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CLINICAL STUDIES

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

Vera Stejskal[a,*], Tim Reynolds[b], Geir Bjørklund[c]

[a] Wenner-Gren Institute for Experimental Biology, University of Stockholm, Stockholm, Sweden
[b] Chemical Pathology, Burton Hospitals NHS Foundation Trust, Burton upon Trent, United Kingdom
[c] Council for Nutritional and Environmental Medicine, Mo i Rana, Norway

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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

* Corresponding author at: August Wahlströms väg 10, 182 31 Danderyd, Stockholm, Sweden. Tel.: +44 2081335166; fax: +44 2087115958.
E-mail address: vera@melisa.org (V. Stejskal).

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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 $P < 0.05/\text{no. of subgroups} = 0.016667$.

Results

The frequency of positive lymphocyte responses to selected metals in patients and controls is shown in Fig. 1. The majority of the patients (87%) showed a positive lymphocyte response to at least one metal and over half of the patients (63%) reacted to two or more of the metals tested. In the control group, less than half of the control subjects (43%) showed a positive lymphocyte response to one metal and only 18% of the controls reacted to two or more metals. The increased metal reactivity in patients compared with controls was highly significant ($P < 0.0001$). The most frequent allergens in the patient group were Hg, Pd, Au, Ni and Ti. Other metals from the