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Harmful Interactions of Non-Essential Heavy Metals with Cells of the Innate Immune System

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Abstract

In trace amounts, some heavy metals are essential for optimum health, while exposure to others, which are non-essential, presents the potential hazard of acute or chronic organ toxicity. Cadmium, mercury, lead, vanadium, platinum and palladium are commonly encountered, non-essential heavy metals which mediate their toxic activities by various mechanisms. All have the potential to interact with extracellular and intracellular protein sulfhydryls, rendering them not only potentially allergenic, but also predisposing to oxidative stress, while displacement of essential elements from their protein carriers may result in deficiency disorders. In addition, several of these metals, especially cadmium, palladium, platinum, and vanadium interact pro-oxidatively with the phagocytic cells of the innate immune system, potentiating the reactivity and toxicity of phagocyte-derived reactive oxygen species. This review is focused on the pro-oxidative/pro-inflammatory interactions of non-essential heavy metals with the cells of the innate immune system, a somewhat under-appreciated mechanism of metal induced toxicity.

Keywords: Cadmium; Lead; Macrophages; Mercury; Neutrophils; Palladium; Reactive oxygen species; Vanadium

Introduction

Heavy metals comprise a heterogeneous group of elements, some of which are essential cofactors for various enzymes, while others are non-essential. The former group includes the trace elements cobalt, copper, iron, manganese, molybdenum, selenium and zinc. Because excessive concentrations of the free metals pose potential health risks, their circulating and tissue concentrations are tightly regulated through interactions with binding proteins. The latter group includes metals such as arsenic, cadmium, lead, mercury, plutonium, tungsten and vanadium [1]. These non-essential metals are potent toxins and gain access to organisms by virtue of physico-chemical properties, such as ionic charge, shared with their essential counterparts [2]. They may enter the body through food, water, air, or by absorption through the skin following inadvertent occupational exposure in the agricultural, manufacturing/industrial settings, or through environmental exposure. The manufacturing/industrial setting is the most significant source of exposure in adults [3].

Notwithstanding their direct cytotoxic effects on eukaryotic cells at high concentrations, interactions of non-essential heavy metals at lower non-cytotoxic levels with cells of the innate immune system, which abound in the airways, skin, gastrointestinal tract, liver, spleen, kidneys and circulatory system, may initiate harmful inflammatory responses with accompanying organ dysfunction and disease. These potentially harmful pro-inflammatory interactions of non-essential heavy metals (toxicants), specifically cadmium, lead, mercury, platinum, palladium and vanadium, with the cells of the innate immune system, and their adverse effects on health, are the topic of this review, which also includes cigarette smoking as a cause of exposure to heavy metals [4]. Of necessity, a consideration of the pro-inflammatory activities of these metals is preceded by a brief consideration of innate cellular defence mechanisms.

Innate Immunity

Innate immunity encompasses effector cells and proteins that serve as a first line of defence against infectious agents, restraining or, in some cases eliminating, microbial or viral pathogens while the host develops an adaptive, antigen-specific immune response [5]. Key cellular components of innate immunity include phagocytes such as neutrophils and monocytes/macrophages, as well as dendritic cells, mast cells, eosinophils, basophils and natural killer cells. Innate defenses also encompass physical barriers such as the epithelial and endothelial cell linings. Although not strictly classified as being cellular elements of the innate immune system, epithelial cells and endothelial cells are key orchestrators of both innate and adaptive immune responses.

Phagocytes accomplish their task at sites of infection by phagocytosing and killing bacteria and fungi, utilizing an arsenal of toxic molecules such as proteolytic enzymes and reactive oxygen species (ROS) (Table 1), as well as bacteriocidal proteins, which synergize/harmonize to eliminate these pathogens [6-7]. Although effective, these antimicrobial systems are indiscriminate and may cause significant inflammation-mediated damage to bystander host tissues if inappropriately and/or excessively activated. Excessive production of ROS may result in damage to lipids, proteins, or DNA, compromising cellular function, with resultant cytotoxicity. Because of this, chronic oxidative stress has been implicated in a number of human degenerative diseases, including cancer, cardiovascular disease, chronic obstructive pulmonary disease, atherosclerosis, neurodegenerative diseases (Alzheimer's disease and Parkinson's disease), rheumatoid arthritis, renal diseases, and ageing [8-9].

Cells of the innate immune system such as neutrophils and macrophages are also able to synthesize pro- and anti-inflammatory

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cytokines, growth factors and chemokines that mediate a wide range of physiological responses, primarily in host defence. In addition to their role in immunity and inflammation, however, overproduction of pro-inflammatory cytokines such as interleukin-1β (IL-1β), tumour necrosis factor (TNF) and interleukin-6 (IL-6), as well as chemokines such as interleukin-8 (IL-8, CXCL8), monocyte chemotactic protein-1 (MCP-1, CCL2) and macrophage inflammatory protein-1alpha (MIP-1∝, CCL3) can trigger a variety of pathophysiological conditions [10].

The major mediators of inflammation released by cells of the innate immune system and their potential pathological effects are shown in Table 2.

Cadmium

Cadmium (Cd) is released into the environment through various industrial and domestic activities. Foremost amongst these are the combustion of fossil fuels (coal, diesel, gasoline etc.), incineration of industrial waste (especially Cd-containing batteries and plastics), metal alloy production, electroplating, and manufacture of phosphate fertilizers [11]. Cd is also present in tobacco, with each cigarette containing 1-3 μg of the metal, predisposing both active and passive smokers to the toxic effects of Cd inhalation [11]. In welders, acute exposure to Cd may lead to pneumonitis, pulmonary edema and death, with a Parkinsonism-like neurological disorder being a late effect of acute toxicity [1].

Notwithstanding predisposition to pulmonary disease, renal dysfunction is the most common adverse health effect of chronic exposure to Cd [12].

Cadmium toxicity may also result in osteoporosis by inducing renal tubular dysfunction with consequent increased urinary losses of calcium and phosphate as well as a direct effect on bone osteoblast and osteoclast activity [13-14]. Heavy metal toxicity in the setting of iron-deficiency anaemia may significantly increase the risk of infection as both iron-deficiency [15] and Cd-toxicity impair host responses to infection. Cadmium and zinc compete for carrier molecule binding sites on cell membranes and high Cd concentrations antagonize Zinc absorption and uptake by cells. Therefore, Cd toxicity may predispose affected individuals to zinc deficiency which can disrupt normal cellular and immunological functions [16-18].

In addition to direct cytotoxic effects of Cd at high concentrations on various cell types, including mononuclear cells and macrophages [19-20], a considerable body of evidence exists which implicates harmful

ABBREVIATION	
Cd	Cadmium
$CdCl_2$	Cadmium chloride
CdS	Cadmium sulphide
Pb	Lead
Hg	Mercury
$HgCl_2$	Mercury chloride
MeHg	Methyl Mercury
MeHgCl	Methyl Mercury Chloride
V	Vanadium
Pt	Platinum
Pd	Palladium
ROS	Reactive Oxygen Species
O_2^-	Superoxide anion
H_2O_2	Hydrogen Peroxide
HOCl	Hypochlorous Acid
OH'	Hydroxyl Radical
NO	Nitric Oxide
iNOS	Inducible Nitric Oxide Synthase
MMP	Matrix Metalloproteinases
LTB4, LTC4 and LTD4	Leukotriene B4, Leukotriene C4 & D4
IL (e.g IL-8)	Interleukin (e.g. interleukin-8)
TNF	Tumor necrosis factor
MCP-1	Monocyte chemotactic protein-1
MIP-1α	Macrophage inflammatory protein-1 alpha
NFκB	Nuclear factor kappa B
AP-1	Activation protein-1
JNK	c-Jun N-terminal kinase
MEK-1	MAP (Mitogen-Activated Protein) Kinase/ERK (Extracellular Signal-Regulated Kinase) Kinase 1
P38 MAPK	p38 mitogen-activated protein kinases
ERK-1	Extracellular Signal-Regulated Kinases 1
PI-3K	Phosphatidylinositol-3-Kinase
FMLP	N-formyl-methionyl-leucine-phenylalanine
PMA	Phorbol-12-myristate-13-acetate
PKC	Protein kinase C
PAF	Platelet Activating Factor
PGE_2	Prostaglandin E 2

Table 1: List of abbreviations.

Mediator	Consequences of Overproduction
Reactive oxidant species: O2-, H2O2, HOCl, OH'	Damage cellular lipids, proteins and DNA. Implicated in cancer, cardiovascular disease, atherosclerosis, neurodegenerative diseases and others [9].
Nitric Oxide Synthase-derived Nitrogen Intermediates & Peroxynitrite	Peroxynitrite implicated in cardio-vascular disease, neurodegeneration, diabetes etc. [123].
Proteases e.g. serine proteases in the azurophilic granules: Cathepsin G, elastase, proteinase 3	Degrades matrix proteins such as elastin; implicated in emphysema, chronic bronchitis and cystic fibrosis [124].
Matrix metalloproteinases e.g. MMP-8 & MMP-9	Cause matrix breakdown in COPD, ARDS, sarcoidosis, and tuberculosis etc [125].
Lipid mediators: LTB_4, LTC_4,LTD_4, Platelet Activating Factor (PAF), PGE_2	Overproduction of LTB_4 associated with leukocyte recruitment is involved in the pathogenesis of inflammatory diseases such as bronchial asthma, rheumatoid arthritis, atherosclerosis, and inflammatory bowel disease [126]. LTC_4, LTD_4 play a role in asthma [127].
Cytokines: Pro-inflammatory cytokines e.g. IL-1, IL-6, TNF, IL-12, & anti-inflammatory cytokines, IL-1 receptor antagonist (IL-1Ra), IL-10	Overproduction of proinflammatory cytokines such as TNF may be involved in septic shock, autoimmunity & inflammatory diseases [128].
Chemokines: IL-8, Macrophage Inflammatory Protein(MIP)-1α & β, monocyte chemotactic protein-1 (MCP-1)	Overproduction of chemokines such as IL-8 may be involved in inflammatory conditions, e.g. COPD, ARDS [129-130].

Abbreviations: LT: Leukotriene; PGE2: Prostaglandin E2; COPD: Chronic Obstructive Pulmonary Disease; ARDS: Acute Respiratory Distress Syndrome

Table 2: Mediators of inflammation released by cells of the innate immune system and their potential pathological effects.

pro-oxidative, pro-inflammatory interactions of Cd with neutrophils and macrophages in the adverse health effects of environmental and/or industrial exposure to this metal. With respect to pro-oxidative activity, Freitas et al. (2010) reported that exposure of isolated human neutrophils to $CdCl_2$ caused increases in the production of ROS, specifically O_2^-, H_2O_2, and HOCl, both spontaneously and following activation of the cells with the phorbol ester, phorbol myristate acetate (PMA) [21]. The latter is a direct activator of protein kinase C (PKC), which in turn activates the superoxide-generating system of phagocytes, NADPH oxidase [21]. These findings were confirmed by others and were extended to include murine macrophages and macrophage cell lines in which exposure of these cells to Cd (10 μM) also resulted in increased production of nitric oxide (NO), an activity which was associated with both increased activity and synthesis of inducible NO synthase (iNOS) [22-24]. Although the exact molecular mechanisms which underpin the pro-oxidative interactions of Cd with neutrophils and macrophages remain to be conclusively established, it is noteworthy that exposure of murine macrophages to Cd at concentrations of 20-500 μM was found to increase cytosolic Ca^{2+} concentrations [25]. Ca^{2+} is a well-recognized second messenger, and increases in the cytosolic concentrations of this cation precede and are a prerequisite for receptor-mediated activation of NADPH oxidase [26].

Other mechanisms of Cd -mediated pro-oxidative activity include: i) inhibition of superoxide dismutase [27]; ii) bonding to sulfhydryl groups, depleting glutathione and protein sulfhydryls, thereby compromising intracellular anti-oxidative defences [28]; iii) activation of 5-lipoxygenase activity, leading to production of the neutrophil/monocyte chemoattractant, leukotriene B_4 (LTB_4), which also sensitizes these cells for increased activity of NADPH oxidase [29]; and iv) oxidative activation of redox-sensitive transcription factors such as nuclear factor kappa B (NFκB) and activator protein 1 (AP-1), which, in turn, activate the expression of genes encoding pro-inflammatory proteins, including iNOS [14,30-32].

The harmful pro-oxidative, pro-inflammatory activities of Cd have also been demonstrated in animal models of experimental pulmonary inflammation. Kataranovski et al. reported that intraperitoneal administration of $CdCl_2$ (0.5 – 2 mg/kg body mass) resulted in a dose-related elevation in the number of circulating neutrophils, as well as those present in lung tissue, which was associated with increased: i) adhesive and pro-oxidative activities of these cells; and ii) circulating levels of the pro-inflammatory cytokines TNF and IL-6, the latter being significantly correlated with the numbers of blood and pulmonary neutrophils [33]. In addition, Kirschvink et al. [34] reported that repeated (x3 weekly for 3-5 weeks) $CdCl_2$ (0.1%) nebulizations resulted in pulmonary inflammation in mice which was characterized by increased influx of neutrophils and macrophages in the setting of elevated levels of the matrix metalloproteinases-2 and -9. Histomorphometric analysis of the lung revealed changes compatible with emphysema, which correlated with the activities of the MMPs.

It is probable that Cd-mediated pulmonary inflammation and damage results from the pro-oxidative interactions of the metal with alveolar macrophages and other resident cells of the innate immune system, resulting in oxidative activation of NFκB and AP-1, leading to synthesis of IL-6, IL-8, and TNF [30,35-36]. In this respect it is also noteworthy that exposure of a murine macrophage cell line to Cd has been reported to activate a Ca^{2+}-ROS-JNK-caspase-3 intracellular signalling pathway which promotes phosphorylation/dephosphorylation of JNK and p38 MAPkinase, modulating cellular mitochondrial activity and proliferation, leading to apoptosis and necrosis [25]. The study by Kirschvink et al. [34] mentioned above is clearly compatible with a pathogenetic link between inhalation of Cd in cigarette smoke, chronic pulmonary inflammation, and development of emphysema. This contention is supported by the observation that relative to non-smokers, concentrations of Cd (and strontium) are elevated in the blood of cigarette smokers [37]. Furthermore, in a study to which 16024 adult humans were recruited, an increasing trend in urinary Cd^{2+} levels from never, through former, to current smokers was observed, which was negatively correlated with forced expiratory volume in 1 second (FEV_1) and the ratio of FEV_1 to forced vital capacity (FVC), both indicative of airflow obstruction [38].

The pro-oxidative and pro-inflammatory effects of Cd on neutrophils and macrophages are depicted in Figure 1.

Lead

According to Hu [39], the worldwide production of lead (Pb) is approximately 5.4 million tons and continues to rise. The major use of lead is in pb batteries, accounting for 78% of reported global consumption in 2003 [40]. This metal is also used in the production of pigments, glazes, solder, plastics, cable sheathing, ammunition, weights, fuel additives, and a variety of other products [39]. The predominant source of worldwide dispersion of pb into the environment for the past 50 years has been the use of pb organic compounds as anti-knock additives in motor vehicle fuels [39]. Emissions from this source have, however, declined with the phasing out of leaded petrol worldwide.

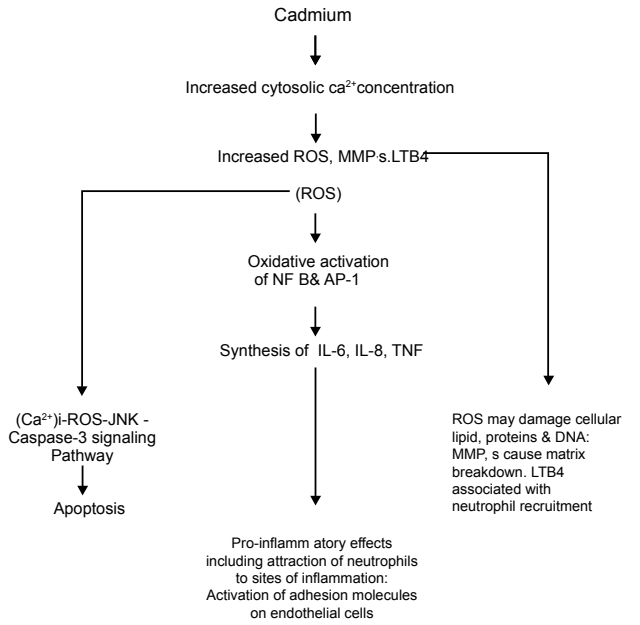

Figure 1: The pro-oxidative and pro-inflammatory effects of Cd on neutrophils and macrophages.

Lead is also present in tobacco, and Zielhuis et al. [41] demonstrated that blood lead levels in male and female students increased with the number of cigarettes smoked per day, although other sources of Pb such as exhaust fumes and food may also play a role. Lead levels among adults with high second-hand smoke exposure were found to be similar to those of smokers [42].

Depending on the dose, pb exposure can cause a wide spectrum of health problems, including neurological, cardiovascular, renal, gastrointestinal, haematological and reproductive disorders. pb accumulates in bone, which may serve as a reservoir for exposure in later life [40]. Children are particularly susceptible to lead intoxication, while at lower blood lead concentrations various neurological and behavioural problems may occur, ranging from a raised hearing threshold to a reduction in intelligence quotient (IQ) [43].

With respect to interactions with cells of the innate immune system, pb has been reported to negatively affect the functions of both neutrophils and macrophages. In the case of neutrophils, chemotaxis, the generation of ROS, and the killing of *Candida albicans* were found to be decreased in workers occupationally exposed to Pb, even in those with blood levels below the currently acceptable biological lower limit [44-48]. On the basis of these observations, Quieroz et al. [46] suggested that immune dysfunction may be a sensitive indicator of exposure to Pb. More recently, Di Lorenzo et al. (2006) reported an association between Pb exposure and the numbers of circulating neutrophils, with the strongest association being observed in occupationally-exposed workers who smoked [49]. This latter observation is not surprising given the well-recognized association of smoking with: i) neutrophilia [50]; and ii) increased levels of Pb in the blood of smokers as described above. Although the authors speculate that increased numbers of circulating neutrophils may represent a mechanism to compensate for Pb-mediated immune dysfunction, they concede that it is more likely to reflect a neuroendocrine response to toxicity/stress. In this setting, increased production of endogenous glucocorticoids and catecholamines may contribute to both neutrophilia and neutrophil dysfunction possibly by interfering with the adhesion of these cells to vascular endothelium [49].

In the case of macrophages, Pb, at non-cytotoxic concentrations of 0.1-10 μg/ml, has been reported to inhibit the production of NO by cytokine-induced cell lines by interfering with induction of iNOS at the level of gene transcription [51,52]. Moreover, exposure to Pb, at a concentration of 1300 ppm, was found to inhibit the adherence of murine peritoneal macrophages to plastic tissue culture dishes, which may underpin the inhibitory effects of the metal on the migratory responsiveness of these cells [53,54]. These effects of Pb on the induction of iNOS and spreading of macrophages are opposite to the effects on cytokine production. Flohé et al. [55] observed that exposure of murine bone marrow-derived macrophages to Pb (0.2-20 μM), prior to activation with bacterial lipopolysaccharide, resulted in augmentation of production of TNF, IL-6 and IL-12, as well as prostaglandin E_2, while production of anti-inflammatory IL-10 was decreased. More recently, Valentino et al. [56], in a study designed to measure the circulating levels of pro- and anti-inflammatory cytokines in workers exposed to very low levels of Pb, found significant increases in the plasma concentrations of TNF and IL-10 relative to those of non-exposed control subjects. They reasoned that the Pb-mediated increase in IL-10 was a biological, anti-inflammatory strategy to counteract the increase in the production of pro-inflammatory TNF [56].

Several mechanisms, including pro-oxidative properties and antagonism of the second messenger function of Ca^{2+} have been proposed to explain the modulatory effects of Pb on the function of cells of the innate immune system. With respect to pro-oxidative activity, the chemical properties of Pb favour interactions with diverse bio-ligands, particularly protein sulfhydryls [57]. Increases in Pb-binding proteins, as well as in glutathione (GSH), occur soon after metal exposure and are believed to protect against Pb toxicity [57]. However, by depleting glutathione and protein sulfhydryls, Pb can compromise host anti-oxidant defences, creating an intracellular environment conducive to the oxidative activation of transcription factors such as NFκB, while promoting oxidant-mediated inhibition of the protective functions of neutrophils and macrophages.

However, the prevailing theory of Pb-mediated modulation of the functions of neutrophils and macrophages is that the metal may affect intracellular Ca^{2+} homeostasis, either by mimicking Ca^{2+} action and/or antagonizing Ca^{2+}-dependent cellular functions. Given the critical second messenger role of Ca^{2+}, exposure to Pb may affect several intracellular signalling pathways, including those involving activation of protein kinase C possibly underpinning interference with neutrophil and macrophage functions as described above [58].

Mercury

Elemental mercury (Hg) is liquid at room temperature, and in this form is less toxic than inorganic or organic bound Hg. Methylmercury (MeHg), the most predominant form of organic Hg, is the form that most commonly poses a health risk, mainly through fish consumption [59]. Between 2700 and 6000 tons of elemental Hg are released into the biosphere annually through degassing from the earth's crust and oceans [60,61]. Occupational (chloralkali plants, production of lamps and batteries, gold mining, dentistry) and environmental (dental amalgams, food) exposures to Hg also occur frequently [62]. According to reports "a single dental amalgam filling with a surface area of only 0.4 cm^2 is estimated to release as much as 15 micrograms of Hg per day primarily through mechanical wear and evaporation." It is also stated that "the average individual has eight amalgam fillings and could absorb up to

120 micrograms of Hg per day from their amalgams" [63,64].

Hg has been found to cause various health problems, including neurological, renal, immunological, cardiac, motor, reproductive and even genetic disorders [65]. Pre- or post-natal exposure to high levels of MeHg causes mental retardation, cerebral palsy, seizures and ultimately death [66], while inorganic Hg is known to induce autoimmune disease in susceptible rodent strains. Additionally, in inbred strains of mice prone to autoimmune disease, Hg can accelerate and exacerbate disease manifestations [67]. Metals such as Hg may also contribute to the pathogenesis of autoimmune diseases by modulating mast cell activity [68].

Hg has been reported to affect the functions of the cells of the innate immune system, including neutrophils, moncytes/macrophages, natural killer cells and dendritic cells as well as epithelial cells. In the case of neutrophils, Jansson et al. [69] reported that exposure of these cells to low micromolar (≤5 μM) concentrations of $HgCl_2$ *in vitro* resulted in significant, albeit variable, increases in the production of superoxide activated by the chemoattractant, N-formyl-L-methionyl-L-leucyl-L-phenylalanine, but not with other activators such as PMA or opsonized zymosan. At approximately the same concentrations, $HgCl_2$ and MeHgCl were found to inhibit spontaneous apoptosis of human neutrophils *in vitro* [70], possibly by a mechanism related to induction of mild oxidative stress. At higher concentrations, however, the metal (in both studies) was found to become abruptly cytotoxic [69,70].

In contrast to the aforementioned studies which addressed the effects of short-term exposure to Hg on neutrophil function and viability *in vitro*, prolonged exposure to the metal in the workplace was reported to be associated with impairment of neutrophil chemotaxis and generation of ROS [71]. These effects persisted following reductions in exposure due to improvements in factory hygiene practices, prompting the authors to propose that exposure to Hg, even at levels considered to be "safe", may lead to impairment of neutrophil function.

With regard to monocytes/macrophages, electron microscopic analysis of cells exposed to MeHg clearly revealed uptake of the metal with deposition in lysozomes and dispersal in the cytoplasm and nuclei [72]. Functional analysis of macrophages exposed to Hg (1-5 μM) demonstrated: i) impairment of their phagocytic and migratory activities, possibly as a consequence of increased production of ROS by metal-exposed cells [72,73]; ii) increased production of LTB_4 [74], possibly resulting from increased activity of p38 MAPK [75], but not NFκB, which was actually decreased [76]; and iii) decreased production of NO in the setting of increased synthesis of the cytokines IL-6 and TNF, which was associated with increased activity of p38 MAPK, but not NFκB [76].

The stimulatory effects of Hg on the production of IL-6 and TNF by macrophages are essentially in agreement with the findings of an earlier study by Villanueva et al. [36]. These authors reported that exposure of human peripheral blood mononuclear leukocytes to $HgCl_2$, as well as to $CdCl_2$ as mentioned earlier, resulted in increased production of both IL-1β and TNF in the setting of decreased production of anti-inflammatory IL-10 and IL-1 receptor antagonist. The cytokines interferon-γ, IL-4 and IL-17 which typify activation of the Th1, Th2 and Th17 sub-populations of $CD4^+$ T cells of the adaptive immune system respectively, were also increased following exposure of mononuclear leukocytes to $HgCl_2$ [77].

In addition to its effects on neutrophils and monocytes/macrophages, Hg, as mentioned above, has been reported to modulate the functions of natural killer cells, and epithelial cells. In the case of the former, dietary intake of MeHg (3.9 μg/gram diet) by mice and rats resulted in suppression (42-44%) of the tumoricidal activity of blood and splenic natural killer cells, as well as proliferation of the T-cells and B-cells of the adaptive immune system [78,79].

More recently, Migdal et al. [80] investigated the effects of thimerosal and other Hg-containing compounds on the spontaneous expression of surface markers of cell activation, as well as on the production of the pro-inflammatory cytokine TNF, and the chemokine IL-8 by human monocyte-derived dendritic cells *in vitro*. The authors observed that exposure of the cells to all of these compounds was associated with over-expression of CD86 and HLA-DR, both of which mediate T-cell activation, as well as increased production of TNF and IL-8. From a mechanistic perspective, these pro-inflammatory interactions of Hg with dendritic cells were associated with increased intracellular oxidative stress, presumably as a consequence of interaction of the metal with glutathione and protein sulfhydryls, and delayed, oxidant-mediated influx of extracellular Ca^{2+} [80,81]. Both of these events (increased intracellular levels of ROS and associated Ca^{2+} influx) result in activation of transcription factors and expression of genes encoding pro-inflammatory proteins.

Epithelial cells are also prone to metal-mediated oxidative stress as described by Han et al. [82]. These authors reported that exposure of the bronchial epithelial cell line (BEAS-2B) to Hg or, as mentioned earlier to Cd in particular, at concentrations of 0-50 μM, resulted in dose-related oxidative stress due to increased intracellular generation of the ROS, superoxide, H_2O_2 and hydroxyl radical [82]. Oxidative stress resulted from metal-mediated depletion of intracellular sulfhydryls, resulting in cytotoxicity.

The pro-oxidative, pro-inflammatory and cytotoxic properties of Hg are clearly similar to those of Cd and Pb, resulting from the interactions of the metal with, and depletion, of intracellular sulfhydryl groups [83]. Oxidative stress, in turn, leads to influx of Ca^{2+} and activation of p38 MAPK, resulting in production of pro-inflammatory cytokines by cells of the innate immune system [25]. Excessive and prolonged oxidative stress, however, results in oxidative inactivation of protective functions such as adherence, migration and phagocytosis in the case of neutrophils and monocytes/macrophages, and ultimately to cell death by apoptosis due to p38 MAPK-mediated activation of caspase 3 and oxidant-mediated necrosis [25].

Vanadium

Metallic vanadium (V) does not exist in nature; rather, V compounds exist in oxidation states ranging from -1 to +5, the most common valences being +3, +4, and +5, with quadrivalent salts being the most stable. Occupational exposure to V is common in the petrochemical, mining, steel, and utilities industries; fossil fuels and some ores contain significant amounts of this metal [84]. Inhalation is the most prevalent route of human exposure to insoluble pentavalent V oxides and soluble salts in urban/occupational settings. Workers exposed to V-bearing dusts or fumes display an increased incidence of several lung diseases (e.g., asthma, bronchitis, pneumonia) [85]. Exposure to V can also take place through the smoking of cigarettes. The concentration of V in cigarettes ranges between 0.49 –5.33 mg/g (average: 1.11 mg/cigarette). About 60% of the vanadium remains in the ash, 8.3% in the cigarette filter and 31.3% in the smoke [4,86].

Like the other metals, V has been found to interact pro-oxidatively with cells of the innate immune system, including neutrophils, macrophages, basophils, as well as epithelial cells. In the case of neutrophils, Fickl et al. [87] reported that exposure of activated human

neutrophils to V (25 μM) in the +2, +3 and +4, but not the +5, valence states promoted hydroxyl radical formation by these cells. This was achieved by a Fenton reaction via interaction of the metal with H_2O_2 generated by active neutrophils. This mechanism may, however, be of greater relevance to macrophages as these cells do not possess myeloperoxidase, negating competition between the metal and the enzyme for H_2O_2. At much higher concentrations (50-1000 μM) than those used in the aforementioned study, Grabowski et al. [88] reported that exposure of rat alveolar macrophages to sodium metavanadate (+5 valence state) resulted in a generalized, dose-related (from 50 μM) increase in the intracellular generation of ROS. Exposure of the cells to the metal resulted in the activation of NADPH oxidase, as well as tyrosine phosphorylation of cellular proteins [88]. The authors propose that a mechanism involving V-mediated generation of ROS in macrophages, and possibly other cell types such as epithelial cells, may result in oxidant-mediated activation of intracellular signalling cascades, leading to synthesis of pro-inflammatory cytokines/chemokines, which, in turn, promote the airway inflammation which accompanies inhalation of the metal. This contention was confirmed in a murine model of experimental airway inflammation in which aspiration of V(+5) into the pulmonary airspace was accompanied by influx of neutrophils, which was associated with increased production of ROS (superoxide, hydroxyl radical, H_2O_2) by alveolar macrophages *ex vivo* [89], as well as increased expression of genes encoding neutrophil and monocyte chemoattractants [84,90,91].

In addition to alveolar macrophages, airway epithelial cells are also involved in V-mediated airways inflammation, again as a consequence of intracellular oxidative stress. In an earlier study, Jaspers et al. [92] reported that exposure of primary human bronchial epithelial cells to V (+4, 12-50 μM) resulted in increased transcription of the IL-8 gene, as well as synthesis of the protein as a consequence of activation of NFκB. These observations are in keeping with the study of Zhang et al. [93] in which it was reported that exposure of the human lung epithelial cell line, A549, to V (+5, 100 μM) resulted in intracellular generation of ROS (superoxide, hydroxyl radical, H_2O_2).

In the case of human basophils, Kitani and co-workers reported that exposure of these cells, as well as rat mast cells and basophilic leukaemia cells to V in the +4 and +5 valence states in combination with H_2O_2 (1 mM), but not the individual agents, caused the release of histamine, which was associated with increased intracellular Ca^{2+} levels, extensive protein tyrosine phosphorylation, and morphological changes. The authors proposed that V in the presence of H_2O_2 may exacerbate allergic reactions [94].

With respect to cellular signalling and activation of transcription factors, V compounds have been found to activate many key effector proteins of the signalling pathways including AP-1, MEK-1, ERK-1, JNK-1, PI-3K and NF-κB [95,96].

Platinum Group Metals: Platinum and Palladium

Palladium, platinum, rhodium, ruthenium, iridium and osmium form a group of elements referred to as the platinum group metals (PGMs). The increasing use of platinum group metals in vehicle catalytic converters leads to the emission of PGMs into the environment, while several other applications (e.g. industrial, jewelry, anticancer drugs, etc.), can also result in exposure to these metals [97]. They are also potent allergens and sensitisers, and are associated with asthma, nausea, increased hair loss, increased spontaneous abortion, dermatitis and other health problems in humans [98].

Platinum (Pt) and palladium (Pd) have been reported to affect the functions of human neutrophils and ciliated respiratory epithelial cells. In the case of neutrophils, both metals, but not rhodium or osmium, were found to potentiate the reactivity, as opposed to the generation of neutrophil-derived oxidants [99]. The potential health risk of these pro-oxidative interactions of the metals with neutrophils was demonstrated in a study in which alpha-1-proteinase inhibitor, the major antagonist of neutrophil elastase, was exposed to activated neutrophils in the absence or presence of Pt or Pd. Exposure to neutrophils in the presence of the metals significantly increased the magnitude of oxidative inactivation of the elastase-inhibitory capacity of alpha-1-proteinase inhibitor. If operative *in vivo,* these pro-oxidative interactions of Pd and Pt with neutrophils in the airways may predispose to pulmonary dysfunction in occupationally- and possibly environmentally-exposed individuals [99].

This latter contention is supported by three additional studies. Firstly, platinum in the +2 and +4 oxidation states was found to increase ROS production by the BEAS-2B bronchial epithelial cell line [100]. Secondly, in a study designed to investigate the role of metals in sino-nasal inflammation in individuals environmentally exposed to dense motor vehicle traffic it was found that platinum levels in the nasal lavage correlated with neutrophilic inflammation, as well as epithelial shedding [101]. Thirdly, Feldman et al. [102] reported that exposure of nasal ciliated epithelium to platinic chloride resulted in slowing of ciliary beating and damage to the structural integrity of the cells. These effects were enhanced in the presence of neutrophils and partially attenuated by catalase, confirming the involvement of neutrophil-derived ROS in platinum-mediated dysfunction of ciliated respiratory epithelium [102].

A summary of the macrophage- and neutrophil-derived inflammatory mediators which are increased following exposure to heavy metals is shown in Table 3.

Heavy Metals and Predisposition to Infection

Cigarette smoking, as mentioned earlier, is a well-documented cause of exposure to heavy metals such as Cd, Pb and V, and a recognised risk for development of respiratory bacterial infection including tuberculosis and severe pneumococcal disease [103-105]. Moreover, cigarette smoke exposure has also been reported to induce the formation of biofilm by various common respiratory and oral pathogens [106,107]. Encasement in biofilm, a self-generated extracellular polymer matrix, is a survival strategy utilised by bacteria to promote persistence by evasion of both host defences and antibiotics, and has been implicated in 60-80% of all microbial infections [108]. Although the exact components of cigarette smoke which promote biofilm formation have not been established, it is noteworthy that nickel, which like Cd, Pb and V is also present in tobacco has been reported to promote biofilm formation by *Escherichia coli* in vitro [109,110]. Alternative sources of exposure to Cd, Pb and V include industrial and environmental pollution, high risk occupations and contaminated food such as fish [111,112].

As mentioned earlier, heavy metals such as Pb and Hg may predispose to infection by promoting oxidative inhibition of the protective functions of neutrophils and monocytes/macrophages. Exposure to Pd may also compromise innate host defences, albeit by

Mediator	Effect	References
ROS	• Increased generation in $CdCl_2$/CdS (0.6μM-1mM) treated neutrophils & macrophages. • Increased generation in MeHgCl(1.25-5μM)-treated monocytes, activated with PMA. • Pt & Pd (0.025-25μM) potentiate reactivity of neutrophil derived ROS. • Vanadium 2+, 3+, 4+ (25μM) mediate hydroxyl radical production from FMLP-& PMA-activated neutrophils. • Increased generation by rat alveolar macrophages treated with sodium metavanadate (50-1000μM).	[21-23] [72] [99] [87] [88]
NO	0.6-10μM $CdCl_2$ cause increased NO production by macrophages.	[23-24]
LTB4	• <30μM $CdCl_2$ augments LTB4 secretion by rabbit alveolar macrophages. • Production increased in neutrophils from lead exposed workers. • Increased generation by rabbit alveolar macrophages exposed to $HgCl_2$.	[29] [45] [74]
Cytokines	• Increased production(or expression of mRNA) of IL-1, TNF, IL-6 by PBMC exposed to $CdCl_2$/CdSO4 (1-10μM). • Increased production of TNF, IL-6, IL-12, PGE2 and decreased production of IL-10 by Pb treated macrophages. • $HgCl_2$ (200nm-5μM) increases the production of IL-1β, TNF, IL-6, IL-8 and downregulates production of IL-Ra & IL-10.	[30,36] [55] [36,77]

Abbreviations: $CdCl_2$, cadmium chloride; CdS, cadmium sulphide; MeHgCl, methyl mercury chloride; Pt, Platinum; Pd, palladium; FMLP, N-formyl-methionyl-leucine-phenylalanine); PMA, phorbol-12-myristate-13-acetate

Table 3: Macrophage- and neurophil-derived inflammatory mediators which are increased following exposure to heavy metals.

an unusual mechanism involving the inactivation of the neutrophil/monocyte chemoattractants, IL8 and the complement cleavage product, C5a. Brief exposure to the metal (25 μM) resulted in either partial (IL-8) or complete attenuation (C5a) of both the Ca2+-mobilizing and chemotactic activities of the chemoattractants for neutrophils [113].

Heavy Metals and Respiratory Disease

Exposure to numerous metals may injure the lung directly or via interaction with cells of the innate immune system. Metal-induced injury may involve the airways, alveoli or interstial tissues of the lung or predispose subjects to bronchial carcinomas. Airway diseases such as bronchitis and bronchiolitis may occur following exposure to metal fumes containing Cd or Hg [114]. Chronic cadmium exposure may cause emphysema by inhibiting the synthesis of plasma α_1-antitrypsin which predisposes to oxidant-mediated tissue injury. Furthermore, exposure to cadmium fumes has been reported to accelerate the progression of emphysema [115]. Not withstanding instead of Non withstanding the mechanisms described above, metals such as platinum, may act as haptens inducing IgE synthesis which, in susceptible individuals may lead to occupational asthma [116].

Metal fume fever is an acute inflammatory response triggered by pulmonary macrophages following exposure to fumes containing metal oxides such as cadmium oxide. Welders are at increased risk and symtoms such as fever, malaise, myalgia, dyspnea and cough begin about 4 – 8 hours after exposure. Bronchial lavage fluid contains high concentrations of tumour necrosis factor-alpha, interleukin-6 and interleukin-8 as well as neutrophils. The course of the illness is self-limiting over 48 hours [116].

A severe form of acute lung injury manifesting as pneumonitis or the acute respiratory distress syndrome (ARDS) known as acute metal fume toxicity may occur following heavy exposure to Cd[117,118] and Hg [114]. These metals are cytotoxic and alveolar damage results in acute lung injury which may progress to respiratory distress.

Mercury in its metallic form may reach the lungs via embolization from the venous system and has been reported in intravenous drug users and in some cases of attempted suicide [119]. An acute inflammatory reaction is elicited in the lung in the form of a foreign body giant cell reaction. Interstitial lung fibrosis may follow the inflammatory response [120].

The carcinogenic potential of metals such as Cd [121] and arsenic [122] may increase the risk of bronchus carcinoma. Smelter workers exposed to these agents were more likely to develop lung cancer. Concomitant exposure to tobacco smoke may accentuate the risk of a pulmonary malignancy.

Conclusion

To minimize the risk of inflammation-mediated tissue damage to bystander tissues, activation of the cells of the innate immune system should be efficient and transient. Occupational and environmental exposure to heavy metals, especially Cd, Pt and V is, however, an important cause of inappropriate activation of these cells, predisposing to oxidant-and protease-mediated tissue damage. Cigarette smoking is a major, albeit eminently avoidable risk, which results in simultaneous exposure to multiple heavy metal toxins and may be compounded both by occupation and proximity to combustion of fossil fuels in the environmental setting. In these latter settings, in which avoidance may be difficult, frequent monitoring and early recognition of symptoms of metal- associated toxicity is recommended, with consideration given to the implementation of anti-inflammatory/anti-oxidative therapy where necessary.

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