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CYTOKINE PROFILES IN HUMAN EXPOSURE TO METALS

(IUPAC Technical Report)

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Abstract: Immunosensitization to metal ions through occupational and environmental exposure has been described in earlier papers from this project. Here we discuss the possible role of cytokine profiling in demonstrating and understanding this phenomenon. The cytokines are a large family of polypeptides exerting autocrine, paracrine, and/or endocrine effects. They include interleukins (ILs), interferons (IFNs), and growth factors. They may be grouped as pro-inflammatory (e.g., IL-1, IL-6, IL-12, IL-18, TNF-α), anti-inflammatory (e.g., IL-10), or those regulating T-helper (TH) cell function. The latter are subdivided into those associated with TH1 (e.g., IL-2, IL-12, IFN-γ, TNF-β) or TH2 (e.g., IL-4, IL-5, IL-13) cell function. Because different types of immune reactions (e.g., immediate reaction vs. delayed-type hypersensitivity) differentially involve TH1 and TH2 cells, measurement of cytokine production in response to metal ions can potentially give insight into underlying immune mechanisms and responses. Examples are given for species of Ni, Cr, Co, Hg, Cd, and Be; and in less detail for species of Fe, Pt, Pd, and Rh. Antibodies are available commercially that allow for the determination of many cytokines, and such measurements are most usefully performed with body fluids, supernatants from stimulated lymphocyte cultures, or lysates of lymphocytes or other biopsied cells. The predominant methods include enzyme-linked immunosorbent assay (ELISA) and flow cytometric measurement of the cytokine, bioassay of its activity in cell culture, and polymerase chain reaction (PCR) assessment of its mRNA level. In practice, levels of individual cytokines are highly variable between individuals, and reliable reference values are generally lacking. Ratios of cytokines are more informative than absolute concentrations, and biological variability in cytokine production dictates that repeated testing is necessary to confirm trends. Determining cytokine profiles is presently of questionable diagnostic utility in individual cases of metal sensitization, but is providing mechanistic insights in a research context.

Keywords: immunosensitization; cytokine profiling; interleukins; interferons; immunosensitization to metals; hypersensitivity.

INTRODUCTION

Cytokine production is altered in most, if not all, disease states. In disorders arising from human exposure to noxious substances, there is an extensive literature on the involvement of cytokines; for a survey, see refs. [1–3]. Consequently, measurements of cytokines have been performed and their use propagated as a diagnostic procedure in a number of relevant disorders [4–8]. In recent years, several methods and test kits have become available for cytokine measurement, including for studying allergic disorders where cytokines have been shown to play an important role. However, cytokine profiles in biological fluids are rather unstable, and are influenced by various exogenous and endogenous factors. Moreover, it has recently become evident that analysis of single cytokines in the blood has little diagnostic relevance. Our purpose here is to consider the basis for, and the meaningfulness of, cytokine measurements, specifically as they relate to exposure to metals.

DEFINITIONS AND BACKGROUND

The superfamily of cytokines typically consists of polypeptides of about 100–200 amino acids, which can be produced and secreted by nearly all cell types, especially those of the immune system [9]. As mediators, they induce either in the same cell (autocrine), in neighboring cells (paracrine), or systemically in distant target cells (endocrine) a tissue- and cytokine-specific signal. Several dozen cytokines are known, which are divided into interleukins (ILs), interferons (IFNs), and growth factors. Some of the most important cytokines are listed in Table 1. Sometimes chemokines are also included in the superfamily of cytokines. There are about 50 chemokines; they are smaller than most cytokines (less than 120 amino acids), and they act locally on different classes of leukocytes to elicit pro-inflammatory and chemotactic responses.

Table 1 Overview of some of the most important cytokines of the innate and the specific immune system, their source and effector functions.

Cytokine	Source	Group of cytokines	Effector reactions
IL-1α	Macrophages/monocytes T-cells (TH1/TH2)	Pro-inflammatory	- Synthesis of acute-phase proteins - Induction of fever - Increase of the cytotoxicity of macrophages/monocytes and NK-cells - Co-stimulator for T- and B-cells - Expression of adhesion molecules on antigen-presenting cells
IL-2	T-cells (TH1), NK-cells	T-cell cytokine	- Growth factor for T-, NK-, and B-cells - Increase of the cytotoxic activity of CD8+ T- and NK-cells
IL-4	T-cells, mast cells, basophils	T-cell cytokine	- Growth and differentiation factor for B-cells - Switch of Ig isotypes from IgG4 and IgE - Inhibition of macrophage activation
IL-5	T-cells, mast cells	T-cell cytokine	- Growth and differentiation factor for B-cells - Regulation of Ig release - Co-stimulation for T-cells
IL-6	Macrophages/monocytes, T-cells (TH2)	Pro-inflammatory	- Synthesis of acute-phase proteins - Growth factor for B-cells - Co-stimulation for T-cells
IL-10	Macrophages/monocytes, T-cells (TH2), B-cells	Anti-inflammatory	- Co-stimulator for B-cells - Increase of Ig production (IgM, IgG, IgA) - Inhibition of cytokine production by macrophages/monocytes and T-cells
IL-12	Macrophages/monocytes	Pro-inflammatory	- Induction of IFNg production by T- and NK-cells - Activation of cytotoxic CD8+ T-cells
IL-13	T-cells (TH1/TH2), mast cells	T-cell cytokine	- Switch of Ig isotype to IgE - Inhibition of cytokine production by macrophages/monocytes

(*continues on next page*)

Table 1 (*Continued*).

Cytokine	Source	Group of cytokines	Effector reactions
IL-18	Macrophages/monocytes	Pro-inflammatory	- Induction of the IFNg production by T- and NK-cells - Co-stimulator for T-cells
GM-CSF	Macrophages/monocytes, T-cells (TH1/TH2)	T-cell cytokine	- Stimulator for macrophages/monocytes and granulocytes - Factor for differentiation of APC - Activation of macrophages
IFN-γ	T-cells (TH1), NK-cells	T-cell cytokine	- Activation of macrophages - Activation and increase of cytotoxicity of NK-cells - Activation of cytotoxic CD8+ T-cells - Increase of the expression of MHC class I and II molecules - Switch of Ig isotypes from IgG1 and IgG3
TNF-α	Macrophages/monocytes, T-cells (TH1), NK-cells	Pro-inflammatory	- Synthesis of acute-phase proteins - Induction of fever - Improvement of the phagocytic activity of neutrophils - Activation of T- and NK-cells
TNF-β	T-cells (TH1)	T-cell cytokine	- Improvement of cytotoxicity of T- and NK-cells - Activation of neutrophils
TGF-β	T-cells (TH3), B-cells	Anti-inflammatory	- Suppressor cells - Inhibition of lymphocyte proliferation - Inhibition of production of IL-1, IL-6, TNFα - Switch of Ig isotype to IgA

Cytokines are the major regulators of both the innate and the specific immune system. They control principal biological processes such as cell growth, differentiation, apoptosis, angiogenesis, and fetal development, and they are responsible for the restoration of immune homeostasis after disturbance by immunological challenge. Their interaction with neuroendocrinological processes can lead to systemic effects such as fever and shivering, and can influence mental and physical states [10–13]. They act through receptors on cell membranes as well as via soluble cytokine receptors. There are functional interactions between the various cytokines but also between cytokines and other mediators (e.g., hormones and neurotransmitters) [11–13], thus conferring a highly integrative physiological role upon the cytokines.

Cytokines can be divided into three groups: pro-inflammatory cytokines, anti-inflammatory cytokines, and cytokines related to T-helper (TH) cell function [14,15]. TH cells are divided into at least two subsets, namely TH1 and TH2 cells, which are characterized in part by their specific cytokine production. The group of TH1 cytokines (*type 1 cytokines*) consists of IL-2, tumor necrosis factor β (TNF-β), and IFN-γ. They activate a cytotoxic mechanism but result in the elimination of intracellular pathogens. A reduced TH1 response, therefore, increases the risk of acquired infections or tumors, whereas an increased TH1 response may predispose to some forms of autoimmune diseases. In contrast, TH2 cells produce *type 2 cytokines*, including IL-4, IL-5, and IL-13, thereby activating the humoral immune response with the production of immunoglobulins (especially IgE), and the release of eosinophils; i.e., they induce an immune response against extracellular pathogens. Hyper-reactivity of TH2 cells can,

however, produce allergic reactions. *Pro-inflammatory cytokines* (TNF-α, IL-1, IL-6, IL-12, IL-18) are mainly produced by macrophages and monocytes. *Anti-inflammatory cytokines* include IL-10, which is released by macrophages, but additionally by dendritic cells, TH2 cells, and regulatory T-cells. The latter are an important class of T-cells responsible for maintaining the balance between self-tolerance and autoimmune reactions. Regulatory T-cells also secrete transforming growth factor beta (TGF-β), which is a very important inhibitor of epithelial and hematopoietic cells.

Cytokine receptors are expressed by a variety of cell types, including immunocompetent cells and also neurons and hormone-producing cells [11,16,17]. Lymphocytes have, in addition to cytokine receptors, receptors for hormones and neurotransmitters. Therefore, the immune system, hormonal signalling, and the central nervous system form a highly integrated network [18]. In summary, different cells and tissues use cytokines for communication and response to exogenous signals, thereby maintaining or re-establishing homeostasis. This implies a key role of cytokines in both acute and chronic disease, and they are, therefore, also involved in immunological disorders arising from occupational and environmental exposure. Thus, there are many reports of the alteration of cytokines in, for example, allergies, hyper-reactive bronchial disorders, silicosis, and asbestosis, and furthermore in functional somatic syndromes such as fibromyalgia, chronic fatigue syndrome, Gulf War syndrome, multiple chemical hypersensitivity, sick building syndrome, etc., as well as in post-traumatic stress syndrome [5,12,13,17,19–29]. However, alterations in cytokine expression pattern or concentrations are not specific for a defined agent or process, but rather reflect the reactivity of the organism to potential harm. Cytokines play an important role in the induction and chronic persistence of some disorders, for instance, in fibrosis. In other diseases, such as autoimmune disorders, the type 1/type 2 cytokine balance is disturbed [4]. To interpret these alterations, it is more helpful to analyze changes in the pattern of cytokines than to determine single cytokine concentrations.

CYTOKINE PATTERNS AND PATHOMECHANISMS

The analysis of cytokine production by immunocompetent cells during processes that follow chemical exposures is of major interest for explaining pathophysiological mechanisms. Since the recognition of TH1/TH2-mediated processes, several allergic reactions which had previously been attributed to different types of reactions [e.g., type I (immediate reaction) vs. type IV (delayed-type hypersensitivity reaction)] can now be related to the activation of different T-cell subpopulations (TH1 or TH2) and the resultant production of specific cytokine patterns [4,5]. Determining whether certain substances induce TH1/TH2-cell-specific cytokines or macrophage/monocyte-derived cytokines can be helpful in predicting probable reactions toward those substances. Most experience in this respect to date has been with drug-induced allergies and allergic contact dermatitis [5,6,30].

Data obtained within the last few years have shown a great variability in cytokine levels in healthy individuals [31] and in disease states [4]. There are only a few diseases in which measurement of cytokines in the blood is of diagnostic relevance (e.g., acute appendicitis in childhood, septic shock, and lymphoma). In environmental diseases, no specific changes in *plasma cytokine-levels* have been documented. Nevertheless, analysis of *intracellular cytokines in lymphocytes* or determination of *cytokines in lymphocyte supernatants* after cultivation of the cells with antigenetic environmental substances may give an indication of sensitization to those substances and the individual's predisposition to exhibit allergic reactions [32,33]. Such measurements, in addition to the skin prick test or determination of specific IgE, may provide further information in very early stages of sensitization [4]. They may also be an indicator for non-IgE-mediated reactions, as is, for example, known for mycotoxins [34], drugs [5], or metals such as cadmium [35].

One can imagine that in the future, cytokines may increasingly be used as control parameters for follow-up during therapy, because it is known that cytokine profiles may be influenced to quite a great extent by different therapeutic regimens. This has been shown, for instance, in typical type 1 cytokine-mediated (e.g., multiple sclerosis, psoriasis) and type 2 cytokine-mediated (e.g., allergic disorders) di-

seases [7,33,36–38]. In type 2-mediated allergic diseases, a shift of immunoreactivity during therapy into a type 1 pattern seems to be beneficial, while type 1-mediated disorders may benefit from an enhancement of type 2 reactivity. However, for many disorders, it is not yet known whether induction of type 1 or type 2 cytokines may be useful.

CYTOKINE MEASUREMENT

Cytokines can be measured in body fluids such as plasma, serum, effusions, and cerebrospinal fluid; in supernatants of lymphocytes; or intracellularly in lymphocytes or other cells using biopsy material or cell cultures. The most important methods for the determination of cytokines are given in Table 2.

Table 2 Methods for determination of cytokines.

Material for study	Methods
Body fluids	ELISA: detection of the protein Bioassay: measurement of activity
Cells in body fluids	PCR: quantification of mRNA Flow cytometry: intracellular cytokine production
Ex vivo stimulated cells (from blood, other body fluids, biopsy material)	ELISA, ELISpot: detection of secreted proteins, demonstration of immunoregulatory properties Bioassays in supernatants: measurement of activity PCR: quantification of mRNA Flow cytometry: intracellular cytokine production, demonstration of immunoregulatory properties

Cytokines in fluids (including supernatants from lymphocytes) are nowadays most frequently measured by a *double sandwich enzyme-linked immunosorbent assay (ELISA)*, which allows quantitative analysis. Test kits or at least monoclonal/polyclonal anti-human cytokine antibody pairs are available from several companies. Microtiter plates are coated with monoclonal anti-human cytokine antibodies and incubated with the fluid [32]. The captured cytokine is detected by the respective polyclonal anti-human cytokine antibody, either directly conjugated with an enzyme or with biotin, which is then recognized by streptavidin, conjugated with enzyme (e.g., horse radish peroxidase or alkaline phosphatase). After addition of a substrate such as *o*-phenylenediamine, the reaction product is measured spectrophotometrically. The amount of bound secondary antibody correlates with the absorbance and can be related to a standard curve allowing a quantitative measurement.

Bioassays using cell lines sensitive toward distinct cytokines are also available for the analysis of cytokines in body fluids. They depend on an assessment of the biological activity of a given cytokine as tested on a target cell. These assays establish dose–response relationships, and many different biological systems are available, for instance, cell proliferation assays using murine thymocytes (for IL-1), cytotoxicity assays (for TNFα, TNFβ), antiviral activity, induction of major histocompatibility class II molecules (for IFNγ), chemotactic activity (for IL-8), and inhibition of cytokine secretion (IFNγ on IL-10 production). A major drawback of these types of bioassays is their lack of specificity due to the fact that several cytokines can exhibit similar or even synergistic effects. Furthermore, these assays require functional integrity of the cytokines and are, therefore, more sensitive to destruction of the cytokines than the ELISA, leading to poor reproducibility [39].

Measurement of intracellular cytokines in peripheral blood mononuclear cells (PBMCs) can be performed with *flow cytometry* either with whole blood or after isolation of lymphocytes and stimulation with different mitogens/antigens [40,41]. The cells have to be stimulated with phorbol 12-myristate-13-acetate (PMA) and ionomycin/brefeldin, and afterwards treated with a permeabilization buffer

containing saponin. The intracellular cytokines can then be stained by dye-conjugated (e.g., fluorescein isothiocyanate or phycoerythrin) anti-human cytokine antibodies. Cells are analyzed by three- or four-color flow cytometry. The measurement of intracellular cytokines by flow cytometry is best standardized and most suitable for IFNγ and IL-4.

Polymerase chain reaction (PCR) is a method to determine the induction of cytokine response by measuring the expression of cytokine mRNA. For this purpose, total RNA is isolated from cells (lymphocytes or tissue) and transcribed into cDNA, which is then amplified using cytokine-relevant sense and anti-sense primer pairs [42]. The PCR products are electrophoresed on agarose gels and can be visualized by ethidium bromide staining. This method can, in principle, be used for all cytokines. However, one has to keep in mind that mRNA of some cytokines (especially the pro-inflammatory ones) is already constitutively expressed; changes in cytokine expression arising from changes in transcript translation will not be detected.

To interpret properly the results of cytokine measurement, it is mandatory to know whether the measurements have been performed on body fluids, tissues, or isolated cells. Moreover, the different methods reflect different stages on the way from cytokine induction to cytokine secretion: PCR indicates the expression of cytokine mRNA, intracellular staining via flow cytometry the translation into protein, but only ELISA or bioassays with supernatants give information on whether the cytokines are, in fact, secreted. In general, the measurement of T-cell-associated cytokines (e.g., IFN-γ, TNF-β, IL-4, IL-10, IL-5) in supernatants of lymphocytes after antigen stimulation, or analysis of intracellular cytokines is more sensitive than their detection in serum or plasma. Only a few pro-inflammatory cytokines/chemokines, such as IL-6, TNFα, or IL-8 can be detected with sufficient sensitivity in the serum. These are of diagnostic relevance because severe inflammatory processes that can occur during infections (e.g., sepsis) can be diagnosed with this method up to 3 h earlier than with clinical-laboratory parameters such as C-reactive protein [43].

Precautions and pre-analytical procedures

For the measurement of cytokines in supernatants of ex vivo-stimulated lymphocytes, blood has to be collected, transported, and stored correctly. Thus, it is essential to use anticoagulated blood (preferably heparinized), transport it at room temperature, and isolate the PBMCs within 24 h. When lymphocytes are incubated with the suspected sensitizing substance, it is important not to use too high concentrations of the substance for lymphocyte stimulation because this may lead to non-specific stimulation, or inhibition of cytokine release. Therefore, different concentrations of the stimulating antigen should be used in one assay. The resulting supernatants may be stored at either –20 °C or preferably at –80 °C for a prolonged time before cytokine measurement is performed by ELISA or bioassay. Repeated freezing and thawing must be avoided. Bioassays are more prone to give a falsely low level because they depend upon the functional integrity of the cytokines, whereas in ELISA measurements cytokines are determined via their more stable antigenicity.

The detection of cytokines released into the supernatant is only a suggestion of immunological reactivity but does not necessarily correlate with a certain clinical manifestation. Moreover, "reference values" hardly exist so that in each test age-matched controls have to be included. Furthermore, it has to be pointed out that it is not alterations in cytokine levels *per se*, but rather in cytokine patterns that are more indicative of a pathological process. Because of the necessity of adequate controls, including cell viability checks, cell proliferation tests, and dilution series, the analyses are rather time-consuming and expensive. They are, therefore, predominantly performed for research purposes.

Quality assurance

The above-mentioned methods can be performed with great precision and reliability with commercially available test kits or anti-human cytokine antibodies. However, reference values are not generally avail-

able. Even when they are available, they are only of limited meaning because of the great inter- and intra-individual variations, both in health and disease. In any case, the high variability renders it difficult to come to a reliable interpretation of results. Repeated measurements or follow-ups are mandatory. Slight alterations or small deviations from reference values do not necessarily indicate an abnormality. Each laboratory has to evaluate the reliability of the method for each parameter on its own, using appropriate standards and controls.

CYTOKINE PROFILES ASSOCIATED WITH EXPOSURES TO METALS

As already mentioned, cytokine production can be influenced by various chemicals. Metals are especially known to influence immunocompetent cells to quite a large extent. This is of special relevance to health because of the ubiquitous exposure to metals in daily life and their specific occurrence in some working places. In the following section, we will summarize the most recent knowledge of the effects of selected metals on cytokines.

Nickel

Early work showed that Ni-specific T-lymphocyte clones could be isolated from peripheral blood of patients with Ni allergy [44]. Activation and cytokine production by these cells required the presence of antigen-presenting cells and were restricted to MHC class II molecules. Isolated clones produced IL-2 and IFN-γ, suggesting a TH1 response. This was confirmed by the finding that most Ni-specific T-lymphocyte clones secrete TNF-α, granulocyte/macrophage colony-stimulating factor (GM-CSF), IL-2, and IFN-γ, but low levels of IL-4 and IL-5 [45,46]. Lindemann et al. [30] used an ELISA-based method to study PBMCs from 60 Ni patch test-positive patients and 19 negative controls and found significant increases in IL-2 and IFN-γ, but also IL-4, following Ni stimulation of the cells from the positive individuals. The results correlated with those of the lymphocyte transformation test. In similar studies with 11 Ni-allergic females, IL-4, IL-5, IL-13, and IFN-γ, but not IL-2, were increased compared to 9 matched controls [6]. Increases in IL-5, but not IL-2, IL-4, TNF-α, or IFN-γ are found following oral challenge of Ni-sensitive individuals with Ni [47]. Consistent with the lymphocyte proliferation data above, reverse transcription-PCR of skin biopsies taken from individuals 24 h after application of a $NiSO_4$ patch showed increased mRNA for IFN-γ, IL-2, and IL-4 in both atopic individuals and non-atopic subjects with previous Ni allergy, while IL-10 mRNA was increased in the non-atopic group only [48]. Higher IFN-γ and lower IL-10 were also noted by Cavani et al. [49] in Ni^{2+}-specific CD4+ cells of Ni-allergic patients. However, lymphocytes from a skin biopsy of a patient with Ni-related contact dermatitis produced high levels of IL-4 and IL-5, with low IFN-γ [50], suggesting that a TH2 response predominates in some instances. Furthermore, Borg et al. [51] found that Ni-stimulated lymphocytes from individuals with Ni allergy secreted increased IL-4 and IL-5, but not IFN-γ or TNF-α, suggesting that IL-4 and IL-5 might be the most useful indicators of Ni sensitivity. However, the clinical utility of these observations is unclear: serum IL-2, IL-4, IL-5, IL-13, and IFN-γ levels were measured in non-allergic and atopic healthy men [52], and while urinary Ni levels were correlated with the presence of specific subtypes of T-cells, only a weak correlation with serum IL-2 was noted. More recently, Moed et al. [53] have found T-cell subtypes in patients with Ni contact allergy that proliferate in response to Ni and produce both type 1 (IFN-α) and type 2 (IL-5) cytokines.

Chromium

Chromium is also an immunosensitizing metal that frequently produces allergies in Ni-sensitive individuals [54], but cytokine profiles have been less extensively studied in Cr allergies than with respect to Ni. Cr(VI) is most readily absorbed, as chromate, and is more likely to be an irritant than Cr(III) species. The same study that noted a weak correlation between urinary Ni and serum IL-2 found a cor-

relation between urinary Cr and serum IL-5 [52]. Interleukin-6 levels were compared between 46 individuals living or working in Hudson County, New Jersey, (a site of ore processing with high soil Cr levels) and 45 college faculty living and working outside the area [55]. Production of IL-6 by pokeweed mitogen-stimulated lymphocytes from the exposed population was only 64 % of that of controls. While the study was controlled for gender, age, and smoking, it has been criticized on the basis that a number of factors affecting immune response were not accounted for and exposure was not confirmed by measurement of urinary excretion of total Cr [56]. Human macrophages/monocytes treated with lipopolysaccharide and Cr showed increased secretion of IL-1β and TNF-α and decreased TGF-β_1 [57]. Similar results were obtained with PBMCs of patients with failed Cr-containing hip replacements [58]. Because TNF-α favors bone resorption while TGF-β enhances bone formation, it has been suggested that this pattern induced by release of Cr metal may be an important contributor to joint failure.

Cobalt

Cobalt, another sensitizing metal [54], occurs together with Cr in alloys used for prosthetic devices. Cytokine production was studied in PBMCs retrieved from patients with loosening Co/Cr-alloy hip prostheses [59]. Compared to control individuals, lymphocytes from the patients secreted increased amounts of TNF-α, decreased amounts of IL-6, and similar levels of GM-CSF. Stimulation of the isolated lymphocytes with Co likewise increased TNF-α secretion, but also slightly increased GM-CSF while not affecting IL-6. Upon stimulation with an extract of powdered Cr metal prepared in culture medium, however, secretion of all three cytokines was increased. Wang et al. [60] on the other hand, studied in parallel T-cell proliferation induced by phytohemaglutinin and B-cell proliferation in response to lipopolysaccharide, in cells from individuals with Ti/Co/Cr alloy joint prostheses. Neither T- nor B-cell proliferation was affected by any of the three metals, though each metal decreased the extent of secretion of IL-2 and IL-6 that could be elicited by phytohemaglutinin. IFN-γ secretion was depressed by both Co and Cr. While the significance of depressed IL-6 secretion, in particular, is unclear, it is interesting to note that a Co/Cr/Mo alloy increased secretion of both IL-6 and TNF-α in macrophages co-cultured with T-lymphocytes exposed to particulates [61]. A Ti alloy had the same effect, and this may represent a response to a relatively inert particulate substance; PBMCs were shown to secrete IL-6 and TNF-α upon exposure to particulate Ti alloy [62], a response suppressed by the TH2 cytokines IL-4 and IL-10.

Mercury

Mercury exposure offers additional insight into cytokine response to a metal. The salt $HgCl_2$ induces IL-4 and IFN-γ (and to a lesser extent, IL-12) in mice, albeit in a strain-specific manner. The Hg^{2+} ion activates protein kinase C by increasing Ca^{2+} influx, and this was shown to lead to increased transcription of IL-4 (that is, an increase in IL-4 mRNA [63]). It is suggested that the balance between TH1 and TH2 response is sensitive to intracellular thiol status, low thiol levels favoring a TH2 response with higher levels favoring a TH1 response in a more reducing environment. Although the effects of exposure to external sources of inorganic mercury are noteworthy in their own right, many studies on the immunological effects of mercury focus on dental amalgam. PBMCs were incubated directly in the presence of Hg amalgam and stimulated with lipopolysaccharide, phytohemaglutinin, or *Staphylococcus* endotoxin [64]. IFN-γ and IL-10 secretion decreased while TNF-α secretion increased. The authors noted that release of Cu^{2+} from the amalgam probably also played a role in suppressing IFN-γ and IL-10, although Cu^{2+} alone had no effect on TNF-α secretion. Mouse spleen lymphocytes secreted increased amounts of IL-2 and IFN-γ upon exposure to $HgCl_2$, either continuously or transiently [65]. Pre-exposure also increased IL-4 secretion. While serum TNF-α has been reported to be decreased in workers chronically exposed to Hg, a follow-up study found no correlation between urinary Hg and TNF-α, GM-CSF, or IL-8 [66].

Soluble mercuric ion is capable of inducing direct effects on cells in its own right. Mouse strains either sensitive or resistant to Hg-induced lymphoproliferation were challenged with oral Hg nitrate [67]. Measurement of mRNA levels in lymphoid tissue revealed increased expression of IL-2, IL-4, and IFN-γ in the sensitive mice, but increased IL-10 in the resistant colony. Resistance was characterized by low IL-2 and IFN-γ expression, neither of which could be increased by Hg exposure. Parallel exposures to Ag nitrate led to the conclusion that sensitivity to both Ag^+ and Hg^{2+} is characterized by increased expression of IL-2, IL-4, and IFN-γ, with decreased IL-10. A common mechanism based on the high affinity of these two ions for thiol groups seems not to have been considered, but perhaps should be in light of another comparative study of the effects of the chloride salts of Hg and Pb [68]. Both $HgCl_2$ and $PbCl_2$ can cause immune-system dysfunction by activating TH2 cells in preference to TH1 cells. This phenomenon was characterized in vivo by measuring increased plasma levels of IL-4, while plasma IFN-γ was decreased. Consistently, in vitro IL-4 production by a TH2 clone was increased while IFN-γ production by a TH1 clone was decreased. Here, TH1 cells are producing IL-2 and IFN-γ, but not ILs 4, 5, 6, or 10. The converse is true of TH2 cells. From this study, the authors conclude that IL-4 and IFN-γ are the most useful markers for defining T-cell subset involvement [68]. Treatment of mouse macrophages with inorganic Hg was also found to increase lipopolysaccharide-stimulated production of TNF-α and IL-6 [69], while IL-4 and TNF-α were increased in mouse T-lymphoma cells [70]. Mercury salts, and also gold salts, have been shown to trigger T-cell IL-4 production, and to a lesser extent, production of IFN-γ, by triggering Ca signals and protein kinase C [71].

Cadmium

Cadmium has a high affinity for thiol groups. As a consequence, it is very toxic and potentially both an immunosensitizer and immunotoxicant. Although IFN activity is unaffected in mice injected with up to 6.25 mg $CdCl_2$/kg [72], other cytokines respond more convincingly to Cd exposure. Failure of IL-2 secretion and reduced IL-2 receptor expression occur in Cd(II)-treated cells [73,74]. It has been proposed that Cd(II) interferes with lymphocyte signal transduction and activation. When renal cells were cultured from kidneys of lipopolysaccharide-primed mice injected subcutaneously (s.c.) with 0.5 mg $CdCl_2$ three times a week for 14 weeks, IL-6 was found to be secreted by mesangial cells [75]. Cadmium chloride in the range of 1–100 μM inhibited phytohemagglutinin-induced production of IL-1 and TNF-α in human PBMCs that were activated in vitro [76]. Decreased mRNA levels of the cytokines indicate suppression at a transcriptional level. Relatively low concentrations of 0.1 μM $CdCl_2$ caused human PBMCs to display increased IL-8 activity and mRNA [77], but decreased expression of IL-6 and its mRNA [78]. Isolated PBMCs were exposed to 5–100 μM $CdCl_2$ in vitro: IL-1 and TNF-α were increased at lower Cd concentrations, but the effect was less noticeable at higher concentrations. IFN-γ was significantly decreased at the higher concentrations. IL-6, on the other hand, was significantly elevated at all concentrations. Previous studies of occupational exposures to Cd have given inconsistent results for various parameters of immune function. In a convincing examination of selected cytokines (IL-1β, IL-2, TNF-α, and IFN-γ), selected as regulators of immune system function, Yücesoy et al. [79] compared workers who had increased blood Pb (59.4 ± 3.2 μg/100 ml) or Cd (8.2 ± 1.4 μg/L). Both exposures significantly decreased serum IL-1β, while neither affected TNF-α or IL-2. Lead appeared to decrease IFN-γ very modestly (from 0.59 to 0.55) and Cd increased IFN-γ only from 0.59 to 0.65 IU/ml (both results statistically significant).

Beryllium

Occupational exposure to Be compounds is known to induce an immunologically based lung disorder, which can be diagnosed with the lymphocyte transformation test [80]. Beryllium sulfate stimulates IL-2, IL-6, IL-10, IFN-γ, and TNF-α production in bronchoalveolar lavage cells from patients with chronic Be disease [81], and activates TH1 cell clones from such patients [82]. In one mechanism,

Be(II) binds to an allelic variant of a human leukocyte antigen, HLA-DPglu69, containing glutamate at position 69, and is presented to the T-cell [83]. Antibodies to HLA-DPglu69 were able to suppress proliferation and IFN-γ release by T-cells from patients with beryllosis, but not secretion of TNF-α, indicating different mechanisms stimulating release [82]. Increased numbers of T-cells producing IL-2 and IFN-γ have been reported in blood of patients with chronic Be disease [84] .

Miscellaneous

Some additional examples bear further upon metal-induced cytokine expression. Hexachloroplatinate catalysts and the chemotherapeutic agent cisplatin modify the release of cytokines, thus allowing identification of the TH type stimulated by these Pt compounds [85]. In lymph node cells isolated from Pt-treated animals and stimulated by concanavalin A, IL-4 release was increased between 12 and 48 h. IL-10 and INF-γ were increased at later times without stimulation, suggesting a TH2 response. Various chloro- and aminochloro-platinum salts stimulated release of INF-γ, , TNF-α, and IL-5 from PBMCs, in a concentration- and species-dependent manner [86,87], whereas related salts of Pt and Rh were inhibitory [87]. Iron overload is commonly cited as a situation of metal poisoning. It has been reported that natural killer T-cells from thalassemic patients with Fe overload produce less INF-α [88]. In Fe-deficient hosts, T-cell secretion of IFN-γ by splenocytes is decreased [89]. Macrophages from Fe-loaded mice produce normal amounts of IL-1α but increased amounts of TNF-α upon lipopolysaccharide stimulation [90].

SUMMARY AND CONCLUSIONS

The involvement of cytokines in environmental disorders associated with disturbances of physical and mental states is well known. This may lead in the future to a better understanding of the pathogenesis of those disorders and open new therapeutic options. The determination of cytokines and analysis of their mode of action is, therefore, of importance for basic and clinical science. However, in individual diagnoses of environmentally mediated disorders, including disorders related to exposures to metals, their usefulness is still questionable. In some cases, they may be helpful for the characterization of TH1- and TH2-subpopulations. Meaningful results will generally be obtained only in specialized centers operating under research conditions. Considering the large variabilities of cytokine production, repeated measurements and follow-ups are mandatory. Research on cytokine profiling will continue to be of immediate interest, and priority should be given to correlating specific profiles with individual exposures. With respect to metal sensitization, metal speciation must be considered. Improved accuracy and precision of analytical methods for cytokine determination will be necessary for more reliable correlations to be drawn.

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