Diagnosis and treatment of metal-induced side-effects

Vera Stejskal[1], PhD; **Romuald Hudecek**[2], DDS; **Jenny Stejskal**[3], MD **& Ivan Sterzl**[1], MD, PhD

[1] Dept. of Immunology and Microbiology, 1st Medical Faculty, Charles University Prague, Czech Republic.
[2] Biomedical Dental Centre, Uppsala, Sweden.
[3] Mörby General Practice, Stockholm, Sweden.

Correspondence to: Vera Stejskal, PhD
August Wahlströmsväg 10
182 31 Danderyd, SWEDEN
TEL/FAX:+46 8 753 23 22
EMAIL: vera@melisa.org

Submitted: December 2, 2006 *Accepted: December 18, 2006*

Key words: **autoimmunity; allergy; chronic fatigue syndrome; genetics; lymphocyte; LTT; MELISA®; patch test; metals; mercury; gold; titanium**

Neuroendocrinol Lett 2006; **27**(Suppl 1):7–16 **PMID:** 17261999 NEL270706A09 ©Neuroendocrinology Letters **www.nel.edu**

Abstract

Environmental factors are recognized as a cause of the increasing frequency of allergic and autoimmune diseases. In addition to external pollutants, metal ions released from dental restorations or from other body implants might trigger inflammation in susceptible subjects. In humans, genes governing metal-induced inflammation and autoimmunity are not yet known.

In clinical praxis, metal-sensitive patients will present various symptoms ranging from oral mucosal changes and skin disease to excessive fatigue and autoimmune diseases. Since genetic markers of genetic susceptibility in man are not known, one has to rely on the phenotypic markers. Such biomarkers might be certain detoxification enzymes but also the presence of metal-specific memory cells in the blood. With the increasing use of metal implants in medicine and dentistry, it is important to have a proper tool for the diagnosis of metal allergy in susceptible subjects.

In addition to patch test, an *in vitro* blood test, an optimized commercially available lymphocyte transformation test (MELISA®) is discussed. Both tests were used for the diagnosis of metal allergy in a selected group of 15 patients who suffered from clinical metal sensitivity in addition to other health problems. The concordance of the two tests was good but MELISA® detected more metal allergies than patch test. The removal of incompatible dental material (RID) resulted in long-term health improvement in the majority of patients. We postulate that *in vivo*, metal ions activate T-cells, initiating systemic inflammation, which, through cytokines, affects the brain and hypothalamus-pituitary-adrenal axis.

The treatment and rehabilitation of metal sensitive patients is based on a firm understanding and recognition of individual susceptibility. RID has to be done with extreme caution and according to standard working protocol. If performed properly, this treatment can result in decreased systemic inflammation and improved health in sensitized patients.

REVIEW ARTICLE

Abbreviations

CFS – chronic fatigue syndrome
RID – removal of incompatible dental material
SH – sulfhydryl
GSTT1 – glutathione s transferase T1
GSTM1 – glutathione s transferase M1
SLS – sodium lauryl sulfate
LTT – lymphocyte transformation test
SI – stimulation index
MRI – magnetic resonance imaging
CNS – central nervous system

Background

Mankind is exposed to toxic particles, such as metals, on a daily basis through contaminated food and air. Environmental toxins can be one of the etiological factors behind the increase of so-called modern illnesses: allergies, autoimmunity and cancer [14, 28, 33, 66, 67]. The role of the organic mercury (Hg) compound thimerosal as a factor contributing to the increased frequency of autistic disorders has also been widely discussed [16, 17, 42]. In addition, metal-based medical devices such as dental restorative materials (e. g. amalgam and gold alloys) release metal ions and contribute to internal pollution.

Dental restorative materials are developed for replacement of tooth substance which is lost and are placed in the teeth for long periods of time. Previously, the majority of such materials were based on various metal alloys, but in modern dentistry, the use of non-metallic dental materials, for example ceramics, is steadily increasing.

Such materials should preferably be free from toxic and/or allergenic substances which might affect the patients' and dental staffs' health. Special care has to be taken in patients with known allergy and/or autoimmunity.

Metals affect the immune system in several ways. In the oral cavity, a high concentration of metal ions may be toxic and act as a local immunosuppressant. This may explain why the oral mucosa contains only a low number of dendritic cells, and why mucosal changes adjacent to dental metal fillings are infrequent [35]. Nielsen and Klaschka [45] have shown that a 5–12 times higher concentration of the allergen has to be applied on the oral mucosa than on the skin to elicit microscopic reaction.

Certain metals stimulate the immune system non-specifically as shown by increased levels of serum immunoglobulins in workers professionally exposed to Hg [5]. Further, an abnormal antioxidant system with reduced levels of glutathione and catalase activity was found in Hg exposed workers [54]. In the general population, anti-oxidant capacity of serum is inversely related to the number of amalgam fillings, as described by Pizzichini and coworkers [49]. Interestingly, glutathione depletion inhibits TH1-associated cytokine production and/or favors TH2-associated responses [46]. This might explain the TH1 to TH2 switch in animals treated with low concentrations of inorganic Hg [19, 50]. In contrast, in some hereditarily predisposed individuals, metals may act as specific allergens [6, 11, 21, 29, 37, 51].

The majority of metals used in dental alloys belongs to the group of transition metals in Mendeleev's periodic table. A general characteristic of these elements is the strong binding capacity to various groups of enzymes and cells in the body. Transition metals form strong complexes with both organic and inorganic ligands [85]. Metals bind to sulfhydryl (SH) and other groups, thus altering the molecular structure of autologous proteins. T-lymphocytes mistakenly recognize metal-modified cells as foreign and start the autoimmune process [20]. The term "allergy" was coined by von Pirquet to describe a deviant immunological reaction – hypersensitivity [48].

It has to be emphasized that metals are only one of several agents which may trigger chronic inflammation and thus significantly contribute to chronic fatigue syndrome (CFS) and autoimmune diseases. The role of other agents, such as microbial [79]or viral, in inflammatory processes is reviewed elsewhere [15].

Biomarkers of harmful effects of metals and other environmental pollutants include detoxification enzymes, such as apolipoprotein E, where the substitution of cystein with arginin – an amino acid lacking SH-groups – predisposes for increased risk for Alzheimer's disease [18] and increases vulnerability to chronic mercury toxicity [91]. Other detoxification enzymes of importance are glutathione s transferase T1 (GSTT1) and glutathione s transferase M1 (GSTM1). As shown by Westphal's group [90], homozygous deletion of GSTT1 and combined deletion of GSTT1-/GSTM1- was markedly more frequent in patients sensitized by thimerosal, than in healthy controls.

Regarding metal susceptibility, measurement of beryllium (Be) specific memory cells in the blood of exposed workers is currently the golden standard for detection of Be-susceptibility [30, 31, 44]. Since clinical reactions to metals, such as local skin reactions or systemic reactions (fever, profound fatigue, multiple chemical sensitivity) are not experienced by all exposed individuals, standard case-control studies with a small number of participants, who are not matched for the susceptible genotype, are of limited value [89]. Instead, a suitable cohort should consist of patients suffering from the same symptoms but selected on the basis of susceptible phenotype; for example, patients suffering from CFS and clinical metal sensitivity [52, 76, 78].

Thus, the best way to study the possible role of metals in the pathogenesis of diseases seems to be first, the selection of susceptible patients from a heterogeneous multi-factorial cohort; second, therapy based on the elimination of the exposure to putative allergen(s); and third, long-term follow-up of patient's health. Finally, it is also important to bear in mind that exposure to metals can originate from all types of metal-containing medical devices, and not only from dental appliances. Other

sources are foods [55], jewelry, cosmetics, vaccines, metallic razors [12] and contaminated air (cigarette smoke, pollution).

T-lymphocytes are key players in inflammation and autoimmunity

T-lymphocytes play a key role in all types of allergic and autoimmune reactions [19, 20, 56]. After contact with an allergen, allergen-specific T-lymphocytes, together with B-cells and macrophages, are activated and inflammation may occur locally or in other parts of the body. The allergen specificity is retained on the surface of memory cells. Memory lymphocytes (Fig.1a) circulate in the blood and the lymph, which explains why allergy is a systemic phenomenon. Exposure to the same or chemically similar (cross-reacting) substance will induce a faster, secondary reaction. Cytokine release by activated lymphocytes and macrophages will result in deregulation of the hypothalamus-pituitary-adrenal axis, as well as in multi-systemic symptoms such as profound fatigue, psychosomatic problems and sleep disturbances [9, 39, 65, 68, 84].

In *vivo* testing

Currently, the patch test is the only test available for routine *in vivo* diagnosis of delayed type hypersensitivity. Although the test is useful in clinical praxis, it has several disadvantages [36, 43, 66, 87]. Direct application of the allergen under occlusion on the skin might boost already existing sensitivity, which might aggravate patients' symptoms [36]. Some allergens, such as gold (Au) salts, may carry the risk of sensitization [43, 58] and positive patch test reactions may persist for months. Patch test results can also be affected by the condition of the skin; fair-haired patients usually have more sensitive skin. In pre-menopausal women, the patch test results may vary depending on the menstrual cycle [80]. Under standard conditions, only 7% out of the total amount of nickel (Ni) applied will penetrate the skin [13]. If the permeability of the skin is increased by local pretreatment by a surfactant such as sodium lauryl sulfate (SLS), the penetration of metals through the skin increases, which improves the accuracy of patch testing [62]. SLS might be beneficial in patch testing but its presence as an ingredient in toothpaste, soap and shampoo should be questioned.

In spite of the fact that the patch test is regarded as golden standard, the test, as such, is for many allergens not standardized. Allergens can be diluted in water or applied in undiluted form in petrolatum.

Finally, the evaluation of patch testing is subjective and depends on the skills of the evaluating specialist. The results of patch testing with skin-irritating substances such as Hg salts or formaldehyde may be unreliable since toxic reactions due to irritation are difficult to discriminate from allergic reactions [12].

In vitro testing

The lymphocyte transformation test (LTT) uses the property of memory cells to be re-stimulated by a specific allergen. Lymphocytes are isolated from peripheral blood on a density gradient and cultivated with metal salts for 5 days in 37°C. If memory cells are present in the blood, they start to divide and differentiate to so-called lymphoblasts (Fig. 1a). Proliferation is measured by the uptake of radiolabeled thymidine into newly synthesized DNA. The proliferation in metal-treated cultures is compared with cells incubated in the absence of metal salts and expressed as an Stimulation Index (SI). SI=counts per minute in metal-treated cultures divided by counts per minute in control cultures. SI $\geq$ 3 is considered as a positive response while SI 2–3 is considered a weakly positive response [73, 74].

In the case of low molecular substances, allergen-specific memory cells are found in the blood of subjects experiencing exposure-related clinical symptoms but not in the majority of healthy non-allergic subjects [30, 60, 64, 69–74, 76, 77].

Memory cells can be detected in the blood of sensitized individuals already prior to the appearance of visible clinical reactions. Thus, workers with Be-specific *in vitro* lymphocyte responses have been diagnosed as Be-allergic even if the symptoms of the chronic lung disease berylliosis were not yet apparent [30, 31, 44]. Following the relocation to a Be-free environment, these workers did not develop berylliosis and remained healthy.

For decades, it has been known that inorganic Hg salt ($HgCl_2$) activated human lymphocytes *in vitro* regardless of the donor's Hg allergy status [8, 61]. Hence, to be able to use LTT for diagnosis of Hg allergy, it was necessary to modify the test in such a way that only lymphocytes from patients with Hg-induced symptoms were activated [74]. This was achieved by reducing the concentrations of Hg salts added to cultures to suboptimal concentrations (0,5 μg per 1 ml culture)[73, 74]. The same turned out to be true for Ni [59, 60, 64] as well as Au and palladium (Pd) salts, where a concentration of 5 μg per culture is routinely used [73]. This modified LTT was named MELISA®, an acronym for Memory Lymphocyte Immuno Stimulation Assay [73]. Another important modification was the increase of the total number of lymphocytes to 1×10^6 cells instead of 2×10^5 cells used in standard LTT. The key importance of lymphocyte concentration for optimal results has been described by Valentine-Thon [86]. Further, since the number of monocytes (Fig. 1b) increased during the preparation procedure, it was necessary to bring the amount of monocytes back to the normal value by partial monocyte depletion. Although necessary for antigen presentation to T-cells, activated monocytes produce prostaglandins [27] which negatively affect lymphocyte activation [57].

At Astra Pharmaceuticals, the MELISA® test was originally used for the diagnosis of occupational allergy to drugs in the pharmaceutical industry [69, 70]. Later

Table 1. The results of patch test and MELISA® in 15 patients with clinical metal sensitivity and temporary worsening of symptoms in connection with dental treatment (2–3 days later).

Pat. nr	Sex	Age (years)	Clinical symptoms	Dental metal exposure	Patch test [i]	MELISA® Stimulation Index (SI)	Therapy	Clinical effect of therapy
BE	M	48	CFS, sinusitis, dry eyes, joint pain	17 AF [ii] 1 Au crown 5 RF [iii] 1 gold-plated pin	Au ++ [iv] PhHg - Hg -	Au ++ (15) PhHg + (3.3) Hg ± (2.9)	Removal of all metals 1 RF with Au pin left	1 yr later: much better, joint pain gone, eye dryness decreased. Sinusitis persists.
EH	F	48	Crohn's disease, abnormal fatigue, oral problems	AF in all teeth MBP [v] Au crowns 10 RF (brass pins)	Au ± Pd -	Au + (10) Pd + (3.3)	1. AF replaced with Au crowns 2. Au replaced to ceramic crowns, RF with brass pins replaced	1. Worsening of health. 2. Health improvement. Decrease in lymphocyte responses to Au (SI 2.7)
GCH	M	53	Urticaria on the back after dental treatment eczema, acne, psychiatric problems, anxiety, depression	25 AF 2 Au restorations brass pin on AF crown 2 RF	Au + Hg -	Au ++ (10) Hg - (1.4)	Replacement of all metals	2 yrs after replacement: decreased skin problems and psychiatric symptoms.
AS	F	45	Neurological symptoms and eczema after insertion of MBP	10 AF 6 MBP crowns RF with AF & Au pin	Ni + Au - Pd - Cu +	Ni ++ (37) Au ++ (11) Pd ++ (10.2) Cu - (1)	Removal of most metals 9 AF left	2 yrs after replacement: some symptoms disappeared and some became worse.
IH	F	62	Polymyalgia rheumatica following replacement of AF with Au crowns and bridges	AF (previously) Au crowns 5 RF with screws	Ni + Au + PhHg - Pd - Hg -	Ni + (9.7) Au ++ (19) PhHg ± (2.7) Pd ± (2.6) Hg - (0.9)	Removal of Au and RF , rests of AF found under the Au crowns	2 yrs after replacement: long term health improvement. Decrease in lymphocyte responses to Au (SI 2) and Ni (SI 4.2)
LL	F	44	CFS, fibromyalgia, oral symptoms, eczema, headache	13 AF 2 Au-plated brass screws in RF	Ni ++ Pd ± Hg - Al ± Au -	Ni+ (7.7) Pd ++(18) Hg+ (4.9) Al – (1.9) Au- (1.6)	Removal of all metals including corroded screws in RF anti-oxidant therapy	1 yr later: partly better, still fatigued, back pain, muscle pain better. 2 yrs later: further health improvement
IB	F	41	Oral symptoms and eczema after insertion of Pd-containing bridge	AF (previously) Au bridge	Ni ++ Pd ± Au - Cr ++	Ni ++ (32) Pd + (9) Au + (3.6) Cr not done	Removal of Au bridge	2 yrs later: marked improvement in oral health
EV	F	52	Thyroiditis with autoantibodies, fatigue, endocrine problems	9 AF	Ni ++ Hg -	Ni ++ (43) Hg ++ (18.8) Sn + (3.5) MeHg +(5.8) PhHg + (6.5)	Removal of AF anti-oxidant therapy	2 yrs later: improved health and decrease of lymphocyte reactivity to Hg (SI 5), Ni (SI 11) and MeHg (SI 1.7). 9 yrs later: good health persists.
DF	F	39	CFS, joint pain, provocation with Hg positive	25 AF MBP Au crowns 3 RF	Hg - Au ± Ni -	Hg + (7) Au - (1.3) Ni - (1.1) PhHg + (5.6) Sn + (3.7)	Removal of all metals	Health improvement
CD	F	66	Oral symptoms after placement of Au bridge, fibromyalgia	AF MBP bridge containing Au, Pd and Ag	Au - Ni - Pd - Ag -	Au + (4.2) Ni + (4.4) Pd ± (1.7) Ag - (1.2)	Removal of all metals	No health improvement
BZ	F	64	Mucosal problems around metal prosthesis, gingivitis	AF (previously) Ti crowns RF with screws Metal prosthesis (Cr, Mo and 2% Ni)	Ni ++ Au ++ Pd ++	Ni ++ (15.5) Au + (3.7) Pd + (4.3)	Removal of metal prothesis and RF	Improvement of oral health

Pat. nr	Sex	Age (years)	Clinical symptoms	Dental metal exposure	Patch test [i]	MELISA® Stimulation Index (SI)	Therapy	Clinical effect of therapy
JK	M	49	CFS, depression, facial eczema after dental treatment	6 AF 2 Au bridges 1 RF	Au + Ni - Hg - PhHg -	Au ++ (14) Ni ++ (27) Hg + (4.6) PhHg + (4.5)	Removal of all metals antioxidant therapy	2 yrs later: successive health improvement
BBR	F	46	Optical neuritis, multiple sclerosis, asthma, known nickel allergy	16 AF	Hg ++ Ni -	Hg + (5.6) Ni + (6.3)	Removal of all metals anti-oxidant therapy	Symptom free for 15 yrs, MRI is normal. Lymphocyte reaction to Hg -
AD	M	46	Optical neuritis and multiple sclerosis, joint pain	AF (previously) Au crowns 1 MBP RF containing N_2 (PhHg)	Au + PhenylHg - Hg -	Au + (3.9) PhenylHg + (3.5) Hg - (1.3)	1. AF replaced with MBP 2. MBP replaced with ceramic material RF filling extracted Anti-oxidant therapy	1. Worsening of health. 2. Improvement of health, no relapse in multiple sclerosis for 15 yrs. Decrease in Au responses *in vitro.*
BML	F	49	Sjögren's syndrome, electrosensitivity, Hashimoto thyroiditis,	AF (previously) 4 RF with Au pins MBP	Au - Pd not done Ni +	Au + (7) Pd + (5.5) Ni ++ (11.9)	Removal of all metals pins in RF were corroded	1 yrs later: health improvement, electrosensitivity disappeared. 3 yrs later: lymphocyte reactivity decreased to Au (SI 3.2) and Pd (SI 3.2)

i) Test done according to Marcusson [35,71]; ii) Amalgam fillings; iii) Root fillings.
iv) + positive reaction, ± weakly positive, - negative reaction;
v) Metal-bound porcelain

on, the test was developed for detection of cell-mediated immune responses to formaldehyde [69], industrial epoxides [69], Kathon CG [71], mercurials [73, 74, 76], as well as for other metals [73, 75, 76]. In addition to type 4 allergy, the test has also been used for the diagnosis of immediate hypersensitivity (type 1 allergy) due to psyllium exposure in geriatric wards [72]. It can also be used for the monitoring of desensitization to insect venoms (unpublished). Recently, the value of LTT in the diagnosis of drug allergy has been reviewed by Pichler and Tilch [47], and for the measurement of Ni allergy by Sanchez and colleagues [60].

Comparison of patch test with MELISA® test

Clinically, patients with intolerance to dental materials often display multiple metal allergies which can be demonstrated by patch test or by MELISA®. From a larger group of patients monitored by us for several years, 15 are described in detail in Table 1. In addition to inorganic Hg, reactivity to Au, Pd and Ni was often present. Patch test results and *in vitro* lymphocyte responses were usually in accord. However, in some patients, patch test was negative despite of positive MELISA® test. The limited value of patch test for diagnosis of Hg allergy has been reported elsewhere [66]. In the majority of patients, replacement of metallic appliances with ceramic and composite materials resulted in long-term health improvement. This is in agreement with previous publications [51, 76, 77, 87, 92]. Together with improved health, lymphocyte responses to dental metals also decreased after RID, reflecting the down-regulation of inflammation *in vivo*.

Undesirable effects of dental materials

Regarding side-effects caused by dental materials, the focus has until now been primarily on local oral problems (Fig. 2). This is despite of the fact that already in 1982, Swedish researchers described that "allergens released in the mouth may result in allergic reactions in other parts of the body, or worsen or maintain such reactions without any local reaction in the oral mucosa" [35]. Only a few reports describe reactions in other parts of the body. For example, Hay [22] describes a case of recurring facial dermatitis after dental treatment. The patient had facial symptoms as well as lichenoid reactions near the dental amalgam; the same reactions were induced by dental gold. The patch test showed a strong reaction to cobalt (Co), Ni, copper (Cu), and Pd – and a

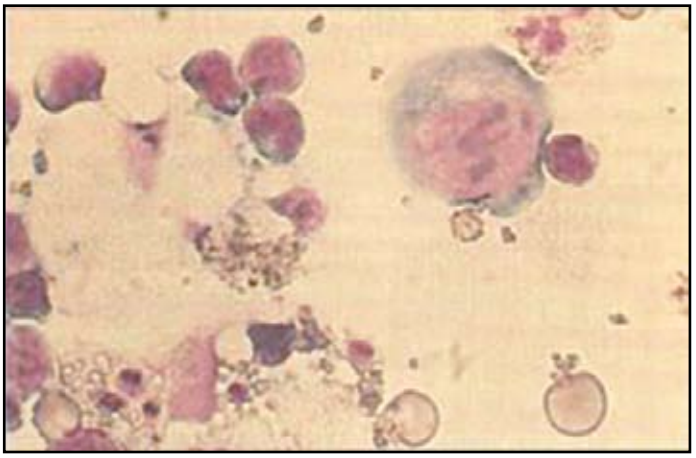

Figure 1a. One large stimulated lymphoblast and small, non-stimulated lymphocytes .

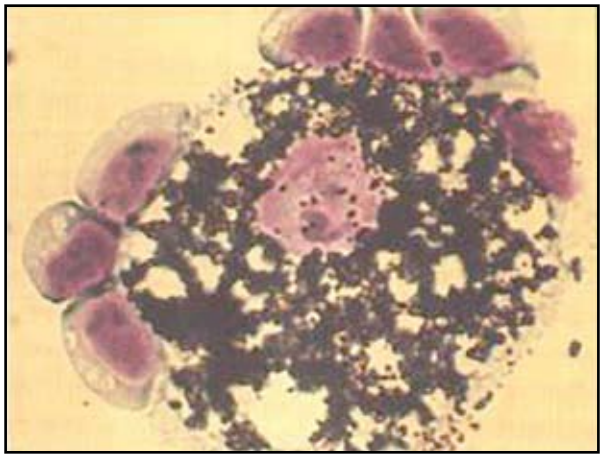

Figure 1b. Macrophage containing titanium dioxide, surrounded by small lymphocytes.

weak reaction to inorganic Hg and Au thiosulphate. The tests were negative to all other materials tested, including latex and acrylic materials (methyl-hydroquinone).

Laine and co-workers in Finland [32] studied allergy to different restorative materials in 118 patients with oral lichenoid changes adjacent to metal fillings. The contact allergy was determined by patch testing. Eighty patients (68%) showed positive results. Seventy-six patients (64%) were reactive to Hg, 11 (9%) to Au, 4 (3%) to Co, and 2.5% reacted to tin (Sn), silver (Ag), or Pd. Allergic reactions to acrylates, a composite component, was not detected. Removal of metal fillings was performed in 62 out of 80 patch test-positive patients and healing of the oral mucosa was observed in almost 50% of the cases. The authors point out that allergy to acrylates may be caused by negligent use of non-hardened acrylate monomers during dental work, but it is not a problem following hardening in oral cavity.

Metallic Au used in dental alloys has previously been regarded as inert and Au-induced contact allergy as a rare phenomenon. This changed when Björkner and co-workers reported that 8.6% of patients referred to the Dermatology Clinics in Malmö, Sweden, reacted positively to Au thiosulfate in patch test [6]. This was later confirmed by Marcusson [37] who tested 397 patients with multi-symptoms suspected to be caused by dental restorative metals. The frequency of patch test-positive patients was 23% for Au, 8% for Pd, and 4% for inorganic Hg and ethyl Hg. In contrast, a study of 2,853 patients in Portugal demonstrated that only 0.8% of the patients – all women – suffered from Au allergy [63]. The authors speculate that the reason for the low frequency of Au allergy is that golden alloys are rarely used in dentistry in Portugal. Swedish researchers [1, 2] further reported that Au-positive patch test correlated with the number of patients' Au restorations, and that the concentration of Au in saliva and serum correlated with the number of Au restorations as well. These findings corroborate the original findings of Drasch and co-workers [10] regarding correlation of Au and Pd in the saliva with the number of Au restorations. Hence, the aggressive environment in the oral cavity, including oral bacteria and acidic pH, as well as the presence of galvanic streams among disparate metals [41, 53, 88] may contribute to increased corrosion and to a higher frequency of Au sensitization.

Titanium allergy: does it exist?

Titanium (Ti) is increasingly used in dental and body implants. It is rapidly oxidized to titanium dioxide, $Ti0_2$, a white coloring agent added to drugs, candy, food, cosmetics, sunscreen, toothpaste and chewing gum [75]. The reactivity of Ti with oxygen is due to its physiochemical properties as a transition element and this reactivity is greatly enhanced by the presence of fluoride ions [83]. Protein reactivity together with the ability to trigger free radicals [23, 81] should be of concern when evaluating the possible adverse effects of Ti in humans.

In dermatology, the allergenic potential of Ti is virtually unknown since patch test invariably turns negative [24, 40]. This could be due to the fact that testing is performed with a suspension of TiO_2 which has only limited diffusion through the skin.

In 650 Swedish patients with clinically verified or suspected metal hypersensitivity, 3% were positive to TiO_2 in MELISA® testing [75]. Valentine-Thon and colleagues [87] found similar results among 700 patients in northern Germany, where 4.2% reacted to TiO_2. Finally, Müller et al. [40] reported on 56 patients who developed health problems after receiving Ti-based implants. In the MELISA® test, more than half responded with increased proliferation to Ti, although they were all patch test negative. In patients who did not respond to Ti *in vitro*, a majority responded to other metals. Clinical symptoms disappeared or improved dramatically after implant replacement.

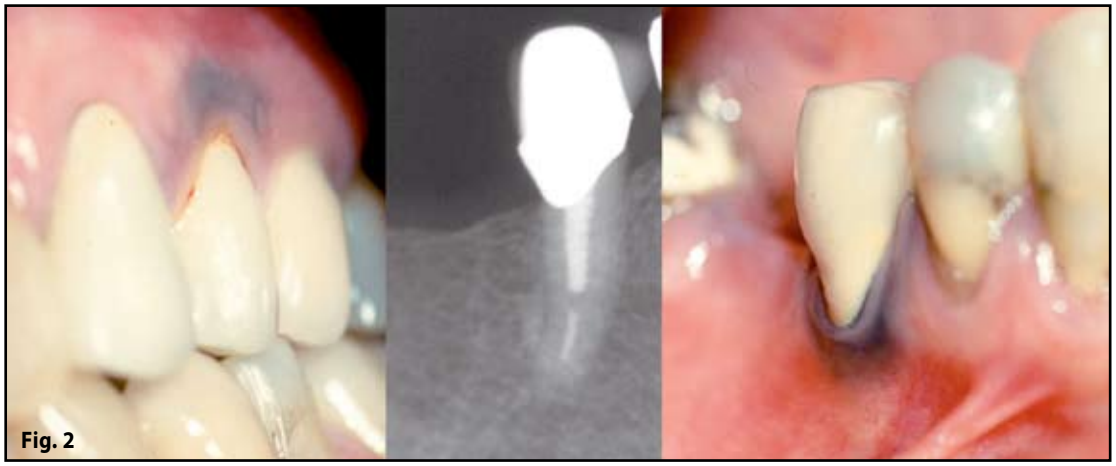

Figure 2. Metal-ceramic crowns on teeth 24 and 45. In the roots of these teeth Ti posts have been placed (centre). Observe the changes in the adjacent mucosa (black coloration of the gingiva) due to corrosion products of metals.

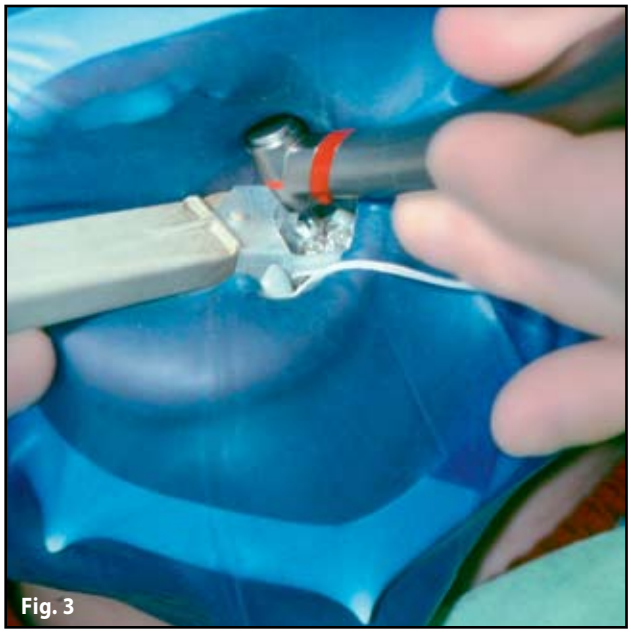

Figure 3. Rubber-dam and Clean-up® applied during amalgam removal.

In vitro responses in patients and controls

The frequency of metal-induced lymphocyte responses was examined in 3,162 patients in three European laboratories (two Swedish and one German) using MELISA® [76]. Patients suffered from oral symptoms such as oral lichen planus, burning and itching and systemic symptoms resembling CFS. In both countries, the most frequent metal allergen found was Ni, followed by inorganic Hg, Au, phenyl Hg, Pd, cadmium and Ti. Positive responses to other metals such as Ag, platinum (Pt) and Cu were only rarely observed. Similar results were later published by others who also validated the MELISA® method [86, 87].

Sterzl et al. [77] reported that lymphocytes from fatigued patients suffering from autoimmune thyroiditis responded more frequently to Ni and inorganic Hg than healthy controls. The increased Ni allergy in CFS patients was also demonstrated by patch testing [37, 38]. Regland et al. [55] have shown that female patients with Ni allergy suffering from fibromyalgia will improve on a low Ni-diet.

Tibbling et al. [82] used magnetic resonance imaging (MRI) to examine 32 patients with central nervous system (CNS) and systemic multi-symptoms suggestive of metal-induced pathology. Metal responsiveness at the lymphocyte level was examined by MELISA® and lymphocyte phenotype was analyzed with flow cytometry. One hundred twenty age-matched patients without CNS symptoms served as controls for the MRI study, 77 healthy subjects with dental amalgam fillings served as controls for MELISA® and 75 served as controls for phenotype determination. Pathological MRI findings were present in 81% of the patients, most of them with signs of degeneration in the basal ganglia, but none was found in the controls. The MELISA® test showed a higher frequency of metal-specific responses in patients than in controls. The difference in metal reactivity was highly significant for inorganic Hg ($p< 0.001$), phenyl Hg ($p<0.002$), and Au ($p<0.005$), weakly significant for lead ($p=0.05$) and not significant for the remaining metals. In both patients and controls, Ni was the most frequent allergen. The lymphocyte phenotype determination was pathological in 58% of patients. Sixty-two of the patients

had atopic diseases and 35% suffered from hypothyroidism. The authors concluded that dental restorative metals may play an important role in the development of the brain lesions in patients with basal ganglia disorders.

The impact of RID

Anneroth and co-workers [3] studied 10 patients who suspected amalgam replacement as a cause of aggravation of their symptoms. Six of 10 patients had contact allergies due to metals; three of them were induced by inorganic Hg. The changes in laboratory tests prior and after amalgam removal indicated that amalgam drilling might have activated the immune system.

The effect of dental metal replacement with metal-free restorations was studied in 111 CFS patients with metal allergy [76]. Following RID, 83 patients (73%) reported long-term health improvement. Twenty-four patients (22%) reported unchanged health and two (2%) reported worsening of symptoms. There was a marked decrease in lymphocyte reactivity to inorganic Hg as well as to other metals used as components or dental alloys. These data have been been confirmed in larger studies [34, 92].

Prochazkova and coworkers [52] were first to show that amalgam removal in Hg-sensitive patients suffering from various autoimmune diseases, such as multiple sclerosis or rheumatoid arthritis, resulted in down-regulation of Hg-specific responses *in vitro* and long-term health improvement. This also correlated with the decrease of anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-Tg) antibodies [78]. In contrast, Hg-sensitive patients who refrained from amalgam replacement did not show any health changes half a year later. In this group, the level of autoantibodies and lymphocyte responsiveness to inorganic Hg *in vitro* remained the same as at the start of study. The authors conclude that removal of dental amalgam in patients with Hg-sensitivity might contribute to successful treatment of autoimmune diseases.

The following case illustrates the importance of proper diagnosis and dental treatment for the rehabilitation of a patient. A female nurse suffered from electrosensitivity, fatigue, depression, and social phobia. She had on several occasions been exposed to broken Hg-containing thermometers, and suspected dental amalgam as the cause of her ill health. Amalgam was replaced with a white Au bridge but health problems persisted. Analysis of the cerebrospinal fluid showed a negligible amount of Hg (0,6 μg/l, reference range <1 μg/l), but a high concentration of Pt (5 μg/l), a component of the bridge. The patient's lymphocytes reacted positively *in vitro* to Pt (SI=5,4), Pd (SI=5,3) and weakly to Sn (SI=2,8). Lymphocyte responses were negative to thimerosal, Ti, Ag, Cu, Au, methyl Hg, phenyl Hg and inorganic Hg. Since Pd, Sn and Pt were components of her white Au bridge, it was replaced with a ceramic bridge. The patient's fatigue, electrosensitivity, depression and social phobia diminished and 10 years on she still enjoys good health.

The dentist's role in the treatment of patients with clinical metal allergy is of utmost importance. All mechanical work in the oral cavity may result in increased exposure to potentially allergenic substances and consequently to temporary worsening of patient's symptoms (Table 1). By strict use of precautionary measures and safety devices it is possible to reduce the exposure to a level where serious side-effects are rare and only occur in particularly sensitive patients.

Precautionary measures during RID is the use of rubber-dam, Clean-up® (CleanDent, Sweden) (Fig. 3), traps that remove Hg vapor from the air, and fresh-air outlets. The choice of instruments, such as the type of drill, drilling speed and the use of manual instruments for removal of fillings, has been described in detail previously [25, 26].

In addition to ceramics and composites, zirconia holds the promise of one of the future's immuno-compatible implant materials [7]. Finally, a patient's detoxification capacity and other genetic factors may play a crucial role in the patient's recovery. During rehabilitation, the patient's physician should consider prescribing supporting therapy, such as antioxidant treatment.

Conclusion

Dental materials and implants can induce sensitization in genetically susceptible individuals. The frequency of metal allergy is significantly higher in patients with autoimmune disorders and CFS than in healthy controls. Metal allergy can be tested by patch test (*in vivo*) and by LTT (*in vitro*). The identification of metal-sensitive patients is the first step to successful treatment, which may involve RID. Many case reports and clinical studies show that the replacement of amalgam or other metal alloys in allergic individuals can lead to dramatic clinical improvement. To avoid side-effects, it is important to follow a strict working protocol, which minimizes the risk of metal exposure for the patient.

Acknowledgement

The authors wish to thank Margit Forsbeck MD and Jan Marcusson MD for the skillful performance of patch tests, and to Linda Nelson for the help with the preparation of the manuscript.

This work was supported by grant NR 9414-3 of the Internal Grant Agency of Ministry of Health, Czech Republic, which is gratefully acknowledged.

REFERENCES

1 Ahlgren C, Ahnlide I, Bjorkner B, Bruze M, Liedholm R, Moller H, et al. K. Contact allergy to gold is correlated to dental gold. Acta Derm Venereol. 2002; **82**(1): 41–4

2 Ahnlide I, Ahlgren C, Bjorkner B, Bruze M, Lundh T, Moller H, et al. Gold concentration in blood in relation to the number of gold restorations and contact allergy to gold. Acta Odontol Scand. 2002 Oct; **60**(5): 301–5.

3 Anneroth G, Ericson T, Johansson I, Mornstad H, Ryberg M, Skoglund A, et al. Comprehensive medical examination of a group of patients with alleged adverse effects from dental amalgams. Acta Odontol Scand. 1992 Apr; **50**(2): 101–11.

4 Bartova J, Prochazkova J, Kratka Z, Benetkova K, Venclikova Z, Sterzl I. Dental amalgam as one of the risk factors in autoimmune diseases. Neuro Endocrinol Lett. 2003 Feb–Apr; **24**(1–2): 65–7.

5 Bencko V, Wagner V, Wagnerova M, Ondrejcak V. Immunological profiles in workers occupationally exposed to inorganic mercury. J Hyg Epidemiol Microbiol Immunol. 1990; **34**(1): 9–15.

6 Björkner B, Bruze M, Möller H. High frequency of contact allergy to gold sodium thiosulfate. An indication of gold allergy? Contact Dermatitis. 1994 Mar; **30**(3): 144–51.

7 Blaschke C, Volz U. Soft and hard tissue response to zirconium dioxide dental implants – a clinical study in man. Neuro Endocrinol Lett. 2006; **27**(Suppl1): 69–72.

8 Caron GA, Poutala S, Provost TT. Lymphocyte transformation induced by inorganic and organic mercury. Int Arch Allergy Appl Immunol. 1970; **37**(1): 76–87.

9 Clauw DJ. The pathogenesis of chronic pain and fatigue syndrome, with special reference to fibromyalgia. Med Hypotheses. 1995 May; **44**(5): 369–78.

10 Drasch G, Muss C, Roider G. Gold and palladium burden from dental restoration materials. J Trace Elem Med Biol. 2000 Jun; **14**(2): 71–5.

11 Everness KM, Gawkrodger DJ, Botham PA, Hunter JAA. The discrimination between nickel-sensitive and non nickel-sensitive subjects by an *in vitro* lymphocyte transformation test. Br J Dermatol. 1990 Mar; **122**(3): 293–8.

12 Feilzer A, Muris J, Valentin-Thon E, Hiller R. Electrical shavers as possible risk factor for metal exposure. Arch Dermatol. 2006 Oct; **142**(10): 1361–2.

13 Fischer T, Maibach H. Recovery of nickel sulfate from a standard patch test. Contact Dermatitis. 1984 Aug; 11(2): 134.

14 Fowler JR, Sexton K. EPA priorities for biologic markers research in environmental health. Environ Health Perspect. 1992 Nov; **98**: 235–41.

15 Fujinami RS, von Herrath MG, Christen U, Whitton JL. Molecular mimicry, bystander activation, or viral persistence: infections and autoimmune disease. Clin Microbiol Rev. 2006 Jan; **19**(1): 80–94.

16 Geier DA, Geier MR. A meta-analysis epidemiological assessment of neurodevelopmental disorders following vaccines administered from 1994 through 2000 in the United States. Neuro Endocrinol Lett. 2006; **27**(4): 401–13.

17 Geier DA, Geier MR. A clinical trial of combined anti-androgen and anti-heavy metal therapy in autistic disorders. Neuro Endocrinol Lett. 2006 Dec;**27**(6): 833–8.

18 Godfrey ME, Wojcik DP, Krone CA. Apolipoprotein E genotyping as a potential biomarker for mercury neurotoxicity. J Alzheimers Dis. 2003 Jun; **5**(3): 189–95.

19 Goldman M, Druet P, Gleichmann E. TH2 cells in systemic autoimmunity: insights from allogeneic diseases and chemically-induced autoimmunity. Immunol Today. 1991 Jul; **12**(7): 223–7.

20 Griem P, Gleichman E. Metal ion induced autoimmunity. Curr Opin Immunol. 1995 Dec; **7**(6): 831–8.

21 Haberman AL, Pratt M, Storrs FJ. Contact dermatitis from beryllium in dental alloys. Contact Dermatitis. 1993 Mar; **28**(3): 157–62.

22 Hay IC, Ormerod D. Severe oral and facial reaction to 6 metals in restorative dentistry. Contact Dermatitis. 1998 Apr; **38**(4): 216.

23 Titanium dioxide induced chemiluminescence of human polymorphonuclear leukocytes. Int Arch Occup Environ Health. 1988; **61**(1–2): 1–6.

24 Holgers KM, Roupe G, Tjellstrom A, Bjursten LM. Clinical, immunological and bacteriological evaluation of adverse reactions to skin-penetrating titanium implants in the head and neck region. Contact Dermatitis. 1992 Jul; **27**(1): 1–7.

25 Hudecek R, Danersund A, Kinigalakis G, Lindvall A. Experiences of Medical Odontological Treatment: Removal of Incompatible Dental Material (RID) in Patients with Intolerance of Dental Materials. Amalgam and Health-New perspective on Risks, Report at Conference for Swedish Council for Planning and Coordination of Research. Stockholm, 14 November 1997.

26 Hudecek R. Dental Materials and Ill-health, part II, Foundation for Metal Biology, Uppsala Sweden, 2005 ISBN 91-631-8284-X (In Swedish).

27 Humes JL, Bonny RJ, Pelus L, Dahlgren ME, Sadowski SJ, Kuehl FA, et al. Macrophages synthesis and release prostaglandins in response to inflammatory stimuli. Nature. 1977 Sep 8; **269**(5624): 149–51.

28 Ionescu JG, Novotny J, Stejskal VD, Latsch A, Blaurock-Busch E, Eisenmann-Klein M. Increased levels of transition metals in breast cancer tissue. Neuro Endocrinol Lett. 2006; **27**(Suppl1): 36–39.

29 Kazantis G. The role of hypersensitivity and the immune response in influencing susceptibility to metal toxicity. Environ Health Perspect. 1978 Aug; **25**: 111–8.

30 Kreiss K, Newman LS, Mroz M, Campbell PA. Screening blood test identifies subclinical beryllium disease. J Occup Med. 1989 Jul; **31**(7): 603–8.

31 Kreiss K, Miller F, Newman LS, Ojo-Amaze E, Rossman MD, Saltini C. Chronic beryllium disease – from the workplace to cellular immunology, molecular immunogenetics, and back. Clin Immunol Immunopathol. 1994 May; **71**(2): 123–9.

32 Laine J, Kalimo K, Happonen RP. Contact allergy to dental restorative materials in patients with oral lichenoid lesions. Contact Dermatitis. 1997 Mar; **36**(3): 141–6.

33 Lewalter J, Neumann HG. Biologische Arbeitsstoff-Toleranzwerte (Biomonitoring). Arbeitsmed Sozialmed Umweltmed 1998; **8**: 352–364.

34 Lindh U, Hudecek R, Danersund A, Eriksson S, Lindvall A. Removal of dental amalgam and other metal alloys supported by antioxidant therapy alleviates symptoms and improves quality of life in patients with amalgam-associated ill health. Neuro Endocrinol Lett. 2002 Oct–Dec; **23**(5–6): 459–82.

35 Magnusson B, Bergman M, Bergman B, Sörenmark R. Nickel allergy and nickel-containing dental alloys. Scand J Dent Res. 1982 Apr; **90**(2): 163–7.

36 Marcusson JA. Psychological and somatic subjective symptoms as a result of dermatological patch testing with metallic mercury and phenyl mercuric acetate. Toxicol Lett. 1996 Feb; **84**(2): 113–22.

37 Marcusson JA. Contact allergies to nickel sulfate, gold thiosulfate and palladium chloride in patients claiming side-effects from dental alloy components. Contact Dermatitis. 1996 May; **34**(5): 320–3.

38 Marcusson JA, Lindh G, Evengård B. Chronic fatigue syndrome and nickel allergy. Contact Dermatitis. 1999 May; **40**(5): 269–72.

39 Moldofsky H. Sleep, neuroimmune functions in fibromyalgia and chronic fatigue syndrome. Adv Neuroimmunol. 1995; **5**(1): 39–56.

40 Muller KE, Valentine-Thon E. Hypersensitivity to titanium: Clinical and laboratory evidence. Neuro Endocrinol Lett. 2006; **27**(Suppl1): 31–35.

41 Muris J, Feilzer AJ. Micro analysis of metals in dental restorations as part of a diagnostic approach in metal allergies. Neuro Endocrinol Lett. 2006; **27**(Suppl1): 49–52.

42 Mutter J, Naumann J, Schneider R, Walach H, Haley B. Mercury and autism: accelerating evidence? Neuro Endocrinol Lett. 2005; **26**(5):439–46.

43 Nedorost S. Wagman A. Positive patch-test reactions to gold: patients' perception of relevance and the role of titanium dioxide in cosmetics. Dermatitis. 2005 Jun; **16**: 67–70.

44 Newman LS. Significance of the blood beryllium lymphocyte proliferation test. Environ Health Perspect. 1996 Oct; 104 Suppl **5**: 953–6.

45 Nielsen C, Klaschka F. Test studies on the mouth mucosa in allergic eczema. Dtsch Zahn Mund Kieferheilkd Zentralbl Gesamte. 1971 Oct; **57**(7): 201–18.

46 Peterson JD, Herzenberg L, Vasques K, Waltenbauch C. Glutathione levels in antigen-presenting cells modulate Th1 versus Th2 response patterns. Proc Natl Acad Sci U S A. 1998 Mar 17; **95**(6): 3071–6.

47 Pichler WJ, Tilch J. The lymphocyte transformation test in the diagnosis of drug hypersensitivity. Allergy. 2004 Aug; **59**(8): 809–20.

48 Pirquet von Cesenatico CP. Studien über Vakzination und vakzinale Allergie. MünchenerMedizinische Wochenschrift, 1906; **53**: 457–1458.
49 Pizzichini M, Fonzi M, Giannieri F, Mencarelli M, Gasparoni A, Rocchi G, et al. Influence of amalgam fillings on Hg levels and total antioxidant activity in plasma of healthy donors. Sci Total Environ. 2003 Jan 1; **301**(1–3): 43–50.
50 Prigent P, Saoudi A, Pannetier C, Graber P, Bonnefoy JY, Druet P, et al. Mercuric chloride, a chemical responsible for T helper cell (Th)2-mediated autoimmunity in brown Norway rats, directly triggers T cells to produce interleukin-4. Clin Invest. 1995 Sep; **96**(3): 1484–9.
51 Prochazkova J, Bartova J, Ivaskova E, Kupkova L, Sterzl I, Stejskal VD. HLA-association in patients with intolerance to mercury and other metals in dental materials. Dis Markers. 2000; **16**(3–4): 135–8.
52 Prochazkova J, Sterzl I, Kucerova H, Bartova J, Stejskal V. The beneficial effect of amalgam replacement on health in patients with autoimmunity. Neuro Endocrinol Lett. 2004; **25**(3): 211–8.
53 Prochazkova J, Podzimek S, Tomka M, Kucerova H, Mihaljevic M, Hana K, Miksovsky M, Sterzl I, Vinsova J. Metal alloys in the oral cavity as a cause of oral discomfort in sensitive patients. Neuro Endocrinol Lett. 2006; **27**(Suppl1): 53–58.
54 Queiroz ML, Pena SC, Salles TS, de Capitani EM, Saad ST. Abnormal antioxidant system in erythrocytes of mercury exposed workers. Exp Toxicol. 1998 Apr; **17**(4): 225–30.
55 Regland B, Zachrisson O, Gottfries CG, Stejskal V. Nickel allergy is found in a majority of women with chronic fatigue syndrome and muscle pain and may be triggered by cigarette smoke and dietary nickel intake. J Chronic Fatigue Syndr. 2001; **8**(1): 57–65
56 Roitt I, Brostoff J, Male D. Immunology. The C.V. Mosby Company, St Louis, Toronto, Gower Medical Publishing, London, NY; 1986.
57 Roth MD, Golub SH. Human pulmonary macrophages utilize prostaglandins and transforming growth factor beta 1 to suppress lymphocyte activation. J Leukoc Biol. 1993 Apr; **53**(4): 366–71.
58 Rytter M, Schubert H. Gold allergy as a result of primary epicutaneous sensitization from a gold ring. Dermatologica. 1971; **142**(4): 209–18.
59 Räsänen L, Kalimo K, Laine J, Vainio O, Kotiranta J, Pesola I. Contact allergy to gold in dental patients. J Dermatol. 1996 Apr; **134**(4): 673–7.
60 Sanchez APG, Maruta CW, Sato MN, Ribeiro RL, Zomignan CA, Nunes RS, et al. Study on lymphocyte proliferation in nickel sensitive patients. An Bras Dermatol 2005; **80**: 149–158.
61 Schöpf E, Schulz KH, Gromm M. Transformation und Mitosen von Lymfocyten in vitro durch Quecksilber(II)chlorid. Naturwissenschaffen 1967; **54**: 568–569.
62 Seidenari S, Motolese A, Belletti B. Pre-treatment of nickel test areas with sodium lauryl sulfate detects nickel sensitivity in subjects reacting negatively to routinely performed patch tests. Contact Dermatitis. 1996 Feb; **34**(2): 88–92.
63 Silva R, Pereira F, Bordalo O, Silva E, Barros A, Goncalo M, et al. Contact allergy to gold thiosulfate – a comparative study. Contact Dermatitis; 1997; **37**: 78–81.
64 Silvennoinen-Kassinen S. The specificity of a nickel sulphate reaction in vitro: a family study and a study of chromium-allergic subjects. Scand J Immunol. 1981; **13**(3): 231–5.
65 Sivri A, Cindas A, Dincer F, Sivri B. Bowel dysfunction and irritable bowel syndrome in fibromyalgia patients. Clin Rheumatol. 1996 May; **15**(3): 283–6.
66 Skoglund A. Value of epicutaneous patch testing in patients with oral mucosal lesions of lichenoid character. Scand J Dent Res. 1994 Aug; **102**(4): 216–22.
67 Subcommittee on Immunotoxiology Committee on Biologic Markers, National Research Council. Biologic markers in Immunotoxicology. Washington DC: National Academy Press; 1992.
68 Stejskal J, Stejskal VD. The role of metals in autoimmunity and the link to neuroendocrinology. Neuro Endocrinol Lett. 1999; **20**(6): 351–364.
69 Stejskal VDM, Olin R, Forsbeck M. The lymphocyte transformation test for diagnosis of drug-induced occupational allergy. J Allergy Clin Immunol. 1986 Mar; **77**(3): 411–26.
70 Stejskal VDM. Allergy to Drugs and Other Chemicals Diagnosed by the presence of specific memory cells in human blood. Realm of Tolerance Pavol Ivanyi (Ed), 1989; 213–224, Springer Verlag Berlin, Heidelberg, New York, London, Paris, Tokyo, Hong Kong.
71 Stejskal VDM, Forsbeck M, Nilsson R. Lymphocyte transformation test for diagnosis of isothiazolinone allergy in man. J Invest Dermatol. 1990 Jun; **94**(6): 798–802.
72 Stejskal V, Slanina P, Olin R, Machado L, Stålenheim G, Forsbeck M. Occupational hypersensitivity to psyllium-allergic or pseudoallergic. Allergy and Immunol.1990; **9**: 77–84
73 Stejskal VDM, Cederbrant K, Lindvall A, Forsbeck M. MELISA – an *in vitro* tool for the study of metal allergy. Toxicol In Vitro 1994; **8**: 991–1000.
74 Stejskal VDM, Forsbeck M, Cederbrant C, Asteman O. Mercury-specific lymphocytes: An indication of mercury allergy in man. Clin Immunol. 1996 Jan; **16**(1): 31–40.
75 Stejskal V. Human hapten-specific lymphocytes: biomarkers of allergy in man. Drug Inform J. 1997; **4**: 379–82.
76 Stejskal VD, Danersund A, Lindvall A, Hudecek R, Nordman V, Yaqob A et al. Metal-specific lymphocytes: biomarkers of sensitivity in man. Neuro Endocrinol Lett. 1999; **20**: 289–298.
77 Sterzl I, Prochazkova J, Hrda P, Bartova J, Matucha P, Stejskal VDM. Mercury and nickel allergy: risk factors in fatigue and autoimmunity. Neuro Endocrinol Lett. 1999; **20**: 221–228.
78 Sterzl I, Prochazkova J, Hrda P, Matucha P, Bartova J, Stejskal VD. Removal of dental amalgam decreases anti-TPO and anti-Tg autoantibodies in patients with autoimmune thyroiditis. Neuro Endocrinol Lett. 2006; **27**(Suppl1): 25–30.
79 Sterzl I, Hrda P, Potuznikova B, Matucha P, Hana V, Zamrazil V. Autoimmune thyroiditis and Helicobacter pylori – is there a connection? Neuro Endocrinol Lett. 2006; **27**(Suppl1): 41–45.
80 Tamer E, Ikizoglu G, Toy GG, Alli N. Comparison of nickel patch test reactivity in phases of the menstrual cycle. Int J Dermatol. 2003 Jun; **42**(6): 455–8.
81 Tengvall P, Hornsten EG, Elwing H, Lundström I. Bacterial properties of titanium-peroxy gel obtained from metallic titanium and hydrogen peroxide. J Biomed Mater Res. 1990 Mar; **24**(3): 319–30.
82 Tibbling L, Thuomas KO, Lenkei R, Stejskal V. Immunological and brain changes in patients with suspected metal intoxication. Int J of Occupat Med and Toxicol. 1995; **4**: 285–294.
83 Toumelin-Chemla F, Rouelle F, Burdairon G. Corrosive properties of fluoride-containing odontologic gels against titanium. J Dent. 1996 Jan–Mar; **24**(1–2): 109–15.
84 Turnbull AV, Rivier C. Regulation of the HPA axis by cytokines. Brain Behaviour and Immunity. Brain Behav Immun. 1995 Dec; **9**(4): 253–75.
85 Vallee BL, Ulmer DD. Biochemical effects of mercury, cadmium, and lead. Annu Rev Biochem. 1972; **41**(10): 91–128.
86 Valentine-Thon E, Schiwara H-W. Validity of MELISA® for metal sensitivity. Neuro Endocrinol Lett. 2003 Feb–Apr; **24**(1–2): 57–64.
87 Valentine-Thon E, Muller KE, Guzzi G, Kreisel S, Ohnsorge P, Sandkamp M. LTT-MELISA(®) is clinically relevant for detecting and monitoring metal sensitivity. Neuro Endocrinol Lett. 2006; **27**(Suppl1): 17–24.
88 Venclikova Z, Benada O, Bartova J, Joska L, Mrklas L, Prochazkova J, Stejskal VD, Podzimek S. In vivo effects of dental casting alloys. Neuro Endocrinol Lett. 2006; **27**(Suppl1): 61–68.
89 Weiss NS, Liff JM. Accounting for the multicausal nature of disease in the design and analysis of epidemiologic studies. Am J Epidemiol. 1983 Jan; **117**(1): 14–8.
90 Westphal GA, Schnuch A, Schulz TG, Reich K, Aberer W, Brasch J, et al. Homozygous gene deletions of the glutathione S-transferases M1 and T1 are associated with thimerosal sensitization. Int Arch Occup Environ Health. 2000 Aug; **73**(6): 384–8.
91 Wojcik DP, Godfrey ME, Christie D, Haley BE. Mercury toxicity presenting as chronic fatigue, memory impairment and depression: diagnosis, treatment, susceptibility, and outcomes in a New Zealand general practice setting (1994-2006). Neuro Endocrinol Lett. 2006; **27**(4):415-23.
92 Yaqob A, Danersund A, Stejskal VD, Lindvall A, Hudecek R, Lindh U. Metal-specific lymphocyte reactivity is down regulated after dental metal replacement. Neuro Endocrinol Lett. 2006 Feb–Apr; **27**(1–2): 189–97.