-immune interactions during aging. Exp Gerontol 32: 415–429

Fagiolo U, Cossarizza A and Scala E et al. (1993) Increased cytokine production in mononuclear cells of healthy and elderly people. Eur J Immunol 23: 2375–2378

Fortes C, Forastiere F and Agabiti N et al. (1998) The effect of zinc and vitamin A supplementation on immune response in an older population. J Am Ger Soc 46: 19–26

Fosmire GJ (1990) Zinc toxicity. Am J Clin Nutr 51: 225–227

Franceschi C, Monti D and Sansoni P et al. (1995) The immunology of exceptional individuals: the lessons of centenarians. Immunology Today 16: 12–16

Frasca D, Pucci S and Goso G et al. (1997) Regulation of cytokine production in aging: use of recombinant cytokines to up regulate mitogen-stimulated spleen cells. Mech Ageing Dev 93: 157–169

Goldstein AL (ed) (1984) Thymic Hormones and Lymphokines. Plenum Press, New York

Grider A and Cousins RJ (1989) Role of metallothionein in copper and zinc metabolism: special reference to inflammatory condition. In: Milanino R, Rainsford KD and Velo GP (eds) Copper and Zinc in Inflammation, pp 21–32. Kluwer, London

Halder RC, Seki S and Weerasinghe A et al. (1998) Characterization of NK cells and extrathymic T cells generated in the liver of irradiated mice with a liver shield. Clin Exp Immunol 114: 434–437

Hamer DH (1986) Metallothionein review. Ann Rev Biochem 55: 913–951

Henney CS, Kurihayashi K and Kern DE et al. (1981) Interleukin-2 augments natural killer cell activity. Nature 291: 335–340

Herberman RB and Ortaldo JR (1981) Natural killer cells: their role in defence against disease. Science 214: 24–30

Hernandez J, Giralt M and Belloso E et al. (1996) Interactions between metallothionein inducers in rat liver and primary cultures of rat hepatocytes. Chem Biol Interact 100: 27–40

Hopper NM (ed) (1996) Zinc Metalloproteases in Heath and Disease. Taylor and Francis, London

Iiai T, Watanabe H and Iwamoto T et al. (1994) Predominant activation of extathymic T-cells during melanoma development of metallothionein/ret transgenic mice. Cell Immunol 153: 412–427

Iwata T, Incefy GS and Tanaka T et al. (1979) Circulating thymic hormone levels in zinc deficiency. Cell Immunol 47: 100–105

Jacob RA, Russel RN and Sandstead HH (1985) Zinc and copper nutrition in aging. In: Watson RR (ed) Handbook of Nutrition in the Aged, pp 77–88. CRC Press, Boca Raton, Florida

Kagi RHJ (1993) Evolution, structure and chemical activity of class I metallothioneins: an overview. In: Suzuki KT, Imura N and Kimura M (eds) Metallothioneins III, pp 29–56. Birkhauser Verlag, Basel

Kagi RHJ and Schaffer A (1988) Biochemistry of metallothioneins. Biochemistry 27: 8509–8515

Kawamura H, Kawamura T and Kokai Y et al. (1999) Expansion of extrathymic T cells as well as granulocytes in the liver and other organs of granulocytes colony stimulating factor transgenic mice: why they host the ability of hybrid resistance. J Immunol 162: 5957–5964

Kelly EJ, Quaife CF and Froelik GJ et al. (1996) Metallothionein I and II against zinc deficiency and zinc toxicity. J Nutr 126: 1782–1790

Kloth DM, Chin JL and Cherian MG (1995) Induction of hepatic metallothionein I in tumour-beraring mice. Br J Cancer 71: 712–716

Kolkenbrock H, Orgel D and Hecker-Kia A et al. (1991) The complex between a tissue inhibitor of metalloproteinases (TIMP-2) and 72kDa progelatinase a metallo proteinase inhibitor. Eur J Biochem 198: 775–781

Kondo Y, Rusnak JM and Hoyt DG et al. (1997) Enhanced apoptosis in metallothionein null cells. Mol Pharmacol 52: 195–201

Johnston CJ, Finkelstein JN and Gelein R et al. (1998) Pulmonary cytokine and chemokine mRNA levels after inhalation of lypopolysaccharide in C57BL/6 mice. Toxicol Sci 46: 300–307

Lazo JS, Kuo SM and Woo ES et al. (1998) The protein thiol metallothionein as an antioxidant and protectant against antineoplastic drugs. Chem Biol Interact 111–112: 255–262

Leblondel G, Mauras Y and Allain P (1986) Tissue distribution of some elements in rats. Biol Trace Element Res 10: 327–333

Li Z, Froehlich J and Galis ZS et al. (1999) Increased expression of matrix metalloproteinase-2 in the thickened intima of aged rats. Hypertension 33: 116–123

Liang Y, Liu YY and Zou J et al. (1999) Inhibitory effect of zinc on human prostatic carcinoma cell growth. Prostate 40: 200–207

Mackay JP and Crossley M (1998) Zinc fingers are sticking together. Trends Biochem Sci 23: 1–4

Maret W and Vallee BL (1998) Thiolate ligands in metallothionein confer redox activity on zinc clusters. Proc Natl Acad Sci USA 95: 3478–3482

Matthews BW (1988) Structural basis of the action of thermolysis and related zinc peptidases. Acc Chem Res 21: 333–340

Miller GG and Strittmatter WJ (1992) Identification of human T-cells that require zinc for growth. Scand J Immunol 36: 269–277

Mills CF (ed) (1989) Zinc in Human Biology. Springer-Verlag, London

Miyazaki K, Funahashi K and Numata Y et al. (1993) Purification and chracterization of a two chain form of tissue inhibitors of metalloproteinase (TIMP) type 2 and a low molecular weight TIMP-like protein. J Biol Chem 268: 14387–14393

Mocchegiani E and Fabris N (1995) Age-related thymus involution: zinc reverses *in vitro* the thymulin secretion effect. Int J Immunopharmacol; 17: 745–749

Mocchegiani E, Santarelli L and Muzzioli M et al. (1995) Reversibility of the thymic involution and of age-related peripheral immune dysfunctions by zinc supplementation in old mice. Int J Immunopharmacol 17: 703–718

Mocchegiani E, Verbanac D and Santarelli L et al. (1997) Zinc and metallothioneins on cellular immune effectiveness during liver regeneration in young and old mice. Life Sci 61: 1125–1145

Mocchegiani E, Muzzioli M and Cipriano C et al. (1998a) Zinc, T-cell pathways, aging: role of metallothioneins. Mech Ageing Dev 106: 183–204

Mocchegiani E, Santarelli L and Tibaldi A et al. (1998b) Presence of links between zinc and melatonin during the circadian cycle in old mice: effects on thymic endocrine activity and on the survival. J Neuroimmunol 96: 111–122

Mocchegiani E, Ciavattini A, Santarelli L, Tibaldi A, Muzzioli M, Bonazzi P, Giacconi R and Fabris N (1999a) Role of zinc and alpha-2 macroglobulin on thymic endocrine activity and on peripheral immune efficiency (natural killer activity and interleukin-2) in cervical carcinoma. Br J Cancer 79: 244–250

Mocchegiani E, Muzzioli M and Gaetti R et al. (1999b) Contribution of zinc to reduce CD4+ risk factor for severe infection relapse in aging: parallelism with HIV. Int J Immunopharmacol 21: 271–281

Monti D, Troiano L and Tropea F et al. (1992) Apoptosis-programmed cell death: a role in the aging process? Am J Clin Nutr 55: 1208–1214

Muzzioli M, Provinciali M and Fabris N (1986) Age dependent modification of natural killer activity in mice. In: Facchini A, Haajiman JJ and Labò G (eds) Immunoregulation in Aging, pp 221–227. Eurage, TNO, The Netherlands

Muzzioli M, Mocchegiani E and Bressani N et al. (1992) *In vitro* restoration by thymulin of the NK activity of cells from old mice. Int J Immunopharmacol 14: 57–61

Nagase H (1996) Matrix Metalloproteinases. In: Hopper NM (ed) Zinc, Metalloproteases in Health and Disease, pp 153–204. Taylor and Francis, London

Orlowski C and Piotrowski JK (1988) Metal composition of human hepatic and renal metallothionein. Biol Trace Elem Res 65: 133–141

Otvos JD, Liu X and Li H et al. (1993) Dynamic aspects of metallothionein structure. In: Suzuki KT, Imura N and Kimura M (eds) Metallothionein III, pp 57–74. Birkhauser Verlag, Basel

Palmiter RD (1994) Regulation of metallothionein genes by heavy metals appears to be mediated by a sensitive inhibitor that interacts with a constitutively active trascription factor, MTF-1. Proc Natl Acad Sci USA 91: 1219–1223

Palmiter RD, Findley SD and Whitemore TE et al. (1992) MT III, a brain specific member of metallothionein gene family. Proc Natl Acad Sci USA 89: 6333–6337

Papageorgiou AC, Collins CM and Gutman DM et al. (1999) Structural basis for the recognition of superantigen streptococcal pyrogenic exotoxin A (Spe A1) by MHC class II molecules and T-cell receptors. EMBO J 18: 9–21

Pawelec G and Solana R (1997) Immunosenescence. Immunol Today 18: 514–516

Penkowa M, Giralt M and Moss T et al. (1999) Impaired inflammatory response to glial cell death in genetically metallothionein I and II deficient mice. Exp Neurol 156: 149–164

Piantanelli L, Basso A and Rossolini G (1994) Modelling of the link between aging rate and mortality rate. Ann New York Acad Sci 719: 136–145

Prasad AS (1998) Zinc and immunity. Mol Cell Biochem 188: 63–69

Prasad AS, Fitzgerald JT and Hess JW et al. (1993) Zinc deficiency in elderly patients. Nutrition 9: 218–224

Prasad AS, Beck FJ and Grabowski SM et al. (1997) Zinc deficiency changes in cytokines production and T-cell subpopulations in patients with head and neck cancer and in non cancer subjects. Proc Ass Am Physician 109: 68–77

Provinciali M, Di Stefano G and Stronati S (1998) Flow cytometric analysis of CD3/TCR complex, zinc and glucocorticoid-mediated regulation of apoptosis and cell cycle distribution in thymocytes from old mice. Cytometry 32: 1–8

Rink L and Seyfarth M (1997) Characteristic of immunological tests in the elderly. Z Gerontol Geriatr 30: 220–225

Saha AR, Hadden EM and Hadden JW (1995) Zinc induces thymulin secretion for human thymic epithelial cells *in vitro* and augments splenocytes and thymocytes response *in vivo*. Int J Immunopharmacol 17: 729–733

Savino W, Huang PC and Corrigan A et al. (1984) Thymic hormone containing cells. V. Immunohistological dection of metallothionein within the cells bearing thymulin (a zinc-containing hormone) in human and mouse thymus. J Histochem Cytochem 32: 942–946

Sbarbati A, Mocchegiani E and Marzola P et al. (1998) Effect of dietary supplementation with zinc sulphate on the aging process: a study using high field intensity MRI and chemical shift imaging. Biomed Pharmacother 52: 454–458

Scortengagna M, Galdziki Z and Rapoport SI et al. (1998) In cortical cultures of trisomy 16 mouse brain the up-regulated metallothonein I/II fails to respond to H_2O_2 exposure or glutamate receptor stimulation. Brain Res 32: 169–181

Shankar AH and Prasad AS (1998) Zinc and immune function: the biology basis of altered resistence to infection. Am J Clin Nutr 68: 447–463

Sobocinski PZ, Canterbury Jr WJ and Mapes et al. (1978) Involvement of hepatic metallothioneins in hypozincaemia associated with bacterial infection. Am J Physiol 234: E399–E406

Souttrp-Jensen L (1989) Alpha-macroglobulins: structure, shape and mechanisms of proteinase complex formation. J Biol Chem 264: 11539–11542

Stettler-Stevenson WG, Liotta LA and Kleiner DE Jr (1993) Extracellular matrix 6: role of matrix metalloproteinases in tumour invasion and metastasis. FASEB J 7: 1434–1441

Sullivan VK, Burnett FT and Cousins RJ (1998) Metallothionein expression is increased in monocytes and erythrocytes of young men during zinc supplementation. J Nutr 128: 707–713

Suzuki KT and Kuroda T (1995) Transfer of copper and zinc from ionic and metallothionein-bound form to Cu, Zn-superoxide dismutase. Res Comm Mol Pathol Pharmacol 87: 287–296

Suzuki KT, Nakajima K and Kawaharada U et al. (1992) Metallothionein in human brain. Acta Histochem Cytochem 25: 617–622

Tachibana I, Imoto M and Adjei PN et al. (1997) Overexpression of the TGF beta-regulated zinc finger encoding gene, TIEG, induces apoptosis in pancreatic epithelial cells. J Clin Invest 99: 2365–2374

Tanaka Y, Schiozava S and Moromoto I et al. (1990) Role of zinc in interleukin 2 (IL-2) mediated T-cell activation. Scand J Immunol 31: 547–552

Taylor GA and Blackshear PJ (1995) Zinc inhibits turnover of labile mRNA in intact cells. J Cell Physiol 162: 378–387

Tsukahara A, Seki S and Iiai T et al. (1997) Mouse liver T cells: their change with aging and in comparision with peripheral T-cells. Hepatology 26: 301–309

Turnlund JR, Durvin N and Costa F et al. (1986) Stable isotope studies on zinc absorption and retention in young and elderly men. J Nutr 116: 1239–1247

USDA (1980) Science and Education Administration, Food and Nutrient Intakes of Individuals in 1 day in United States, Vol 4, pp 45–61. Dept of Agriculture, Washington, DC

Vallee BL and Auld DS (1990) Zinc coordination, function and structure of zinc enzymes and other proteins. Biochemistry 29: 5647–5659

Vallee BL and Falchuk KN (1993a) The biochemical basis of zinc physiology. Phys Res 73: 79–118

Vallee BL and Maret W (1993b) The functional and potentional functions of metallothioneins. A personal perspective. In: Suzuki KT, Imura N and Kimura M (eds) Metallothionein III, pp 1–27. Birkhauser Verlag, Basel

Wellinghausen N, Kirchner H and Rink L (1997a) The immunobiology of zinc. Immunol Today 18: 519–521

Wellinghausen N, Martin M and Rink L (1997b) Zinc inhibits interleukin 1-dependent cell stimulation. Eur J Immunol 27: 2529–2535

West AK, Stallings R and Hildebrand CE et al. (1990) Human metallothionein genes: Structure of the functional locus at 16q3. Genomics 8: 513–518

Yu CW, Chen JH and Lin LY (1997) Metal-induced metallothionein gene expression can be inactivated by protein kinase C inhibitor. FEBS Lett 420: 69–73

Yurkow EJ and Makhijani PR (1998) Flow cytometric determination of metallothionein levels in human peripheral blood lymphocytes: utility in enviromental exposure assessement. J Toxicol Environ Health 54: 445–457

Zambenedetti P, Giordano R and Zatta P (1998) Metallothioneins are highly expressed in astrocytes and microcapillares in Alzheimer's disease. J Chem Neuroanat 15: 21–26

Zorning M, Schmidt and Karsunky H et al. (1996) Zinc finger protein GFI-1 cooperates with myc and pin-1 in T cell lymphonagenesis by reducing the requirements for IL-2. Oncogene 12: 1789–1801