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MELISA—AN *IN VITRO* TOOL FOR THE STUDY OF METAL ALLERGY

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Abstract—The sensitizing properties of metals widely used in medical and dental care have been studied with the help of an optimized lymphocyte proliferative assay, MELISA. MELISA (memory lymphocyte immuno-stimulation assay) was originally developed for the screening of allergenic epitopes of drugs and other chemicals of low molecular weight, but has recently been adapted for the study of metal-induced sensitization. The patients studied suffered from various oral mucosal problems which were suspected to be caused by the release of metal ions from dental restorations. They were also troubled by chronic fatigue persisting over many years. One patient was also occupationally exposed to metals while working in a dental practice. Healthy subjects without any discomfort due to metal devices served as controls. In addition to metals used in dentistry, lymphocyte responses to organic mercurials used widely as preservatives in vaccines, eye/nose drops and contact lense fluids were studied. The results indicated that mercurials, as well as other metals such as gold or palladium, induce strong lymphocyte proliferative responses in patients with oral or systemic symptoms, but not in similarly exposed unaffected subjects. The results of MELISA performed with a pair of identical twins with chronic fatigue syndrome (CFS) indicated that metal-specific responses may be dependent on the genetics of the patient. Thus, many metals that are today accepted for use in medicine and dentistry carry a definite sensitizing risk for certain genetically predisposed individuals. Therefore, the use of these metals should be limited in the future.

INTRODUCTION

Metals play a dual role in our lives. Some metals, such as iron, copper, cobalt and zinc, are, in low doses, essential for life. Other metals, like mercury, cadmium, lead and arsenic, are harmful to all living organisms (Goering *et al.*, 1992; Vallee and Ulmer, 1972). There is abundant evidence in the literature concerning the allergy-inducing potential of some metals such as nickel or chromium (Langård and Hensten-Pettersen, 1981; Maibach and Menné, 1989) but other metals such as gold, palladium or mercury, although frequently used in medical care and dentistry, have not been evaluated thoroughly in this respect. Studies in laboratory animals indicate that mercury as well as gold salts can induce autoimmunity in certain genetically susceptible strains (Hultman *et al.*, 1992; Pelletier *et al.*, 1990). In this communication, the antigenic properties of metals used either as dental implants or as bactericidal agents were studied by an optimized lymphocyte proliferation assay, MELISA (memory lymphocyte immuno-stimulation assay). The results indicate that mercurials and transition metals such as gold or palladium can induce strong specific lymphocyte responses in subjects with oral mucosal symptoms who also suffered from chronic fatigue syndrome (CFS). Such responses were not seen in exposed healthy subjects. These results may have impact on the future limitation of metals used in medical and dental practice.

Abbreviations: CFS = chronic fatigue syndrome; MELISA = memory lymphocyte immuno-stimulation assay; MHC = major histocompatibility complex; SI = stimulation index.

MATERIALS AND METHODS

Patients and controls

The patients described in this communication were referred for MELISA testing from various Medical Clinics in Stockholm and Uppsala. Blood from patients with mucosal changes adjacent to dental metals were obtained from the Dermatology Clinics at Södersjukhuset, Stockholm, where patch tests were also performed. Patients with CFS were part of the project performed at the Department of Clinical Metal Biology, The University Hospital, Uppsala. They had suffered from long-term debilitating fatigue or easy fatiguability for at least 6 months and fulfilled the criteria of CFS (Holmes *et al.*, 1988). Healthy control subjects exposed to dental metals were also recruited. One control had never had any metal alloys in the oral cavity.

MELISA

MELISA, based on a protocol originally used in our laboratory (Stejskal *et al.*, 1986 and 1990), was adapted for the study of lymphocyte reactivity to various metals.

Venous blood was collected in sterile vacutainer tubes containing polystyrene beads (Becton Dickinson, England) and defibrinated by shaking. Lymphocytes were isolated on Ficoll–Paque (Pharmacia, Sweden) and washed with RPMI 1640 (Gibco, Scotland) containing 10 mM HEPES, 8 mg gentamicin/litre and 4 mmol L-glutamine/litre. The monocyte content was reduced by plastic adherence for 30 min at 37°C in the presence of 20% AB+ serum. Non-adherent cells were recovered and diluted to 1×10^6 cells per ml in complete RPMI 1640 with 10% inactivated AB+ serum. The cells were cultivated in 1-ml macrocultures, containing 1×10^6 lymphocytes, in 48-well plates (Costar, Holland) which were precoated with metal salts in the following dilutions: $HgCl_2$, 0.004–9 μg/ml; phenylmercuric acetate, 0.007–1 μg/ml; $SnCl_2 \times 2H_2O$, 12.5–50 μg/ml; $CuSO_4 \times 5H_2O$, 0.5–0.25 μg/ml; $CH_3COOHAg$, 2.5–5 μg/ml; $Na_3Au(S_2O_3)_2$, 1.5–50 μg/ml; $PdCl_2$, 1.56–6.25 μg/ml; $Pt(SO_4)_2 \times 2H_2O$, 12.5–25 μg/ml; $Pb(NO_3)_2$, 12.5–25 μg/ml; $CdCl_2 \times 2H_2O$, 1.5–6 μg/ml; TiO_2, 12.5–50 μg/ml; CH_3HgCl, 0.1–0.5 μg/ml; CH_3CH_2HgCl, 0.1–0.5 μg/ml; thiomersal, 0.1–0.5 μg/ml; thimerfonate, 0.06–0.5 μg/ml; $NiCl_2 \times 6H_2O$, 1–10 μg/ml; $CrCl_3 \times 6H_2O$, 1.5–50 μg/ml; $CoCl_2 \times 6H_2O$, 0.2–6.3 μg/ml. All salts used were of 'pro analysi' grade. Three to six control cultures, without antigens, provided information about the spontaneous proliferation of the lymphocytes. PPD (Purified Protein Derivative, tuberculin, Statens Seruminstitut, Denmark) was used as a positive control antigen for the estimation of cell-mediated immunity. Following 5 days of incubation, 600 μl from each well was transferred to a new plate supplemented with 30 μCi [*methyl*-^{3}H]thymidine (Amersham, England, sp. act. ca 3.2 TBq/mmol) and incubated at 37°C for 4 hr. The cell cultures were harvested in an automatic cell-harvester (Inotech, Switzerland) and the radioactivity was measured in a liquid-scintillation counter (LKB/Wallac, Finland).

The increase in [^{3}H]thymidine incorporation in the cultures was expressed as counts per minute (cpm) and a stimulation index (SI) was obtained as:

$$SI = \frac{\text{cpm in antigen-treated cultures}}{\text{mean cpm of control cultures.}}$$

Morphology

100 μl cell suspension was taken from each culture following 5 days of cultivation for smear preparation in a cytocentrifuge (Shandon, England). The smears were stained with May–Grünwald/Giemsa (Stejskal *et al.*, 1986). Cell preparations provide valuable information about the state of cultures and complete objective results of DNA incorporation.

MELISA evaluation

The threshold for a positive response was set at $SI \geqslant 3$ since this value excluded the majority of control subjects. SI values between 2 and 3 were considered as weakly positive. The maximal stimulation index indicates proliferation obtained at the optimal concentration of a given metal salt. Positive results obtained by [^{3}H]thymidine incorporation were confirmed by the presence of lymphoblasts on cell smears (data are not shown).

Patch tests

Patients with oral mucosal changes were patch tested with a dental screening series (Chemotechnique Diagnostics AB, Sweden) which included mercury 0.5% in petrolatum, cobalt chloride 0.5% in petrolatum, goldsodiumthiosulfate 0.5% in petrolatum, nickel sulfate 5.0% in petrolatum, palladium chloride 2.0% in petrolatum, phenylmercuric acetate 0.01% in water thiomersal 0.1% in petrolatum, thimerfonate 0.1% in petrolatum. Patch tests were performed with Finn chambers (Epitest Helsinki, Finland) on Scanpor. The test substance was applied for 48 hr on the patient's back and read after 72 hr. Erythema, induration and papules or vesicles were considered as a positive reaction.

RESULTS AND DISCUSSION

Cellular reactivity induced by mercurials

Dental amalgam is an alloy of 50% metallic mercury in combination with other metals such as tin, copper, silver and zinc. Since dental personnel are continuously exposed to metallic mercury at work and sometimes also to other mercurials such as phenyl-Hg (component of N2 root fillings) we have studied lymphocyte reactivity in dental staff.

A 42-yr-old atopic female (S28) worked as a dental nurse during the period 1977–87. She attended the Dermatology Clinics in 1987 because of the occurrence of lichenoid changes adjacent to amalgam fillings, verified by PAD, and chronic eczematous eruptions on her hands. She had a history of manual handling of amalgam in the past. The patient's lymphocytes responded *in vitro* to a wide range of inorganic Hg concentrations but also to organic phenyl-Hg (Fig. 1). The patch test was positive to phenyl-Hg but negative to metallic Hg. Since this patient obviously suffered from systemic sensitization to mercury, she was advised to replace her amalgam fillings and stop working as a dental nurse. Amalgam removal was completed in 1989. The patient also found another job. Her oral problems disappeared and her skin problems significantly improved.

Since lymphocyte responses to low molecular weight chemicals such as penicillins or isothiazolonines are usually present in patients with symptoms of hypersensitivity but not in exposed healthy individuals (Stejskal, 1989; Stejskal *et al.*, 1986) we wanted to see if this phenomenon was valid for mercury compounds. Thus, we studied the lymphocytes from another dental nurse (L7) who was exposed to amalgam through her own fillings and at work but did not have any skin or mucosal problems. L7's lympho-

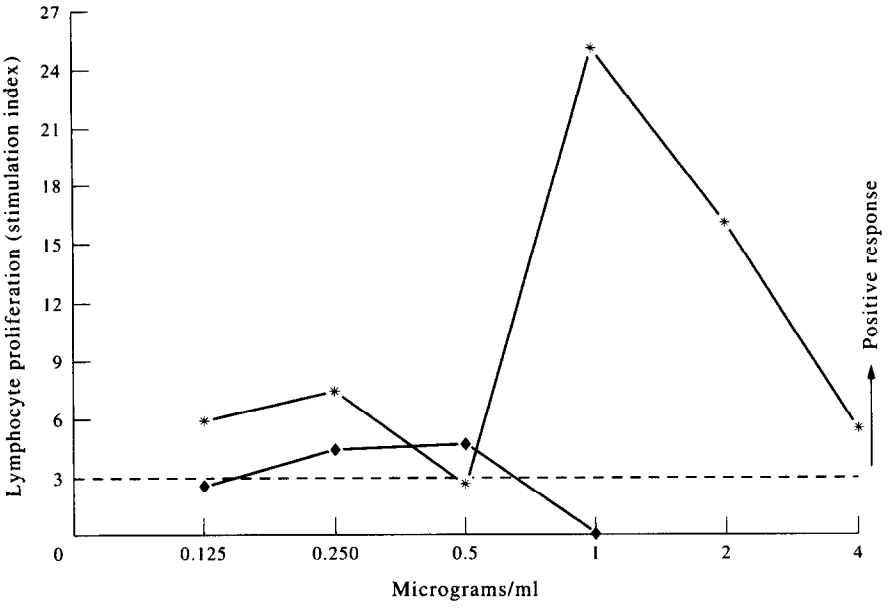

Fig. 1. MELISA with $HgCl_2$ (*) and phenylmercury acetate (◆) in a 42-yr-old atopic dental nurse (S28) with oral lichen and a positive patch test to phenylmercury. Control cpm = 1345.

cytes did not respond to inorganic Hg (0.009–9 μg/ml) or to phenyl-Hg (0.007–0.5 μg/ml) at any of the concentrations tested. Similar results were found when lymphocytes from another atopic dental nurse (TL43), who was free from amalgam restorations, were studied ($HgCl_2$: 0.125–4 μg/ml; phenyl-Hg: 0.06–0.5 μg/ml).

Comparatively lower responses induced by phenyl mercury in comparison with inorganic Hg, which were observed in patient S28, were not a regular finding in all of the patients studied. Lymphocytes from a 48-yr-old male (SK15) suffering from CFS responded vigorously to phenyl-Hg but only slightly to inorganic Hg (Fig. 2). The phenyl-Hg-specific sensitization was additionally demonstrated by positive patch test which also showed a positive reaction to thiomersal. The MELISA results were reproducible in several consecutive experiments (data not shown). On interviewing the patient about a possible source of sensitization it was found that he had been

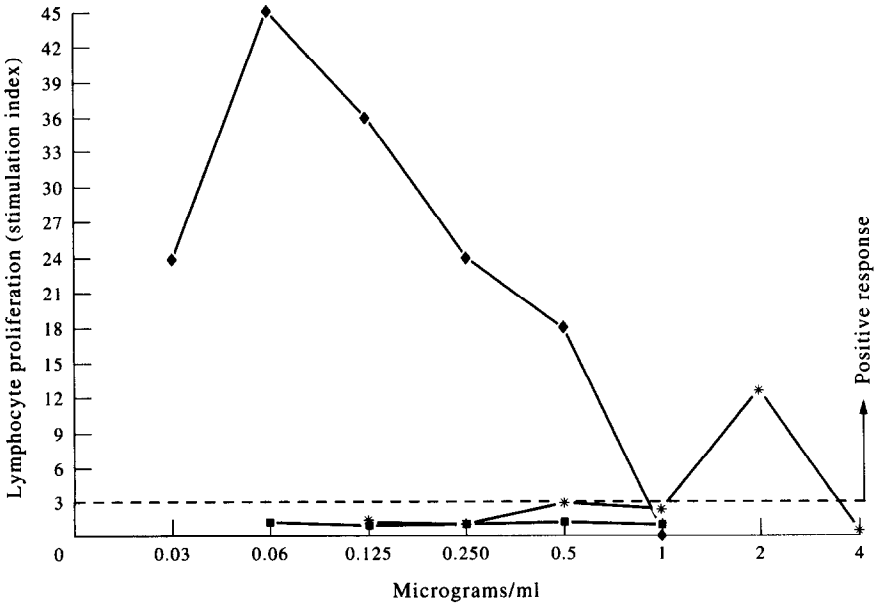

Fig. 2. MELISA with $HgCl_2$ (*) phenyl-Hg (◆) and thiomersal (■) in a 49-yr-old male (SK15) with CFS and a positive patch test to these compounds. Control cpm = 2396.

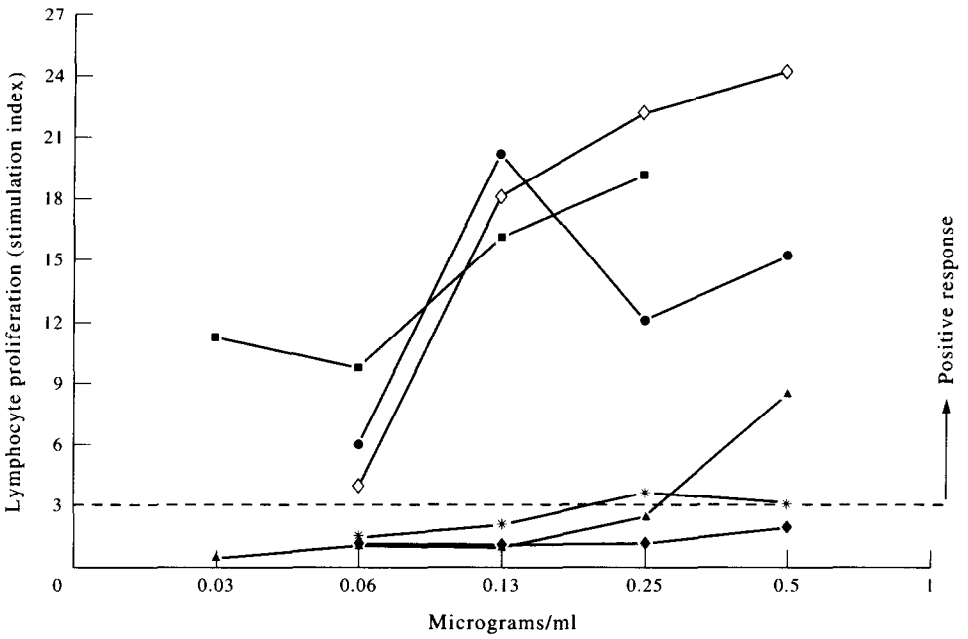

Fig. 3. MELISA with various mercury compounds in a 52-yr-old female (TL87) exposed to thiomersal containing vaccines at work: ▲, methyl-Hg; ■, thiomersal; ●, ethyl-Hg; ◇, thimerfonate; ◆, phenyl-Hg; *, $HgCl_2$.

exposed to organic mercurial pesticides in his youth while gardening in Chile. Interestingly, he also reported the appearance of conjunctivitis induced by the intake of large amounts of citrus fruits. It is possible that the citrus fruits may have been treated with pesticides containing phenyl-Hg, and that the conjunctivitis could be the result of a positive provocation.

Another organic mercurial, thiomersal (sodium ethyl mercurithiosalicylate) is widely used in Sweden and worldwide as a preservative in vaccines, soft contact-lense fluids and immunoglobulin preparations because of its excellent bactericidal properties. We wanted to examine the possible sensitizing properties of thiomersal at the lymphocyte level. A 52-yr-old female technician (TL87) was exposed to thiomersal while employed in vaccine production for several years. She was also treated with thimerfonate-containing anti-D-globulin following delivery of each of her two children. Past medical history revealed atopy and severe intolerance to fish and egg protein. While working as an animal attendant she developed joint oedema and dyspnoea and had to be relocated to an animal-free laboratory. However, RAST against various laboratory animals, including mice, was negative. The patient suspected animal food containing fish protein as a cause of her problems. The results of lymphocyte studies are shown in Fig. 3. TL87's lymphocytes responded strongly to ethyl-Hg, one of the epitopes of thiomersal (Pirker *et al.*, 1993), to thiomersal and to thimerfonate. Thimerfonate is another ethyl-Hg derivative used in vaccines for its bactericidal properties. A low degree of reactivity was demonstrated to methyl-Hg and to inorganic Hg while no response was detected to phenyl-Hg (data not shown). A patch test with ethyl-Hg showed a strong reaction at 0.05% concentration, and redness with possible infiltration at 0.1%. A strong reaction was also observed with thimerfonate but the results with these compounds were considered as toxic. A patch test with inorganic Hg showed a weak redness and was considered negative. Within 4 hr of the patch test the patient experienced headache, chills and itching on the back under the patch tests. The itching later spread over the extremities and finally all over the body. These symptoms, together with a debilitating fatigue, lasted for 3 days.

It is generally accepted that genetics plays a decisive role in immune responsiveness of the organism to low molecular substances (Barna *et al.*, 1984; Hultman *et al.*, 1992; Ishii *et al.*, 1990; Landsteiner *et al.*, 1939). We had an opportunity to study lymphocyte reactivity to metals in two pairs of identical twins. One pair of twins had had a diagnosis of CFS; the other pair was healthy. The former pair of 46-yr-old female identical twins (AD144 and AD147) were referred for MELISA testing because of suspected metal sensitivity such as intolerance to metal earrings and an aggravation of illness following dental treatment. They had suffered from debilitating fatigue for several years and fulfilled the criteria of CFS. The results of MELISA performed with various metal salts are shown in Fig. 4(a,b). Positive lymphocyte responses were detected in cultures incubated with inorganic Hg and phenyl-Hg and with gold and nickel salts. The lymphocyte reactivity pattern was

identical in both twins, suggesting the role of genetics in the sensitization process. AD144 showed a positive patch test to gold and mercury. AD147 experienced worsening of symptoms after drilling in amalgam fillings. None of them had any mucosal problems. Another pair of identical twins studied (TL26 and TL27; Fig. 5) were 52-yr-old males employed as chemists and exposed to amalgam and gold alloys through dental restorations. As shown in Fig. 5, their lymphocytes did not respond to any of the metals tested. The control values and proliferation responses to tuberculin (PPD) were similar in both twin-pairs as shown by the following cpm values: AD144, 3171 and 52 316 (PPD); AD147, 3010 and 158 321 (PPD); TL26, 2917 and 321 044 (PPD); TL 27, 2401 and 416703 (PPD).

The confirmation of the clinical relevance of *in vitro* lymphocyte responses by skin test was sometimes not possible since the patients refused to participate in testing because they were afraid of aggravation of symptoms. Therefore, another CFS patient with a known positive patch test to several metals was tested with MELISA in December 1992 (Fig. 6). This patient (S85) attended the Dermatology Clinics at the beginning of 1980. A patch test was performed and was found to be strongly positive to nickel and cobalt. In 1984, the patient was re-admitted because of itching and burning in the oral cavity in the vicinity of many amalgams and gold crowns. She also complained of general weakness and jewellery intolerance. Following dental treatment she became bedridden for 2 wk and her

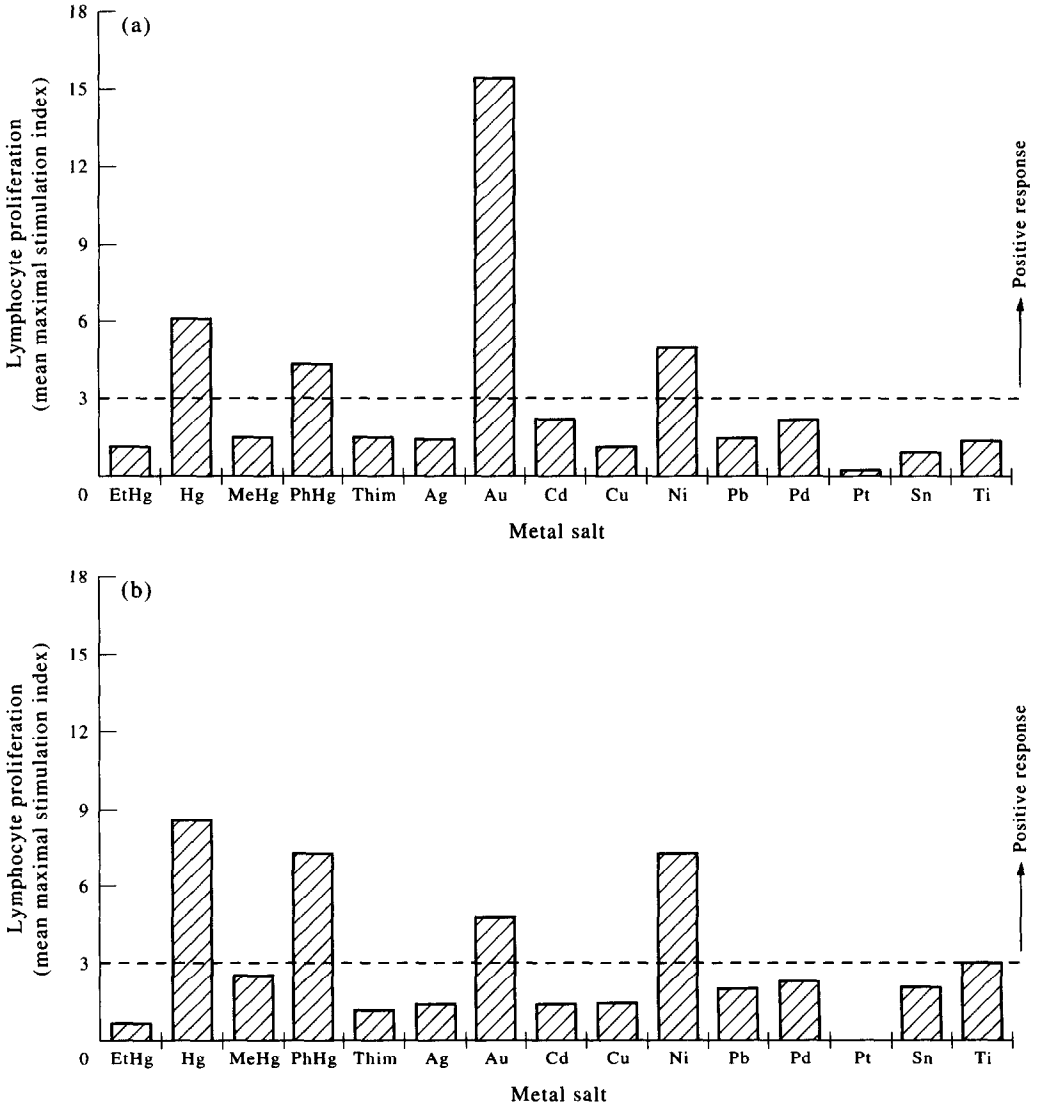

Fig. 4. MELISA with various metal salts in 46-yr-old female identical twins, (a) AD144 and (b) AD147, with CFS. The twins were exposed to dental amalgam, and had known earring intolerance. The mean stimulation index of two consecutive maximal values is shown.

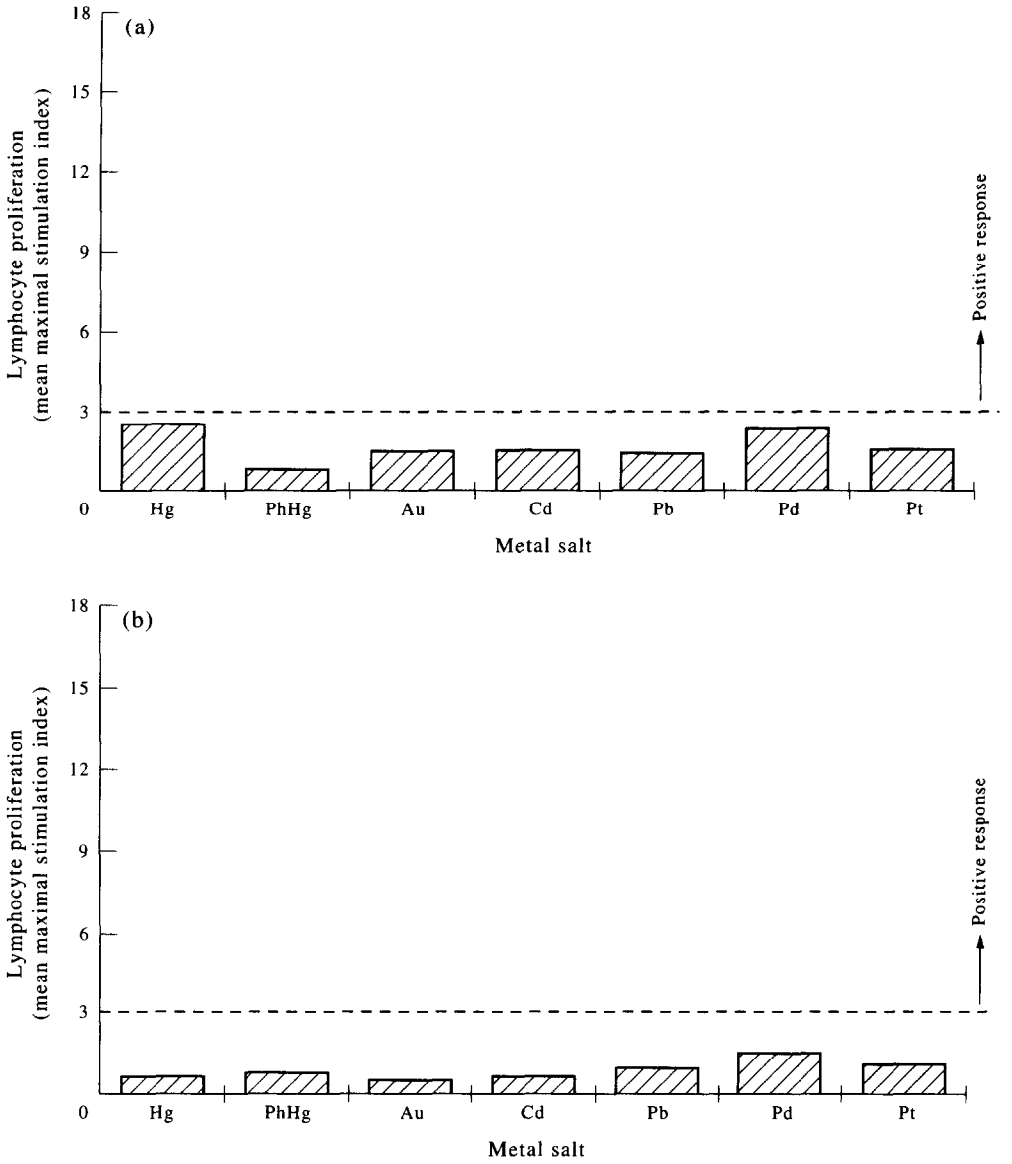

Fig. 5. MELISA with various metal salts in 55-yr-old male identical twins, (a) TL26 and (b) TL27. The twins were exposed to amalgam, dental gold and root fillings. The mean stimulation index of two consecutive maximal values is shown.

depression and asthma increased. A new patch test, performed in September 1990, was positive to palladium, gold, nickel and cobalt. A palladium-positive patch test is not uncommon among people with a very strong nickel reaction (Aberer *et al.*, 1993; Camarasa and Serra-Baldrich, 1990), as in this case. The results of MELISA confirmed strong lymphocyte reactivity to nickel and gold salts and a positive response to cobalt. The results were negative with another transition metal, chromium. The patient's dentist replaced her gold and amalgam fillings with ceramic crowns which resulted in the disappearance of oral symptoms. Her general health status improved as well.

Palladium, another transition metal frequently used in dental gold alloys, may induce sensitization as reported previously (Aberer *et al.*, 1993; Camerasa and Serra-Baldrich, 1990). A 39-yr-old female (AD190) had suffered from CFS since 1970. She was unable to wear jewellery and had developed intolerance to electromagnetic fields. Following the insertion of two gold constructions in February 1991 she became seriously ill with mucosal problems and general fatigue. She also had oral lichen adjacent to amalgam fillings. A patch test performed in December 1991 showed a positive reaction to nickel, cobalt, palladium and gold. The results of MELISA are shown in Table 1. The patient's lymphocytes responded to gold, palladium, nickel, inorganic Hg and phenyl-Hg. She did not respond to other components of amalgam such as tin, copper and silver, or to other components of metal

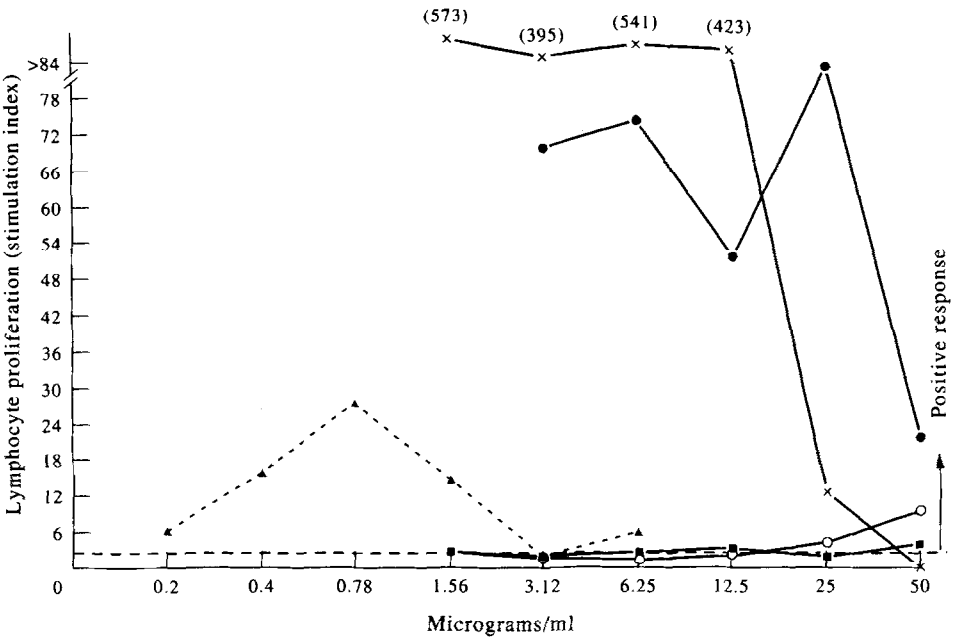

Fig. 6. MELISA with Au (●), Ni (×), Co (▲) and Cr (■) in a 51-yr-old female (S85) with positive patch tests to these metals. MELISA with Au in a healthy male control (TL100) exposed to gold fillings is also shown. (○) Control cpm in untreated cultures were 712 (S85) and 6239 (TL100).

alloys such as platinum, cadmium and titanium. The responses to ethyl- and methyl-Hg salts and to cobalt were also negative. Since July 1992 she has been metal-free in the oral cavity and her mucosal problems have regressed. For comparison, results obtained with lymphocytes from a control donor TL100 are shown in Table 1. This 52-yr-old male, in good health, had been exposed to amalgams, gold crowns and gold bridges. He also had many root fillings. Despite this considerable metal exposure, his lymphocyte responses to metals remained negative.

DISCUSSION

The results presented in this communication indicate that mercury, as well as gold and palladium, behave as unique antigenic entities and are able to induce a state of sensitization in humans. Studies concerning sensitization properties of metals have been ongoing in our laboratory since 1987 and the data presented are representative cases from a large group of patients with oral lichen and/or CFS. The data compiled from these studies are now being prepared for publication. During these investigations, it became clear that the use of low concentrations of metal salts, particularly while testing with inorganic mercury, nickel, gold and palladium is of the utmost importance for the discrimination of the reactivity between lymphocytes from the patients and those from healthy subjects. For example, many healthy subjects may show lymphocyte reactivity to $HgCl_2$ at doses exceeding 0.5 μg per ml. The same is true for gold thiosulfate and $PdCl_2$ at concentrations over 6 μg per ml (V. D. U. Stejskal *et al.*, unpublished data, 1994).

Table 1. MELISA results in patient AD190 and control TL100

Test substance	AD190 cpm (mean ± SD)	AD190 SI	TL100 cpm (mean ± SD)	TL100 SI
Control	1695 ± 848	1.0	6239 ± 2618	1.0
PPD	436,022	257	505,770	81.0
$HgCl_2$	13,350 ± 4536	7.9	6448 ± 588	1.4
Phenyl-Hg	14,790 ± 479	8.7	2322 ± 366	1.4
$SnCl_2$	1342 ± 286	0.8	2202 ± 737	0.5
$CuSO_4$	718 ± 130	0.4	3163 ± 1064	0.6
$AgCOOCH_3$	3095 ± 46	1.8	2612 ± 2883	0.6
$Au(S_2O_3)_2$	2701 ± 1395	1.6	5211 ± 642	1.1
$PdCl_2$	85,651 ± 12,522	50	4661 ± 2012	1.2
$Pt(SO_4)_2$	2407 ± 540	1.4	2652 ± 903	0.6
$Pb(NO_3)_2$	2468 ± 892	1.4	11,292 ± 1685	2.4
$CdCl_2$	5838 ± 5902	3.4	8298 ± 5020	2.1
TiO_2	1945 ± 469	1.1	3704 ± 142	0.8
Methyl-Hg	2600 ± 978	1.5	3779 ± 127	0.8
Ethyl-Hg	1079 ± 382	0.6	2676 ± 1337	0.6
Thiomersal	903 ± 1798	0.8	3704 ± 142	0.8
$NiCl_2$	103,162 ± 67,762	62	4887 ± 2295	1.0

SI = stimulation index

Several investigators have demonstrated that metallic ions (corrosion products) from restorations, crowns, clasps or root fillings penetrate into the adjacent tissues such as dentin and enamel of the teeth (Lappalainen and Yli-Urpo, 1987; Sörenmark *et al.*, 1968; Winter, 1976). The release of metallic ions from seemingly inert precious metals such as palladium or gold is potentiated by inflammatory processes in the oral cavity. Thus, stimulated neutrophiles, monocytes and macrophages produce strongly oxidizing agents such as peroxide or hydroxide radicals (Contrino *et al.*, 1988; Halliwell *et al.*, 1988; *Lancet*, 1985) and contribute, together with the oral microbial flora, to the generation of corrosion products. Metal ions are taken up by immunological

scavangers, monocytes and macrophages and distributed through the lymph and blood throughout the entire body (Coleman *et al.*, 1973; Willert and Semlitsch, 1976; Winter, 1976). Metal-containing phagocytes may be trapped in several organs such as joints or brain and metal material released may bind *in situ* to thiol groups of proteins. Heavy metals such as mercury, lead or cadmium interact strongly with SH-groups and disulfide bridges as reviewed extensively by Boyer (1959). One could postulate that this process can occur not only in the skin or mucosa but also in other organs and tissues such as brain, muscles or joints. Strong binding to protein ligands may change the tertiary structure of autologous proteins, thus making them foreign and therefore vulnerable to the attack of lymphocytes. Since metal-sensitized lymphocytes circulate freely in the blood and lymphoid system, metals could be implicated as aetiological agents in many inflammatory degenerative diseases.

Although mercury may lack specificity in inhibiting sulfur-containing enzymes, it may show selectivity in the whole organism by binding to specific organs and may be concentrated in specific cell components such as in lysosomes (Bolewska *et al.*, 1990). Among other groups that attract heavy metals, are phosphates, cysteinyl and histidyl residues of proteins, purines, pteridins and porphyrins. Such binding often leads to enzyme inhibition (Vallee and Ulmer, 1972). Like mercury, other metals such as gold and palladium are frequently used as components in dental alloys. These elements belong to the transition metals which means that they exist with only a partially filled electron shell, either in their elemental state or in one of their common oxidation states. Transition metals occupy the central block of the periodic table filling the three rows from scandium to copper, zirconium to silver and hafnium to gold. The properties that distinguish transition metals from other metals are their variable oxidation states and their very great propensity to form strong complexes with both inorganic and organic ligands (Clark, 1981). Palladium is increasingly used in dentistry, and also in jewellery and in industry. Recently, the sensitization rate to palladium was reported to be 8.3% (Aberer *et al.*, 1993). The prevalency of positive patch tests to gold is also increasing (Björkner *et al.*, 1994). Specific lymphocyte responses in patients with hypersensitivity were previously reported for nickel (Hutchinson, 1972), gold (Aro *et al.*, 1993; Denman and Denman, 1968) and beryllium (Kreiss *et al.*, 1989). The proliferation in response to palladium by lymphocytes from palladium-sensitized patients has to our knowledge not been described previously.

Apart from such artificial situations as metal implants, transition metals never occur in very large amounts in any organism. The majority of them have no biological function and indeed, the soluble salts of all of them are toxic in even moderate amounts. At concentrations above those found naturally, they will bind to the active sites of enzymes, disrupt membrane functions and disturb the equilibrium controlling other metals (Clark, 1981).

Fortunately, not everyone is genetically susceptible to develop immunologically mediated disorders caused by metals. When treated with metal salts, only certain, genetically identical (inbred) strains of animals developed autoimmunity (Druet *et al.*, 1982; Mathieson *et al.*, 1992) while others did not. In experiments with human cells it has been found that nickel created a new antigenic determinant by interacting with a peptide bound to the major histocompatibility complex (MHC) molecule (Romagnoli *et al.*, 1991). Furthermore, the same group has provided data suggesting that gold can alter MHC–peptide complexes (Romagnoli *et al.*, 1992). Taken together, these studies show that the genetic constitution of an individual may play the ultimate role in the responsiveness of the immune system to low molecular weight compounds.

The impact of genetics on the development of metal sensitivity is much more difficult to study in outbred populations such as mankind. However, it is possible to examine HLA-identical populations by studying identical twins. In the research reported here, studies of two pairs of identical twins indicated that the reactivity pattern of metal-specific lymphocytes is similar for genetically identical individuals who have had the same metal exposure in the past.

A frequently used method for the diagnosis of delayed type hypersensitivity is the patch test. However, this method has several disadvantages. Since many chemicals such as mercury can be harmful to the skin, false positive irritation reactions are quite common. Thus, the prevalence of mercury allergy based on the positive patch test varies widely (Finne *et al.*, 1982; Laine *et al.*, 1992; Mobacken *et al.*, 1984). In this study, patient TL87 showed a strong inflammatory reaction to ethyl-Hg which was interpreted as an irritative effect of mercury on the skin. However, the results of MELISA confirmed a true sensitization to this compound by a high specific lymphoproliferative response. The ability to discriminate between toxic and allergic reactions with the help of hapten-specific memory cells has earlier been described for isothiazolinones (Stejskal *et al.*, 1990). Another disadvantage of the patch test is that each application of test substance on the skin may increase the immune reactivity of the individual to a given antigen (boosting effect) and thus aggravate the allergic symptoms. Systemic problems following patch testing of metal-sensitive individuals have often been reported by patients and were recently confirmed by a double-blind study (Marcusson, 1994).

It has been generally anticipated that inorganic mercurials such as mercuric chloride function as mitogens for human and animal lymphocytes *in vitro* (Caron *et al.*, 1970; Schöpf *et al.*, 1967). The discrepancy between published data and the results presented in this study may be explained by

methodological variations in the performance of MELISA versus the conventional lymphocyte transformation test (Nordlind and Lidén, 1993). These details are, however, well beyond the scope of this communication.

In conclusion, the data presented in this study indicate that metals generally accepted for long-term use in medicine and dentistry carry a definite sensitizing risk for an at present unknown part of the human population and should be avoided in the future. Therefore, mercury-based bactericidal agents and metal-containing dental materials should be replaced with non-mercury preservatives and with metal-free restorative materials.

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