Systemic nickel allergy syndrome (SNAS): A review

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SUMMARY

Epidemiological studies in different countries showed that nickel is the most common sensitizing agent in contact allergy, with a mean prevalence of 15-20% in the general population, particularly in women. Immunological studies in animals show that it is possible to induce a state of tolerance to metal by repeated administration of nickel salts by oral route, and that this state of tolerance is mediated by T lymphocytes with suppressive activity. The existence of Ni-specific Treg lymphocytes has been demonstrated in healthy humans, but not in allergic subjects. Studies of nickel metabolism show significant excretion by urine. Contact allergy is the most frequent clinical pattern in nickel-sensitized individuals, but many clinical elements demonstrate that the systemic absorption of nickel, e.g. by oral route, is able to elicit gastrointestinal (e.g. abdominal pain, diarrhoea and/or constipation, nausea and/or vomiting), atypical systemic manifestations (e.g. headache, chronic fatigue) and chronic dermatological symptoms (e.g. urticaria-angioedema), that are called Systemic Nickel Allergy Syndrome (SNAS). At the immunological level, a significant increase of CD45RO+ "memory" cells in the gastrointestinal mucosa is observed in these patients, as well as an increase of IL-5. The oral hyposensitization with increasing dosages of nickel sulphate, associated with a nickel-free diet, has been successfully performed in some clinical studies, with control groups consisting of patients on dietary regimen alone. A significant reduction of the clinical severity of the disease was observed in treated patients, compared to controls, confirmed by a significant reduction of nickel reactivity evaluated by nickel oral challenge. Also, the use of rescue medications (antihistamines, corticosteroids) was significantly reduced in the treated versus control patients. With regard to genetic aspects, some specific HLA haplotypes are associated to several

immune-mediated diseases, such as celiac disease, systemic nickel allergy and respiratory allergic diseases. The analysis of the genetic makeup in patients with nickel allergy often reveals the typical characteristics of celiac disease, even though in the absence of symptoms related to gluten reactivity, suggesting the necessity of other dietary recommendations other than nickel avoidance.

Key-words: *Nickel allergy, immunological tolerance, SNAS, nickel metabolism, hyposensitization, MHC, haplotype, celiac disease.*

1. INTRODUCTION

Prevalence of nickel allergy

Contact allergy is the outcome of a type-IV sensitization to low molecular weight haptens, usually induced by skin contact. There are some risk factors which favour the onset of contact allergy. First of all, the inherent sensitization potential of the hapten, but also the high frequency and time of exposure, occlusion, the presence of skin penetration enhancing factors and altered skin barrier function. Contact allergy affects nearly 15-20% of the general population[1].

Nickel is a hard, silvery white metal used in many industrial and consumer products, as stainless steel, metal plating, coins, magnets, many alloys and cheap jewellery. Nickel dermatitis was first described in 1889 in platers and until the 1930s it was predominantly an occupational pathology in the plating industry. From the 1930s it became increasingly frequent in women, related to consumer items, such as earrings and jewellery, which release nickel ions. In the 1970s nickel dermatitis was recognized as the main dermatological condition in the female general population, often associated with hand eczema.

Nowadays, nickel is the most important worldwide contact sensitizer and in the past decades a constant increase of nickel dermatitis, with positive patch test, especially among female patients, has been observed. In Sweden, the prevalence increased from 7% to 29% in the period from 1962 to 1997[2]. A comprehensive review of all the epidemiological surveys conducted from 1966 to 2007, in Europe and USA, revealed a prevalence of nickel allergy ranging from 2.5% (Germany, 1966) to 17.6% (Norway, 2007)[3]. It is noteworthy that the proportion of nickel contact allergy prevalence, in relation to contact allergy prevalence to at least one allergen, showed an almost linear increase from 5% (1966) to 65% (2007)[3]. Nickel allergy prevalence is higher among women than men (mean 17.1% versus 3%, respectively).

In 1994, the alarming concern caused by this trend pushed the European Union to approve legislation prohibiting products that released more than 0.5 $\mu g/cm^2$ of nickel per week[4]. As a consequence, a significant decrease in nickel sensitization has been observed in the past few years. In Denmark, there was a decrease of nickel allergy prevalence, in patients below 18 years of age, from 24.8% to 9.2% over the period 1985-1998[5]. In Germany, nickel sensitization decreased from 36.7% to 25.8% among women below 30 years over a 9-year period[6]. In spite of the decrease due to the above-mentioned measures, nickel allergy still remains the primary determinant of contact allergy prevalence in the general population, both among children and adults[3].

Systemic symptoms in nickel allergy

In sensitized subjects, nickel is able to elicit cutaneous symptoms independent from the direct skin contact, as demonstrated by some cases of generalized eczema and urticaria in patients with dental[7] and orthopaedic[8] prostheses. Nickel is also present in many foods, especially vegetables, and it has been demonstrated that the con-

sumption of a nickel-rich diet may elicit eczematous skin lesions in sensitized subjects; a phenomenon called "systemic nickel contact dermatitis"[9] or "haematogenous contact eczema"[10]. The content of nickel in foods is reported in Table 1. The following systemic reactions may be elicited by nickel systemic absorption: eczematous, vasculitic, mucosal, respiratory, urticarial, gastrointestinal[11]. There is an increasing interest on the gastrointestinal reactions related to the systemic absorption of nickel, such as nausea, pyrosis, meteorism, abdominal pain, diarrhoea and constipation. The association of cutaneous and gastrointestinal symptoms is now considered a real syndrome, designated by "Systemic Nickel Allergy Syndrome" (SNAS)[12,13]. The clinical features of SNAS are described in detail in Chapter 3.

Table 1. *Nickel content in foods*

High
Foods with >1000 µg of Nickel/Kg (approximately): *Peanuts, Oats, Cacao (Chocolate, etc.), Tomato concentrate, Lentils, Almonds, Walnuts and Hazelnuts*
Medium
Foods with 200-1000 µg of Nickel/Kg (approximately): *Foods preserved or cooked in metal vessels, Asparagus, Cabbage and Cauliflower, Bean and French Bean, Wholemeal Bread, Dry Yeast, Margarine, Mussels and Oysters, Potatoes, Peas, Tomatoes and Spinach, Dried Plums.*
Low
Foods with 50-199 µg of Nickel/Kg (approximately): *Apricots, Lobster, Broccoli, Onions, Maize, Pear, Grape raisin, Avocado, Carrots and Lettuce, Figs, Mushrooms, Buckwheat, Liquorice, Herrings, Tea, Rhubarb.*

Induction of immunological tolerance

Many experimental studies have been made in animal models to study the induction of immunological tolerance to some haptens, included nickel, by repeated oral administration, and in particular the involvement of suppressor T cells. The first historical study was made in 1911 with vegetable proteins in guinea pigs, and a state of antigen-specific tolerance after repeated oral administration was observed[14]. A subsequent study, in 1946, demonstrated that the preventive treatment by repeated oral administration of chloro-2,4-dinitrobenzene rendered guinea pigs unable to become subsequently sensitized[15].

The induction of tolerance to nickel has been the object of experimental studies in guinea pigs, to which nickel and chromium powder, or corresponding metallic salts, were administered by oral route, incorporated into the pelleted feed. The treated animals failed to react to a subsequent sensitizing immunization, therefore, showing a state of tolerance, while the control animals became clearly hypersensitive[16]. The results of these studies were confirmed in mice. After oral administration of nickel sulphate ($NiSO_4$) in the drinking water for 10 weeks, the treated mice were tolerant towards the subsequent sensitization with $NiSO_4$, in comparison to the controls. This tolerance was mediated by CD4negCD8+ T cells[17]. Also in the mouse model both the preventive and the desensitizing effect of nickel administration have been studied. To study the preventive effect, naïve animals have been treated with a 4-week course of oral administration of 10 mM nickel chloride ($NiCl_2$), which was able to induce tolerance, and so preventing the subsequent induction of hypersensitivity for a period of at least 20 weeks[18]. To study the desensitizing effect, mice experimentally sensitized to nickel were treated with continuous $NiCl_2$ administration, showing long term desensitization. When splenic T cells or lymph node cells of orally-tolerized mice donors were transferred to naïve recipients, even after a treatment-free interval of 20 weeks they specifically prevented sensitization of the recipient mice. The lymph node cells of such donors were anergic, because *in vivo* sensitization with $NiCl_2$ and *in vitro* restimulation with the hapten did not induce the enhanced proliferation and IL-2 production that was seen in lymph node cells of mice not tolerant before sensitization.

Taken together, the studies in animal models opened the prospect to induce a tolerance state in patients sensitized to nickel. Many preliminary clinical studies, reviewed in Chapter 4, show that such a prospect is possible by

hyposensitization, consisting in the repeated administration of small amounts of nickel salts.

The immunological study of an Italian research group focused on the differences in T-cell response to nickel between normal subjects and nickel-allergic patients is particularly interesting because it addresses the possible mechanism of hyposensitization[19], namely the capacity of CD25+ T regulatory cells (Treg) to modulate T cell responses to nickel. CD4+ cells isolated from the peripheral blood of healthy (that is, non nickel-allergic) individuals showed a limited capacity to proliferate in response to nickel *in vitro*. However, the response was strongly increased (+ 240%) when CD25+ Treg cells were depleted, confirming that these cells in healthy subjects were able to suppress nickel-specific responses of peripheral blood CD4+T cells. On the contrary, the CD25+ T cells isolated from peripheral blood in the nickel-allergic patients showed a limited or absent capacity to suppress metal-specific CD4+ and CD8+ T cell responses. The results of this study indicate that in healthy individuals CD25+ Treg can control the activation of both naïve and effector nickel-specific T cells.

Recent studies on the Treg role in the course of specific immunotherapy for common inhalant allergens showed a significant response of these cells, as demonstrated by the high IL-10 secretion by allergen-stimulated T cells[20]. It is very probable that Treg-mediated tolerance could play an important role also in nickel hyposensitization.

2. NICKEL METABOLISM AND IMMUNOLOGICAL RESPONSE

Sensitization to nickel can occur through different mechanisms: (*i*) skin contact with nickel may induce Allergic Contact Dermatitis (ACD); (*ii*) food ingested nickel can cause gastrointestinal symptoms and/or chronic dermatopathies defining the "Systemic Nickel Allergy Syndrome" (SNAS) or Systemic Contact Dermatitis (SCD).

Systemic contact dermatitis is a term that defines an inflammatory skin disease occasionally seen as a flare-up of previous eczema or *de novo* eczema similar to allergic contact dermatitis, when sensitive patients are systemically exposed to nickel[21].

The pathogenesis of these skin disorders is not completely known. The histopathology of the flare-up eczema in patients with SNAS after oral nickel challenge is similar to the reactions in allergic contact dermatitis, but because SCD starts a few hours or up to 1-2 days following nickel ingestion, more than one type of hypersensitivity mechanism may be involved.

Nickel metabolism

Nickel blood concentrations vary greatly in different reports of oral challenge with the metal. It is known that many factors, including diet, stress, age and seasonal variation, may influence serum nickel levels. However, the most recent studies show similar serum nickel concentrations in allergic patients and controls, both before and after metal ingestion.

Nickel is excreted equally well via urine and faeces. Under normally dietary conditions, 1 to 2% of ingested nickel is absorbed, and the unabsorbed nickel is excreted with faeces. In rat intravenously injected with nickel chloride, 90% is eliminated in the urine within 4 days post-injection and only 3% is excreted by faecal discharge[22].

Nickel urinary excretion is rapid, not dose-dependent and its elimination appears to follow first-order kinetics[23]. Estimates of the half-life of urinary removal of nickel range from 20 to 60 hours[24-25].

Nickel (Ni) absorption and/or excretion seem to be more elevated in atopic Ni-sensitized patients than in non-atopic patients. Urinary Ni excretion after ingestion of 1 mg of Ni sulphate is higher in Ni-sensitized atopic subjects than in Ni-sensitized non-atopic or non-Ni-allergic subjects[26].

Serum and urine Ni of non-Ni-allergic and Ni-allergic women did not significantly differ. Serum and urine Ni levels determined before oral Ni challenges were in the range of the reference values recently reported by other authors (0.2 to 2.0 μg/L of serum or urine). Ni was greatly augmented in urine and serum 4 hours after the challenge

(43 to 264 μg/L urine Ni and 15 to 52 μg/L serum Ni). Twenty-four hours after Ni ingestion, urine Ni was 41 to 153 μg/L and serum Ni 4 to 17 μg/L[27].

Diet and in-vivo challenges

Systemic contact dermatitis is seen when sensitized individuals are systemically exposed to a hapten, that is orally, transcutaneously, intravenously, subcutaneously, intramuscularly or by inhalation[21]. Nickel is found in many foods and its daily intake has been estimated between 200 and 600 μg. Most ingested nickel remains unabsorbed within the gastrointestinal tract, only 1-10% being absorbed. In healthy subjects, serum nickel concentrations vary from 1.6 to 7 μg/L and urinary nickel excretion from 2 to 5 μg per day[28]. Continuous exposure to nickel may lead to oral tolerance mechanisms that modulate nickel sensitivity[29].

A low nickel diet should be prescribed to patients with widespread, chronic, allergic-type dermatopathies and contact sensitization to nickel when a link between diet and clinical manifestations is clear. Low nickel diet and subsequent Ni oral exposure may also be useful in order to demonstrate the relationship between Ni ingestion and the onset of symptoms.

Oral challenge experimental trials have been used to show that oral intake of nickel can elicit a SNAS in nickel-sensitive individuals. The oral challenge was performed in the morning at doses varying from 0.3 to 10 mg. A definite dose-response reaction pattern to oral nickel exposure was observed among nickel-sensitive subjects[30]. The authors showed that the number of nickel-sensitive patients who reacted to oral exposure to 4.0 mg nickel is statistically significantly higher than those given placebo. This was not the case for nickel-sensitive subjects who received 0.3 and 1.0 mg nickel. However, unequivocal cutaneous reactions were seen when nickel-sensitive patients were exposed to 0.3 and 1.0 mg nickel and to placebo, in addition to the nickel exposure from normal dietary intake.

Reactions that occur in patients exposed to nickel include: itching, urticaria, oedema, eruptions of previously unaffected skin such as flexural dermatitis, a maculopapular rash and vasculitis-like lesions, flare-up reactions at previous sites of contact dermatitis, including the flare-up of current or former hand eczema and flare-up reactions of previously positive nickel patch tests. Abdominal pain, diarrhoea and headache have also been described.

These tests were in no way dangerous and the triggers generally elicited a relapse of varying intensity of the previous clinical manifestations.

The administration of nickel has also been proposed for treatment of such diseases[31,32] and there are no reported cases of severe side effects. The double-blind, placebo-controlled challenge test is the gold standard for this type of disease, being highly recommended and generally necessary for a correct diagnosis.

Experimental studies

In a previous study[12], we examined blood and intestinal mucosa lymphocyte subpopulations as well as cytokine production in SNAS patients compared to healthy controls and in patients suffering from ACD only. The study demonstrated a reduction of NK cell activity in sensitized patients in respect to normal subjects.

The lamina propria of normal gastrointestinal mucosa exhibits 60% of CD45RO+ and 40% CD8+ T lymphocytes. In the gastrointestinal epithelium of allergic patients sensitive to oral nickel, as well as in skin biopsies from nickel patch test reactions, we found a lower infiltration of CD8+ lymphocytes. Furthermore, biopsies in nickel allergic subjects sensitive to food-ingested nickel show higher levels of CD45RO+ cells in the lamina propria.

The exact role of specific cytokines in nickel allergy has not been elucidated in sufficient detail, but available data do suggest its central role.

Patients who reacted to oral nickel exposure had higher serum IL-2 and IL-5 before nickel challenge than healthy non-allergic controls and nickel-sensitive patients who did not react to oral nickel challenge[33-35].

In a previous study[33], we found statistically significant increased serum levels of IL-5, after oral exposure, while the changes of IL-2, IL-4, IL-13 and INF-γ were not sta-

tistically significant. A decrease of blood "naive" CD4-CD45RO- lymphocytes in non-allergic subjects was seen 24 hours after Ni administration. This modification may be explained by the transformation of these cells into CD4-CD45RO+ "memory" lymphocytes. However, these inflammatory alterations cannot be interpreted as specific for nickel because recurrent contact with any antigen can induce an increase in CD45RO+ "memory" cells.

An increase in CD8+ lymphocytes, 24 hours after Ni ingestion in non-allergic subjects was also observed. Such data are difficult to explain since it is known that Ni does not appear to have direct effects on CD8+ lymphocytes and it binds to class II proteins of the major histocompatibility complex of antigen presenting cells that interact with CD4+ lymphocytes.

"Non-responder" and "responder" Ni-sensitized women showed higher values of CD19+ and CD5-CD19+ lymphocytes. This immune modification may be referred to as an activation of the immune system without increase of serum IgE that is produced by B lymphocytes.

Szepietowski et al.[36] and Ulfgren et al.[37] studied cutaneous biopsies performed at the site of patch tests. In the first study, cytokine production at the site of the patch test and at another site in the same patient was evaluated by mRNA extraction. The results documented a statistically significant increased expression of mRNA for INF-γ, IL-2, IL-4, IL-10 after nickel exposure. A limitation of this study was the small number of patients. In the second study, Ulfgren et al.[37] evaluated cytokine production by immunohistochemical analysis of cutaneous biopsies taken at the site of the patch test and at sites of application of irritants. Cytokine production was the same in both groups: increase of IL-2 and IL-4. Only INF-γ production was greater in the biopsies taken at the sites of application of irritants.

Borg et al.[38] described cytokine release from $NiSO_4$-stimulated peripheral blood mononuclear cells (PBMC) from nickel allergic patients. Nickel-stimulation did not give rise to significant differences in the cytokine ratios for IFN-γ and TNF-α, but induced a relevant increase of IL-4 and IL-5. This was confirmed by the study of Budinger et al.[39] in $NiSO_4$-stimulated PBMC and also by Jakobson et al.[40] that also observed a higher production of IL-4, IL-5, IL-13 and INF-γ from $NiSO_4$-stimulated PBMC of nickel allergic patients.

These findings showed that nickel stimulation of the PBMC obtained from nickel-allergic individuals induces secretion of both Th1 and Th2-type cytokines. This is in contrast with the results of some studies with similar design that showed that a Th1 cytokine profile developed in such individuals[41-43]. Further studies are needed to elucidate the precise involvement of these cytokines in the disease mechanisms of SNAS.

In conclusion, a definite dose-response reaction pattern to oral nickel exposure was observed in nickel-sensitive individuals. Nickel-sensitive individuals who had cutaneous reactions to the oral challenge with nickel showed a significant increase of CD45RO+ in the gastrointestinal mucosa, suggesting maturation of T lymphocytes from naïve into memory cells. This immune reaction also involves CD8+ lymphocytes. IL-5 (able to activate eosinophils) also seems to be an important cytokine involved in the SNAS, indicating an activation of Th2 lymphocytes in peripheral blood.

3. SYSTEMIC NICKEL ALLERGY SYNDROME

In the seventies, some authors noted that a considerable number of nickel-sensitive patients had dermatitis at sites other than those that were in direct contact with nickel-plated items. Christensen[44] was the first author to suspect that ingested nickel could be responsible for these reactions. The most common clinical manifestations were eczematous lesions at elbow and knee flexures, eyelids, neck and inner thighs, recurrent vesicular dermatitis of the palms, sides of the fingers and/or soles of the feet, symmetrical nummular eczema, anogenital eczema. It was also noticed that the hand eczema, that so often followed the sensitization to nickel, usually starting some years after the first signs of metal sensitivity, most commonly appeared as volar, vesicular, symmetric pompholyx and showed its own activity independent of metal handling. These sys-

temic cutaneous manifestations were referred as "Systemic Contact Dermatitis"[21].

Analytical studies of different food items have shown that nickel is present in whole wheat, rye, oats, cocoa, tea, gelatin, baking powder, kippered herrings, soy, red kidney beans, green beans, peas, peanuts, hazelnuts, soy, sunflower seeds, spinach, strong licorice and dried fruits, regardless of the nickel content of the soil in which they are grown.

Various other nickel-containing foods and drinks can aggravate nickel eczema even though the nickel content of these foods may be low. These include beer, herring, mackerel, tuna, tomato, onion, carrot, lettuce, maize and certain fruits in particular pears and citrus fruits (juice)[45].

It has been estimated that the average human daily intake of nickel is approximately 200 µg and that a nickel dietary requirement of about 50 µg per day is important in human nutrition[46]. Most ingested nickel remains unabsorbed within the gastrointestinal tract and only about 1 to 10% is absorbed, so serum concentrations vary from 1.6 to 7 µg/L and urinary nickel concentration from 2 to 5 µg/L. Nickel concentration in sweat is high, ranging from 7 to 270 µg/L, thus sweating may provide an important route for the excretion of nickel from the body. Furthermore, sweat, which may contain up to 20 times as much nickel as plasma, may influence the amount of nickel that reaches the skin[47].

It has been shown that urine is the most reliable parameter to follow after oral intake of nickel even though both serum and urinary levels of nickel reflect the nickel intake[48].

Some studies reported the importance of systemic nickel sensitization with the ingestion of nickel containing foods[49-50] as well as the changes in the gastrointestinal mucosa in patients with nickel allergy[51].

Sensitization to nickel introduced by the diet started to be considered a model of cellular food hypersensitivity[52], although few studies have attempted to describe the immunological mechanism underlying the reactions elicited by systemic oral nickel exposure[13].

In our clinical experience, we began to notice that patients with a positive history of allergic contact dermatitis to nickel could present systemic clinical manifestations such as cutaneous symptoms, gastrointestinal symptoms, as well as other signs and symptoms correlated with the ingestion of nickel containing foods, in what we referred to in these cases as "Systemic Nickel Allergy Syndrome" (SNAS)[53].

The cutaneous manifestations include signs of systemic contact dermatitis as described above such as cutaneous rashes and urticaria-angioedema. The gastrointestinal symptoms include recurrent aphtosis, abdominal bloating and distension, recurrent abdominal pain, diarrhea and/or constipation, nausea and or vomiting, and eventually endoscopic findings of chronic gastroduodenitis, while other atypical systemic clinical manifestations include headache, chronic fatigue, postprandial dyspnea, cystitis and/or vulvovaginitis, acne, and iron deficiency anaemia.

The diagnosis of SNAS is made after thoroughly evaluating the patients presenting with the former symptoms.

Clinical suspicion is confirmed by symptom improvement after a low nickel diet for at least one month. A nickel patch test is then performed and, if positive, a provocation test with nickel sulphate is performed after a 3 to 4 weeks interval.

The provocation test consists of administering a capsule containing talc as placebo, followed after 1 hour by a capsule containing 0.6 mg of nickel sulphate. The oral challenge is performed in the morning, in individuals who have been fasting for 12 hours. If the test is still negative, a further dose of 1.25 mg of nickel is administered after a 1 hour interval from the last dose.

The skin status and systemic symptoms are evaluated and recorded 24 hours after the challenge. Positive reactions include eruptions of previously unaffected skin, flare-up at previous sites of contact dermatitis, including the flare-up reactions of previously positive nickel patch-test, urticaria, and any systemic symptoms such as headache, marked fatigue and gastrointestinal symptoms.

The diagnosis of SNAS is then confirmed and hyposensitization to nickel suggested.

Nowadays, there is no doubt that food allergy is a disease with great socioeconomic impact, affecting quality

of life in a profoundly negative way[13]. SNAS is an allergic disease due to sensitization to nickel contained in foods and therefore the need for dietary treatment should also be taken into account by health professionals and dieticians when evaluating patients with food related disorders, not only to make a correct diagnosis but also to consider nickel hyposentization treatment.

4. HYPOSENSITIZATION TO NICKEL

Several studies have clearly demonstrated that tolerance to nickel may be induced in sensitized guinea pigs and mice, through oral administration of nickel[54-55].

The efficacy and safety of hyposensitization to nickel in humans was initially evaluated in patients affected solely by contact allergy. In 1987, Sjovall *et al.*[32] performed two controlled studies, each including 24 patients with contact allergy to nickel and with oral administration of 5.0 mg nickel sulphate once a week for six weeks. The degree of contact allergy, measured by patch tests before and after nickel administration, was significantly lowered. Troost *et al.*[56] tested the efficacy of subcutaneous treatment with weekly injections of increasing doses (10^{-6}-10^{-3} mol/L) of a nickel sulphate-containing solution. During the follow up period, testing did not show any statistically significant results when compared to the control group. On the other hand, Morris[57] reported clinical improvement in 85% of patients who completed a sublingual hyposensitization treatment, but this observation was not supported by improvement of tolerance to nickel during challenge tests. In a double-blind placebo controlled study performed by Bagot *et al.*[58], patients who ingested 5 mg capsules of nickel sulphate per week for seven weeks did not show significant improvement of contact allergy as demonstrated by the comparison of the intensity of nickel patch test reaction (with concentrations of 2.4, 0.8, 0.2 and 0.05%) between the treated and placebo groups.

On the basis of these experiences in oral hyposensitization, Panzani et al.[59] successfully performed a therapeutic protocol with increasing doses of oral nickel sulphate associated with an elimination diet, in order to induce tolerance in patients with both local and systemic symptoms with nickel ingestion ("Systemic Nickel Allergy Syndrome", SNAS). Sixty-one patients affected by SNAS were enrolled in this study: 51 patients underwent oral hyposensitization (with 14 drop-outs for various reasons) and 10 patients were treated only with a nickel-free dietary regimen (controls). Treated patients received granules of a nickel sulphate preparation according to the following schedule: 1 granule every other day for 1 month, 1 granule every day for 2 months, 2 granules every day for 1 year, 1 granule a day and 1 granule every other day for 2 or 3 years. After 6 to12 months on a nickel-free diet, patients were allowed to gradually reintroduce prohibited foods. Twenty-nine of the 37 (78.3%) and one of the 37 patients who completed therapy achieved, respectively, a total remission and a partial remission; 7/37 (18.9%) left the study because reactivation of symptoms. In all ten controls, the symptoms that had disappeared during the nickel-free diet, reappeared with the reintroduction of prohibited foods. Oral challenge tests, performed before and after desensitization, showed an overall increase in tolerance in patients successfully desensitized. Patch testing, however, showed no variation in 20 cases, a decrease in 5 cases and disappearance of reactions in 5 cases.

These preliminary clinical results were confirmed by a subsequent study performed by Schiavino *et al.*[60], in which 231 patients with systemic nickel allergy were enrolled: 136 and 95 patients were randomly assigned to a treatment group (protocol in Table 2) and a control group (nickel-free dietary regimen) respectively. Forty-two of the 136 patients (30.9%) interrupted the treatment because of lack of benefits. Ninety-four of the 136 (69.1%) completed the treatment protocol with the following results when they returned to an unrestricted dietary regimen: 64 (47.0%) reported a complete remission of symptoms, 23 (16.9%) had symptoms improvement greater than 80% but less than 100% and 7 (5.2%) reintroduced only some prohibited foods (limited diet). In the control group, 78/95 patients

Table 2. *Protocol of desensitization used by Schiavino et al. in 2006[60] (1 granule = 0.1 ng)*

1 granule every other day for 45 days
1 granule/day for 45 days
1 granule/2 granules on alternate days for 45 days
2 granules/day for 45 days
1 granule/2 granules on alternate days for 45 days
1 granule/day for 45 days
1 granule every other day for 45 days

During the second phase (progressive dose decrease) patients gradually reintroduced nickel containing foods

(82.1%) presented a relapse of the pre-existing systemic symptoms when nickel-containing foods were reintroduced. The resolution rates were 69.1% and 17.9% in the treated and control groups, respectively, with an absolute risk reduction of 51.2% and a relative risk reduction of 74.1% in treated patients. According to their results, two treated patients were required to have one positive result (number needed to treat or NNT). Statistical analysis revealed a significant improvement in treated versus control patients (OR: 8.29; CI: 4.07-16.89). Patch tests and oral provocation tests were performed in both groups before and after desensitization. Control patients did not show any modification in reactivity either to nickel patch or to nickel oral challenge. In treated patients, reactivity to nickel patch test showed no variation in 68 cases (72.3%), decreased in 17 (18%), increased in 1 (1.1%) and turned negative in 8 patients (8.6%). The oral challenge test showed an increase in tolerance to nickel in the majority of cases: 29 (30.9%) did not react, 47 (50%) reacted to a higher dose, 17 (18%) to the same dose, while 1 patient (1.1%) showed a decrease of threshold dose.

Minelli et al.[61] designed a study aimed to evaluate the efficacy and safety of an oral nickel hyposensitizing treatment using higher doses than in the study of Schiavino *et al.*[60]. They enrolled 36 patients with SNAS who were randomly allocated; 24 patients received treatment and 12 diet alone (control group). The treatment, which started one month later than the low-nickel diet, consisted in an incremental dose phase (0.3-3000 ng/week) and a 12-month maintenance phase (1500 ng/week) (Table 3). After 4 months, prohibited foods were gradually reintroduced. Treatment significantly reduced clinical severity of disease (evaluated by Visual Analogic Scale, VAS): at the final visit

Table 3. *Protocol of desensitization used by Minelli et al. in 2008 (in press)[61]. After 4 months, prohibited foods were gradually reintroduced*

Incremental Phase	*Monday*	*Wednesday*	*Friday*
1st week	*0.1 ng (1 cps)*	*0.1 ng (1 cps)*	*0.1 ng (1 cps)*
2nd week	*2 x 0.1 ng (2 cps)*	*2 x 0.1 ng (2 cps)*	*2 x 0.1 ng (2 cps)*
3rd week	*1 ng (1 cps)*	*1 ng (1 cps)*	*1 ng (1 cps)*
4th week	*2 x 1ng (2 cps)*	*2 x 1ng (2 cps)*	*2 x 1ng (2cps)*
5th week	*10 ng (1 cps)*	*10 ng (1 cps)*	*10 ng (1cps)*
6th week	*2 x 10 ng (2 cps)*	*2 x 10 ng (2 cps)*	*2 x 10 ng (2 cps)*
7th week	*100 ng (1 cps)*	*100 ng (1 cps)*	*100 ng (1 cps)*
8th week	*2 x 100 ng (2 cps)*	*2 x 100 ng (2 cps)*	*2 x 100 ng (2 cps)*
9th week	*500 ng (1 cps)*	*500 ng (1 cps)*	*500 ng (1 cps)*
10th week	*2 x 500 ng (2 cps)*	*2 x 500 ng (2 cps)*	*2 x 500 ng (2 cps)*
Constant Phase	*One cps of 500 ng 3 times weekly for 12 months*		

(16 months after the start of the study) VAS showed a value of 0.99 for treated and 6.46 for controls (p = 0.001). Use of rescue medication (antihistamines, topical steroids) was also significantly reduced when comparing treated versus controls at the final visit (4.3% vs 83%: p<0.001). Twenty out of 23 patients (87%) remained symptom-free after reintroduction of nickel-containing diet. The results also showed an excellent safety profile. No patient showed any long term side-effect; 21 patients completely tolerated the maximal weekly dose of 1500 ng; 2 patients did not tolerate the highest doses and were treated with lower dosages (600 and 300 ng/weekly); 1 patient dropped out for gastrointestinal symptoms.

Mechanisms underlying the treatment are not known and SNAS is not mediated by IgE. Artik *et al.*[18] demonstrated that T lymphocytes taken from nickel desensitized mice were unable to produce IL-2 and proliferate after *in vitro* stimulation with $NiCl_2$.

In humans, CD4 lymphocytes have a limited proliferative response to nickel *in vitro* stimulation. However Treg depletion strongly enhances CD4 proliferative response (+240%), therefore desensitization should increase Treg cells[19].

In conclusion, complete nickel avoidance is extremely difficult (especially in the Mediterranean diet) and, if prolonged, may have nutritional consequences (e.g. iron deficiency). However, oral desensitizing treatment is effective in reducing symptoms in SNAS, but not in contact dermatitis alone. The dosage of 0.1 ng and 500 ng have similar efficacy: symptom-free patients after reintroduction of nickel-containing diet were 92.5% and 87% respectively. Surprisingly 500 ng seems to be better tolerated than 0.1 ng: side effects were 14.2% vs 21.5%, respectively, but further studies are needed.

5. SYSTEMIC NICKEL ALLERGY. GENETICAL ASPECTS AND RELATED DISEASES

There is a wide consensus about the genetic contribution to the development of immunopathological diseases[62]. The major histocompatibility complex (MHC) molecules play a key role in the basic regulation process of the acquired immunity. In humans, the MHC is named human leucocyte antigen (HLA) system. MHC molecules are expressed in a co-dominant manner and the complexes of class-I plus class-II MHC genes, expressed on a single chromosome, are defined as haplotypes. The haplotype is transferred to the progeny as a single unit. Some specific HLA haplotypes are associated to genetic susceptibility or protection regarding a great number of immune-mediated diseases, including celiac disease[63]. With regard to the latter, its family aggregation is evident and its distinguishing feature as a complex multifactorial pathology (mainly caused by gluten in the diet) characterized by genetic susceptibility (supported by multiple genes). In particular, the HLA genes encoding for the DQ2 and DQ8 histocompatibility molecules are today clearly identified as the genetic basis of the celiac disease[64]. It is worthwhile to note that the same polymorphism changes in the HLA system, as well as being considered the main genetic background of gluten-dependent enteropathies, can be involved in other clinical disorders, as in skin and respiratory allergic diseases[65-67].

Association between MHC and allergic diseases

In recent studies[68], the frequency of the HLA DQA1/DQB1 haplotypes which codify for the DQ2 and DQ8 heterodimers have been evaluated in 121 patients suffering from various allergic diseases not including patients with nickel allergic response (Table 4). A control group of 116 healthy patients was included in the study. The expression of the HLA DQ2 and/or DQ8 molecules was significantly higher in the allergic group, regarding each of the DQ2 and DQ8 single molecules and also when considering DQ2-DQ8 together (Table 5).

Table 4. *Frequencies of the DQ2 + DQ8 heterodimers in healthy patients vs allergic patients*

Control group (116)	*33/116 (28.3%)*
Allergic patients (121)	*64/121 (53.7%)*

Table 5. *Frequencies of the DQ2, DQ8, DQ2-DQ8 heterodimers in control group vs allergic patients*

	DQ2	*DQ8*	*DQ2-DQ8*
Control group	*24.98%*	*2.58%*	*0.86%*
Allergic patients	*47.1 %*	*4.13%*	*2.48%*

More precisely, it has been ascertained that, within the DQ2 heterodimer range, the HLA DQA1*05/DQB1*02 haplotype is more frequent in the allergic group than the other haplotypes which codify for the DQ2 (Table 6). This analysis (χ^2 test for statistical significance) shows that the DQ2 and DQ8 molecules, already correlated to immune-mediated diseases as the celiac disease, are also involved in allergic disorders, with particular relevance of the DQ2 heterodimer coded by the HLA DQA1*05/DQB1*02 haplotype.

As far as nickel is concerned, preliminary evaluations performed in monosensitive patients, complaining of systemic complex clinical pictures, showed that the allergic sensitivity to this metal also has genetic control and part of this control may be modulated by the class-II HLA polymorphism. More specifically, results from these studies suggest that the DQB1*0202 allele in the DR7-DQ2 haplotype is the main candidate for the presentation of the immunoactive peptide to the CD4+ T cells[69].

Conclusions

In all the clinical pictures studied so far, the role of genetic makeup appears to be highlighted by the presence of haplotypes common to other disorders, such as celiac disease and allergic diseases[70-71]. Also, in patients suffering from allergic diseases, the coexistence of gluten sensitivity may often be under-diagnosed and, as a consequence of the lack of proper dietary recommendations, the clinical picture may become chronic or show insufficient therapeutic response, despite the use of effective drugs.

We are currently conducting some observational studies to evaluate the clinical efficacy of a dietary regimen designed to limit the intake of both gluten and nickel. The patients submitted to this regimen have documented nickel allergy and a genetic makeup analysis with typical characteristics of celiac disease, even though in the absence of classic symptoms of the gluten reactivity of celiac disease. In these patients, pharmacologic treatment and dietary recommendations limited to nickel avoidance frequently show only partial clinical improvement and are often limited in time.

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Table 6. *Frequencies of the HLA DQA1/DQB1 DQ2 haplotypes in control group vs allergic patients*

	*DQA1*05/DQB1*02*	*DQA1*05-DQA1*0201/DQB*02*	*DQA1*0201/DQB1*02*
	DQ2.5	***DQ2trans***	***DQ2.2***
Control group	*7.75%*	*11.2%*	*6.03%*
Allergic patients	*23.1%*	*13.2%*	*10.7%*

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