

Nickel-induced allergy and contact dermatitis: does it induce autoimmunity and cutaneous sclerosis? An experimental study in Brown Norway rats

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Abstract Nickel sensitization is a growing problem and the most common cause of allergic contact dermatitis. The aim of this study was to investigate whether nickel chloride can induce autoimmunity and cutaneous sclerosis in immunosensitive rats. Nickel chloride, in a dose of 4.5 mg in 0.2 ml NS, was administered by the oral and subcutaneous routes to 20 Brown Norway rats. Autoantibodies (ANA, anti-RNP, anti-SCL70 and anti-centromere) were measured and compared in pre- and post-challenge serum samples. Histological studies were also performed in skin biopsies obtained from six positively responding rats and compared

with an equal number of control rats at the 14th week post-challenge. Serum ANA was high in a significant number of rats in both the oral ($P < 0.005$) and subcutaneously nickel-treated groups ($P = 0.02$), while the anti-SCL70 was high in a significant number of rats in only the orally nickel-treated group ($P = 0.04$). Histologically, subcutaneous and oral nickel-treated groups showed sclerodermic features of the skin ($P = 0.22$, $P = 0.5$), respectively. It may be concluded that nickel chloride can induce scleroderma-related autoantibodies and cutaneous sclerosis. More prolonged duration of exposure is probably associated with greater risk. This is the first study showing the potential risk of nickel in triggering the development of cutaneous sclerosis in susceptible hosts.

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Introduction

There is abundant evidence in literature concerning the allergy-inducing potential of some metals such as nickel. Nickel is a ubiquitous trace element that occurs in soil, water and air. Major dietary sources of nickel include plant foods such as cereals and green vegetables. Other dietary animal sources include fish, egg, milk and fats. Foods with high nickel content, irrespective of its source, are whole wheat, cocoa, nuts, chocolate, tea, gelatin and baking powder [1]. Nickel is the fourth most frequently used metal in the world and the most common cause of allergic contact dermatitis [1]. The prevalence of nickel sensitization in the general population is estimated at around 7–10% with a predominance in women (female:male ratio of 7–14:1). Nickel is responsible for 35% of occupational and

non-occupational allergic contact dermatitis. The average age for its onset is 21–30 years [2].

Most of the nickel produced worldwide is used for the manufacture of stainless steel, which is mostly used to produce food-processing equipment and containers, and also for fashion jewelry, machine parts, coins, invasive medical tools, prosthetic implants and metal eyeglasses [1, 3, 4]. Last, but not the least, nickel may be found as a component of dental crown and bridges [5, 6]. The majority of nickel allergies are now thought to be caused by ear piercing and wearing of nickel-plated jewelry [7].

The risks of nickel hypersensitivity were related to ear piercing and the number of holes in the earlobes [8]. Important prerequisites for causing a nickel allergy are the presence of nickel-releasing materials, sufficient duration of contact, the nature of skin contact (duration, temperature, pH of the skin) and the status of the epidermal barrier (e.g., inflamed skin) as well as other conditions that can increase the bioavailability of nickel ions [9].

There is enough evidence of the role of nickel in inducing skin allergy [1–8]; however, data supporting an association of nickel with autoimmune disease such as scleroderma are lacking. The objective of this study was to investigate the potential of nickel chloride in inducing autoimmunity and sclerosis of skin in Brown Norway rats.

Materials and methods

This study was conducted at the Research Center, College of Medicine, King Khalid University Hospital, King Saud University, Riyadh, Saudi Arabia. Brown Norway (BN) rats were purchased from Charles Rivers Laboratories, Wilmington, USA. They were kept in polycarbonate metabolism plastic cages covered with stainless steel in the animal house in the College of Medicine. They were maintained under 12 h dark–12 h light cycles and kept under observation for 3 weeks. No evidence of sickness was observed. All rats were 8–11 weeks old at the onset of the experiment.

Treatment

As much as 20 rats with an average weight of 157 g were randomized into two groups each consisting of ten rats. One group received single dose of nickel chloride 4.5 mg [= 0.029 gm/kg = 125 micromol/kg] [10]. The dose was dissolved in sterile 0.9% NaCl solution and 0.2 ml was given subcutaneously (SC) on the dorsal side. The remaining ten rats received the same dose of nickel chloride orally (PO) on the first day of the experiment. Pre-challenge serum autoantibody results were compared with post-challenge results.

An extra six rats were used as parallel control group for the post-challenge histopathological studies. Their average

weight was 155 g. Three received subcutaneous normal saline (NS) and another three received oral NS of the same volume (0.2 ml).

Blood sampling

Blood samples were obtained from the retroorbital vein plexus and collected in heparinized tubes prior to intervention and at the 7th and 14th weeks post-intervention, respectively.

Serological studies

Serum ANA was detected by indirect immunofluorescence (IF) using the Immco Diagnostic, Inc. (60 Pineview Drive, Buffalo, NY 14228) test kit. ANA/HEP-2 test system is a pre-standardized kit designed for the quantitative and semi-qualitative detection of anti-nuclear antibodies in rats. In this test, rat serum samples were initially diluted 1:10 with phosphate buffer saline (PBS) by adding 20 µl of sera to 180 µl PBS as screening dilution. Slides were removed from storage and allowed to warm to the room temperature of 20°–25°C. Slides containing tissue culture substrates were used as an antigen source. Each well was identified with the appropriate sera and controls (positive and negative). With a suitable dispenser, one drop of approximately 20 µl of test sera and controls was added to the cells in the respective wells and spread over the entire area of the well. Slides were incubated in a moist chamber at room temperature (20°–25°) for 30 min and removed at one time and gently rinsed with a stream of PBS, avoiding direct streams of PBS into the test wells. The slides were then placed in the staining dish and washed in PBS for 5-min intervals with change of buffer PBS. A magnetic stirrer was used for mixing the setup. Slides were removed from PBS one at a time and blotted (with blotters provided) by wiping the reverse slide with an absorbent wipe. One drop (approximately 20 µl) of conjugate [rat IgG F(ab)₂ (H + L)] fragment affinity purified fluorescein conjugate (AP 136 G Chemicon International Temecula, CA 92590 lot No. 12036741) was added and the slides incubated and washed as previously mentioned. Three to five drops of mounting media were applied to each slide and cover-slipped. The slides were examined immediately with an appropriate fluorescent microscope. Detection of apple green fluorescence was considered as positive. The results were evaluated against positive and negative controls. Interpretation of the result depended on the pattern observed and any titer above 1:10 was considered to be significant (1:10 the baseline titer).

Other serum autoantibodies including anti-RNP, anti-SCL70 and anti-centromere were detected by ELISA using the Immco Diagnostic, Inc. test kit (60 Pineview Drive,

Buffalo, NY 14228). Immulisa is an in vitro diagnostic enzyme-linked immunosorbent assay (ELISA), designed for the detection and quantitation of IgG antibodies to (anti-RNP, anti-SCL70, anti-centromere) in rat serum. Purified specific antigens were bound to the wells of a polystyrene microwell plate under conditions that would preserve the antigen in its natural form. The unreacted sites of the polystyrene were blocked to reduce nonspecific binding. Serum samples and test reagents were allowed to come to room temperature (20°–25°C) before starting with the test procedure. To separate wells, 100 µl of pre-diluted positive and negative controls and 1:100 serum dilutions (5 µl of rat serum + 500 µl of serum diluents) were added. The plates were incubated for 30 min at room temperature (to allow antibodies to bind to the immobilized antigen). Unbound samples were washed away from microwells using an automatic washer, as per the manufacturer's instruction. Anti-rat IgG Alk. Phos. Conjugate (AP 136 A) from Chemicon International Temecula, CA 92590 (lot No. 0606033637) was diluted at 1:5,000 (as an optimal working dilution), 100 µl of which was added and incubated for 30 min at room temperature. Microwells were washed as previously mentioned. As much as 100 µl of substrates was added to each well in the same order and timing as for the conjugates and incubated for 30 min. The reactions were stopped by the addition of stop solution using the same order and timing. Absorbance values were read within 1 h from adding the stop solution.

Interpretation of results

In this study, the significant cut-off value for the immunofluorescent (IF) test of serum ANA was taken as 1:10 positive fluorescence [11]. However, significant reading for the ELISA test was taken as 0.205 dilution for anti-RNP, 0.296 for anti-SCL70 and 0.120 for anti-centromere. Since we could not find enough evidence for the cutoff values of anti-RNP, anti-SCL70 and anti-centromere titers in literature, these cutoff values were based on our own baseline readings where almost all serum autoantibody levels were undetectable.

Histopathology

Histopathological examination was performed in six rats with positive autoantibody responses, three from the subcutaneous nickel group and three from the oral nickel group. A parallel control group consisted of six rats, which received subcutaneous and oral normal saline at a dose of 0.2 ml. At 14-week post-challenge after the last blood sample, all skin biopsies were done from the right thigh away from the subcutaneous challenge site on the dorsal side. Examination of the skin biopsy slides was done, blindly, by

a histopathologist. Skin biopsies, prior to intervention in the test groups, were avoided to prevent the possibility of infection or local inflammation.

Skin biopsies were obtained from six rats showing positive autoantibody responses. The sections from each rat were bisected and fixed in 10% buffered formalin for a period of 24 h. The tissue was then processed in the Tissue-Tek Vacuum Infiltration Processor and stained using hematoxylin and eosin stain. The slides were then examined using a light microscope.

Immunofluorescence

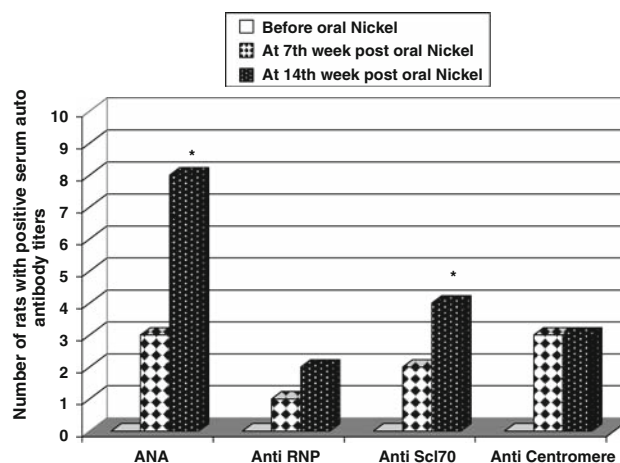
The tissues are processed as a frozen section and stained with various antibodies. The antibodies used were IgA, IgG, IgM, C3, C1q and fibrinogen. Once the slides were ready, they were examined using immunofluorescent microscope.

Statistical analysis

Statistical differences between nickel-treated and corresponding control groups were calculated using the Fisher's exact tests. *P* values of <0.05 were considered to be significant.

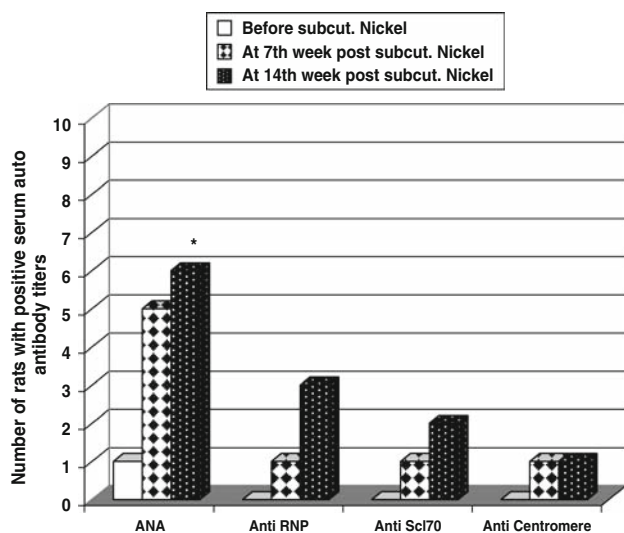
Results

The baseline (pre-challenge) results of serum autoantibodies are shown in Figs. 1 and 2, including ANA, anti-RNP, anti-SCL70 and anti-centromere. All readings in the two groups were negative except for one rat in the subcutaneous group (1/10; 10%) with positive serum ANA titer. These data were taken as a baseline and compared with the



* significant difference between pre oral nickel challenge and at 14th week post oral nickel challenge.

Fig. 1 Number of rats with positive serum auto antibody titers prior and post-oral nickel challenges



* significant difference between pre subcut.nickel challenge and at 14th week post subcut. nickel challenge.

Fig. 2 Number of rats with positive serum auto antibody titers prior and post-subcutaneous nickel challenges

post-challenge results. As in Fig. 1, for the orally treated group, 3/10 rats (30%) ($P = 0.10$) at the 7th week, and 8/10 rats (80%) ($P < 0.005$) at the 14th week showed significant titer of serum ANA. Anti-RNP was detectable only in only one rat 1/10 (10%) ($P = 0.5$) at the 7th week and in 2/10 rats (20%) ($P = 0.23$) at the 14th week.

Anti-SCL70 was positive in two rats 2/10 (20%) ($P = 0.23$) at the 7th week and 4/10 rats (40%) ($P = 0.04$) at the 14th week post-oral nickel treatment. Serum anti-centromere level was detectable in three rats 3/10 (30%) ($P = 0.10$) both at the 7th and 14th week post-challenge.

Results for the subcutaneously treated group are shown in Fig. 2. Serum ANA titers were high in 5/10 rats (50%) ($P = 0.07$) at the 7th week compared to one rat in the pre-challenge group. At the 14th week, 6/10 rats (60%)

($P = 0.02$) showed high ANA titer. Anti-RNP was positive in only one rat 1/10 (10%) ($P = 0.5$) at the 7th week and 3/10 rats (30%) ($P = 0.10$) at the 14th week, while serum anti-SCL70 showed detectable level in only one rat 1/10 (10%) ($P = 0.5$) at the 7th week and two rats 2/10 (20%) ($P = 0.23$) at the 14th week post-challenge. Serum centromere was positive in only one rat 1/10 (10%) ($P = 0.5$) at the 7th and 14th week post-challenge.

In the parallel control group, serum autoantibodies including ANA, anti-RNP, anti-SCL70 and anti-centromere titers at baseline (pre-challenge) and 7th and 14th week post-challenge were all undetectable. Histopathological results are shown in Table 1. Sclerodermic features were considered to be negative if all of the parameters shown in Table 1 were negative, otherwise it was positive. Positive sclerodermic features (see Fig. 3) were seen in 2/3 of the subcutaneous nickel group ($P = 0.22$) and only 1/3 of the oral nickel group ($P = 0.5$), compared with the negative results of the corresponding control group (Fig. 4). Immunofluorescent results were all negative for immunoglobulin and complement deposition. The serum autoantibody titers of the rats in which histopathology was performed are shown in Table 2.

Discussion

The risk of nickel hazards is increasing enormously. Allergy to nickel is the commonest cause of contact dermatitis [12]. Previous studies showed strong evidence of nickel-induced contact dermatitis with immune system irritability [12–17]. Contact dermatitis is caused by CD8⁺ and CD4⁺ T-cell-mediated delayed hypersensitivity reaction to hapten-modified MHC–peptide complex [17]. Type 1 IgE-mediated reactions due to inhalation of nickel dust have

Table 1 Histopathological features of nickel-tested and control groups

Skin biopsy of the following rats	Loss of subcutaneous fat and loss of periadnexal fat	Loss of adnexal structures	Dermal inflammation and peridermal atrophy	Sclerosis
Nickel SC1	Mild (1)	-ve (0)	-ve (0)	Mild (1)
Nickel SC2	-ve (0)	-ve (0)	-ve (0)	-ve (0)
Nickel SC3	Moderate (2)	Mild (1)	-ve (0)	Moderate (2)
Nickel PO1	-ve (0)	-ve (0)	-ve (0)	-ve (0)
Nickel PO2	-ve (0)	-ve (0)	-ve (0)	-ve (0)
Nickel PO3	Mild (1)	-ve (0)	-ve (0)	Mild (1)
Control SC1	-ve (0)	-ve (0)	-ve (0)	-ve (0)
Control SC2	-ve (0)	-ve (0)	-ve (0)	-ve (0)
Control SC3	-ve (0)	-ve (0)	-ve (0)	-ve (0)
Control PO1	-ve (0)	-ve (0)	-ve (0)	-ve (0)
Control PO2	-ve (0)	-ve (0)	-ve (0)	-ve (0)
Control PO3	-ve (0)	-ve (0)	-ve (0)	-ve (0)

SC subcutaneous, PO per os (oral), -ve negative

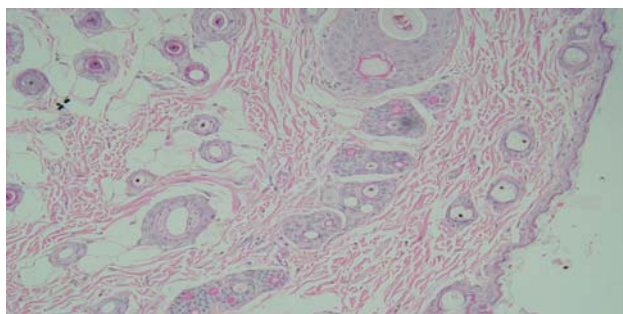


Fig. 3 Rat skin with hematoxylin and eosin stain ($\times 200$ magnification). The section from the rat skin (*control*) shows a normal-looking epidermis and dermis. The dermis reveals multiple adnexal structures such as hair follicles and sweat glands with surrounding periadnexal fat

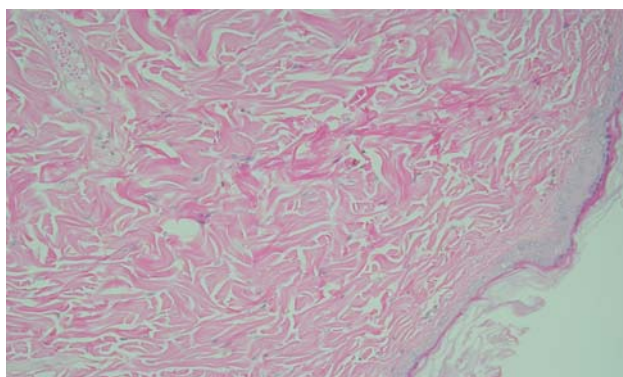


Fig. 4 Rat skin with hematoxylin and eosin stain ($\times 200$ magnification). The section from the rat skin shows loss of adnexal structures and associated periadnexal fat in the dermis. The superficial and mid-dermis is replaced by sclerotic and fibrotic tissue. The epidermis appears normal

been reported [9]. Sunderman et al. [10] (REF) demonstrated that a subcutaneous injection of nickel chloride solution to rats caused nickel uptake and activation of alveolar macrophages, which was manifested by increased

cAMP concentration and diminished 5-nucleotidase activity, followed by reduced phagocytic function.

One study demonstrated the correlation between nickel and development of ANA [18]. However, studies investigating the correlation between nickel and induction of scleroderma-related autoantibodies and skin sclerosis are lacking.

Data in this study showed that a significant number of rats treated with nickel, orally or subcutaneously, showed high serum ANA titers. High titers of serum anti-SCL70 appeared only in a significant number of rats following oral administration of nickel. Other serum autoantibodies, including anti-RNP and anti-centromere, were detected in low titers and probably may need more time to achieve significant levels.

By analogy to the skin-sensitizing potential of nickel leading to contact dermatitis, autoimmune cutaneous sclerosis was more prominent in the group treated with nickel subcutaneously. However, rats in the group treated orally with nickel also developed mild skin reaction, probably due to processing of nickel chloride in the gastrointestinal system by M-cells, which are specialized cells found in the follicular-associated epithelium of intestinal peyer patches [19]. It may be relevant to mention that immunohistochemical staining did not reveal any deposition of immunoglobulin or complement in the nickel-treated group. This could be due to the short exposure period of the study. Although the number of rats examined for histology was small, yet most of them showed cutaneous sclerodermic features correlating with autoantibodies production. It is relevant to mention that in the control groups, neither autoantibodies nor histological changes were observed.

The occurrence of anti-SCL70 and anti-centromere was not consistent with positive histology. This could be explained by the fact that even in established human limited

Table 2 Serum antibody titers of the rats in which histopathology was performed

Skin biopsy of the following rats	ANA (significant titer $\geq 1/10$)	Anti-RNP (significant titer ≥ 0.205)	Anti-SCL70 (significant titer ≥ 0.296)	Anti-centromere (significant titer ≥ 0.120)
Nickel SC1	1/20	0.451	0.760	0.244
Nickel SC2	1/80	-ve	-ve	-ve
Nickel SC3	1/80	0.859	-ve	-ve
Nickel PO1	1/160	-ve	2.00	-ve
Nickel PO2	1/20	0.396	-ve	0.202
Nickel PO3	1/80	-ve	-ve	0.182
Control SC1	-ve	-ve	-ve	-ve
Control SC2	-ve	-ve	-ve	-ve
Control SC3	-ve	-ve	-ve	-ve
Control PO1	-ve	-ve	-ve	-ve
Control PO2	-ve	-ve	-ve	-ve
Control PO3	-ve	-ve	-ve	-ve

SC subcutaneous, PO per os (oral), -ve negative

scleroderma patients, positive titer of serum anti-SCL70 antibody and anti-centromere antibody occur in 31.3 and 26.3%, respectively [20]. Our study suggests that it may be relevant to assess immunological function of patients with an implant or in close contact with a nickel-releasing material prior and post-nickel exposure. It may also be interesting to assess the role of nickel sensitization in patients with autoimmunity and scleroderma.

In conclusion, nickel chloride is probably a potential risk factor for autoimmunity and possible development of cutaneous sclerosis. Further studies are needed to investigate the effect of prolonged contact with nickel and the underlying immunopathogenic mechanism of autoimmunity and end-organ reaction.

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Conflict of interest statement No conflict of interest with any institution/organization.

References

- Sharma AD (2007) Relationship between nickel allergy and diet. *Indian J Dermatol Venereol Leprol* 73:307–312. doi:[10.4103/0378-6323.35733](https://doi.org/10.4103/0378-6323.35733)
- Purello D'Ambrosio F, Bagnato GF, Guarneri B, Musarra A, Di Lorenzo G, Dugo G, Ricciardi L (1998) The role of nickel in foods exacerbating nickel contact dermatitis. *Allergy* 53(suppl 46):143–145. doi:[10.1111/j.1398-9995.1998.tb04986.x](https://doi.org/10.1111/j.1398-9995.1998.tb04986.x)
- Cracchiolo A III, Revell P (1982) Metal concentration in synovial fluids of patients with prosthetic knee arthroplasty. *Clin Orthop Relat Res* (170):169–174
- Corazza M, Maranini C, Aleotti A, Virgili A (1998) Nickel contact dermatitis due to the needle of an infusion pump, confirmed by microanalysis. *Contact Dermatitis* 39:144. doi:[10.1111/j.1600-0536.1998.tb05875.x](https://doi.org/10.1111/j.1600-0536.1998.tb05875.x)
- Sterzl I, Prochazkova J, Hrda P, Bartova J, Matucha P, Stejskal V (1999) Mercury and nickel allergy: risk factors in fatigue and autoimmunity. *Neuroendocrinol Lett* 20:221–228
- Strauss FG, Eggleston DW (1985) IgA nephropathy associated with dental nickel alloy sensitization. *Am J Nephrol* 5:395–397. doi:[10.1159/000166973](https://doi.org/10.1159/000166973)
- Shah M, Lewis FM, Gawkrödger DJ (1998) Nickel as an occupational allergen. A survey of 368 nickel-sensitive subjects. *Arch Dermatol* 134:1231–1236. doi:[10.1001/archderm.134.10.1231](https://doi.org/10.1001/archderm.134.10.1231)
- Christensen OB (1990) Nickel dermatitis. An update. *Dermatol Clin* 8:37–40
- Tanko Z, Diepgen TL, Weisshaar E (2008) Is nickel allergy an occupational disease? Discussion of the occupational relevance of a type IV allergy to nickel (II) sulfate using case reports. *J Dtsch Dermatol Ges* 6:346–349. doi:[10.1111/j.1610-0387.2008.06672.x](https://doi.org/10.1111/j.1610-0387.2008.06672.x)
- Sunderman FW Jr, Hopfer SM, Lin SM, Plowman MC, Stojanovic T, Wong SH et al (1989) Toxicity to alveolar macrophages in rats following parenteral injection of nickel chloride. *Toxicol Appl Pharmacol* 100:107–118. doi:[10.1016/0041-008X\(89\)90095-1](https://doi.org/10.1016/0041-008X(89)90095-1)
- Al-Mogairen SM, Al-Arfaj AS, Meo SA, Adam M, Al-Hammad A, Gad-El-Rab MO (2009) Induction of autoimmunity in Brown Norway rats by oral and parenteral administration of sodium silicate. *Lupus* 18:413–417
- Panzani RC, Schiavino D, Nucera E, Pellegrino S, Fais G, Schinco G, Patriarca G (1995) Oral hyposensitization to nickel allergy: preliminary clinical results. *Int Arch Allergy Immunol* 107:251–254
- Hindsen M, Bruze M, Christensen OB (1999) Individual variation in nickel patch test reactivity. *Am J Contact Dermat* 10:62–67. doi:[10.1016/S1046-199X\(99\)90001-5](https://doi.org/10.1016/S1046-199X(99)90001-5)
- Sivertsen TS, Tchachtchine V, Lund E (2002) Environmental nickel pollution: does it protect against nickel allergy? *J Am Acad Dermatol* 46(3):460–462
- Muris J, Feilzer AJ (2006) Micro analysis of metals in dental restorations as part of a diagnostic approach in metal allergies. *Neuroendocrinol Lett* 27(suppl):49–52
- Gawkrödger DJ, Lewis FM, Shah M (2000) Contact sensitivity to nickel and other metals in jewelry reactors. *J Am Acad Dermatol* 43(1 Pt 1):31–36. doi:[10.1067/mjd.2000.107235](https://doi.org/10.1067/mjd.2000.107235)
- Kosboth M, Chin-Loy A, Lyons R, Wesson SK, Reeves WH (2007) Malar rash caused by metal allergy in a patient with systemic lupus erythematosus. *Nat Clin Nat Rheumatol* 3:240–245. doi:[10.1038/ncprheum0464](https://doi.org/10.1038/ncprheum0464)
- Pollard KM, Pearson DL, Hultman P, Hilderbrandt B, Kono DH (1999) Lupus-prone mice as models to study xenobiotic-induced acceleration of systemic autoimmunity. *Environ Health Perspect* 107(suppl 5):729–735. doi:[10.2307/3434334](https://doi.org/10.2307/3434334)
- Miller H, Zhang J, Kuolee R, Patel GB, Chen W (2007) Intestinal M cells: the fallible sentinels? *World J Gastroenterol* 13:1477–1486
- Walker UA, Tyndall A, Cziráák L, Denton C, Farge-Bancel D, Kowal-Bielecka O et al (2007) Clinical risk assessment of organ manifestations in systemic sclerosis: a report from the EULAR Scleroderma Trials and Research Group database. *Ann Rheum Dis* 66(6):754–763. doi:[10.1136/ard.2006.062901](https://doi.org/10.1136/ard.2006.062901)