Immunological effects of occupational exposure to lead (Review)

CONCETTINA FENGA[1], SILVIA GANGEMI[1], VALENTINA DI SALVATORE[2], LUCA FALZONE[2] and MASSIMO LIBRA[2]

[1]Department of Biomedical, Odontoiatric, Morphological and Functional Images, Section of Occupational Medicine, 'Policlinico G. Martino' Hospital, University of Messina, I-98125 Messina; [2]Department of Biomedical and Biotechnological Sciences, Laboratory of Translational Oncology and Functional Genomics, Section of General and Clinical Pathology and Oncology, University of Catania, I-95124 Catania, Italy

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Abstract. It is well-known that occupational and environmental exposure to several factors, including benzene, heavy metals, chemicals and mineral fibers, is associated with the risk of developing a great number of diseases. Numerous studies have been carried out in order to investigate the mechanisms of toxicity of these substances, with particular regard to the possible toxic effects on the immune system. However, little is known about the influence of heavy metals, such as lead, on the immune system in human populations. Lead is a heavy metal still used in many industrial activities. Human exposure to lead can induce various biological effects depending upon the level and duration of exposure, such as toxic effects on haematological, cardiovascular, nervous and reproductive systems. Several studies demonstrated that exposure to lead is associated to toxic effects also on the immune system, thus increasing the incidence of allergy, infectious disease, autoimmunity or cancer. However, the effects of lead exposure on the human immune system are not conclusive, mostly in occupationally exposed subjects; nevertheless some immunotoxic abnormalities induced by lead have been suggested. In particular, *in vivo*, *in vitro* and *ex vivo* lead is able to improve T helper 2 (Th2) cell development affecting Th1 cell proliferation. Further studies are required to better understand the mechanisms of lead immunotoxicity and the ability of lead to affect preferentially one type of immune response.

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Correspondence to: Dr Silvia Gangemi, Department of Biomedical, Odontoiatric, Morphological and Functional Images, Section of Occupational Medicine, 'Policlinico G. Martino' Hospital, University of Messina, Via Consolare Valeria 1, I-98125 Messina, Italy
E-mail: silviagangemi@hotmail.com

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1. Introduction

It is well-known that occupational and environmental exposure to several factors, including benzene, heavy metals, biological substances and chemicals, may induce adverse effects on human health by promoting the develop of specific disorders and diseases, such as haematological, immunological, cardiovascular, reproductive, neurodegenerative diseases and cancer (1-8). Epidemiological and experimental studies have been carried out in order to investigate the mechanisms of toxicity of these substances, with particular regard to the possible toxic effects on the immune system (9-13). However, little is known about the influence of heavy metals, such as lead, on the immune system in human populations (14).

Lead is a heavy metal which has been widely used in various industrial and domestic settings, due to its physical and chemical properties. Lead is found in pottery, pipes, boat building, manufacture of windows, arms industry, pigments and printing of books. Although its use in several settings has been restricted due to its toxicity to humans, lead and its compounds are still used in many industrial activities such as in production of paints, storage batteries, ceramics, in manufacturing of motor and electric vehicles, building and construction industries, electronic technologies, smelters and welding (15). It is also present in gasoline, in drinking water and in tobacco smoke (16).

In developing countries, many workers continue to be exposed to toxic effects of lead due to lack of knowledge on its safe handling; certain groups of individuals in the general population are still at risk from lead exposure, including pregnant women and their foetuses (17). In this regard, occupational and environmental contamination and exposure to lead still represent a serious issue for human health.

Inhalation and ingestion represent the main routes of exposure, especially for occupationally exposed subject. After absorption, lead distributes and accumulates in blood, bones

and soft tissues, especially liver and kidney. Therefore, these organs are particularly sensitive to lead toxicity (18).

Human exposure to lead can induce various biological effects depending upon the level and duration of exposure, such as toxic effects on haematological, cardiovascular, nervous and reproductive systems (19-24). Although the occurrence of adverse health effects has been largely reduced, the incidence of subclinical effects of chronic exposure to low-doses of lead has been increasing (16).

Furthermore, lead has been classified as possible human carcinogen (group 2B) (25) and its inorganic compounds as probable human carcinogens (group 2A) (26) by the International Agency for Research on Cancer (IARC). Lead exposure has been associated with increased risk of lung, stomach and bladder cancer in diverse human populations (27-31). The proposed mechanisms of lead carcinogenicity include mitogenesis, alterations in gene transcription, oxidative damage and several indirect genotoxicity mechanisms (32,33). Several studies demonstrated that lead is capable of inducing genotoxic and mutagenic effects, but the results on these topics are still contradictory (34,35). Furthermore, lead can cause epigenetic modifications (36,37); however, the role of environmental exposure in epigenetic alterations remains to be fully investigated as, it may be difficult to identify the cause-effect links among environmental factors, epigenetic changes and diseases (38).

In addition, a number of experimental studies have shown that acute and chronic exposure to lead may influence immune responses, even at subclinical doses in animals and also *in vitro* systems, which may increase the incidence of allergy, infectious disease, autoimmunity or cancer (39,40). *In vitro*, lead exposure enhances both B cell and T cell production and major histocompatibility complex (MHC) activity (41). *In vivo* and *ex vivo* animal studies and studies *in vitro* show that lead can activate responses mediated by T helper 2 (Th2) cells and suppress the production of Th1 cells (42). Th cells represent two polarized forms of the $CD4^+$ Th cell-mediated specific immune responses, helper cells can be divided into distinct subsets, including Th1, Th2, Th17 and T regulatory (Treg) cells (43). However, the effects of lead exposure on the human immune system are not conclusive, mostly in occupationally exposed subjects; nevertheless some immunotoxic abnormalities induced by lead have been suggested (44,45). Probably the immunotoxic effects of lead vary according to form of lead, route, dose, time of exposure, and host age and genetic susceptibility (46-48).

2. Mechanisms of lead-induced toxicity

Recent epidemiological studies have demonstrated that exposure to lead is correlated to several diseases such as blood pressure increased levels, kidney disease, neurodegenerative disease and cognitive disorders (46,49-52), which are all associated to oxidative stress. Oxidative stress is known as an imbalance between the production of reactive oxygen species (ROS) and antioxidants in favour of free radicals (ROS) (53,54). Several studies reported that lead can induce oxidative stress in occupationally exposed workers as well as in general population, by two different mechanisms, the first is the pro-oxidative effect of δ-aminolevulinic acid dehydratase (δ-ALAD) and the second is connected with the direct effect of lead on the lipid composition of cellular membranes (55,56). The inhibition of δ-ALAD by lead accounts for the accumulation of its substrate δ-ALA, that can be rapidly oxidized to generate free radicals as superoxide ion ($O_2^{\bullet-}$), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2). Lead has also the capacity of stimulating ferrous ion initiated membrane lipid peroxidation (56).

The decrease of the levels of glutathione (GSH) and protein bound sulfhydryl groups and the changes in the activity of various antioxidant enzymes indicative of lipid peroxidation have been implicated in lead-induced oxidative tissue damage (57). Lead is known to have toxic effects on membrane structure and functions by altering changes in the fatty acid composition of membrane which are more susceptible to lipid peroxidation. This effect is particular evident on erythrocyte membranes because erythrocytes have a high affinity for lead and are more vulnerable to oxidative damage than many other cells (55).

Besides, lead is able to dysregulate the antioxidant defenses, including the antioxidant enzymes and the non-enzymatic antioxidants, such as uric acid (58).

Another mechanism of lead-induced toxicity implicates the immune system. Epidemiological and experimental studies suggest that lead can influence levels of immunoglobulins, alterations in the numbers of lymphocytes, peripheral blood mononuclear cells (PBMCs) and macrophages, impaired responses to mitogens and depression of neutrophil functions (15,59,60).

In vitro studies demonstrated that the treatment of macrophages with lead induces the disregulation of the production of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1α (IL-1α) and IL-6, and promote the synthesis of Th1 cytokines [interferon (IFN)-γ and IL-2] (42,61).

$CD4^+$ Th cell function is mostly sensitive to the immunotoxic effects of lead. Several studies have demonstrated that lead is able to improve Th2 cell development and affect Th1 cell proliferation (42,62). The differential influence of lead on Th1 vs. Th2 activation may be at the level of the antigen-presenting cells (APCs) or the T cell itself. Lead may induce the differential activation of Th subsets by modulating the activity of APC through the modulation of antigen density, a change in the expression of costimulatory molecules on APC, and/or a change in membrane fluidity of the APC (42).

On the contrary, little is known about the possible associations between lead exposure and the function of Th17 and Treg cells.

Besides, lead exerts proinflammatory properties, as reported in studies conducted particularly on occupationally exposed populations (15,46,63).

It has been suggested that lead can also influence the production and the activity of other cells, such as granulocytes and monocytes. The functional activities of these cells are actually regulated by a number of cytokines and by nitric oxide (NO), having both a central role in innate and adaptive immune responses. This hypothesis suggests that lead can influence the cellular and humoral immune response and decrease host resistance (64). However, the exact mechanisms of lead interactions with the immune system in humans still remain unclear (65).

3. Lead exposure and Th1 type cytokines

Th1-mediated responses promote cellular cytotoxic immunity by producing IFN-γ, IL-2 and TNF-α, which have effect on the production of opsonizing and complement-fixing antibodies by B cells, activation of macrophages, cell cytotoxicity, and induction of cell-mediated immunity (CMI). Th1-dominated immune responses predominantly produce a phagocyte-dependent inflammation (43,66).

Production of IFN-γ is frequently used to assess Th1 responses. Lead exposure has been demonstrated to inhibit IFN-γ production *in vivo*, *in vitro* and *ex vivo*. Heo *et al* examined lead effects on the modifications of the Th-derived cytokines IL-4 and IFN-γ *in vitro*, *ex vivo* and *in vivo*. Their results demonstrated that *in vitro*, *in vivo* and *ex vivo* lead inhibits IFN-γ production from a Th1 clone and enhance IL-4 production from a Th2 clone. The plasma IFN-γ levels in mice exposed to lead were significantly lower, on the contrary, plasma IL-4 and IgE levels were elevated, suggesting a preferential activation of Th2 cells and the occurrence of autoimmune responses. Also, lead effects were investigated on expression of IFN-γ mRNA, secretion of IFN-γ protein, proteosomal processing, and kinetics of IFN-γ protein biosynthesis. The results of this study confirmed that *in vitro* IFN-γ biosynthesis is suppressed by lead exposure of Th1 cells at a post-transcriptional stage, indicating that lead inhibitory role is at the translational stage of IFN-γ biosynthesis (67).

In a recent study conducted on three population groups with different period of exposure, the authors highlighted significant higher levels of IFN-γ, IL-2 and IL-12 in workers chronically exposed to lead (13±10 years) compared to workers exposed to lead for 36 to 44 days and to a group of not exposed subjects (68).

It has been demonstrated that lead causes a significant increase of TNF-α production, both *in vitro* and *in vivo* studies (61,69,70). In particular, lead induces an increase of TNF-α at low-doses and a decrease at higher-doses, as demonstrated in human peripheral blood mononuclear cells, in rats, and in exposed workers compared to controls (71).

To evaluate the effects of lead exposure on the activity of TNF-α, rabbit pulmonary macrophages were exposed *in vitro* to particulate lead oxide (PbO) for periods of up to 72 h and then assayed for the activity of TNF-α released after stimulation with lipopolysaccharide (LPS). The levels of TNF-α obtained from PbO-treated cells were decreased in a dose-dependent manner as compared with metal-free control cells for each time-point examined. In addition, incubation of cell-free TNF-α with PbO resulted in a decrease of macrophage cytotoxicity directed against TNF-α-sensitive tumor target cells (72).

As regard the influence of lead on the immune system in humans, Yücesoy *et al* evaluated the levels of serum IL-1β, IL-2, TNF-α and IFN-γ in a group of workers occupationally exposed to lead and cadmium. The results reported that chronic lead and cadmium exposure in humans resulted in significant reduction of the serum IL-1β levels, but did not influence IL-2 and TNF-α levels. A decrease in IFN-γ levels was found in lead exposed group (46).

A great number of studies have shown that lead exposure can produce higher levels of the inflammatory cytokines, such TNF-α, IL-6 and IL-1β both *in vitro* and *in vivo* (15,73,74). In particular, Liu *et al* demonstrated that antioxidant molecules could inhibit the lead-induced production of several proinflammatory cytokines, mainly IL-1β, TNF-α and IL-6. The synthesis of proinflammatory cytokines involves the activation of mitogen-activated protein kinases (MAPKs) and nuclear factor-κB (NF-κB) (75).

4. Lead exposure and Th2 type cytokines

Th2-mediated responses are responsible for humoral immunity and Th2 cells produce IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13, which induce strong antibody responses, including IgE, eosinophil differentiation and activation, but inhibit several functions of phagocytic cells, thus promoting a form of phagocyte-independent inflammation (43).

Epidemiological and experimental studies have been conducted in order to evaluate the effects of lead on Th2 cytokines. It has been reported that exposure to lead promotes IL-4 production and inhibits IFN-γ synthesis in wild-type BALB/c mice, and enhances delayed-type hypersensitivity (DTH) responses in IFN-γ deficient mice (76).

Iavicoli *et al* investigated the effects of a broad range of blood lead concentrations in mice, obtained over an entire lifespan (from conception to sacrifice) on serum levels of two type 1 cytokines (IL-2 and INF-γ) and one type 2 cytokine (IL-4). At higher dietary Pb levels (40 and 400 ppm), a significant increase in IL-4 production was associated with a profound decrease in INF-γ and IL-2 synthesis. At the lowest Pb diet level (0.02 ppm), which resulted in a blood lead level of 0.8 μg/dl, which is below background (2-3 μg/dl) values in humans, increases in INF-γ and IL-2 production along with a significant decrease in IL-4 production were observed. These findings provided evidence of a different trend of lead-induced cytokines availability depending on different blood lead concentration. As blood lead level increases, there is a skewing toward Th2, while Th1 production is promoted at lower blood lead values (48).

In another study, Valentino *et al* assessed plasma levels of nitrites and nitrates (NOx), IL-2, IL-4, IL-6, IL-10, TNF-α and INF-γ in healthy workers with very low and low lead exposure compared to a control group of not exposed workers. Subjects exposed to low levels of lead showed significantly higher plasma levels of IL-10 and TNF-α, compared to non-exposed workers. The significant positive correlation between lead and IL-10 levels seems to suggest a dose-dependent mechanism. The study suggests that cytokine balance is modified with an increase of plasma TNF-α and IL-10 levels. Interestingly, it is well-documented that these two cytokines have different biological activity. So the authors hypothesised that the system of immunophlogosis of exposed workers in response to low lead levels shows an increase of proinflammatory cytokines, such as TNF-α, with a consequent increase of other cytokines, such as IL-10, considered a T cell cross-regulatory factor (71).

Hsiao *et al* assessed whole blood Pb levels and changes in levels of key Th1 and Th2 cytokines in school children from Taiwanese communities with different levels of lead exposure depending upon their living area (urban area with new homes, urban area with old homes, rural site with old homes and area located near an oil refinery) and the absence of respiratory and allergic disease. Refinery children had significantly increased

Pb levels and children with allergies had serum Th2 cytokine levels (IL-4 and IL-5) significantly higher and Th1 cytokine levels (IFN-γ and IL-12) significantly lower than their healthy counterparts. Healthy refinery students did not show altered Th1 or Th2 cytokine levels. The authors concluded that substantially increased whole blood Pb levels may induce Th cell dysregulation and affect the production of key Th1 and Th2 cytokines, which could also contribute to development of respiratory allergic disorders (40).

Recently, it has been hypothesized that lead can influence count and function of red blood cells (RBCs), platelet (PLT) and leukocyte (WBC) indices by acting on immune system. In a study on 37 males occupationally exposed to lead for a short period of time (36-44 days), the authors evaluated peripheral blood morphology and levels of several cytokines, including IL-7 and IL-9, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), hepatocyte growth factor (HGF), stem cell factor (SCF), platelet-derived growth factor (PDGF), and platelet endothelial cell adhesion molecule-1 (PECAM-1). The results of the study highlighted decreased haemoglobin level, probably due to the lead induced inhibition of ALAD, and increased counts of WBC and PLT. The measurement of cytokines showed a significant decrease in IL-7 levels, while the level of IL-9 did not change significantly. According to the authors, a short-term lead exposure could be associated to a decrease in levels of cytokines related to haematopoiesis (58).

5. Th17 type cytokines and Treg cells

Th17 cells are responsible for host defence against infections by bacteria and fungi and in tissue inflammation. Th17 cells produce IL-17 and IL-22, which activate mononuclear phagocytes and also recruit neutrophils, thus inducing epithelial antimicrobial responses (66). Treg cells were originally identified as $CD4^+CD25^+$ and are able to induce immune-suppressive responses (77). Unfortunately, there are few studies on the effects of lead on these Th cells and their results are not conclusive. As regard IL-17, Dobrakowski *et al* demonstrated that short-term exposure to lead did not influence IL-17 levels, while increased levels of IL-17 were reported in workers chronically exposed to lead (68).

In vivo, $Foxp3^+$ Tregs were increased in both the thymus and peripheral lymphoid organs of rats exposed to lead, although conventional $CD4^+$ T cells were decreased. The mechanism by which lead exposure can induce Treg number increase remains unclear. It has been demonstrated that lead enhances rather than inhibits suppressor cell activity (78). In contrast, this study suggest the hypothesis that lead exposure can lead to immunosuppression by upregulating production of Tregs.

6. Conclusion

It is well-established that exposure to lead can promote the development of several diseases and disorders through different mechanisms of toxicity. The hypothesis that the immune system represents a critical target for lead-induced toxicity has been suggested by recent epidemiological and experimental studies. In particular, lead can affect both cellular and humoral immune response by altering Th cell function and increasing susceptibility to the development of autoimmunity and hypersensitivity (44). In the context of hypersensitivity, the use of cyclophosphamide may affect the immune response by reducing the expression levels of several cytokines as occurred in our previous *in vivo* experience (79,80). A great number of epidemiological and experimental studies are available, but results are often contradictory because of some limitations, such as the presence of possible confounding factors, the size of the samples, the dose and time of exposure to lead, and the use of safety measurement in occupational environment. Furthermore, few studies evaluated the effects of lead exposure on subjects occupationally exposed to low-dose of lead. Further studies are needed in order to investigate the mechanisms of lead immunotoxicity and the ability of lead to affect preferentially one type of immune response.

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