

Key words

Nickel • Allergy • Contact allergic dermatitis • Immune system dysregulation • Systemic Nickel Allergy Syndrome

The possible role of Nickel in the pathogenesis of contact allergic dermatitis, immune dysregulation and Systemic Nickel Allergy Syndrome

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Nickel (Ni) represents the principal hapten involved in the determinism of allergic contact dermatitis (ACD). Many studies have demonstrated a role of nickel in the dysregulation of the immune system and in the pathogenesis of the systemic manifestations of the allergy. Ni is the most common contact allergen in both professional and non-professional fields because it is commonly present in metal objects, colorants for porcelain, glass, pottery, detergents and food. It is known that Ni-ACD has a high prevalence in the general population. The exposure to Ni can occur not only through the contact with the skin but also by the gastrointestinal absorption. The Ni allergy was associated to immune dysregulation with prevalent immunosuppressive action, gastrointestinal symptoms, cutaneous manifestations, recurrent infections and increase in oxidative stress. The extracutaneous and cutaneous manifestations in areas not in contact with Ni are the principal manifestations of the Systemic Nickel Allergy Syndrome. Nevertheless the data of literature don't consent to reach definitive conclusions. In this review we want to clarify the potential immunomodulatory action of nickel and the possible clinical manifestations of the allergy.

Introduction

Nickel (Ni) is a ubiquitous metal, hard, malleable, silvery-white, soluble in acid, alkaline corrosion-resistant, easily deposited by electrolysis. The origin of the name comes from the German word "old nick" that was the informal name of Satan. Ni is also known as 'kupfernickel' that means devil's copper. Since the nineteenth century many countries have adopted Ni as coinage metal, although this practice was known in China since the early second century BC. In 2003 the European Community has commissioned Danish Environmental Protection Agency to document an estimate of objective risk linked to use of Ni in the Euro coins. The outcome of the investigation stated that "there is no evidence associating exposure to the current coinage with the induction of allergic contact dermatitis (ACD) induced by Ni in those who handle the coins

for work and in ordinary consumers". In 2004 an experimental model showed that coins of 1 and 2 Euro determine a positive patch test and so that they release Ni after a prolonged contact with the skin ¹.

It is known that Ni-ACD has a high prevalence in the general population ². In this review we'll describe the possible role of Ni on the immune system and the pathogenesis of some non-cutaneous manifestations.

Allergic contact dermatitis

Overall the prevalence of ACD in the general population is estimated to be from 2 to 8,1%. Ni is the most common contact allergen in both professional and non-professional fields because it is commonly present in metal objects, colorants for porcelain, glass,

pottery, detergents and food ². The prevalence of hypersensitivity to Ni in the general population is estimated to be 7-10%, with a predominance of the female sex and mean age of onset between 21 and 30 years ³.

The pathogenesis of ACD recognizes as basic mechanism a delayed-type hypersensitivity and the diagnosis is made, according to international guidelines, by patch test. Other diagnostic tests, although not universally accepted, have been used to assess the possible sensitization to Ni. The lymphocyte proliferation test (LPT) or Lymphocyte Transformation Test (LTT) is based on the assumption that sensitive individuals have Ni-sensitized T lymphocytes able to recognize Ni if it is presented as an antigen. This test has recently been optimized and renamed LTT-MELISA (Memory Lymphocyte immunostimulation assay) but it can't yet be assimilated to the results obtained with patch test ⁴. The oral nickel challenge is not very sensitive and specific, therefore about its validity there is absolutely no agreement in the international scientific community. In fact it can present numerous confounding factors: there may be, for example, uncertainties on the amount of Ni that actually reaches the target cells, on the type and amount of food present in the intestine at the time of ingestion of Ni and finally on the real degree of patients' sensitivity to the metal and on its bioavailability. In clinical studies the oral nickel challenge showed good sensitivity in the systemic symptoms evaluation ^{5,6}.

Metal ions, due to their small size, are incomplete antigens (haptens), which must bind to endogenous peptides to become real antigens. Ni, in fact, binds to cellular proteins and those of the matrix of the skin inducing in this way an immune response. In the development of Ni-ACD it's possible to recognize two phases: Induction Phase (or Sensitization) and Activation Phase (or Re-exposure) ^{7,8}.

The sensitization phase lasts from 1 to 3 weeks after the contact with the skin, provided that the amount of Ni is sufficient to determine a damage to the skin and that the temperature, or other predisposing conditions, such as race, sex and age induce a susceptibility and accelerate the sensitization. The haptens, once penetrated into the skin, interact with carrier proteins, becoming complete and so immunogenic antigens. Once formed, the complete antigen is captured by dendritic cells and dermal Langerhans cells, processed and expressed on the cellular surface in association with molecules of the system MHC class I and II. Langerhans cells are dendritic cells, originated from the bone marrow, that are present in particular in the suprabasal layer of the epidermis and in all layers of the atopic skin. The number of Langerhans cells is decreased in the skin chronically exposed to the sun: this fact may explain the reduced incidence of delayed hypersensitivity in subjects exposed to the sun. The Langerhans cell, afterward, leaves the skin and goes to the

regional lymph nodes. In the paracortical region of the lymph nodes takes place antigen presentation to virgin T lymphocytes with consequent activation and proliferation of T lymphocytes with a specific receptor for the complex molecule HLA / immunogen peptide. The CD4+ T cells (Th0) differentiate into Th1 and Th2 cells. Th1 cells, predominant in the ACD, produce IFN- γ and IL-2. Consequently occurs a clonal expansion of specific T lymphocytes, effectors and memory ones, controlled by IL-2 and the corresponding receptor (IL-2R). The role that the epidermis plays in the ACD is not only limited to the antigen processing, but it is possible that the interaction between Langerhans cells and T lymphocytes also occurs in the skin (peripheral presentation) and not only in the lymph nodes (central presentation). The IL-2, despite being produced by sensitized CD4+ T cells, stimulates in a non-specific way CD4+ and CD8+ T lymphocytes ^{9,10}.

The phase of re-exposure gives rise to the expression of clinical ACD. In sensitized subjects memory T-lymphocytes, specific for the hapten, recirculate in both epidermis and dermis. It has been demonstrated that T-lymphocytes specific for Ni express surface molecules both CD8 and CD4. The CD8+ T-lymphocytes can directly damage the keratinocytes. CD8+ cells, together with CD4+ T-lymphocytes (Th1 and Th2), release lymphokines that promote the synthesis by keratinocytes of proinflammatory cytokines (IL-1, TNF- α and GM-CSF) and chemokines (IP-10, Mig, I-TAC, MCP-1, RANTES), as well as the de novo expression of adhesion molecules (ICAM-1) and molecules MHC class II. Furthermore, the up-regulation of molecules MHC class I, induced by IFN- γ , allows an increase in CD8+-mediated cytotoxicity ^{9,10}. In specimens there is a characteristic infiltration of mononuclear cells in both dermis (spongiosis) and epidermis.

The cutaneous lesion is easily recognizable: it appears as an eczematous area in the point where nickel-plated objects are in close contact with the skin.

The non-professional sensitization to Ni was first noted in subjects wearing, in direct contact with the skin, articles of clothing with buckles, zips or metallic clips. Recently the cases of ACD induced by Ni have increased because of the practice of piercing: in fact it makes use of hooks containing Ni, inserted in the hole, in order to prevent its closure during the cicatrization.

The eczema, that is the clinical expression of Ni-hypersensitivity, may present with primary lesions including papules, erythematous macules and vesicles, which may coalesce to form patches and plaques. In severe forms of eczema may predominate secondary lesions, such as exudation and development of crusts. Chronic forms of eczema are often dry and characterized by thickening and desquamation of the skin (lichenification).

Nickel and nutrition

The exposure to Ni can occur not only through the contact with the skin but also by the *gastrointestinal absorption*. In fact, many foods have a high content of Ni. Vegetables represent the main dietary source. Ni is mainly concentrated in the reproductive part of plants (hazelnuts, peanuts, wheat grains, legumes). In a normal diet, 50% of the daily requirement of Ni is provided by cereals and legumes, followed by fats, dairy products and fruit. Recent studies about the contribution given by stainless steel kitchen utensils have shown that it is negligible. The average daily intake of Ni is approximately 400-600 µg. Only 1-10% of the ingested Ni is absorbed in the gastrointestinal tract, the remainder is excreted in the feces. The absorbed Ni is mainly excreted in the urine and sweat. Recent studies have demonstrated that Ni plays an important role in the nutrition. First of all it facilitates the absorption of trivalent iron, particularly in the case of low blood concentration of iron, stimulating the synthesis or the maintenance of the integrity of a lipophilic complex involved in the transport of the oxidized iron through the mucosa. Furthermore, it activates in a non-specific way different enzymes as tyrosinases, arginases and deoxyribonucleases.

Nickel and immune system

Several evidences prove that Ni, in experimental animal models, can induce negative effects on the immune system, but the data on immunomodulation are contradictory: among the effects most frequently encountered prevails the suppression of the immune response.

Some studies have observed that in experimental animals Ni caused a depression in the interferon production both in vivo and in vitro and that, in test animals treated with Ni, there was an inhibition of phagocytic activity of macrophages and a suppression of the antibody response ¹¹.

Animal model studies, conducted both in vivo and in vitro, have shown an immunomodulatory effect of Ni, demonstrating a reduction in the activity of NK cells in both rats and monkeys ¹¹. Zerominski et al. have tested, in a human model, the effect deriving from the addition of Ni in cell cultures (PBMC), finding that the salts of Ni were able to suppress in vitro the cytotoxic activity of NK cells ¹².

In Ni sensitive women with cutaneous lesions caused by the Ni intake, Di Gioacchino et al. have evaluated the modifications produced by the oral nickel challenge in *lymphocyte subsets* of the peripheral blood and the gastrointestinal mucosa. In the peripheral blood of these patients the authors found a reduction

of all the lymphocyte subsets and in the intestinal epithelium and lamina propria (duodenal mucosa) the presence of a lymphocyte inflammatory infiltrate characterized by CD4+ T-helper, in particular CD45RO+ "memory", with a poor representation of CD8+ cells. These data would validate the hypothesis of a maturation of "naive" lymphocytes (CD4+, CD45RO-) in memory T lymphocytes (CD4+, CD45RO+) that would accumulate in the intestine in response to antigenic stimulation. Further in vitro studies, upon incubation with Ni of mononuclear cells from patients nickel-sensitive, confirm the increase in CD45RO+ "memory" lymphocytes accompanied by a reduction of CD45RO-"naive" ¹³.

The results of cytokine production of clones of T-lymphocytes after nickel-mediated activation are very confusing. In fact, in some studies the clones of nickel-specific T-lymphocytes isolated from peripheral blood and from cutaneous lesions of patients sensitive to Ni and not, have shown to have a typical profile of "T-helper-1-like activity" (IFN-γ, IL-2, TNF-α); conversely, in other studies was observed a predominant Th0-like activity (IL-4, IFN-γ) or Th2 (IL-4) ¹⁴⁻¹⁶.

Falsafi-Amin et al. have demonstrated a difference between nickel-allergic subjects and healthy controls regarding the synthesis of DNA and the production of cytokines after stimulation with Ni sulfate of PBMC. They have observed an increase in DNA synthesis after stimulation with Ni together with a high concentration of some cytokines (IL-2, IL-4, TNF-α). Other authors have studied, by ELISA, the production of cytokines in cultures of PBMC after stimulation with Ni sulfate and they have demonstrated in allergic patients a synthesis of IL-4 and IL-5 significantly higher than in healthy controls. No significant differences of IFN-γ and TNF-α serum concentration have been shown ¹⁷.

Jackobson et al. have evaluated the production of IL-4, IL-5, IL-13 and INF-γ in PBMC after stimulation with Ni observing significantly higher values in nickel-sensitive subjects compared to healthy ones ¹⁸.

Buchvald et al. have shown an increase in DNA synthesis and production of IL-2 and IL-5 in PBMC of patients with Ni-ACD compared to healthy controls and patients with atopic dermatitis ¹⁹.

Ishihara et al. have shown that ions of Ni chloride incubated for three days with rat eosinophils were able to reduce eosinophil survival by inducing apoptosis through an increase in the percentage of annexin V+ PI-. However, further studies are needed to clarify the role of eosinophils in ACD nickel-related ²⁰.

Condevaux et al. have evaluated the in vitro effects of Ni chloride on the NK cell activity, both in rats and monkeys cynomolgus, showing that, at high concentrations, Ni chloride induces a significant decrease in NK cell activity ²¹.

Salsano et al. have demonstrated that nickel-sensitive patients showed a considerable decrease in the NK cell activity, but not

in the NK cell quantity, and that the addition of Ni sulfate to the PBMC cultures of non-sensitized subjects induces a reduction in the release of IL-5, INF- γ and TNF- α . The measurement of NK cell activity has often been considered as an efficient support for the immunotoxicological evaluation of xenobiotics, including environmental chemicals and drugs. Methods used to study NK cells include morphological techniques (such as flow cytometry for NK cell count) and functional analysis. Although the flow cytometry has been recently proposed, the Chromium-51 release assay is still the most widely used technique, especially in rodents ²².

Carbone et al. have given a further demonstration of how much complex and still unclear is the NK cell function in nickel-sensitive patients. These authors have supposed that in the pathogenesis of Ni-ACD is involved a specific NK cell subset (CD56 high CD16-CD62L-) isolated from cutaneous lesions. This subtype would act in two ways: production of proinflammatory cytokines with consequent activation of CD4+ helper T cells and direct induction of keratinocyte apoptosis. This hypothesis would support the importance, for the full manifestation of the ACD, of the interaction between NK-mediated innate response and adaptive one, providing new inputs for further studies ²³.

Kim et al. have observed that murine splenic NK cells, incubated with Ni sulfate for 24 hours, release IFN- γ and have hypothesized that this cytokine may be early involved in the activation of dendritic cells that produce costimulatory signals for T lymphocytes ²⁴. Conversely, Carbone et al. have observed that NK cells of nickel-sensitive patients, exposed in vitro to the metal, didn't proliferate and didn't release INF- γ . On the other hand, the production of INF- γ by the NK cells was quickly induced in the presence of activated T cells secreting IL-2 ²³.

Systemic Nickel Allergy Syndrome

Nickel-hypersensitivity can induce not only ACD but also a more complex disease known as Systemic Nickel Allergy Syndrome (SNAS), characterized by a combination of manifestations caused by the intake of Ni with the diet ^{5 25}. Gastrointestinal symptoms, cutaneous manifestations in areas not in contact with Ni, recurrent infections and an increase in oxidative stress are the principal manifestations of the SNAS.

In the seventies, some researchers have found that a high number of patients with nickel-hypersensitivity showed a persistent dermatitis in areas distant from those that were in direct contact with the metal. The symmetrical eczematous lesions have been attributed to the systemic introduction of Ni (by parenteral introduction or gastrointestinal absorption) and have been called "secondary

eruptions". In fact, it has been demonstrated that the amount of Ni present in a normal diet is sufficient to trigger a pre-existing ACD. The most typical areas of these secondary eruptions are the folds of the elbow, the lateral cervical regions and the root of the inferior limbs. Peculiar forms of secondary eruptions are represented by recurrent vesicular dermatitis localized on the palms, soles or lateral surfaces of the fingers, by symmetrical nummular eczema, eyelid or anogenital eczema and keratotic eczema of elbows. Recently particular attention has been paid to the possible influence of Ni present in the diet on the clinical course of the dermatitis. For this reason, medical doctors have formulated a nickel-poor diet, to follow for a minimum period of 4 weeks (up to a maximum of 6 months) and noted an improvement in about 50% of patients.

In 2009, Rosato et al. have demonstrated that nickel-hypersensitivity is associated with a significant increase in susceptibility to viral, bacterial and fungal infections. In nickel-sensitive patients in fact a higher incidence of recurrent herpes labialis, urinary tract infections, genital candidiasis, and upper respiratory tract infections was detected. Moreover, the same authors have found a reduction in the number of annual episodes of herpes labialis in patients following nickel-poor diet ²⁶. A successive study conducted by Rosato et al. in 2011 on pediatric patients, showed a higher incidence of recurrent upper respiratory tract infections and recurrent otitis media in children with Ni-ACD. This susceptibility, according to the authors, could be determined by a reduction of the NK cell activity caused by the immunomodulatory effects of Ni in allergic subjects ²⁷.

Regarding the headache and the gastrointestinal symptoms, the data of literature are conflicting and not efficiently supported. However the gastrointestinal symptoms data seem to be more accurate than headache ones ²⁸.

An increase in oxidative stress in nickel-sensitive patients was demonstrated in 2009 by Gangemi et al. Serum concentrations of protein carbonyl groups (PCGs) and nitrosylated proteins (NPs), biomarkers of oxidative stress, were measured before and after oral nickel challenge in thirty-one women with diagnosis of Ni-ACD and in thirty-one healthy controls. Although both nickel-allergic patients and controls presented similar serum levels of PCGs, NP values in nickel-sensitive patients appeared higher than in controls and tended to decrease after the challenge ²⁵.

Finally, pivotal clinical studies, monocentric non-randomized and non-double-blind controlled, have preliminarily shown that the oral hyposensitization procedure, obtained with the administration of increasing doses of Ni sulfate, could be suitable to induce immune-tolerance and symptoms improvement ^{29 30}. However, further large randomized clinical trials are needed to confirm these preliminary and suggestive clinical data. However, further

randomized clinical trials are needed to evaluate the effects of hyposensitization therapy.

Conclusions

In conclusion, Ni could be involved in the immune system dysregulation. Several studies have shown a negative effect of Ni on the NK cells that would consist in a functional deficit rather than in a quantitative one.

Orange has spoken of NK cells functional deficit (FNKD), referring to the studies conducted by various authors on the increased incidence of infections caused by cytopathic viruses and intracellular pathogens in subjects with a normal number of NK cells but with a reduced functional activity²²⁻³¹. This functional impairment of the NK cells could explain the increased incidence of infections in nickel-sensitive patients²⁶⁻²⁷. Remains to be determined if the reduced natural cytotoxicity is primary or secondary to the Ni exposure and if it is reversible.

In epidemiological studies, Ni represents the hapten with the highest prevalence in the pathogenesis of ACD. The episodes of ACD exacerbation decrease in patients who succeeded in avoiding cutaneous contact with Ni. While it is now undisputed that Ni represents an etiological agent in the determinism of ACD, its role in the pathogenesis of extracutaneous manifestations need further data. In fact remains to be clarified the role of nickel-poor diet in the control of the secondary eruptions (i.e. developed without contact with the hapten) and of the other systemic manifestations.

The authors declare no conflicts of interest.

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