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REVIEW ARTICLE

Inflammation, genes and zinc in ageing and age-related diseases

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Abstract Lifelong antigenic burden determines a condition of chronic inflammation, with increased lymphocyte activation and pro-inflammatory cytokine production. A large number of studies have documented changes in Zn metabolism in experimental animal models of acute and chronic inflammation and in human chronic inflammatory diseases. In particular, modification of zinc plasma concentration as well as intracellular disturbance of antioxidant intracellular pathways have been found associated to age-related inflammatory diseases, like atherosclerosis. Zinc deficiency is extremely diffused in aged people that are educated to avoid meat and other high Zn-content foods due to fear of cholesterol. Rather, they increase consumption of refined wheat products that lack of Zn, magnesium and other critical nutrients in consequence of refining process. On the other hand, plasma concentration of metallic ions like Zn is influenced by pro-inflammatory cytokines production. A major target of Zn may be NF-kB, a transcription factor critical for the expression of many pro-inflammatory cytokines whose production is finely regulated by extra- and intracellular activating and inhibiting factors interacting with regulatory elements on cytokine genes. Moreover, this factor is regulated by the expression of specific cellular genes involved in inflammation. So it is not surprising that Zn deficiency is constantly observed in aged patients affected by infectious diseases. On the other hand, cytokine genes are highly polymorphic and some of these polymorphisms have been found associated to age-related diseases as atherosclerosis. Therefore, Zn deficiency in individuals genetically predisposed to a dis-regulation of inflammation response, may play a crucial role, in causing adverse events and in reducing the probability of a successful aging.

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Immunosenescence

Immunosenescence, i.e. the alteration in innate and clonotipic immunity described in the elderly, is the consequence of the continuous attrition caused by chronic antigenic stress. Lifelong chronic antigenic load represents the major

driving force for immunosenescence, which impacts on human lifespan by reducing the number of virgin antigen non-experienced T cells, and, simultaneously, filling the immunological space with expanded clones of memory and effector, antigen-experienced T cells. Besides, this continuous antigenic load seems the main factor contributing to the age-associated increase of circulating inflammatory mediators. On the other hand, the inflammation is a non-specific response of the immune system against different pathogens that facilitates the return to physiological homeostasis and permits survival. However, if tissue organization is not restored the body establishes a low-grade irritation and inflammation becomes a chronic condition that continuously damages the surrounding tissues. Accordingly, a genetic predisposition for strong pro-inflammatory responses increases the probability to survive early fatal infections, and is positively selected by the evolutionary program. However, reduced pathogen load, improved medical care and quality of life in present day prosperous societies allow the majority of the people to survive over 50 years, an age where pro-inflammatory traits acquired during evolution results into detrimental effects in terms of chronic inflammation and decreased chance of long life survival. Chronic inflammation indeed is considered to be involved in the pathophysiology of Alzheimer's disease, atherosclerosis, diabetes, sarcopenia, cancer, infections and other age-related diseases with relevant inflammatory components (Candore et al. 2006a, b; Licastro et al. 2005; Vasto and Caruso 2005). As an example of a possible strategy to counteract the major effects of immunosenescence we have among others, dietary factors, which despite their ''extrinsic'' nature, are able to interact with the genome. This interaction may result in changes of gene expression that, in turn, affects many aspects of health including immune system (Mocchegiani et al. 2006a). In this review, a special emphasis has been given to zinc because its emerging role as second messenger and transducer of stress signal into gene expression might be strategic to counteract detrimental changes of the immune system during ageing (Bao et al. 2003). Many genes have been found in determining longevity both in men and animals. Some of them affect the immune functions and are related to Zn ion bioavailability such as p53. Also cytokines are modulated by Zn influencing some ''longevity genes'' which may be involved in prolonging the human lifespan related to the inflammatory status, in particular pro-inflammatory interleukins (IL), such as IL-1, IL-6 and tumour necrosis factor (TNF)-α, and anti-inflammatory ones, IL-10 (Table 1) (for references in Table 1 see: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?DB=pubmed, for reference on human longevity see bibliography).

Zinc metabolism

Zinc (~3 g) is the second most abundant trace element in the body after Fe (4 g) and considerably more abundant than Cu (Solomons et al. 1985). The body does not store Zn and a constant dietary intake is essential. Based on 1989 USA guidelines, the recommended daily allowances of this metal are 5 mg/day for infants, 10 for children, 15 for teenagers, adults and pregnant women and 16–19 for lactating women. Zn is normally obtained from red meat and other animal proteins, which not only have a high Zn content but in these meats Zn is bound to ligands which facilitate its absorption (Solomons et al. 1985). Other sources of Zn are sea foods, dairy foods, cereals and nuts (Dreosti et al. 1993). Most vegetables (including corn and rice) are not ready sources of Zn due to the presence of phytate, a component of plants, which chelates Zn and inhibits its absorption (Arsenault and Brown 2003). Diets with low content in animal protein and rich in phytate contribute to high incidence of Zn deficiency in many developing countries. Another cause of human primary Zn deficiency can be low Zn content of soils (e.g. on which cows are raised; Lambein et al. 1994). Inadequate intake of Zn is accompanied by a number of clinical manifestations, which may prove fatal unless treated. On the other hand, excess of this metal interferes with macromolecular synthesis and function and it is toxic to cells (Cunningham-Rundles et al. 2005). Zn balance is maintained through regulated intestinal uptake, faecal excretion, and renal

Table 1 Genes involved in life expectancy in different species

Gene	Species	Role	Comments
Genes that extend life expectancy or maximum lifespan			
Daf-2 and age-1	Nematodes	Insulin/IGF-I like receptor pathway and PI-3-kinase activity	Mutations in the daf-2 insulin receptor-like gene or the downstream age-1 phosphoinositide 3-kinase gene extend adult lifespan by two- to threefold. The nervous system is the key tissue involved in longevity by this pathway (Wolkow et al. 2000)
sir-2	Nematodes	NAD+ dependent histone deacetylase (DNA silencing)	Overexpression increases life expectancy, whereas sir-2 yeast mutants are short-lived (Tissenbaum and Guarente, 2001)
eat-2	Nematodes	Nicotinic acetylcholine receptor subunit	Loss of function extends lifespan 20–30% because of defects in pharyngeal function that possibly mimic caloric restriction (Lakowski and Hekimi, 1998). Eat-2 further extends lifespan of daf-2, but not clk-1, mutants
daf-16	Nematodes	Forkhead transcription factor	Loss of function allele shortens lifespan and overexpression increases modestly life expectancy and resistance to stress. It is required for life expectancy extension of daf-2, daf-23 and old-1 mutants (Henderson and Johnson 2001)
old-1 and old-2	Nematodes	Receptor tyrosine kinases	Overexpression increases life expectancy and resistance to stress (Rikke et al. 2000)
clk-1	Nematodes	Required for ubiquinone biosynthesis; may regulate replication of mitochondrial DNA	Overexpression shorten lifespan, whereas clk-1 mutants are long lived when fed *E. coli* that supply coenzyme Q_8 (Jonassen et al. 2001)
mev-1	Nematodes	Succinate dehydrogenase cytochrome b	Loss of function shortens life span to 66% of wild type and accelerates accumulation of aging-associated biomarkers such as protein carbonyls and fluorescent materials (Ishii et al. 1998)
Drop-dead	Fruit flies	Unknown	Drop-dead mutants die early in adult life, displaying gross neuropathological lesions in the brain (Buchanan and Benzer 1993)
InR	Fruit flies	Insulin/IGF receptor	InRE19/InRp5545 transheterozygous females show an 85% increase in life span (Tatar et al. 2001). No significant change in life span is reported in male animals
chico	Fruit flies	Insulin receptor substrate	Mutation of chico results in life-span extension of up to 48% in homozygous recessive female animals and 36% in heterozygous females. Male heterozygotes live 13% longer than wild type, but male homozygous recessive animals have a shortened life-span (Clancy et al. 2001)
indy	Fruit flies	Homologous to mammalian sodium dicarboxylate cotransporter	Flies heterozygotic for a disruption have extended lifespan (mean 87–92% longer, max 45% longer). Homozygotes for the disruption show only 10–20% increase in mean lifespan (Rogina et al. 2000)
hsp70	Fruit flies	Heat shock protein 70 kDa	Overexpression increases lifespan (Tatar et al. 2001)
sod1	Fruit flies	Cu/Zn-superoxide dismuase (antioxidant enzyme)	Overexpression increases lifespan, whereas total ablation reduce lifespan. Expression need occur only in motor neurons (Phillips et al. 2000)
mth	Fruit flies	Member of the 7-TM protein superfamily	Partial loss of function increases life expectancy and resistance to stress (Lin et al. 1998)
pcmt	Fruit flies	Protein carboxyl methyltransferase	Overexpression increases life expectancy at high temperature (Chavous et al. 2001)
Pit1	Mice	Transcription factor required for pituitary development	Mutations increases life expectancy, delay immune functions and collagen aging with also defects in growth hormone production (Flurkey et al. 2001)

Table 1 continued

Gene	Species	Role	Comments
Prop1	Mice	Paired-like homeodomain transcription factor important for anterior pituitary development	Mutations increase life expectancy with a phenotype similar to pit1 mice (Bartke et al. 2001)
Ghr	Mice	Growth hormone receptor	Mice homozygous for disruption of GHR/BP have a life-span that is 40–50% longer than wild type or heterozygous mice with high circulating levels of GH (Coschigano et al. 2000)
Ghrhr	Mice	Growth hormone releasing hormone receptor	Mutants have a life span that is 20% longer than wild type with low circulating levels of GH (Flurkey et al. 2001)
Klotho	Mice	Has homology to beta-glucosidases and is involved in undefined signalling pathways	Increased age-related pathologies (atherosclerosis and osteoporosis) (Kuro-o et al. 1997)
p66	Mice	The p66shc gene encodes an alternative splicing variant of signalling adaptor Shc, which is involved in EGF receptor signalling in the mitogen-activated protein kinase pathway	Mutations enhance resistance to apoptosis and increase life expectancy (Migliaccio et al. 1999)
Sod2	Mice	Mithocondrial Superoxide dismutase (antioxidant enzyme)	Premature death by cardiomiopathy (Li et al. 1995); increased sensitivity of cardiomiocytes to apoptosis but without affecting lifespan (van Remmen et al. 2001)
MsrA	Mice	Methionine sulfoxide reductase	MsrA–/– homozygous mutant mice have a 40% shorter life-span than wild type or heterozygote animals (Moskovitz et al. 2001)
Upa	Mice	Urokinase-type plasminogen activator	Overexpression in the hypothalamus decreases appetite and increases life expectancy (approximately 20%) perhaps by mimicking caloric restriction (Miskin and Masos 1997)
p53	Mice	Tumour suppressor protein	Partial deletion of gene leads to decreased cancer incidence with a premature aging phenotypes, and shortened life span (Tyner et al. 2002)
Genes associated with longevity and the risk of pathologies/disease in humans			
APOE	Human	Lipoprotein metabolism	Early onset of Alzheimer's disease (E4 variant), cardiovascular disease and other age-related diseases (Makley and Rall 2000). Centenarians display more frequently the E2 variant
ACE	Human	Angiotensin-converting enzyme (plays a role in regulating blood pressure)	A variant of ACE which predisposes to coronary heart disease is surprisingly more frequent in centenarians (Schachter et al. 1994)
PAI1	Human	Plasminogen-activator inhibitor1	Centenarians display significantly higher frequency of the 4G allele and of the homozygous 4G4G genotype associated with high PAI-1 levels which, in turn, is considered a predictor of recurrent myocardial infarction in young men (Mannucci et al. 1997)
HLA-DR	Human	Histocompatibility locus antigen	DR7, DR11 and DR13 variants are frequent in centenarians (Caruso et al. 2000, 2001)
WRN	Human	Processes both DNA helicase and exonuclease activity	Gene responsible for Werner's syndrome. Loss of WRN promotes aberrant mitotic recombination (Prince et al. 2001)

Table 1 continued

Gene	Species	Role	Comments
Hutchinson Gilford gene	Human	Components of nuclear laminae	Premature cardiovascular disease (Gilford 1904; Sandre-Giovannoli et al. 2003)
B3AR	Human	B-3 adrenergic receptor	Allelic form present affects time of onset of type II diabetes (Walston et al. 1995)
MTHFR	Human	5, 10-methylenetetrahydrofolate reductase	Deficiency leads to increased levels of homocysteine and DNA hypomethylation, with increased risk of cardiovascular disease and cancer (Heijmans et al. 2000)
KLOTHO	Human	Sequence similarity to family 1 glycosidases	The KL-VS allele of the human KLOTHO gene is more common in infants than in elderly people. Individuals homozygous for KL-VS have a 2.6-fold greater chance of dying by age 65 than individuals that are homozygous for the wild type KLOTHO gene (Arking et al. 2002)
Tumour suppressor gene RB, E2F-1 and BRCA1	Human	DNA repair/cell cycle regulation	Development of retinoblastoma and osteosarcoma/increased risk of developing breast and ovarian cancer (Wang et al. 2000; Yamasaki et al. 1998)
IGF-I/PI3KCB	Human	IGF-I response pathway	Genotype combinations at IGF-IR and PI3KCB genes affect free IGF-I plasma levels and longevity (Bonafe et al. 2003)
PON1	Human	Paraoxonase 1 (antioxidant enzyme)	PON 192Q/R polymorphism seems to have an impact both on increased mortality due to coronary heart disease (Christiansen et al. 2004) and on survival advantage (Rea et al. 2004)
ATM	Human	ATM (gene involved in ataxia-telangiectasia) codes for a checkpoint kinase required for phosphorylation of multiple proteins, including p53, BRCA1 and nibrin	Patients with ataxia-telangiectasia show decreased life span, with the maximum of 52 years (Broder 1985)
CETP	Human	Cholesteryl ester transfer protein is involved in the regulation of cholesterol transport and high-density lipoprotein (HDL) levels	Homozygosity for the I405V variant of CETP is associated with exceptional longevity among Ashkenazi Jews (Barzilai et al. 2003)
Trisomy 21	Human	Comparison of molecular breakpoints and detailed clinical evaluations led to a "phenotypic map" that was not consistent with a single region being responsible for most of the features of the syndrome	People with Down's syndrome develop the neuropathalogical lesions of Alzheimer's disease significantly earlier than those without (Wisniewski et al. 1985) and have a shorter life span
GH-1	Human	Human growth hormone	Mean life-span in 11 untreated, affected individuals homozygous for a deletion at the GH-1 locus was significantly shorter ($P < 0.05$) than in unaffected siblings or the general population (Besson et al. 2003)
MTP	Human	Microsomal transfer protein	An unknown locus on chromosome 4, recently identified as MTP (Geesamen et al. 2003), was previously found to be associated with long life-span among centenarians (Puca et al. 2001)

Table 1 continued

Gene	Species	Role	Comments
GR	Human	Glucocorticoid receptor	The ER22/23EK variant of nuclear receptor subfamily 3 group C member 1 (glucocorticoid receptor) is correlated with longevity in males (van Rossum et al. 2004)
SIRT3	Human	NAD-dependent protein deacetylase localized to mitochondrial	In a study of individuals from Calabria, Italy, the TT genotype of a silent G/T transversion (G477T) in the SIRT3 gene was found to correlate with longevity in males, but not females (Rose et al. 2003)
p53	Human	Tumour suppressor gene	Despite having a 2.5-fold increased cancer incidence, the p53 codon 72 Pro/Pro individuals (in a prospective study of individuals age 85 or older) exhibited a significant 41% enhanced survival compared to codon 72 Arg/Pro and Arg/Arg individuals (van Heemst et al. 2005). These results suggest that p53 protect against cancer but at a cost of longevity
P66(shc)	Human	Involved in the mitogen-activated protein kinase pathway (oxidative stress response)	Cells from centenarians present a peculiar regulation of p66(shc) compared to elderly and young that was affected by p53 codon 72 polymorphism (Pandolfi et al. 2005)
TLR4	Human	Lipopolysaccaride receptor (toll-like receptor 4)	TLR4 ASP299GLY polymorphism shows a significantly lower frequency in patients affected by myocardial infarction compared to controls, whereas centenarians show a higher frequency (Balistreri et al. 2004)
IL-6	Human	Interleukin 6 (pro-inflammatory cytokine)	The proportion of homozygotes for the G allele at –174 locus decreases in centenarian males (Bonafe et al. 2001). However, a follow-up study found that frequency of –174 allele G was clearly higher in the survivors of a cohort of nonagenarians (Hurme et al. 2005) These conflicting results may be the consequence of interaction among anti-inflammatory drugs, interleukin-6 –174 C/C genotype, and longevity (Reiner et al. 2005)
IL-10	Human	Interleukin 10 (anti-inflammatory cytokine)	High production of interleukin 10, a condition associated to –1082GG genotype, was found to be protective for acute myocardial infarction and a determinative parameter for longevity (Lio et al. 2004)
TNF-alpha	Human	Tumour necrosis factor (TNF) alpha (pro-inflammatory cytokine)	TNF –308 G > A promoter gene polymorphism seems a risk factor in age-related dementia and longevity (Bruunsgaard et al. 2004). The evaluation of combined IL-10 and TNF-genotypes showed that there was a significant increase of the "anti-inflammatory" (IL-10 –1082GG/TNF –308GG) genotype in centenarian men over controls (Lio et al. 2003)

reabsorption. Often Zn deficiency is found in elderly hospitalized patients, and very often associated with respiratory infections, cardiac failure and atherosclerosis (Beattie et al. 2004). Today, the elderly tend to avoid meat and other high Zn-content foods due to fears of cholesterol. Rather, they increase consumption of refined wheat products. Wheat was a main source of Zn in the historical diet, but today Zn, magnesium and other critical nutrients have been depleted by the refining process (Cunningham-Rundles et al. 2005).

Plasma contains, 0.1% whole-body Zn, it is the natural medium for Zn transport, and so plasma Zn is turned over very rapidly (about 150 times per day in human subjects) (King et al. 2000). The most labile Zn in human plasma is bound to albumin (about 80% plasma total Zn), with the remainder being more firmly associated with higher molecular mass proteins, predominantly α-2-macroglobulin (Chesters et al. 1998). Tissues, such as liver, also contain mobile reserves of Zn, but the kinetics of Zn turnover are slower than those in plasma and this tissue Zn is regarded as a separate pool in compartmental models of Zn metabolism (Lowe et al. 1993). Zn shows a rapid uptake into endothelial cells and may involve receptor-mediated endocytosis of albumin-bound Zn (Rowe and Bobilya 2000). The lability of albumin-bound Zn and the ease with which it is transported into endothelial cells suggests that the vascular endothelium may be particularly influenced by changes in plasma Zn levels, which are affected both by whole-body Zn homeostasis (Hambidge and Krebs 2001). Of interest, it is the intracellular Zn distribution and transport by special Zn-binding proteins termed metallothioneins whose task and function are extensively herein described in a specific chapter (see below).

Zinc functions

It is quite important to know that Zn is required for structural and functional integrity of several transcription factors (Coleman 1992) and more than 300 enzymes (Vallee and Falchuk 1993); therefore, almost every signalling and metabolic pathway in some way depends on at least one, and often several, Zn-requiring proteins. Many Zn-finger proteins bind Zn relatively tightly and have not been regarded as sensitive to changes in cellular total Zn concentrations, which are in the upper milliMolar to lower microMolar concentration range (Valle and Falchuk 1993). However, recent evidence suggests that ''free'' Zn, which is most likely to influence activation of Zn finger proteins and enzymes, is present at fM concentrations (Outten and O'Halloran 2001). Hence, small, localised increases in ''free'' or labile Zn within cells may have disproportionate influences on Zn-dependent protein activation in relation to the overall cellular Zn concentration. In addition, localised intracellular oxidative stress may enhance the lability of Zn within Zn fingers (Webster et al. 2001).

The bulk of body Zn is tightly bound within cellular metallo-enzymes and Zn finger proteins (Vallee and Falchuk 1993). This fixed pool of Zn turns over very slowly and is mainly responsible for housekeeping functions in cellular metabolism and gene expression. The remaining 10–15% of Zn (labile Zn) comprises of more dynamic pools that are readily depleted in Zn deficiency. While fixed Zn is distributed uniformly throughout the body, labile Zn is concentrated in certain tissues and in specific regions within tissues. The most populated class of Zn-binding proteins is hydrolase, transferase and k voltage-gated channels (Andreini et al. 2006)

An intriguing point is the involvement of Zn in NF-kB transcription factor, which in activated cells translocates to the nucleus and it is critical for the expression of many pro-inflammatory cytokines (Aggarwal et al. 2004). In fact, NF-kB is a key transcription factor that regulates the expression of many genes and signalling pathways related to apoptosis and the immune and inflammatory responses by increasing the expression of specific cellular genes, including cytokines, chemokines and the major histocompatibility complex. In this context, the effects of Zn on translocation of this factor have been attributed to suppression of phosphorylation and degradation of the inhibitory proteins that normally sequester NF-kb in the cytoplasm (Jeon et al. 2000). Besides, NF-kB can stimulate the expression of enzymes including the inducible form of

nitric oxide synthase (iNOS), which is in turn codified by Zn finger motifs (Kroncke et al. 2001). iNOS generates nitrous oxide (NO) and the inducible cyclo-oxygenase (COX-2) that generates prostanoids (Tsatsanis et al. 2006). These metabolites contribute to the patho-physiology of the inflammatory process and inflammatory diseases. Therefore, Zn levels may play a central role in the inflammatory signal transduction.

Metallothionein

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 Zn and copper (Bremner and Beattie 1990). MTs bind Zn with high binding affinity and distribute cellular Zn because Zn undergoes rapid inter- and intra-cluster exchange (Otvos et al. 1987).

The biological role of MTs is crucial in antioxidative and immune responses during ageing and age-related diseases (Nath et al. 2000). Among cytokines, IL-1, IL-6, TNF-α and interferon γ have the ability to induce MTs. Interestingly, Zn is also involved in gene expression of MTs (Sato et al. 1992).

Among the different functions, MTs constantly retrieve Zn from plasma and tissues. This, induce low Zn ion bioavailability and, consequently, impair immune responses (Mocchegiani et al. 1998). Thus, MTs may not be the donors of Zn in ageing but rather sequesters of Zn. On the other hand, increased MT levels induce down-regulation of many other biological functions related to Zn, such as metabolism, gene expression and signal transduction. Furthermore, it is suggested that low Zn ion bioavailability is crucial for entire immune efficiency in ageing (Mocchegiani et al. 1998). Moreover, because of the preferential binding of MTs with Zn rather than copper in ageing (Hamer 1986; Mocchegiani 2002a), different roles of MTs (from protective to dangerous) may be further supported because of Zn deficiency in ageing, and Zn ion bioavailability is essential for immunity in ageing and age-related disease (Wellinghausen et al. 1997). On the other hand, the inflammation process provokes consistent Zn loss by urine and faeces (Wapnir et al. 2000), but, at the same time, pro-inflammatory cytokines production, which in turn induce MT-RNA (Andrews et al. 2000). As a result, the crude Zn balance is negative, the Zn ion bioavailability is lower and MT increases sequestering the remaining Zn ions, as reported in young mice during transient inflammation (Mocchegiani et al. 2002b). This phenomenon is more marked in chronic inflammation, such as in ageing. So apparently, metallothioneins are sequestering Zn rather then make it available for the body function.

Atherosclerosis

In the past decade, atherosclerosis has come to be recognized as active and inflammatory rather than simply a passive process of lipid infiltration or a reparative process after endothelial injury. In general, atherosclerosis can be considered as an intramural chronic inflammation resulting from interactions between modified lipoproteins, monocyte-derived macrophages, lymphocytes, and the normal cellular elements of the arterial wall. The process of inflammation occurs in response to functional and structural injury through a variety of known and unknown stimuli and is active over years and decades. In this disease, lesion progression depends on genetic make-up, gender and certain well-recognized risk factors as well as a number of non-traditional risk factors (Licastro et al. 2005; Hansson et al. 2005; Candore et al. 2006a–c). The impact of trace metal deficiencies on this process is less well defined and studied, although the influences of Se and Cu on atherogenesis and heart disease have attracted much attention (Kohrl et al. 2000; Alissa et al. 2003). Zinc deficiency may also be the cause of hypertension, via nitric-oxide pathway, which is a relevant risk factor for atherosclerosis (Lyon et al. 2003). Ripa and Ripa 1994 argued that the antioxidative action of Zn prevents oxidation of LDL and consequently stops the main mechanism of atherogenesis. Zn also affects the activity of lipase (Kettle et al. 2000), which is an enzyme

involved in cholesterol production (Braschi et al. 1998). In fact, low Zn is correlated with high total cholesterol (Abu-el-Zahab et al. 1991). Therefore, the Zn ion bioavailability is very important in atherosclerosis because a lack of this trace element may be the cause of the development of the atherogenic pathological process (Beattie and Kwun 2004) in its various aspects: from the increased LDL peroxidation, to hypertension, up to hypercholesterolemia. In fact, Zn requirement is increased during inflammatory condition, such as atherosclerosis, by the vascular endothelium where apoptosis is prevalent. It is possible that certain lipids and fatty acids can interact with TNF-mediating endothelial apoptotic cell death, where Zn has the function to inhibit the pathway of the signal transduction leading to apoptosis (Henning et al. 1999). Furthermore, Zn binds to metallothionein (MT), which has been described with antioxidant function (Miles et al. 2000), and seems to play an influential role on Zn metabolism during periods of stress (Carey et al. 2000) as well as in atherosclerosis (Giacconi et al. 2004).

IL-6 and Zn

Pro-inflammatory cytokines are believed to play a pathogenetic role in age-related diseases. Genetic variations located within their promoter regions (mostly SNPs) have been shown to influence the susceptibility to age-related inflammatory diseases, such as AD and atherosclerosis, by increasing gene transcription and therefore cytokine production and reciprocally influencing the longevity. IL-6 plays a pivotal role in acute phase response and in the balancing of the pro-/anti-inflammatory pathways. Its –174CG polymorphism associated to different cytokine production has been studied in age-related diseases and longevity (Candore et al. 2006c; Rea et al. 2006).

Although IL-6 appears to be a predictive factor in future cardiovascular events, the role of this most common IL-6 SNP with respect to cardiovascular diseases is far from clear. A number of studies report markedly divergent results for the association of the –174 high IL-6 producer allele with cardiovascular risk or with other important parameters such as arterial responsiveness or CRP serum levels (Candore et al. 2006c; Giacconi et al. 2004).

Concerning longevity, there is a trend for a reduction in GG homozygosity in elderly males in three countries across Europe. That seems intriguing since it appears to confirm in different study populations and with a different study design, the earlier findings obtained in the Italian population. However, in later studies no difference in the IL-6 Á174 C/G promoter allelic and genotypic frequencies between centenarians and controls was reported but the number of subjects enrolled in these studies was low. Conversely, a modest, but significant, increase in the frequency of IL-6 –174 GG homozygotes with age was noted in a large group of Danish subjects, though no analysis was carried out for gender. The reason for the discrepancies, regarding the association between longevity and the IL-6 –174 C/G polymorphism, is unclear. The ethnic difference as well as the lifestyle and cultural difference among these populations could play a role, as well as other unidentified factors (Rea et al. 2006).

On this basis, Giacconi et al. (2004) screened atherosclerotic male patients for the two IL-6 –174 genotypes (C+ and C–) in relation to Zn ion bioavailability, MTs homeostasis and NK activity in comparison with healthy elderly and nonagenarians. Patients with a C– genotype showed impaired NK activity, a higher degree of serum inflammatory cytokines IL-6 and TNF-α, lower Zn ion bioavailability and increased MTmRNA when compared to patients with the C+ genotype. The same trend was observed in healthy elderly C–, whereas in nonagenarians, the presence of C+ genotype seemed to improve NK immune performances. These findings suggest that chronic inflammation by high persistent levels of IL-6 provokes, especially in patients with the C– genotype, a more marked sequestering of intracellular Zn ions by MTs coupled with a still more impaired immune efficiency. The subsequent limited capacity of Zn release by MTs in chronic inflammation (Malavolta et al. 2006) suggests that Zn ion bioavailability, via IL-6 and MTs homeostasis, may be crucial in atherosclerosis.

TNF-α and Zn

The TNF gene cluster located in the HLA region on chromosome 6, encodes the inflammation-related proteins TNF-α. Genetic variations in the –308G/A SNP in TNF-α gene affect cytokine production, being A+ carriers high producers (Candore et al. 2002). Inconclusive results have been obtained on the role of this SNP in age-related diseases and longevity (Rea et al. 2006; Candore et al. 2006c). However, by the innovative approach to study potential susceptibility genes for cardiovascular diseases, to use of centenarians as healthy controls, Lio et al. (unpublished) have recently demonstrated that A+ genotype is significantly overrepresented in cardiovascular disease patients and underrepresented in centenarians. So these results suggest a negative effect of the A+ carrier status in longevity. In any case, genetic variation in the –308 SNP seems to affect the clinical outcome of some infectious diseases (Cipriano et al. 2005). On this basis, Cipriano et al. 2003 have screened healthy young and old subjects, nonagenarians and old patients affected by the acute phase of chronic obstructive bronchitis and bronchopneumonia of bacterial origin for this locus, by grouping them as either A+ or A–. They evaluated the MT and pro- and anti-inflammatory cytokine gene expressions, plasma Zn levels and NK activity in these subjects stratified according to the SNP. The frequency of the A allele was increased in infected patients in comparison with healthy old controls. No differences existed between A+ and A– young adult, old and nonagenarian controls in tested parameters. Conversely, A+-infected patients displayed elevated IL-6, TNF-alpha and MTmRNA, low IL-10 coupled with impaired NK cell cytotoxicity and lower Zn ion with respect to A– patients. Therefore, the –308A polymorphism at the locus of TNF-alpha may be one of the susceptibility factors for infectious diseases in old persons, particularly considering its association to the increased release of pro-inflammatory cytokines and to the reduction of Zn release and MTs synthesis involved in the control of the inflammatory response.

Conclusions

It is quite clear that antioxidant and micronutrients in the diet, such as Zn, influence the development and function of immune cells, the activity of stress-related proteins and antioxidant enzymes and help to maintain genomic integrity and stability. All these functions occur through the action of proteins involved in the regulation of Zn homeostasis, such as MT, which bind Zn with high affinity but, at the same time, release free Zn ions in response to oxidative/nitrosative stress to modulate the expression of Zn-dependent genes and to activate antioxidant enzymes and impact immune response. Thus, the role of Zn is mainly to transducer oxidative stress and other signals converging at the production of nitric oxide into a specific intracellular response, suggesting an intriguing task of ''signal transducer'', similar to that found for calcium in the past. However, many aspects of this model are still unexplored, because the intracellular mechanisms involved in the regulation of Zn homeostasis have been poorly studied in ageing (Mocchegiani et al. 2006b).

However, in this paper, we focused our attention on the pivotal role played by the Zn–gene interaction in affecting some relevant cytokines (IL-6 and TNF-alpha) in ageing, successful ageing (nonagenarians) and in some age-related diseases (atherosclerosis and infections). The polymorphisms of the genes codifying these proteins are predictive in longevity, such as IL-6 –174G/C locus, or in worsening atherosclerosis or severe infections, such as TNF-alpha –308G/A locus. Taking into account that longevity has a strong genetic component but, at the same time, is affected by the life style and environmental factors, the analysis of these polymorphisms in association to some immune parameters (NK cell cytotoxicity) and nutritional factors (Zn) is a useful tool to unravel the role played by these genetic factors in longevity and in the appearance of age-related diseases. Indeed, these polymorphisms are associated with chronic inflammation, low Zn ion bioavailability, depressed innate immune response and high gene expression of MT, which have a limited Zn release for an optimal innate

immune response in ageing. Therefore, the nutrient (Zn)–gene (IL-6, TNF-alpha) interaction is pivotal to keep under control the inflammatory/immune response, indicating these genes as ''robust'' for ''healthy ageing''.

Improving our knowledge of the many factors related to the control of Zn homeostasis and immune efficiency in ageing is necessary in order to develop frailty prevention programmes based on Zn supplementation. In fact, it is clear that Zn is of extraordinary and diverse importance in human biology and nutrition but, especially in ageing, Zn research is still in a relatively immature stage of development. New knowledge on the function of MT, Zn transporters and on the effect of the genetic background has been acquired or is emerging, based on innovative methods and technologies for the assay of Zn ion availability and the related activity of Zn-dependent enzymes (Mocchegiani et al. 2006b).

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