ELSEVIER

FEMS Immunology and Medical Microbiology 34 (2002) 1–7

FEMS Immunology and Medical Microbiology

www.fems-microbiology.org

MiniReview

Effects of chromium on the immune system

Richa Shrivastava, R.K. Upreti, P.K. Seth, U.C. Chaturvedi *

Biomembrane Division, Industrial Toxicology Research Centre, Mahatma Gandhi Marg, Lucknow 226001, India

Received 11 May 2002; accepted 20 May 2002

First published online 1 August 2002

Abstract

Chromium is a naturally occurring heavy metal found commonly in the environment in trivalent, Cr(III), and hexavalent, Cr(VI), forms. Cr(VI) compounds have been declared as a potent occupational carcinogen among workers in chrome plating, stainless steel, and pigment industries. The reduction of Cr(VI) to Cr(III) results in the formation of reactive intermediates that together with oxidative stress oxidative tissue damage and a cascade of cellular events including modulation of apoptosis regulatory gene p53, contribute to the cytotoxicity, genotoxicity and carcinogenicity of Cr(VI)-containing compounds. On the other hand, chromium is an essential nutrient required to promote the action of insulin in body tissues so that the body can use sugars, proteins and fats. Chromium is of significant importance in altering the immune response by immunostimulatory or immunosuppressive processes as shown by its effects on T and B lymphocytes, macrophages, cytokine production and the immune response that may induce hypersensitivity reactions. This review gives an overview of the effects of chromium on the immune system of the body.

Keywords: Chromium; Immune response; T lymphocyte; B lymphocyte; Macrophage; Apoptosis; Cytotoxicity; Micronutrient

1. Introduction

A large number of biologically active substances, including heavy metals, may have direct, primary or secondary effects on the immune system and are of interest to pathologists, immunologists and toxicologists. Various metals are responsible for many biochemical, immunological and physiological activities of the body as micronutrients. But some of them can give rise to disordered functions of the immune system resulting in increased susceptibility to infection, a variety of hypersensitivity reactions, autoimmune diseases and neoplasia. Heavy metals are of significant importance in altering the immune response by immunostimulatory or immunosuppressive mechanisms.

Chromium is a naturally occurring heavy metal found in the environment commonly in trivalent, Cr(III), and hexavalent, Cr(VI), forms. The reduction of Cr(VI) to Cr(III) results in the formation of reactive intermediates that contribute to the cytotoxicity, genotoxicity and carcinogenicity of Cr(VI)-containing compounds. The major non-occupational source of chromium for humans is food such as vegetables, meat, urban air, hip or knee prostheses and cigarettes [1,2]. Cr(VI) is a widely used in industrial chemicals, extensively used in paints, metal finishes, steel including stainless steel manufacturing, alloy cast irons, chrome and wood treatment. On the contrary, Cr(III) salts such as chromium polynicotinate, chromium chloride and chromium picolinate (CrP) are used as micronutrients and nutritional supplements and have been demonstrated to exhibit a significant number of health benefits in animals and humans [3].

Chromium enters the body through the lungs, gastrointestinal tract and to a lesser extent through skin. Inhalation is the most important route for occupational exposure, whereas non-occupational exposure occurs via ingestion of chromium-containing food and water. Regardless of route of exposure Cr(III) is poorly absorbed whereas Cr(VI) is more readily absorbed. Further, absorption of Cr(VI) is poorer by oral route, it is thus not very toxic when introduced by the oral route. But chromium is very toxic by dermal and inhalation routes and causes lung cancer, nasal irritation, nasal ulcer, hypersensitivity reactions and contact dermatitis. All the ingested Cr(VI) is reduced to Cr(III) before entering in the blood stream. The main routes for the excretion of chromium are via kidney/urine and the bile/feces [1,2,4]. Cr(III) is unable

* Corresponding author.
E-mail address: uchaturvedi@yahoo.com (U.C. Chaturvedi).

0928-8244/02/$22.00
PII: S0928-8244(02)00345-0

to enter into the cells but Cr(VI) enters through membrane anionic transporters. Intracellular Cr(VI) is metabolically reduced to Cr(III). Cr(VI) does not react with macromolecules such as DNA, RNA, proteins and lipids. However, both Cr(III) and the reductional intermediate Cr(V) are capable of co-ordinate, covalent interactions with macromolecules. Chromium is an essential nutrient required by the human body to promote the action of insulin for the utilization of sugars, proteins and fats. CrP has been used as nutritional supplement; it controls blood sugar in diabetes and may reduce cholesterol and blood pressure levels. Chromium increases insulin binding to cells, insulin receptor number and activates insulin receptor kinase leading to increased insulin sensitivity [3].

But high doses of chromium and long term exposure of it can give rise to various, cytotoxic and genotoxic reactions that affect the immune system of the body. However, the mechanism of the Cr(VI)-induced cytotoxicity is not entirely understood. A series of in vitro and in vivo studies have demonstrated that Cr(VI) induces oxidative stress through enhanced production of reactive oxygen species (ROS) leading to genomic DNA damage and oxidative deterioration of lipids and proteins. A cascade of cellular events occur following Cr(VI)-induced oxidative stress including enhanced production of superoxide anion and hydroxyl radicals, increased lipid peroxidation and genomic DNA fragmentation, modulation of intracellular oxidized states, activation of protein kinase C, apoptotic cell death and altered gene expression [5]. Some of the factors in determining the biological outcome of chromium exposure include the bioavailability, solubility of chromium compounds and chemical speciation, intracellular reduction and interaction with DNA. The chromium genotoxicity manifests as several types of DNA lesions, gene mutations and inhibition of macromolecular synthesis. Further, chromium exposure may lead to apoptosis, premature terminal growth arrest or neoplastic transformation. Chromium-induced tumor suppressor gene p53 and oxidative processes are some of the major factors that may determine the cellular outcome. Studies have utilized these approaches to understand the interrelationship between chromium-induced genotoxicity, apoptosis and effects on immune response. This review gives an overview of the effects of chromium on the immune system of the body. Due to constraint of space only a limited number of studies have been cited.

2. Effects of chromium on lymphocytes

The effect of chromium on lymphocytes has been investigated in several studies. Borella et al. [6] investigated the in vitro effect of toxic metals including Cr(III) and Cr(VI) on phytohemeagglutinin-induced blastogenesis in human lymphocytes. Cr(VI) shows a biphasic pattern, with a stimulatory effect at the lowest concentrations tested and an inhibitory effect at higher concentrations. Faleiro et al. [7] described the effect of cobalt–chromium–molybdenum (CoCrMo) disc samples on the CD3-mediated in vitro response of human peripheral blood T lymphocytes. Inhibition of lymphocyte proliferation is observed in the presence of CoCrMo disc samples. Ultrastructural studies using scanning electron microscopy revealed that the differences in the number of blast cells on CoCrMo discs from a 4-day culture are consistent with the results observed in the proliferation experiments. The proliferation of both T and B cells and the production of immunoglobulins by lipopolysaccharide-stimulated B cells are significantly inhibited by cobalt–chromium particles after intraperitoneal injection in mice or in in vitro experiments on murine lymphocytes. The data indicate that the metal-induced immunosuppression may be an important factor in the development of implant-associated infection in patients with a prosthesis [8].

On the other hand, several studies have shown that chromium salts/alloys have no effect on cells of the immune system. Yucesoy et al. [9] investigated the immunotoxic effects of lead, cadmium, nickel and chromium on natural killer (NK) cell activity in vitro. None of the metal salts have any effect on NK cell function. Similarly no effect is seen in stainless steel welders exposed to chromium and nickel contained in welding fumes, for the kinetics of cell division in culture of peripheral blood lymphocytes [10].

3. Effects of chromium on macrophages

The inhalation of chromium does not affect lung morphology, but macrophages are enlarged, multinucleated or vacuolated and accumulate in intra-alveolar spaces as nodules. Higher doses of Cr(VI) depress the phagocytic activity of alveolar macrophages and the humoral immune response, whereas lower doses of Cr(VI) stimulate phagocytic activity of the alveolar macrophages and increase the humoral immune response [11]. Macrophages can be induced to produce nitric oxide (NO), which is important for various functions. Tian and Lawrence [12] studied the effect of various metals including chromium and reported that chromium does not modulate NO production by cytokine (IFN-γ, TNF-α)-stimulated murine macrophages. Chromium moderately suppresses inducible NO synthase, which suggests that it may directly modify enzyme or cofactor activity. Thus, metals may be pathogenic via inhibition or enhancement of NO production by suppression of defense mechanisms or induction of hypersensitivity.

Howie et al. [13] reviewed the literature on animal and cellular models used to study the response to wear and corrosion products of cobalt–chrome alloy implants. Injections of large numbers of particles in a single bolus lead to acute inflammation and necrosis, followed by a chronic inflammatory response. Macrophages are the predominant

cell type and may persist in the tissues for years. In vitro studies show that cobalt–chrome alloy particles induce the release of inflammatory mediators from macrophages before causing cell death. These mediators have significant effects on osteoblast-like cells, besides inducing bone resorption. Lee et al. [14] have shown dose-dependent effects of chromium chloride and CrP on glucose uptake, superoxide anion ($O2^-$) production, activity of glucose-6-phosphate dehydrogenase and phagocytosis of *Escherichia coli* by incubation of pulmonary alveolar macrophages in medium in the presence or absence of insulin.

Gatta et al. [15] studied the effects of dietary chromium yeast supplementation on the immune response of rainbow trout (*Oncorhynchus mykiss*). A positive influence is observed on serum lysozyme activity in fish maintained on the high-chromium diet. Significant differences are found in the ability to phagocytose and level of respiratory burst elicited by macrophages of fish fed supplemented chromium. Jain and Kannan [16] demonstrated that chromium supplementation inhibits TNF-α secretion in U937 monocytes cultured in high -glucose medium, which appears to be mediated by its antioxidative effect.

4. Effects of chromium on cytokines

The effect of chromium on cytokines has been studied either by administration of chromium or implantation of prosthesis containing chromium alloy. Myers et al. [17] studied the effect of dietary CrP and recombinant porcine growth hormone, somatotropin (rPST) administration on growth performance and cytokine production in Landrace-Poland China gilts. They showed that CrP-treated swine have very high IL-6 levels while no differences are seen in plasma IL-6 from pigs treated with the rPST and rPST+CrP. Peripheral blood mononuclear cells from CrP-treated animals produce more IL-2 than from all other groups.

Shumilla et al. [18] reported that pretreatment of A549 human lung carcinoma cells with non-toxic levels of Cr(VI) inhibits TNF-α-stimulated expression of the endogenous gene for IL-8 and of an NF-kappaB-driven luciferase gene construct, but not expression of urokinase, a gene with a more complex promoter. Chromium does not inhibit TNF-α-stimulated IkappaBalpha degradation or translocation of NF-kappaB-binding proteins to the nucleus. These data indicate that non-toxic levels of Cr(VI) selectively inhibit NF-kappaB transcriptional competence by inhibiting interactions with coactivators of transcription rather than DNA binding.

Chromium effects immune functions of urban women who are exposed to chromium by vehicular traffic. Serum IgE and spontaneous in vitro production of IL-4 and IFN-γ are higher by mononuclear blood cells of women who are exposed to an urban environment [19].

Several studies have investigated the effect of prosthesis on the cytokine responses of the body. Horowitz et al. [20] elucidated the mechanisms by which cobalt–chromium particulate wear debris contribute to the aseptic loosening of total joint prostheses. Incubation of macrophages with cobalt chromium lead to release of TNF-α and PGE2, but not of IL-1β or IL-6. Macrophages play a role in the initiation of bone resorption at the interface through the phagocytosis of cobalt chromium particles and subsequent release of TNF-α and PGE2.

Prabhu et al. [21] have examined a number of basic biological responses of the J774A.1 cell line, including cell proliferation, apoptosis, cytokines secreted into the culture supernatant (TNF-α, IL-1α, IL-6, and IL-12) and mRNA expression of the cytokines (TNF-α, IL-1α, IL-6, IFN-α, M-CSF and TGF-β) in response to cobalt–chrome alloy particles (CoCr). The results indicate that the relative contribution of CoCr particles in J774A.1 activation is negligible and a change in metabolic activity of J774A.1 cells is observed only at higher concentrations of CoCr particles.

Granchi et al. [22] determined whether the serum levels of bone-resorbing cytokines (IL-1β, TNF-α, IL-6, GM-CSF) are altered in patients with aseptic loosening of a total hip prosthesis. The results suggest that a CoCr implant releases a large amount of metal ions which could mediate the priming or the renewal of a cell-mediated hypersensitivity reaction. The prevalence of circulating Thl lymphocytes accounts for both the significant increase of TNF and the significant decrease of IL-6 in unstimulated PBMC of patients, as well as the significant increase of the 'index of cytokine release' after challenge with metal ions [22].

5. Effects of chromium on immune response

Burton et al. [23] studied the effects of supplemental dietary chromium on immune responses of dairy cows subjected to physical and metabolic stresses. They showed that supplemental chromium elevates anti-ovalbumin-antibody responses and mitogen-stimulated blastogenic responses of peripheral blood mononuclear cells. Another study shows that supplemental chromium had no beneficial effect on health status, mastitis-related parameters or neutrophil phagocytic activity of dairy cows [24]. Van de Ligt et al. [25] have studied the effect of chromium tripicolinate supplementation of diet on porcine immune response during the postweaning period and found no effect on the performance and immune status.

Khangarot et al. [26] studied the effects of subtoxic levels of chromium on humoral and cell-mediated immune responses, blood parameters, susceptibility to bacterial (*Aeromonas hydrophila*) infection and macrophage activity in the freshwater air-breathing Asian catfish, *Saccobranchus fossilis*. Fish exposed to chromium have lower spleen weight, lower antibody titer, reduced numbers of splenic

plaque-forming cells and higher counts of splenic lymphocytes. Differential leukocyte counts revealed that chromium exposure causes a significant decrease in large and small lymphocytes, whereas neutrophils and thrombocytes increase. Concanavalin A-induced proliferation of splenic and pronephric lymphocytes is decreased. The eye-allograft rejection time is increased. Fish exposed to chromium exhibit higher susceptibility to *A. hydrophila* infection. The phagocytic activity of splenic and pronephros macrophages is significantly decreased. Arunkumar et al. [27] injected African mouth breeder *Oreochromis mossambicus* (Peters) intraperitoneally with Cr(III) and Cr(VI) and subsequently immunized with bovine serum albumin. Both forms of chromium suppress the antibody response, with Cr(VI) being more suppressive than Cr(III). Reduction in spleen weight, splenocyte number and the percentage of blood lymphocytes is observed following administration of both forms of chromium. An early study done on human cells by Borella et al. [28], who studied the effects of Cr(VI) and other metals on cultured human lymphocytes found that chromium induces reductions in both blastogenesis and immunoglobulin production in relation to its capability to enter the cells.

6. Chromium-induced hypersensitivity reactions

Chromium induces two types of hypersensitivity reactions: type I, anaphylactic type, and type IV, the delayed-type hypersensitivity. Chromium is one of the most common skin sensitizers in the general population. Such exposure, for example, involves handling cement, tanning of leather, chromium plating. Cr(VI) readily transverses cell membrane and undergoes intracellular reduction to Cr(III) by forming a conjugate with proteins to act as complete antigen. Circulating antibodies against Cr(III) but not against Cr(VI) have been identified in sensitized animals [29]. Dermal exposure of chromium produces irritant and allergic contact dermatitis [29,30–33]. It is observed that keratinocytes are the first target cells affected by chromium in causing contact dermatitis. These cells can be directly activated through the expression of the membrane antigen ICAM-I, a ligand of the leucocytes antigen LFA-I and the production of cytokines including a significant release of TNF-α. These findings indicate that chromium may be able to induce an aggressive cellular effect and may play a major role in keratinocyte activation during contact dermatitis [34]. Development of allergic contact dermatitis by exposure to chromium has been reported in several studies. Thomas et al. [35] reported a case of a 37-year-old man who developed an aseptic intolerance reaction to a chromium–cobalt alloy. Skin testing gave a delayed-type reaction to dichromate. Immunohistology reveal a monocytic and dense T-cell infiltrate. The latter, instead of being random, showed an oligoclonal T-cell receptor rearrangement. The actual tissue mRNA expression for IL-4, IL-6, and IFN-γ was visualized by reverse transcription-polymerase chain reaction. This indicated a Th1-type mediator expression (IL-6 and IFN-γ but not IL-4). Chromium may also be less commonly involved in immediate or type I hypersensitivity reactions. Cases of asthma with an immediate or delayed response have been observed following occupational exposure to chromium [36]. Hassmanova et al. [37] have studied occupational diseases caused by chromium and its compounds. They have reported perforations of the nasal septum, bronchial asthma, allergic rhinitis and contact allergic eczemas and an exceptional finding of a chromium ulcer (pigeonneaux) on the lower extremity of a builder.

7. Chromium-induced cell death

Chromium is known to have cytotoxic effects on cells. Vasant et al. [38] have shown that apoptosis is the mode of cell death of human lymphocytes in the presence of both Cr(V) and Cr(VI). Pretreatment of cells with antioxidants, before exposure to chromium(V) complexes, reverses apoptosis partially. The possibility has been suggested for the formation and implication of reactive oxygen species in Cr(V)-induced apoptosis of human lymphocyte cells. The studies of Carlisle et al. [39] show that chromium-induced apoptosis of normal diploid human lung fibroblasts is p53 dependent.

8. Mechanisms of action of chromium on cells of immune system

Easy availability, convenient to handle and maintain in vitro in culture make lymphocyte and macrophage ideal cells to study the mechanism of cytotoxicity and genotoxicity and, thus, the mechanism of effects on immune functions of body. Initial studies used DNA strand breaks and sister-chromatid exchange (SCE) in blood lymphocytes to investigate the toxicity of chromium. Gao et al. [40] incubated human lymphocytes with Cr(VI) at 37°C for 3 h and showed a dose-dependent increase in DNA strand breaks without concurrent cytotoxicity. In contrast, Cr(III) fails to induce DNA strand breaks at subcytotoxic concentrations. Gennart et al. [41] determined SCE in blood lymphocytes in 26 male workers occupationally exposed to chromium, cobalt and nickel dust and in 25 controls matched for age and smoking habits. An analysis of variance on the SCE rank values reveal that both exposure status (exposed persons vs. controls) and smoking habits (smokers and former smokers vs. never smokers) have a statistically significant effect.

Katsifis et al. [42] and Lai et al. [43] compared the effect of chromium and Ni–Cr on SCE in lymphocytes to obtain an understanding of the mutagenic effect of Cr(VI) in humans. The data indicate that antagonism may occur

when human lymphocytes are exposed simultaneously to Ni(II) and Cr(VI), suggesting an explanation for epidemiological studies reporting conflicting results for cytogenetic effects in lymphocytes of workers exposed to chromium and nickel. On the other hand, additive damaging effect of chromium and curcumin on DNA of human lymphocytes and gastric mucosa (GM) has been reported by Blasiak et al. [44] by using the alkaline single cell gel electrophoresis (comet assay). Moreover, curcumin itself can damage DNA of these cells and the total effect of chromium and curcumin is additive.

Blasiak and Kowalik [45] compared the effects of Cr(III) and Cr(VI) on the DNA damage in human lymphocytes using the comet assay. The effect is dose dependent. Treated cells recover within a 120-min incubation. Cr(III) causes greater DNA migration than Cr(VI). Catalase, an enzyme inactivating hydrogen peroxide, decreases the extent of DNA damage induced by Cr(VI), but not that induced by Cr(III). Lymphocytes exposed to Cr(VI) and treated with endonuclease III, which recognizes oxidized pyrimidines, displayed a greater extent of DNA damage than those not treated with the enzyme. Such an effect is not observed when Cr(III) is tested. The results obtained suggest that reactive oxygen species and hydrogen peroxide may be involved in the formation of DNA lesions by Cr(VI). The comet assay did not indicate the involvement of oxidative mechanisms in the DNA-damaging activity of Cr(III) and due to its binding to cellular ligands it may play a role in its genotoxicity. Trzeciak et al. [46] studied chromium-induced damage to DNA in both the GM cells and lymphocytes by the comet assay. The effect induced by K2Cr2O7 in GM cells is similar to that seen in the lymphocytes.

Recent in vitro and in vivo studies have investigated the effect of Cr(VI) on human PBMC, chronic myelogenous leukemic K562 cells and J774A.1 murine macrophage cells and on female C57BL/6Ntac and p53-deficient C57BL/6TSG p53 mice using comet assay, laser scanning confocal microscopy and flow cytometry. [5,47]. The findings demonstrate that chromium induces an oxidative stress that results in oxidative deterioration of biological macromolecules. Chromium undergoes redox cycling, resulting in enhanced production of reactive oxygen species such as superoxide ion, hydroxyl radicals and hydrogen peroxide. These reactive oxygen species result in increased lipid peroxidation, enhanced excretion of urinary lipid metabolites, modulation of intracellular oxidized states, DNA damage, membrane damage, altered gene expression and apoptosis. Enhanced production of nuclear factor-kappaB and activation of protein kinase C occur. Furthermore, the p53 tumor suppressor gene is involved in the cascade of events associated with the toxicities of these cations. Taken together, oxidative stress and oxidative tissue damage and a cascade of cellular events including modulation of apoptosis regulatory gene p53 are involved in Cr(VI)-induced toxicity and carcinogenesis [5,47].

9. Conclusions

Chromium is a naturally occurring heavy metal and is an essential micronutrient required to promote the action of insulin in body tissues so that the body can use sugars, proteins and fats. Clinical and laboratory evidences indicate that hexavalent chromium, Cr(VI), is responsible for most of the toxic actions. Chromium is very toxic by inhalation and dermal route and causes lung cancer, nasal irritation, nasal ulcer and hypersensitivity reactions like contact dermatitis and asthma. Chromium affects various components of the immune system and may result in immunostimulation or immunosuppression. The reduction of Cr(VI) to Cr(III) results in the formation of reactive intermediates that together with oxidative stress and oxidative tissue damage and a cascade of cellular events including modulation of apoptosis regulatory gene p53 contribute to the cytotoxicity, genotoxicity and carcinogenicity of Cr(VI)-containing compounds. Exposure to Cr(VI) can result in various point mutations in DNA and to chromosomal damage, as well as to oxidative changes in proteins. The relative importance of the chromium ions and of the free oxidizing radicals that may generate in causing cancers and allergic sensitization remain to be elucidated.

Acknowledgements

U.C.C. is an Emeritus Scientist of the Council of Scientific and Industrial Research, New Delhi (Project No. 21(0478)/00/EMR-II).

References

[1] Hertel, R.F. (1986) Sources of exposure and biological effects of chromium. IARC Sci. Publ. 71, 63–77.
[2] US Environmental Protection Agency (1984) Health effects assessment for hexavalent chromium. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria, EPA/540/1-86-019 Cincinnati, updated 1998.
[3] Anderson, R.A. (2000) Chromium in the prevention and control of diabetes. Diabetes Metab. 26, 22–27.
[4] Kerger, B.D., Paustenbach, D.J., Corbett, G.E. and Finley, B.L. (1996) Absorption and elimination of trivalent and hexavalent chromium in humans following ingestion of a bolus dose in drinking water. Toxicol. Appl. Pharmacol. 141, 145–158.
[5] Bagchi, D., Bagchi, M. and Stohs, S.J. (2001) Chromium (VI)-induced oxidative stress, apoptotic cell death and modulation of p53 tumor suppressor gene. Mol. Cell. Biochem. 222, 149–158.
[6] Borella, P., Manni, S. and Giardino, A. (1990) Cadmium, nickel, chromium and lead accumulate in human lymphocytes and interfere with PHA-induced proliferation. J. Trace Elem. Electrolytes Health Dis. 4, 87–95.
[7] Faleiro, C., Godinho, I., Reus, U. and de Sousa, M. (1996) Cobalt-chromium-molybdenum but not titanium-6aluminium-4vanadium alloy discs inhibit human T cell activation in vitro. Biometals 9, 321–326.

[8] Wang, J.Y., Wicklund, B.H., Gustilo, R.B. and Tsukayama, D.T. (1997) Prosthetic metals impair murine immune response and cytokine release in vivo and in vitro. J. Orthop. Res. 15, 688–699.
[9] Yucesoy, B., Mirshahidi, S., Yucesoy, C. and Karakaya, A. (1999) In vitro effects of various metals on natural killer cell activity in cultured human lymphocytes. Immunopharmacol. Immunotoxicol. 21, 599–607.
[10] Myslak, M. and Kosmider, K. (1997) Kinetics of cell division in peripheral blood lymphocytes of stainless steel welders. Med. Pr. 48, 261–264.
[11] Glaser, U., Hochrainer, D., Kloppel, H. and Kuhnen, H. (1985) Low level chromium (VI) inhalation effects on alveolar macrophages and immune functions in Wistar rats. Arch. Toxicol. 57, 250–256.
[12] Tian, L. and Lawrence, D.A. (1996) Metal-induced modulation of nitric oxide production in vitro by murine macrophages: lead, nickel, and cobalt utilize different mechanisms. Toxicol. Appl. Pharmacol. 141, 540–547.
[13] Howie, D.W., Rogers, S.D., McGee, M.A. and Haynes, D.R. (1996) Biologic effects of cobalt chrome in cell and animal models. Clin. Orthop. 329, S217–232.
[14] Lee, D.N., Yen, H.T., Shen, T.F. and Chen, B.J. (2000) Chromium-induced glucose uptake, superoxide anion production, and phagocytosis in cultured pulmonary alveolar macrophages of weanling pigs. Biol. Trace Elem. Res. 77, 53–64.
[15] Gatta, P.P., Thompson, K.D., Smullen, R., Piva, A., Test, S. and Adams, A. (2001) Dietary organic chromium supplementation and its effect on the immune response of rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol. 11, 371–382.
[16] Jain, S.K. and Kannan, K. (2001) Chromium chloride inhibits oxidative stress and TNF-alpha secretion caused by exposure to high glucose in cultured U937 monocytes. Biochem. Biophys. Res. Commun. 289, 687–691.
[17] Myers, M.J., Farrell, D.E., Evock-Clover, C.M., Cope, C.V., Henderson, M. and Steele, N.C. (1995) Effect of recombinant growth hormone and chromium picolinate on cytokine production and growth performance in swine. Pathobiology 63, 283–287.
[18] Shumilla, J.A., Broderick, R.J., Wang, Y. and Barchowsky, A. (1999) Chromium(VI) inhibits the transcriptional activity of nuclear factor-kappaB by decreasing the interaction of p65 with cAMP-responsive element-binding protein-binding protein. J. Biol. Chem. 274, 207–212.
[19] Boscolo, P., Di Gioacchino, M., Sabbioni, E., Di Giacomo, F., Reale, M., Volpe, A.R., Di Sciascio, M.B., Conti, P. and Giuliano, G. (2000) Lymphocyte subpopulations, cytokines and trace elements in asymptomatic atopic women exposed to an urban environment. Life Sci. 67, 1119–1126.
[20] Horowitz, S.M., Luchetti, W.T., Gonzales, J.B. and Ritchie, C.K. (1998) The effects of cobalt chromium upon macrophages. J. Biomed. Mater. Res. 41, 468–473.
[21] Prabhu, A., Shelburne, C.E. and Gibbons, D.F. (1998) Cellular proliferation and cytokine responses of murine macrophage cell line J774A.1 to polymethylmethacrylate and cobalt-chrome alloy particles. J. Biomed. Mater. Res. 42, 655–663.
[22] Granchi, D., Ciapetti, G., Stea, S., Savarino, L., Filippini, F., Sudanese, A., Zinghi, G. and Montanaro, L. (1999) Cytokine release in mononuclear cells of patients with Co-Cr hip prosthesis. Biomaterials 20, 1079–1086.
[23] Burton, J.L., Mallard, B.A. and Mowat, D.N. (1993) Effects of supplemental chromium on immune responses of periparturient and early lactation dairy cows. J. Anim. Sci. 71, 1532–1539.
[24] Chang, X., Mallard, B.A. and Mowat, D.N. (1996) Effects of chromium on health status, blood neutrophil phagocytosis and in vitro lymphocyte blastogenesis of dairy cows. Vet. Immunol. Immunopathol. 52, 37–52.
[25] van de Ligt, J.L., Lindemann, M.D., Harmon, R.J., Monegue, H.J. and Cromwell, G.L. (2002) Effect of chromium tripicolinate supplementation on porcine immune response during the postweaning period. J. Anim. Sci. 80, 449–455.
[26] Khangarot, B.S., Rathore, R.S. and Tripathi, D.M. (1999) Effects of chromium on humoral and cell-mediated immune responses and host resistance to disease in a freshwater catfish, *Saccobranchus fossilis* (Bloch). Ecotoxicol. Environ. Saf. 43, 11–20.
[27] Arunkumar, R.I., Rajasekaran, P. and Michael, R.D. (2000) Differential effect of chromium compounds on the immune response of the African mouth breeder Oreochromis mossambicus (Peters). Fish Shellfish Immunol. 10, 667–676.
[28] Novey, H.S., Habib, M. and Wells, I.D. (1983) Asthma and IgE antibodies induced by chromium and nickel salts. J. Allergy Clin. Immunol. 72, 407–412.
[29] Bruynzeel, D.P., Hennipman, G. and Van ketel, W.G. (1988) Irritant contact dermatitis and chromium passivated metal. Contact Dermatitis 19, 175–179.
[30] Kanerva, L., Jolanki, R., Estlander, T., Alanko, K. and Savela, A. (2000) Incidence rates of occupational allergic contact dermatitis caused by metals. Am. J. Contact Dermatitis 11, 155–160.
[31] Gueniche, A., Viae, J., Lizard, G., Charveron, M. and Schmitt, D. (1994) Effect of various metals on intercellular adhesion molecule-I expression and tumor necrosis factor alpha production by normal human Keratinocytes. Arch. Dermatol. Res. 286, 466–470.
[32] Estlander, T., Jolanki, R. and Kanerva, L. (2000) Occupational allergic contact dermatitis from trivalent chromium in leather tanning. Contact Dermatitis 43, 114–116.
[33] Kvitko, E. (2001) Occupational contact dermatitis in the tanning industry. Contact Dermatitis 45, 256–259.
[34] Thomas, P., Summer, B., Sander, C.A., Przybilla, B., Thomas, M. and Naumann, T. (2000) Intolerance of osteosynthesis material: evidence of dichromate contact allergy with concomitant oligoclonal T-cell infiltrate and Th1-type cytokine expression in the peri-implantar tissue. Allergy 55, 969–972.
[36] Leroyer, C., Dewitte, J.D., Bassanets, A., Boutoux, M., Daniel, C. and Clavier, J. (1998) Occupational asthma due to chromium. Respiration 65, 403–405.
[37] Hassmanova, V., Vaneckova, J. and Bousova, K. (2000) Occupational diseases caused by chromium and its compounds. Acta Med. (Hradec Kralove) 43, 33–36.
[38] Vasant, C., Balamurugan, K., Rajaram, R. and Ramasami, T. (2001) Apoptosis of lymphocytes in the presence of Cr(V) complexes: role in Cr(VI)-induced toxicity. Biochem. Biophys. Res. Commun. 285, 1354–1360.
[39] Carlisle, D.L., Pritchard, D.E., Singh, J. and Patierno, S.R. (2000) Chromium(VI) induces p53-dependent apoptosis in diploid human lung and mouse dermal fibroblasts. Mol. Carcinog. 28, 111–118.
[40] Gao, M., Binks, S.P., Chipman, J.K., Levy, L.S., Braithwaite, R.A. and Brown, S.S. (1992) Induction of DNA strand breaks in peripheral lymphocytes by soluble chromium compounds. Hum. Exp. Toxicol. 11, 77–82.
[41] Gennart, J.P., Baleux, C., Verellen-Dumoulin, C., Buchet, J.P., De Meyer, R. and Lauwerys, R. (1993) Increased sister chromatid exchanges and tumor markers in workers exposed to elemental chromium-, cobalt- and nickel-containing dusts. Mutat. Res. 299, 55–61.
[42] Katsifis, S.P., Kinney, P.L., Hosselet, S., Burns, F.J. and Christie, N.T. (1996) Interaction of nickel with mutagens in the induction of sister chromatid exchanges in human lymphocytes. Mutat. Res. 359, 7–15.
[43] Lai, J.S., Kuo, H.W., Liao, F.C. and Lien, C.H. (1998) Sister chromatid exchange induced by chromium compounds in human lymphocytes. Int. Arch. Occup. Environ. Health 71, 550–553.
[44] Blasiak, J., Trzeciak, A., Malecka-Panas, E., Drzewoski, J., Iwanienko, T., Szumiel, I. and Wojewodzkam, M. (1999) DNA damage and repair in human lymphocytes and gastric mucosa cells exposed to chromium and curcumin. Teratog. Carcinog. Mutag. 19, 19–31.
[45] Blasiak, J. and Kowalik, J. (2000) A comparison of the in vitro

genotoxicity of tri- and hexavalent chromium. Mutat. Res. 469, 135–145.

[46] Trzeciak, A., Kowalik, J., Malecka-Panas, E., Drzewoski, J., Wojewodzka, M., Iwanenko, T. and Blasiak, J. (2000) Genotoxicity of chromium in human gastric mucosa cells and peripheral blood lymphocytes evaluated by the single cell gel electrophoresis (comet assay). Med. Sci. Monit. 6, 24–29.

[47] Stohs, S.J., Bagchi, D., Hassoun, E. and Bagchi, M. (2001) Oxidative mechanisms in the toxicity of chromium and cadmium ions. J. Environ. Pathol. Toxicol. Oncol. 20, 77–88.