The role of metals in autoimmunity and the link to neuroendocrinology

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Abstract

Current available literature indicates a risk for metal-induced autoimmunity in man. Metal pathology may be due to toxic or allergic mechanisms where both may play a role. The main factors decisive for disease induced by metals are exposure and genetics which determine the individual detoxifying capacity and sensitivity to metals. This paper reviews the possible mechanisms which may play a role in metal-induced autoimmunity with the emphasis on multiple sclerosis (MS), rheumatoid arthritis (RA) and amyotrophic lateral sclerosis (ALS). We also discuss the role of inflammation-induced changes in the hypothalamus-pituitary-adrenal (HPA) axis as a possible explanation of fatigue, depression and other psychosomatic symptoms observed in these diseases. The increased knowledge about individual sensitivity based on genotype and phenotype variability together with the use of biomarkers for the diagnosis of this individual susceptibility seems to be the key in elucidation of the operating mechanisms. Since metal-induced sensitization may be induced by chronic low-dose exposure, the conventional toxicological approach comparing concentrations of metals in brain autopsies, organ biopsies and body fluids in patients and controls may not provide answers regarding the metal-pathology connection. To address this issue, longitudinal studies of metal-sensitive patients are preferable to the traditional case-control studies.

INVITED NEL REVIEW

Abbreviations

ALS	amyotrophic lateral sclerosis
ANA	anti-nuclear antibodies
ANoA	anti-nucleolar antibodies
APC	antigen-presenting cells
CFS	chronic fatigue syndrome
CNS	central nervous system
CSF	cerebrospinal fluid
DMSA	dimercaptosuccinic acid
D-pen	D-penicillamine
FALS	familial amytrophic lateral sclerosis
GFAP	glial fibrillary acidic protein
GLU	glutamate
HLA	human leukocyte antigen
HPA	hypothalamus-pituitary-adrenal
Ig	immunoglobulin
IL-1	interleukin 1
LFA-1	lymphocyte function-associated antigen-1
m-Ab	monoclonal antibodies
MBP	myelin basic protein
MCS	multiple chemical sensitivity
MHC	major histocompatibility complex
MELISA®	Memory Lymphocyte Immuno Stimulation Assay
MND	motor neuron disease
MS	multiple sclerosis
MT	metallothioneins
NK	natural killer
OH-	hydroxyl radical
PLC	phospholipase C
PLP	proteolipid protein
PNS	peripheral nervous system
RA	rheumatoid arthritis
ROS	reactive oxygen species
SH	sulfhydryl
SLE	systemic lupus erythematosus
SOD	superoxide dismutase
TNF	tumor necrosis factor

Introduction

Our demands for a high living standard create an increasingly artificial environment. We are subjected to accumulating pollution from industry, exhausts, magnetic fields (from computers, cellular phones, lamps), implants (such as bone screws, silicone breasts, dental fillings), food (preservatives, food colorings) and psychological stress. In parallel, many modern-time diseases are steadily increasing in frequency. These diseases include allergies, multiple chemical sensitivity (MCS) [1], chronic fatigue syndrome (CFS), sensitivity to electro-magnetic fields, depressions and the vast group of autoimmune diseases.

Possible factors in the pathogenesis of auto-immunity

Environmental factors that are implicated in the development of autoimmune diseases include bacteria, viruses, and xenobiotics such as chemicals, drugs and metals. Many cases of autoimmunity debut after an infection. However, it seems that despite persistent research efforts, no conclusive evidence has linked certain microorganisms or viruses to the pathogenesis of autoimmune disease. That subject is, however, beyond the scope of this article which will focus on the current knowledge concerning the possible etiological role of metals in the pathogenesis of autoimmune disease.

Several factors have been studied concerning the induction of autoimmunity. Certain types of major histocompatibility complex (MHC) are associated with an increased risk for autoimmunity in animal models. In man, the human lymphocyte antigen (HLA) linkage to susceptibility has only a relative predictive value, thus indicating that other factors also contribute to the development of autoimmunity. Obvious is the overrepresentation of female patients in certain autoimmune diseases (for example, in SLE, the female predominance ratio is 10–20:1, for MS 10:1 [2]), indicating that sex hormones may play a role in the pathogenesis. It is also common that autoimmune disease debuts in women of child-bearing age, when the levels of estrogen and progesterone peak. The observation that monozygous twins do not always develop the same disease (e.g. the concordance rate of autoimmune diabetes is around 30% [2]) further indicates that there are other factors involved in the pathogenesis of autoimmune disease. In one study [3], the results of HLA typing of metal-sensitive patients showed higher frequencies of certain HLA antigens, among others HLA DR 4 and HLA B27. In another study [4], HLA DR 4 antigen was significantly increased in palladium-sensitive patients.

The effects of metals in biological systems: toxicity and allergy

Available literature clearly demonstrates metal-induced autoimmunity in animal systems [5]. Reports that link metal exposure to the development of autoimmunity in man include epidemiological studies, occupational exposure to metals, and a high prevalence of side-effects following treatment with metal chelators and colloidal gold.

Metals in nature occur bound to sulfur groups in metal ores in the ground. When extracted for indus-

trial use, they are purified and thereby lose their chemical stability. Some transition metals such as iron, cobalt, zinc, selenium, molybdenum, magnesium, chromium, manganese and copper are essential for life. Others, such as titanium, chromium, iron, nickel, copper, palladium, silver, platinum, gold and mercury are widely used in industry and in various implants. Except for chromium, iron and copper, those metals have no established function in man.

In living organisms, metals exert their effects in different ways. They avidly bind to sulfhydryl (SH) groups but also to -OH, NH_2 and Cl groups in proteins, enzymes, co-enzymes and cell membranes. The metal binding interferes with cellular processes, changing membrane charge, permeability, and the antigenicity of autologous structures. Metals in ionic form reach cell membranes attached to circulating blood proteins, particularly the water-soluble component of lipoproteins. Here, the affinity is strongest for SH-containing molecules such as methionine, cysteine and glutathione. It is this feature that allows ionic metals to exchange freely between lipoprotein and the macromolecules of ligands of cell membranes, including red blood cells. The hemoglobin of red blood cells is particularly rich in SH groups which further explains how ionic metals reach the various cell membranes via blood. Since metals in ionic form are lipophilic, they readily pass the blood brain barrier. For example, mercury vapor readily oxidizes in brain and nervous tissue to its ionic form, where ionic mercury binds with SH groups of cell membranes, protein and brain enzymes [6].

The toxic effects of metals are mediated through free radical formation, cell membrane disturbance or enzyme inhibition, among others. By binding to cell membranes, metals alter the membrane charge, which may result in changed membrane permeability, calcification and cell death. Metals also bind to mitochondria, thereby impairing cellular respiration [7]. Depending on genetically determined detoxification systems, an individual may tolerate more or less exposure to toxic metals before showing adverse effects.

The immunological effects of metals are either non-specific such as immunomodulation or antigen-specific such as allergy and autoimmunity. Metals may act as immunosuppressants (cytostatically) or as immunoadjuvants (non-specific activation of the immune system). One example of immunomodulation is the ability of metals to modify cytokine production *in vitro* and *in vivo*. The resulting imbalance between Th1 and Th2 activation can result in immunodysregulations leading to impaired cell-mediated immunity and/or aberrant humoral immunity that may culminate in autoimmune disease. Heo *et al.* found that lead and mercury enhanced IL-4 production by a Th2 clone (and inhibited Th1 proliferation) *in vitro* and *in vivo*. This suggests that these metals may induce an autoimmune response by dysregulating the balance between Th1 and Th2, which could enhance the production of antibodies to self-antigens [8]. Another example is the enhancement of the intensity and duration of antigen-specific IgE responses by gold salts [9], mercury, platinum and aluminum [10, 11].

Metals may also induce allergy in genetically susceptible individuals. Most of these are of type 4 (delayed-type hypersensitivity, such as contact dermatitis) but immediate-type reactions are sometimes also observed [12-14]. It can be anticipated that cellular reactions triggered by metals may operate elsewhere in the body where metals are deposited. Traditionally, metal allergy has been diagnosed by patch test. This method has, however, several drawbacks; objective interpretation is difficult, application of allergen onto skin may aggravate an existing allergy and, finally, it harbors the risk of *de novo* sensitization. Recently, Penz *et al.* compared the diagnostic efficiency of the expression of CD69 activation markers, cytokine release and lymphocyte stimulation test (LST) in nickel allergy. Of the tests, LST had the highest diagnostic efficiency (87%) for the diagnosis of nickel sensitization [15]. LST has been used in immunology diagnostics for delayed-type hypersensitivity for decades [2]. Memory Lymphocyte Immuno Stimulation Assay (MELISA®) has been found particularly useful for diagnosis of metal allergy *in vitro* [16–19].

Several mechanisms are proposed for how metals act within the immune system and induce autoimmunity. Metals bind to SH and other groups, thereby modifying self-proteins which via T-cells may activate B-cells and render the altered self-protein target for autoantibodies. Due to cross-reactions, the T-cells may also react to the native protein. Metal-binding directly to MHC II without prior processing by antigen-presenting cells or even directly to the T-cell receptor is also proposed. Another possibility is described in a study of scleroderma where autoantigens possess metal-binding sites, which after binding will generate free radicals. Free radicals will fragment the auto-antigens, thereby exposing cryptic epitopes which may then trigger autoimmunity [20]. In this case, the metal is not a part of the autoimmune epitope.

In his excellent review on metal-induced autoimmunity, Bigazzi [21] provides further evidence that metals may cause aberrant MHC II expression on target cells, inhibit T-suppressor cells, cause alterations in the idiotype-anti idiotype network and induce heat-shock proteins. These and other factors may play a role in metal-induced autoimmunity.

Autoantibodies

Autoantibodies occur in systemic and organ-specific autoimmune disease. Since autoantibodies sometimes occur before the onset of disease, they can be used as predictive markers. Some are disease-specific markers and used to establish a diagnosis, to record progression and predict outcome of the disease. Both drugs and heavy metals are known to induce autoantibodies [22]. Monestier *et al.* found that treatment with D-penicillamine (D-pen) or quinidine, two lupus-inducing drugs in humans, resulted in production of autoantibodies against chromatin antigens in genetically susceptible mice [23]. The authors found that the V_h chains of several D-pen or quinidine-induced monoclonal antibodies (mAb) are most similar to those of anti-nucleolar mAb obtained from mercury-injected mice. The authors refer to a study showing that cross-reactive idiotypes are shared by autoantibodies induced by heavy metals, D-pen and in graft-vs.-host reactions.

The potential of heavy metals to induce autoantibodies has been investigated in animal models. Originally described by Druet [24], it has since then been confirmed by other groups. Eneström *et al.* showed that both mercury and silver induced anti-nucleolar antibodies (ANoA), targeted against fibrillarin, in genetically susceptible mice [25, 26].While mercury furthermore induced systemic immune complex deposition and polyclonal activation of B- and T-cells, silver did not. Pollard and colleagues, who demonstrated the same results with regards to mercury, ANoA and fibrillamin, propose that mercury binds to the thiols in the cysteine group of fibrillamin, thereby changing its antigenicity and subsequently evoking the production of autoantibodies [27]. In patients with systemic scleroderma, ANoA in about half of the patients reacted with fibrillamin. After exposure to mercury, certain strains of rats produced high levels of antibodies to laminin [21].

In a recent article [28], El-Fawal and co-workers studied the immune status of metal-exposed workers and experimental animals. Antibodies to neuronal cytoskeletal proteins, neurofilaments and myelin basic protein (MBP) were frequently present in the sera of male workers exposed to lead and mercury. The titers correlated with blood and urinary concentrations of those metals. Similar results were obtained in animal systems. In rats exposed to metals, histopathology showed central nervous system (CNS) and peripheral nervous system (PNS) changes as well as astrogliosis. The authors conclude that autoantibodies can be used to monitor the neurotoxicity of environmental chemicals and that immune mechanisms may be involved in the progression of neurodegeneration.

Rheumatoid Arthritis

The joint inflammation in rheumatoid arthritis (RA) is characterized by invasion of T-cells in the synovial space and proliferation of activated macrophages and fibroblasts in the synovial intima. Further, in many cases, plasma cells producing rheumatoid factor can be detected. The localized CD4+ T-cells show strong signs of activation, and trigger macrophages and immunoglobulin (Ig)-producing cells in the joint. These macrophages produce proteolytic enzymes and pro-inflammatory cytokines such as IL-1 and TNF that contribute to cartilage and bone destruction. The T-cell activating antigen is presently unidentified. RA is linked to HLA-DR4, indicating that antigens presented on this HLA type may be important in the pathogenesis of the disease [29].

At many sites in the synovium, the histopathologic findings resemble those of a classic delayed-type hypersensitivity reaction [30]. The majority of synovial T-cells are of the memory type and express activation antigens like HLA-DR and transferrin receptors on their surface. Large, strongly HLA-DR positive macrophages and dendritic cells form close contact with the T-cells. In comparison to normal synovial lining cells, rheumatoid synovial dendritic cells are extremely efficient in allogenic T-cell activation [30]. Furthermore, most data on humans are consistent with the hypothesis that RA is not caused by antibodies to type 2 collagen but that the inflammatory response is amplified by production of these antibodies [30].

The rheumatic joint also shows an increased activity of macrophages and leucocytes which are producing reactive forms of oxygen, so-called reactive oxygen species (ROS) or free radicals [31]. Transition metals are known to catalyze free radical formation (e.g. the Fenton reaction) [32]. It is shown that ROS degrade cartilage components and activate leukocyte collagenase [33]. Additionally, free radicals mediate lipid peroxidation and oxidize Ig, which is also found in the rheumatic joint [33].

Pedersen *et al.* discuss the link between the exposure to heavy metals in paint pigments and

the development of RA [34]. The causal relationship between metals and the development of RA is reviewed by Kusaka [35]. Notably, the treatment of RA includes the administration of gold salts, penicillamine, antioxidants and sulfa-based drugs. The frequency of side-effects induced by gold and penicillamine treatment is high, and the symptoms in the affected patients resemble those of chronic metal exposure.

Colloidal gold is a routine treatment of RA. The effects of gold drugs include inhibition of monocyte-induced proliferation of lymphocytes [36]. Further, gold accumulates in lysosomes of macrophages and stabilizes lysosomal membranes, leading to reduced production of free radicals [37]. It is known that therapeutic gold can sometimes exacerbate the disease, and that phospholipase C (PLC) and arachidonic acid are increased in RA patients. Gold interacts with selenium *in vivo*, decreasing the amount of this essential trace element [38, 39]. Goldberg *et al.* found that in contrast to other metals, gold in low concentrations stimulates leukocyte collagen synthesis while higher concentrations decrease collagen synthesis [38]. Gold also induces the production of metallothioneins [40].

The fact that gold allergy today is frequent [41] should be taken into account in the treatment of RA patients with gold preparations, as demonstrated by one study [42]. In this study, an intramuscular test dose of gold sodium thiomalate induced a flare-up of previously positive epicutaneous and intradermal test sites, with a histological and immunohistochemical picture compatible with an allergic contact dermatitis. Several studies have reported debut of gold allergy as determined by positive patch test after colloidal gold treatment in RA [42–44]. Of drugs causing cutaneous reactions, gold salts used in the treatment of RA are the most frequent [42].

Another drug used in the treatment of RA is D-penicillamine (D-pen). The total frequency of side-effects is high [45–47] and amounts to 30-60%, of which acute hypersensitivity reactions constitute 2-10%. Severe side-effects include toxicity and autoimmune phenomena. The toxic effects include thrombocytopenia and leukocytopenia (5-15%), gastrointestinal disturbances (10-30%), changes or loss of taste (5-30%), loss of hair (1-2%), proteinuria (5-20%) [35] and skin pigmentation [48]. Autoimmune side-effects occur in about 1% of treated patients and include pemphigus, SLE (systemic lupus erythematosus), polymyositis [46], membranous glomerulopathies and myasthenia. Since D-pen is a thiol (containing an SH-group), it has long been used as a chelating agent for various forms of metal toxicity. Metals are also routinely used for the detection of D-pen and

protein thiols [49]. The mechanism of penicillamine action has been studied by several groups [45, 50]. Interestingly, in one study [47] the histological findings of mercury-induced glomerulonephritis are virtually indistinguishable from the picture induced by D-pen. This implicates that the latter case is perhaps not caused by D-pen *per se*, but rather by the mobilized metal. Halliwell [51] showed that the chelating agent Desferal prevents iron-dependent formation of hydroxyl radicals involved in the destruction of the inflamed joint. The same author discusses the role of free radicals in RA [51, 52]. D-pen oxidation is catalyzed by transition metals, i.e. the metal is simultaneously reduced [53].

Swollen and aching joints, among other systemic symptoms, are reported by some women with silicone breast implants. The alleged offending material, silicone, is a synthetic polymer containing a silicon-oxygen backbone [54]. The authors claim that the polymeric and hydrophobic characteristics of silicone and the presence of electrostatic charges and organic side groups make silicone a potentially ideal immunogen. Since silicon (Si) is an essential constituent of proteoglycans, it could cross-react with connective tissues. In one study comprising 46 patients and 45 controls, 35% of women with health problems attributed to silicone breast implants had anti-collagen antibodies (to collagen type I and II), while only 8.8% of the control group did [55].

Multiple Sclerosis

In multiple sclerosis (MS), an autoimmune T-cell attack on the CNS myelin sheath results in demyelinated plaques. The periventricular white matter, medulla oblongata and the optic nerves are most commonly affected, but any part of the CNS can be involved. The plaques commonly surround venules. In active plaques, a disrupted blood-brain barrier and some edema can often be seen. Inflammatory cells, including activated T-cells, plasma cells and macrophages are prominent and accumulate around centrally located vessels, and in the periphery where myelin loss occurs. Microscopical changes include loss of myelin; however, axons are relatively spared.

The demyelinization causes the common symptoms of MS, such as disturbances in vision, coordination, speech, strength, sensation and bladder control, among others. Genetically, MS is linked to HLA-DR2 [2]. The relatively low concordance of monozygotic twins to develop the disease (25-30%) [56] suggests that the myelin basic protein (MBP)-specific T-cell repertoire may be shaped differently even in monozygotic twins [57], and also that other factors may operate in the pathogenesis of the dis-

ease. Robinson *et al*. discuss a connection between MS and genes encoded within or closely linked to the TCR (T-cell receptor) beta chain gene complex [58].

Several epidemiological studies link environmental metal exposure to the subsequent development of MS. Ingalls *et al* describe the outbreak and clustering of MS and other demyelinating diseases as well as myasthenia gravis following pollution of the environment with large concentrations of heavy metal wastes in sewage and river water in one area [59, 60]. Irvine *et al*. find that areas with soils low in copper, iron and vanadium, but high in lead, nickel and zinc, and with drinking waters low in selenium and sulfate may predispose to MS [61].

Can metals cause demyelinization? Schwyzer *et al*. discuss how exposure to toxic low- molecular weight substances cause modification of protein or glycoprotein in the myelin sheath [62]. This induces the formation of autoantibodies and phagocytosis of the damaged myelin will lead to the formation of plaques. Simultaneously, MBP-specific lymphocytes are present in the blood of MS patients [63]. In rats, exposure to methylmercury will generate antibodies to neurotypic and gliotypic proteins such as MBP and GFAP (glial fibrillary acidic protein) [64]. Metals are used for the staining of brain and nervous tissue in histopathology [65]. Following a disrupted blood-brain barrier (e.g. after injury), metals can enter the CNS, bind to proteolipid protein (PLP) or MBP in myelin and evoke an autoimmune response. The disruption of the blood-brain barrier is not the only way that metals can enter the CNS. Chang [66] injected radioactively-labeled mercury into rodents and subsequently demonstrated the deposition of radioactivity in the myelin sheath of the brain. Interestingly, several studies show that there is no difference between the amount of heavy metal deposition in autopsies of MS subjects compared to controls [67–69]. Further, investigators found no difference between the number of amalgam fillings between MS patients and controls [70, 71], nor between blood and urine levels of mercury and lead [71].

Siblerud *et al*. [72] compare laboratory measurements of MS patients with dental metal fillings with MS patients with metal fillings removed. The metal-exposed MS patients had significantly lower levels of red blood cells, hemoglobin, hematocrit, thyroxine, total T-cells and CD8+ suppressor cells than the unexposed MS patients. The exposed MS group showed significantly higher blood urea nitrogen and hair content of mercury, than the unexposed group. The metal-exposed MS group also had significantly more (33.7%) exacerbations during the past 12 months compared to the unexposed group.

Another aspect of the role of heavy metals in the development of MS is the interaction between zinc and other divalent cations. It has been shown that zinc stabilizes the association of MBP with brain myelin membranes by promoting its (Zn) binding to proteolipid protein [73]. Another study confirms this finding, and also investigates the potency of other divalent cations to interfere with the binding of zinc to MBP [74]. The ions most effective to interfere with zinc binding were cadmium, mercury and copper. However, MBP aggregation was not inhibited by copper.

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a systemic motor neuron disease that affects corticospinal and corticobulbar tracts, ventral horn motor neurons and motor cranial nerve nuclei [75]. Most cases of ALS are sporadic with a male predominance, begin in midlife, and run a course of 2 to 6 years. Approximately 10% of cases are familial and these have been linked to a point mutation in the gene coding for Cu/Zn superoxide dismutase (SOD) [75]. In a recent review Multhaup [76] suggests that the most convincing evidence so far for a link between neurological disorders and oxygen radical formation is the strong association between Familial ALS (FALS) and mutations in the Cu/Zn superoxide dismutase gene. It was observed that some pedigrees of autosomal dominant FALS have missense point mutations in the gene located on chromosome 21, encoding cytosolic Cu/Zn superoxide dismutase 1 (SOD1). Mice transgenic for mutated SOD1 develop symptoms and pathology similar to those in human ALS [77]. This study indicates that mutant SOD1 toxicity is mediated by damage to mitochondria in motor neurons, and this may trigger the functional decline of motor neurons and the onset of ALS in mice. In rats administration of antioxidant coenzyme Q10 increases brain mitochondrial concentration and exerts neuroprotective effects [78]. The role of free radicals and mitochondrial mutations in ALS pathology was recently reviewed by Cassarino and Bennett [79].

One feature of ALS is dysregulation of the excitatory amino acid glutamate (GLU) in the extracellular space in CNS and in plasma [80]. Mercury has been shown to inhibit glutamate uptake in astrocytes [81]. The results of another study [82] demonstrate that mercury binds to SH-groups in the astrocyte membrane and disturbs GLU transport.

Recent evidence supports the role of autoimmune mechanisms in the pathogenesis of ALS. One review finds that inflammatory cell infiltration in the CNS of ALS victims may be more common than previously suspected [83], especially referring to findings

of both CD4+ and CD8+ around degenerated corticospinal tracts. In another study, Kerkhoff *et al.* found no difference between the number of T-cells in the peripheral nervous system (PNS) between patients with MND and controls [84]. However, increased MHC II expression on denervated Schwann cells and macrophages in nerves with axonal degeneration was found. The group also demonstrated that the inflammatory cell infiltrate was not secondary to axonal degeneration. Finally, this review refers to studies demonstrating IgG in motor neurons in the spinal cord and in the motor cortex of ALS patients, compared to controls that did not show IgG presence. Activated macrophages have been found in the spinal cords of ALS cases [84] and in one study [85], 75% of ALS patients had antibodies to calcium channels compared to controls. The antibody titers correlated with disease progression. In ALS, among other nervous system disorders, autoantibodies against neural proteins are evident at some stage of the disease. In one study [86], lead-exposed mice subsequently developed autoantibodies against neural proteins, including MBP and GFAP. To our knowledge, there is no published study addressing the issue of metal hypersensitivity in ALS. In our laboratory, 12 out of 13 ALS patients tested showed positive lymphocyte responses to metals *in vitro* [87].

The observation that in two different studies of 120 and 90 monozygous twin pairs only 2 pairs respectively none were concordant for ALS development points to the role of an environmental factor in the pathogenesis of ALS [88]. It is well known that neurotoxins, including heavy metals, induce selective death of certain groups of nerve cells [89]. Heavy metals have been linked to the development of ALS through environmental [59, 90–93] and occupational [94–97] exposure. One case study [96] describes the development of a syndrome resembling ALS after occupational exposure to mercury. The syndrome resolved when the exposure was terminated. In another study of 77 ALS cases and 80 controls [91], exposure to heavy metals was connected to a high relative risk for the development of ALS. However, other studies did not show any correlation between occupational heavy metal exposure and the development of ALS [98, 99]. Schwarz *et al.* [100] describes the development of ALS in a young nurse accidentally exposed to mercury from a thermometer plunged into the palm of her hand. Concentrations of mercury in blood and urine were in the normal range. The authors conclude that relatively small amounts of mercury may cause ALS without other signs of mercury intoxication. In one case study, the removal of metal fillings led to complete

remission of ALS [101].

Metals may enter the CNS through the circumventricular organs and because of lipophilic properties cross the blood-brain barrier. A disrupted blood-brain barrier increases this passage. Skeletal trauma and participation in sports are reported risk factors in ALS [102]. Mercury vapor is continuously released from amalgam fillings and readily crosses the blood-brain barrier. Within the brain it is oxidized to inorganic forms. While the half-life of mercury vapor in the blood is very short, the half-life of mercury stored in the brain can be over 20 years. Mercury has been found in nerve cells in autopsy analysis 16 years after mercury exposure [103].

Pamphlett and co-worker determined the fate of inorganic mercury injected intraperitoneally in mice [104]. Mice were injected with mercuric chloride (0.05-2 micrograms/g body weight) and studied between 5 days and 18 months after injection. Five days after injection mercury granules were detected in motor neurons of the spinal cord and brain stem. Mercury was still present in motor neurons 6-11 months after injection. The authors conclude that since low doses of inorganic mercury are selectively taken up and retained by motor neurons, mercury is a good candidate for a cause of sporadic motor neuron disease. Regarding metallic mercury, as little as 12 hour exposure to 25 microgram mercury/m^3 resulted in deposition of mercury granules in spinal motor neurons where it remained for 30 weeks after exposure [105].

Animal studies clearly show that most divalent cations (such as cadmium, mercury and lead) are bound to proteins in plasma (e.g. albumin, transferrin) [106] and taken up by non-specific (fluid phase) endocytosis and retrogradely transported along the axon to the soma of the neuron [107]. The same author describes that metals enter through ion channels, for example lead through calcium channels and mercury through both sodium and calcium channels. In this way, certain toxins may bypass the blood-brain barrier and accumulate in neurons. After injections of iron, cadmium and mercury in the tongues of mice, these metals were detected in the hypoglossal nuclei [107]. Injections of iron and mercury into the vibrissae area of mice resulted in the deposition of these metals in the facial nuclei. After application of gold particles to the nasal mucosa, gold was localized within the axoplasm, in the mitochondria of the olfactory nerve. The gold particles reached the olfactory bulb 30-60 minutes after inoculation. The same was shown for silver. In these experiments, no morphological evidence of nerve cell degeneration was demonstrated, nor did the animals show any signs of neurologic dysfunction. Olfactory

dysfunction is demonstrated in cadmium-exposed workers [107]. It remains to be seen if the genetics of the experimental animals or yet another factor is responsible for differences in the outcome.

Trace element analysis of ALS patients show variable results. One study found significantly higher levels of selenium, and significantly lower levels of manganese in red blood cells of ALS patients, compared to controls and to one group consisting of patients with other neurological disorders [108]. Another study found significantly lower levels of mercury and selenium in plasma and red blood cells, compared to controls [109]. A third study found higher levels of mercury, and lower levels of selenium in the hair of ALS patients compared to controls [110]. Analyses of calcified substance in the frontal cortex of ALS cases showed significantly higher levels of aluminum and calcium than in control subjects [111, 112]. It is known that mercury modifies Ca transport [113] and metal-induced calcification has been demonstrated in the degenerated areas of CNS tissue in ALS [114]. One of the possible explanations of accumulation of trace metals in the brain may be deficiency in detoxification systems [115]. One of the important enzyme systems in this respect is sulfoxidation. Generally, patients with neurodegenerative diseases such as Parkinson's disease, ALS and Alzheimer's disease have deficient sulfoxidation. The rate-limiting step is the conversion of cystein to sulfate due to low activity of cysteine dioxygenase. Low sulfate availability could also reduce an individual's capacity to detoxify metals.

Metallothioneins are discussed in the context of ALS. Metallothioneins (MT) are a group of low molecular weight metal-binding proteins [116]. In the single chain polypeptide, 20 out of 61 amino acids are cysteines. In humans, MT exhibit a complex polymorphism, with at least 12 MT genes mapped on chromosome 12. MT have been attributed a major role in metal metabolism and homeostasis, including functioning in detoxification, storage of heavy metals, regulation of cellular copper and zinc metabolism, free radical scavenging, inflammation and cell proliferation [116]. In vertebrates, the highest MT concentrations have been found in liver, kidneys, intestines, lung and testis [117]. In human CNS, immunoreactivity for MT is mainly limited to astrocytes. The cerebral cortex and basal ganglia stain more strongly for MT than other areas of the brain [117]. Areas of the brain containing high concentrations of Zn such as the retina, pineal gland and hippocampus synthesize unique isoforms of MT continuously [118]. One proposed function of MT in CNS is to supply neurons with essential ions such as Zn

and Cu and protect them against toxic ones. MT synthesis is induced by copper, cadmium, mercury, gold [40], and also by glucocorticoids, interferons, IL-1, endotoxins, ethanol [119] and stress. Only intracerebral administration of metals increases brain MT levels, while systemic administration does not [120, 121]. Sanders *et al.* note that the relative affinities of metals for MT based on *in vitro* studies (i.e., $Hg^{2+} > Ag^{1+} > Cu^{1+} > Cd^{2+} > Zn^{2+}$) provide an indirect mechanism for induction of MT via zinc displacement and concomitantly allow these more toxic metals to be sequestered while the less toxic zinc is released [122]. The concentration of Zn has been shown to be altered in an extensive number of disorders of the CNS, including alcoholism, Alzheimer-type dementia, ALS, Down's syndrome, epilepsy, Guillaine-Barre's syndrome, hepatic encephalopathy, MS, Parkinson's disease, Pick's disease, retinitis pigmentosa, retinal dystrophy, schizophrenia and Wernicke-Korsakoff's syndrome. Since several of these disorders are associated with oxidative stress, and since MT is able to prevent the formation of free radicals, it is believed that cytokine-induced induction of MT provides a long-lasting protection to avert oxidative damage [118].

The choroid plexus protects the cerebrospinal fluid and CNS against toxic metals. After the administration of lead, mercury and arsenic compounds, these metal ions accumulated in the lateral choroid plexus at concentrations that were 70-, 95- and 40-fold higher than those found in the CSF [123]. One proposed mechanism is that the content of metal-binding cysteines is four-fold greater in the choroid plexus than in the cerebral cortex [123]. Methyl-mercury has been found in astrocytes [124] and further, heavy metals can induce CNS toxicity by impairing the astrocytic mitochondrial DNA [125]. In ALS cases, increased MT expression is found in spinal cord gray matter protoplasmic astrocytes [126, 127], and significantly increased MT levels are found in ALS liver and kidney [126] compared to controls.

Psychoneuroimmunology aspects in autoimmune disease

It is generally recognized that serious fatigue is one of the characteristics of autoimmune diseases as well as of allergic diseases. In addition, the other frequently presenting symptoms are neuropsychiatric symptoms. These symptoms are also found in other diseases such as CFS, fibromyalgia or MCS. CFS patients often have a central down-regulation of the hypothalamus-pituitary-adrenal (HPA) axis resulting in mild hypocortisolism [128]. Magnetic

resonance imaging (MRI) has demonstrated areas of high signal in white matter more often than in healthy control subjects [129-131]. One hypothesis is that these lesions represent sites of inflammation and/or demyelinization. Similar brain abnormalities can also be seen in single photon emission computed tomography (SPECT) [132, 133].

Many studies provide evidence of chronic immune activation in CFS and related diseases. The most prominent findings are an increased number of CD8+ cytotoxic T-cells that show activation markers [134]. Another finding is a decreased function of natural killer (NK) cells [135–137]. One group investigated the association between affective and neuroendocrine abnormalities in MS patients [138] and found that the disorders were related to inflammatory activity in these patients. The possibility of chronic metal-induced inflammation triggered by occupational and dental metal exposure was recently investigated by Sterzl *et al.* [18]. In this study, patients with fatigue and with or without autoimmune thyreoiditis exhibited significantly higher *in vitro* lymphocyte responses to inorganic mercury and nickel as compared to healthy controls. As shown previously, mercury has been found in the thyroid gland [139]. Patients with psoriasis and atopic eczema improved following the reduction of metal exposure by diet low in metal ions or by dental metal replacement in metal-sensitive patients [140, 141]. In another Japanese study [142] lymphocyte stimulation test is used to identify the causative metals. These studies confirm the data of the Swedish scientists on the beneficial effects of removal of incompatible dental materials in metal-sensitive patients with CFS-like symptoms [19, 87]. In Swedish as well as in Czech patients, concordant decrease of lymphocyte reactivity to dental metals was observed following the replacement of metallic restorations.

Metals are just one of the environmental agents which may induce T-cell mediated delayed-type hypersensitivity and thus trigger the multi-symptoms observed in the above-mentioned disorders. Other low-molecular weight compounds which may operate similarly are pharmaceuticals or chemicals such as formaldehyde and isothiazolinones [143, 144]. The effects of environmental toxins on the dysregulation of the HPA-axis has been studied in animal systems [145] and in man [146]. Metals may disturb the endocrine axis by binding to crucial sites in the HPA-axis. Significant accumulation of mercury in the pituitary gland is reported by Weiner *et al.* [147] and Maas *et al.* [148]. The accumulation of mercury in neurosecretory neurons in the hypothalamus of rodents is described by Villegas *et al.* [149].

Discussion

In the light of current knowledge, it seems plausible that metals are directly or indirectly involved in the induction or promotion of autoimmunity. At least four different mechanisms could be involved in the induction or promotion of metal-induced pathology: free radical formation, local toxic effects, calcification and inflammation.

Current available literature indicates a potential risk for the induction of autoimmunity by metals in man. Based on animal studies, this risk seems to be regulated by genetic factors, among others. For example, certain strains of mice develop ANA antibodies to metals while others do not. In man, the susceptibility to the effects of xenobiotics may be due to the genetically determined detoxification systems, including the acetylator-, sulfoxidizer-, aromatic hydrocarbon receptor-, P450- and MT-phenotypes [21]. Certain MHC structures may present antigens to helper T-cells more efficiently then others and thus facilitate the development of autoimmunity [56]. Thus the ability to detoxify xenobiotics together with the individual susceptibility to the metal are probably the most important factors in the outcome of metal exposure.

Although animal systems may be important for clarification of several autoimmune mechanisms, they only partly reproduce the clinical disease in man. In man, both organ and systemic autoimmune diseases persist for years, while in experimental animal systems, autoimmunity is a transitional phenomenon. To explain this discrepancy, the differences in biochemistry between man and experimental animals must be taken in account. Animals used in experimental studies produce their own vitamin C [6], which might neutralize the pathologic effects of metals. Animals produce under non-stress condition between 5-40 grams of vitamin C per day. Under stress, the production of vitamin C rises proportionately. The lack of the critical enzyme L-gulonolactone oxidase (GLO) which catalyzes the last step in the synthesis of L-ascorbic acid from D-glucose, prevents several species, including guinea pigs, monkeys, apes and man, to synthesize the vitamin. This may be one factor which makes man more vulnerable not only to the effects of metals, but to other free radical generating substances as well. Possibly, animals not synthesizing vitamin C and thus with a biochemistry more similar to man in this respect might be more suitable for study of autoimmunity.

In a recent study, Saxe *et al.* [69] measured the concentration of mercury in the brains of Alzheimer and MS patients and compared them with the data of controls. The authors concluded that since there

was no difference in the mercury deposition in the brains of patients vs. controls, mercury cannot be a factor in the development of those diseases. Similar findings were published by Fung *et al.* [67] and Clausen [68]. If allergic rather than toxicologic mechanisms operate in Alzheimer's and MS disease, the interpretation of these studies may be questioned. In contrast to the toxic effects of metals, the concentration of the metal in a sensitized individual is of minor importance. Minute concentrations of an allergen can induce systemic reactions in sensitized individuals. In such a situation, metal-induced inflammatory reactions in the brain or elsewhere could be triggered despite low concentrations detected in body fluids or locally. The role of immunologically mediated inflammation in the abovementioned diseases is well established. This is the reason why Saxe's and Fung's studies cannot be used as evidence of the absence of metal-induced pathology in MS and Alzheimer's disease.

Considering the complexity of the immune system and its interaction with the nervous and endocrine systems [66], it is obvious that a combination of mechanisms is responsible for the induction of autoimmunity. One of the most decisive factors seems to be individual sensitivity based on the genetic constitution. Other factors include nutrition or may be psychological, such as stress. Infectious agents may through immunomodulation compromise the immune system and thus render the individual more sensitive to the effects of environmental agents. The synergistic effects of these factors may play a role in the precipitation of autoimmune disease.

Conclusions

This review can be summed up in a few crucial points. The data indicates that metals have the potential to induce or promote the development of autoimmunity in man. Chronic metal-induced inflammation may dysregulate the HPA-axis and contribute to fatigue and other non-specific symptoms characterizing autoimmune diseases.

The majority of studies until now are designed from a toxicological approach, including epidemiological studies and measurements of concentrations of metals in tissue and body fluids. Although these studies establish exposure, they show no significant differences in metal load between patient and control groups. The increased knowledge about individual sensitivity based on genotype and phenotype variability together with the use of biomarkers for the diagnosis of this individual sensitivity seems to be the key in elucidation of the operating mechanisms. In the case of metal pathology in autoimmu-

nity, future studies should be longitudinal studies of metal-sensitive patients rather than traditional case-control studies.

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