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CLINICAL STUDIES

Increased frequency of delayed type hypersensitivity to metals in patients with connective tissue disease

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ABSTRACT

Background: Connective tissue disease (CTD) is a group of inflammatory disorders of unknown aetiology. Patients with CTD often report hypersensitivity to nickel. We examined the frequency of delayed type hypersensitivity (DTH) (Type IV allergy) to metals in patients with CTD.
Methods: Thirty-eight patients; 9 with systemic lupus erythematosus (SLE), 16 with rheumatoid arthritis (RA), and 13 with Sjögren's syndrome (SS) and a control group of 43 healthy age- and sex-matched subjects were included in the study. A detailed metal exposure history was collected by questionnaire. Metal hypersensitivity was evaluated using the optimised lymphocyte transformation test LTT-MELISA® (Memory Lymphocyte Immuno Stimulation Assay).
Results: In all subjects, the main source of metal exposure was dental metal restorations. The majority of patients (87%) had a positive lymphocyte reaction to at least one metal and 63% reacted to two or more metals tested. Within the control group, 43% of healthy subjects reacted to one metal and only 18% reacted to two or more metals. The increased metal reactivity in the patient group compared with the control group was statistically significant ($P < 0.0001$). The most frequent allergens were nickel, mercury, gold and palladium.
Conclusions: Patients with SLE, RA and SS have an increased frequency of metal DTH. Metals such as nickel, mercury and gold are present in dental restorative materials, and many adults are therefore continually exposed to metal ions through corrosion of dental alloys. Metal-related DTH will cause inflammation. Since inflammation is a key process in CTDs, it is possible that metal-specific T cell reactivity is an etiological factor in their development. The role of metal-specific lymphocytes in autoimmunity remains an exciting challenge for future studies.

Introduction

Connective tissue disease (CTD), which includes systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and Sjögren's syndrome (SS), is a group of systemic autoimmune disorders characterised by a broad spectrum of clinical features and multi-system involvement [1]. In all CTD, symptoms vary among individuals. SLE affects multiple organs, including skin, joints, kidneys, heart, and brain, and symptoms can range from mild rashes, through arthritis to severe life-threatening organ involvement [2,3]. Rheumatoid arthritis is characterised by a chronic persistent and progressive fluctuating synovial inflammation that can lead to loss of joint function due to cartilage destruction [4,5]. Sjögren's syndrome attacks immune cells and destroys exocrine glands producing tears and saliva, causing dry eyes and mouth, which can result in difficulty swallowing and dental damage [6,7]. Sjögren's syndrome may occur alone (primary SS) or with other rheumatological conditions (secondary SS). Thirty percent of patients with SLE and RA suffer secondary SS [7]. Symptoms of all CTD are variable and may include chronic fatigue. Mercury (Hg) and nickel (Ni) hypersensitivity have been linked to this symptom [8]. Finally, whilst the causes of autoimmune diseases are unknown, genetic, environmental, and lifestyle factors will play a role.

The pathological effects of metal exposure may be induced through toxic and/or allergic mechanisms. Mercury and gold (Au) have been shown to induce autoimmunity in genetically susceptible animals [9–11] and can induce or promote the development of autoimmunity in humans [12–14]. Various etiological factors, including silicon and cigarette smoke, have been implicated in the

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Table 1
Baseline characteristics of CTD patients.

	Systemic lupus erythematosus	Sjögren's syndrome	Rheumatoid arthritis
No. of Participants	9 (8 female, 1 male)	13 (12 female, 1 male)	16 (15 female, 1 male)
Dental metal exposure	9/9 data available 9 amalgam 6 gold 1 stainless steel plate 2 orthodontic braces	13/13 data available 13 amalgam 11 gold 1 orthodontic brace	14/16 data available 14 amalgam 8 gold 2 stainless steel plates
Environmental and pharmaceutical exposure to metals[a]	9/9 data available 5 exposed	9/13 data available 5 exposed	7/16 data available 5 exposed
Body implants	9/9 data available 1 silicone breast implant 1 titanium plate	11/13 data available 2 silicone breast implants	9/16 data available 2 orthopaedic implants 1 silicone breast implant
Smokers (including active and passive smoking)	7/9 data available 2 smokers 3 ex-smokers 2 non-smokers	10/13 data available 2 smokers 2 ex-smokers 6 non-smokers	9/16 data available 3 smokers 2 ex-smokers 4 non-smokers
Metal intolerance[b]	9/9 data available 7 metal intolerant	9/13 data available 7 metal intolerant	10/16 data available 9 metal intolerant

[a] Patients were exposed through environment e.g. lived near factory, motorway, etc. or through family e.g. husband worked as welder, father was dentist, etc. Pharmaceutical exposure: e.g. taking titanium dioxide-coated pills or treatment with gold salts.
[b] Metal intolerance was reported by doctors and/or by patients through questionnaires; e.g. worsening of symptoms 2 days after dental treatment, dermal reactions to nickel-containing earrings, jeans buttons or jewellery.

causation of CTD. Rheumatoid arthritis and SLE are known to be associated with tobacco smoking [15], which is also linked to Ni sensitisation [16]. Other risk factors in CTD include traffic pollution [17] and occupational exposure to silica and mineral oils [18] – all of which contain metals such as Ni, Hg and palladium (Pd). Increased frequency of SLE has been described in a community exposed to petroleum products and Hg [19].

We examined the incidence of delayed type hypersensitivity (DTH) (Type IV allergy) to metals to which the patients were exposed.

Materials and methods

Patients and controls

Thirty-eight patients, 35 females and three males (mean age 51 years, range 22–77 years) participated in the study and gave their informed consent. Of these patients, nine had SLE, 16 had RA, and 13 had SS. The CTD patients were referrals to the laboratory performing LTT-MELISA testing during the period 1991–2006 (Toxicology Laboratory, Astra Pharmaceuticals, Sweden). Anamnesis was taken by the referring doctor as well as through questionnaires filled in by the patients. Patients were diagnosed by rheumatology specialists according to the American College of Rheumatology classification criteria for SLE (1997) [20,21], RA (2010) [22] and SS (2002) [23]. Patients' demographic information is shown in Table 1. All patients had amalgam fillings, either at the time of the study or previously. Many also had gold dental restorations. Most reported intolerance to costume jewellery or other Ni containing items. Some patients reported worsening of their health after dental treatment.

The control group consisted of 42 healthy subjects; 37 females and five males (mean age of 52, range 26–78). Controls were selected to match the age and gender balance of the patient group and were tested during the same period. Questionnaires were not available for the control group, but as the age and gender were similar to that of the patient group we have assumed that the overall metal exposure was similar.

DTH testing (LTT-MELISA test)

Delayed type hypersensitivity to metals was investigated in all patients and controls using the optimised lymphocyte transformation test LTT-MELISA (Memory Lymphocyte Immuno Stimulation Assay), which is an in vitro assay for memory T-cells [14,24–27]. Metals were tested based on the subjects' exposure history. The test menu included inorganic Hg, organic Hg (phenyl Hg, methyl Hg, thimerosal), tin (Sn), copper (Cu), silver (Ag), Au, Pd, Ni, cadmium (Cd), lead (Pb) and titanium (Ti) (as titanium dioxide). Table 2 shows the metals tested according to patient group.

Lymphocytes were isolated from a citrate blood sample and cultivated with metal salts for five days. Lymphocyte proliferation was measured by the uptake of radioactive thymidine by stimulated lymphocytes (lymphoblasts) and was reported as a Stimulation Index (SI): counts per minute (cpm) in metal-treated cultures divided by the mean cpm of the control cultures. An $SI \geq 3$ was considered a positive response. For statistical evaluation, the two maximum stimulation indices obtained for each metal were used. Positive responses were confirmed by morphological evaluation of lymphoblasts on stained smears [14,25].

Statistical evaluation

The significance of the results was evaluated by calculating and plotting 2-sided 95% confidence intervals for the proportion of patients/controls [28]. To assess the statistical significance of the differences of proportions, *Z*-scores were calculated and assessed using a 2-tailed hypothesis and a standard method [29]. For the all patients vs. controls statistical testing, the critical *P* value for significance was defined as $P < 0.05$. For the sub-group analyses, a Bonferroni correction was applied; such that the *P* value for significance was defined as $P <$

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Table 2
Metals tested using LTT-MELISA: patients and healthy controls.

	Healthy controls	Patients (total)	Patients (SLE)	Patients (SS)	Patients (RA)
Hg	41	38	9	13	16
Phenyl-Hg	37	33	9	12	12
Methyl-Hg	28	25	7	9	9
Ethyl-Hg	20	16	5	5	6
Thimerosal	25	28	5	4	9
Sn	29	26	6	9	11
Cu	24	24	5	7	12
Ag	22	24	5	9	10
Au	33	34	8	12	14
Pd	20	31	8	11	12
Pt	21	19	4	7	8
Pb	29	25	6	8	11
Cd	31	29	7	9	13
Ti	27	22	5	7	10
Ni	30	33	8	11	14

range tested (Table 2) only elicited a positive response in a small number of cases, which were clearly insufficient to be statistically significant and were, therefore, excluded from further analysis.

Mercury

Approximately half of the patients (18 of 38 (47%)) and four of the 41 healthy controls (10%) reacted positively to Hg (Fig. 2). This was statistically significant ($P < 0.0001$). In the sub-group analysis, all subgroups were significantly different from the controls (SLE, $P = 0.0012$; SS, $P = 0.0155$; RA, $P = 0.0008$).

Palladium

Eleven of 31 patients (35%) and one of 30 controls (3%) responded to Pd (Fig. 3). This was statistically significant ($P = 0.00158$). In the sub-group analysis, SLE and SS subgroups were significantly different from controls (SLE, $P = 0.0005$; SS, $P = 0.0042$; RA, $P = 0.031$ (NS)). The RA subgroup would have been statistically significant if the Bonferroni-corrected P threshold had not been chosen for subgroup analysis.

Gold

Seven of 34 patients (21%) and two out of 33 controls (6%) showed positive lymphocyte responses to Au (Fig. 4). This was not statistically significant ($P = 0.08186$). Therefore, no subgroup analysis was performed.

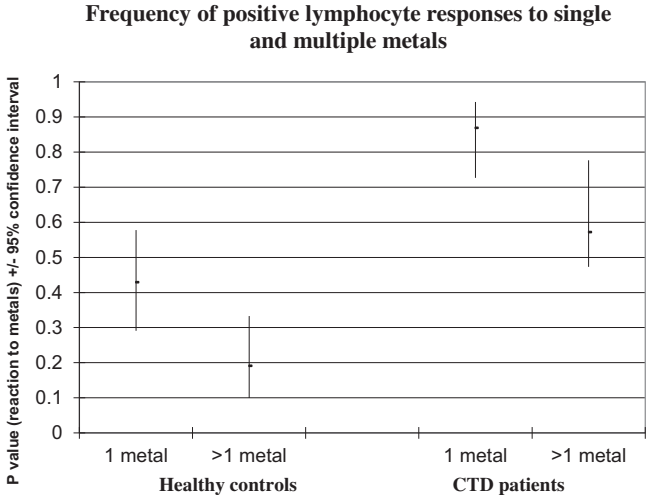

Fig. 1. Frequency of positive lymphocyte responses to single and multiple metals in healthy controls $n = 42$ and CTD patients $n = 38$; $P < 0.0001$.

Nickel

Seventeen of 33 patients (52%) and eight of 30 controls (27%) reacted positively to Ni (Fig. 5). The difference is statistically not significant but was on the borderline of significance ($P = 0.05614$). Due to the high frequency of reactivity in the control group, the group sizes in the subgroups were insufficient for statistically significant differences to be demonstrated.

Titanium

Six of 22 patients (27%) and one of 27 controls (4%) had a positive reaction to Ti (Fig. 6). This was statistically significant ($P = 0.01878$). In the sub-group analysis, the SLE and SS subgroups were not statistically significantly different from the controls but, it was of interest that five of 16 patients (31%) with RA reacted to Ti ($P = 0.0117$). This did reach the Bonferroni-corrected significance and were therefore statistically significant. A larger study is, therefore, necessary to investigate whether Ti allergy is clinically linked to RA.

Discussion

The results of this study demonstrate that DTH to Hg, Pd, Au, Ni and Ti is frequent in patients with CTD. In the following paragraphs, we will describe sources of exposure for metals which may cause hypersensitivity, and also discuss possible mechanisms which may lead to the development of CTD.

Mercury

All the patients studied have been exposed to Hg through amalgam restorations. The release of Hg from dental amalgam fillings is the main source of exposure to inorganic Hg and metallic Hg in the human population [30]. Dental amalgam consists of a metal powder (most commonly 70% Ag, 25% Sn, 1.6% Cu and 0–2% zinc (Zn)), which is mixed with Hg in approximately equal amounts [31]. Both inorganic and organic forms of Hg can induce local or systemic reactions. For example, lichen planus may develop adjacent to amalgam fillings [32]. In addition to DTH, Hg can function as an immunostimulant or an immunosuppressant, depending on the dose and individual susceptibility. For immune-mediated reactions, there is no-observed-adverse-effect level (NOAEL) [33]. In June 2014, the US Food and Drug Administration updated their information regarding the use of amalgam, stating "If you are allergic to any of

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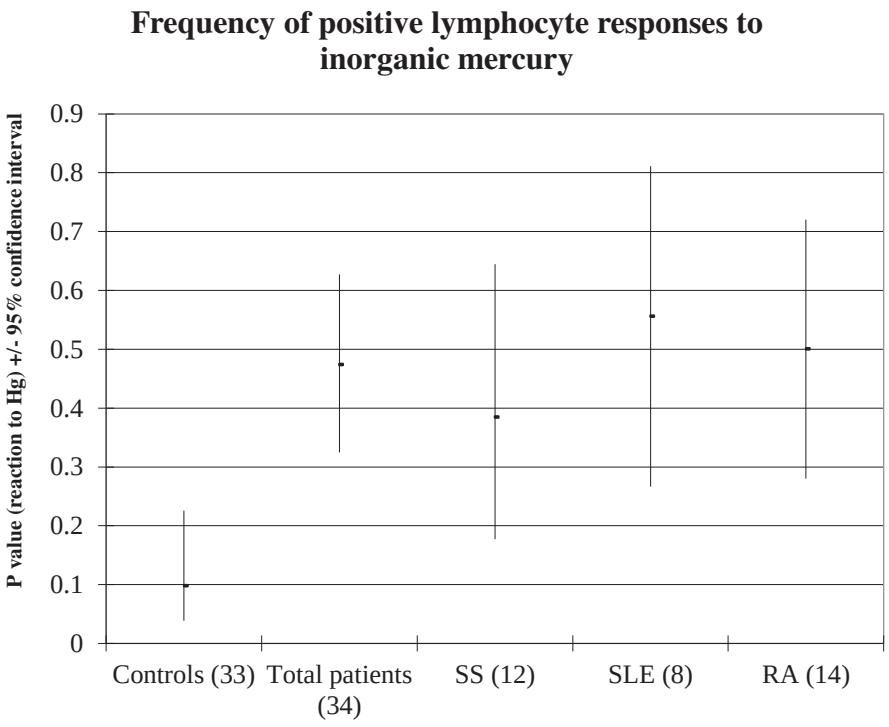

Fig. 2. Frequency of positive lymphocyte responses to inorganic mercury in healthy controls $n = 33$ and CTD patients $n = 34$; $P < 0.00002$.

the metals in dental amalgam, you should not get amalgam fillings" [34].

Tibbling et al. demonstrated a strong correlation between neuropathological changes, T-lymphocyte pathology and metal-specific lymphocytes in patients with symptoms resembling intoxication from dental amalgam fillings [35]. Health improvements have been reported in patients with various CTD following removal of dental amalgam fillings in Hg allergic subjects [13,14].

Palladium

Most of the patients in our study had Pd-containing gold-based dental restorations. As Pd is increasingly used in industry, jewellery and dentistry this increases human exposure. Palladium and Ni might partly cross-react, and Pd sensitisation is usually seen together with sensitisation to Ni [36] or other metals [37].

Since Pd is present in dental alloys, teeth brushing may cause Pd release [36,38–40]. The association between Pd-containing dental alloys, and oral and systemic complaints has been investigated at the Academisch Centrum Tandheelkunde Amsterdam (ACTA): Oral complaints including 'burning mouth', 'dry mouth' and 'persistent metallic taste' and systemic complaints including fatigue and joint and muscle pain were identified in patients who also had increased Pd- specific DTH as measured by lymphocyte responses and patch testing [36].

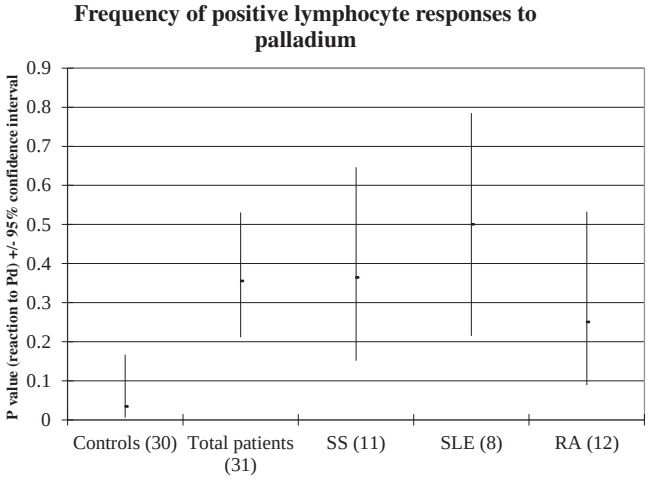

Fig. 3. Frequency of positive lymphocyte responses to palladium in healthy controls $n = 30$ and CTD patients $n = 31$; $P < 0.00158$.

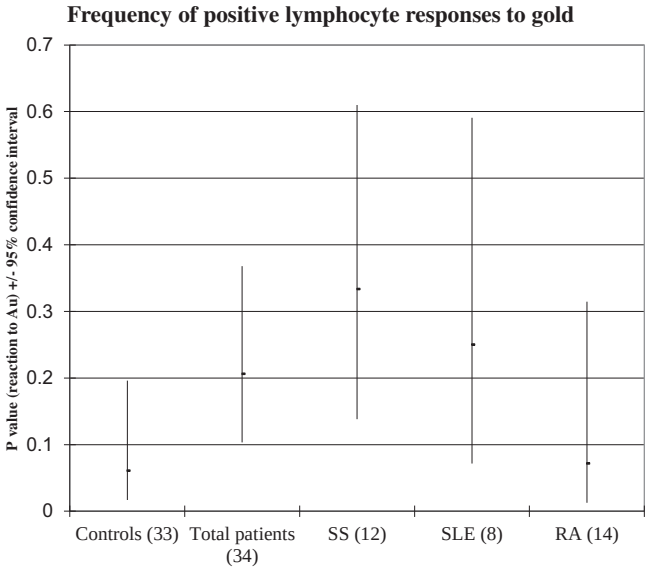

Fig. 4. Frequency of positive lymphocyte responses to gold in healthy controls $n = 33$ and CTD patients $n = 34$; $P < 0.08186$.

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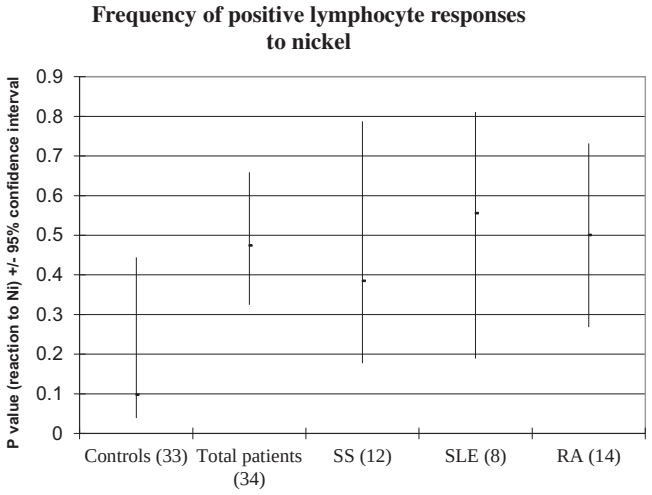

Fig. 5. Frequency of positive lymphocyte responses to nickel in healthy controls $n = 30$ and CTD patients $n = 34$; $P < 0.05614$.

Gold

Lymphocytes from 7 of 34 patients with CTD (21%) and 2 out of 33 controls (6%) responded to Au in vitro. These results were not statistically significant. However, some patients were being treated with steroids at the time of the study and this could have decreased lymphocyte responses.

Most of the patients were exposed to dental gold, together with amalgam (Table 1). It is well known that a mixture of dental alloys containing metals with different electric potential increases the corrosion rate and exposure to metal ions. Since Au-based dental alloys consist of 65–97% Au mixed with other metals such as Cu, Pd, Pt, Ag, Sn, iridium (Ir) and indium (In), the lymphocyte reactivity to these metals was tested as well.

Dental alloys, jewellery (including piercings) and Au salt therapy are the main causes of Au-contact dermatitis [35,41]. The risk for Au sensitisation is greatest when, as in piercing, there is permanent contact with live tissue [35] and in occupations where Au is used. Women more frequently show Au sensitisation than men [35,41]. One estimate of the prevalence of Au sensitisation worldwide is 13%, as confirmed by patch testing [41]. Gold compounds have been used in the treatment of RA [42] and Au-sensitisation is common in patients with RA especially in those where Au salts have been used [41,43].

Regarding dental restorations, blood concentration of Au correlates with the number of Au-containing dental fillings [44,45] and Au-induced DTH significantly correlates with the presence of dental Au restorations [46,47]

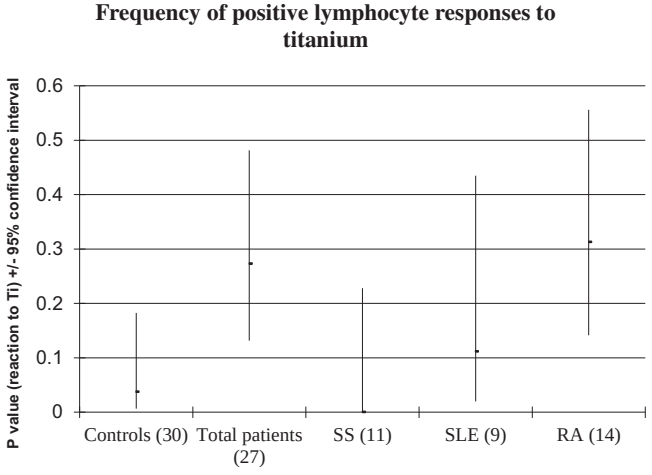

Fig. 6. Frequency of positive lymphocyte responses to titanium in healthy controls $n = 30$ and CTD patients $n = 27$; $P < 0.01878$.

Nickel

Nickel in jewellery, clothing and coins is the most common cause of contact sensitisation in the western world [47–51]. Therefore, it is not surprising than many of the patients in this study reacted to Ni. Nickel is used in Au and Ni-plating and many alloys; e.g. white gold, stainless steel and silver-like Cu alloys [52]. It is also found as an impurity in dental amalgams and alloys [52]. Other sources of Ni include food, cigarette smoke, some detergents, soaps and cosmetics [53–55]. The prevalence of Ni sensitisation is approximately 20% in the European general population [55], but the prevalence is variable [52,56]. Contact reactions to Ni are more common in women, possibly due to sensitisation through Ni-containing earrings, although other explanations are also plausible [57,58]. Nickel can induce scleroderma-related autoantibodies and cutaneous sclerosis in rats [59]. Systemic Ni sensitisation has also been implicated in complex chronic diseases such as chronic fatigue syndrome [60] and fibromyalgia [61].

Titanium

For the overall CTD group and for a subgroup of RA patients, the lymphocyte reactivity to Ti was significantly higher than in the control group. Titanium-based implants and prostheses are often used in patients with CTD. Titanium corrosion products are disseminated in the blood and organs [62] and can still be present in the body over a decade after removal [63]. Concerns have been raised because of the unusually high incidence of side-effects in paediatric spinal surgery using Ti-based instrumentation. One report identified 74 complications in 54 patients, a complication rate of 137% per patient and 40% per surgery [64].

Only one patient in this study was exposed to Ti through an implant; but many were exposed to Ti-coated drugs on a daily basis. Titanium dioxide is a white pigment widely used in medicines, sunscreens, cosmetics, toothpastes, foods (as E171) and other everyday items. Thus, there might be a considerable risk for latent sensitisation to Ti especially in susceptible individuals. In the last decade, case reports of Ti hypersensitivity have been described [65–68]. Since patch testing has not yet been developed for Ti [69], the actual frequency of Ti sensitisation in the general population is unknown and might be underestimated. However, it is important to note that the Ti production process does not completely remove all traces of Ni [70]. Hence, patients with strong Ni sensitivity might experience problems with Ti implants containing minute traces of Ni.

This study clearly demonstrates that DTH to Hg, Pd, Au, Ni and Ti is frequent in patients with CTD. Chronic low-dose exposure to these transition metals may trigger local and systemic inflammation in susceptible patients and initiate or exacerbate CTD. There are, however only a few studies on the role of heavy and transition metals in CTD reported in the medical literature.

In Brown Norwegian rats, low dose Hg administration induces a systemic autoimmune response and lupus-like oral lesions [71]. This strain of rat is known to be prone to developing metal-induced autoimmune diseases, which suggests a crucial role for genetics mediating a host reaction to metal exposure.

Federmann et al. reported a female patient who developed autoantibody-negative SLE after the implantation of chromium-Ni alloy plates containing 2.8% molybdenum (Mo). After their removal, her symptoms decreased but recurred after re-exposure to Mo

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through patch testing, which confirmed sensitisation to Mo. A lymphocyte transformation test indicating DTH was also positive to Mo. The authors suggested that Mo hypersensitivity might be an environmental trigger for SLE [72].

Güner et al. investigated the role of allergic contact dermatitis in the development of discoid lupus erythematosus (DLE) [73]. Thirty patients with DLE and 40 controls were patch tested. Fifty percent of the patients reacted to Ni compared with 25% of the control group, making Ni one of the most frequent sensitisers in both groups.

It is known that occupational exposure to metals can induce granuloma formation [74], and there are innumerable reports dealing with granulomas and cytokine release. It is also well recognised that cytokines released as part of the inflammatory process deregulate the hypothalamic–pituitary–adrenal (HPA) axis and can trigger non-specific systemic symptoms such as chronic fatigue, sleep disturbances and psychiatric symptoms; all well-known comorbidities found in patients with CTD [17,75]. Reducing metal exposure by replacing dental and surgical metal implants with non-metallic materials and avoiding cigarette smoke will result in decreased inflammation and improved health in sensitised subjects [13,14,25,61]. This also applies to patients suffering from CTD [13]. Prochazkova and colleagues reported that 10 out of 15 SLE patients, all sensitised to Hg, showed long-term health improvement after the removal of amalgam fillings. In this study, some of the patients reported onset of disease after dental treatment, as well as health improvement following the removal of sensitising metals.

Regarding the possible role of metals in CTD, we would like to put forward the following hypothesis. Transition metals may bind to sulphur group in collagen and other connective tissues thereby modifying the antigens to create a new epitope that the body recognises as "foreign" and therefore attacks. This 'hapten effect' is well known and is used, for example, in the production of vaccines [76]. In the case of transition metal haptenisation, the modified structures are then recognised by metal-sensitised lymphocytes and destroyed. The involvement of lymphocytes is important: in RA, joint inflammation is characterised by the invasion of T cells in the synovial space and the histopathological findings resemble those of a classic DTH reaction [77]. Rheumatic joints also show increased macrophage and leucocyte activity, which produces reactive oxygen species (ROS) and other free radicals [78]. Furthermore, transition metals are known to catalyse free radical formation and ROS degrade cartilage components and activate leucocyte collagenase [79]. Thus, not only may transition metals initiate an immune reaction by creating new autoantigenic 'foreign' epitopes, but they may then perpetuate the response by catalysing the creation of damaging reactive oxygen species.

The other possible explanation is that lymphocytes from patients with CTD exhibit higher inflammatory potential and respond to metals non-specifically. If so, it would be expected that lymphocytes from the individual patients would show similar frequencies of metal responses. Instead, as our data show (Figs. 2–6), the rate of metal reactivity is different in individual patients. Therefore, it seems reasonable to start from the working hypothesis that metal-triggered autoimmunity is the cause of CTD, and not that CTD causes hypersensitivity to metals. Many patients with CTD, especially RA, develop severe joint disease and require joint replacement implants. The presence of such implants would increase the likelihood of metal-reactivity in those individuals.

Finally to our knowledge, this is the first study reporting increased DTH to dental and environmental metals in CTD. This was a small study, and although it was possible to identify linkage between CTD and metal sensitivity, the sample group was too small to investigate the link with disease severity. A further larger study is, therefore, required which can not only investigate this link in more depth but also to review whether there is any link between the intensity of DTH reaction and disease severity.

Conclusion

This study demonstrates that patients with SLE, RA and SS show an increased frequency of metal DTH, especially to Hg, Pd, Au, Ni and Ti. In the group of patients described in this article, the exposure to Hg, Pd and Au was related to dental restorations while Ni and Ti exposure occurred from the patients' environment. Regardless of the mechanisms which triggered metal hypersensitivity, it seems that metal-sensitised CTD patients will benefit from metal-free implants. Metal-free dental restorations are already available. It is our hope that this study will encourage the research and development of more immuno-compatible medical devices. Further research is clearly indicated.

Conflict of interest

Vera Stejskal is the owner of the LTT-MELISA trademark and receives royalties from testing. She does now own any laboratories performing testing.

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