Metal Allergens of Growing Significance: Epidemiology, Immunotoxicology, Strategies for Testing and Prevention

G. Forte*, F. Petrucci and B. Bocca

Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy

Abstract: Metal-induced allergic contact dermatitis (ACD) is expressed in a wide range of cutaneous reactions following dermal and systemic exposure to products such as cosmetics and tattoos, detergents, jewellery and piercing, leather tanning, articular prostheses and dental implants. Apart from the well known significance of nickel in developing ACD, other metals such as aluminium, beryllium, chromium, cobalt, copper, gold, iridium, mercury, palladium, platinum, rhodium and titanium represented emerging causes of skin hypersensitivity. Despite the European Union directives that limit the total nickel content in jewellery alloys, the water soluble chromium (VI) in cement, and metals banned in cosmetics, the diffusion of metal-induced ACD remained quite high. On this basis, a review on the epidemiology of metal allergens, the types of exposure, the skin penetration, the immune response, and the protein interaction is motivated. Moreover, *in vivo* and *in vitro* tests for the identification and potency of skin-sensitizing metals are here reviewed in a risk assessment framework for the protection of consumer's health. Avenues for ACD prevention and therapy such as observance of maximum allowable metal levels, optimization of metallurgic characteristics, efficacy of chelating agents and personal protection are also discussed.

Keywords: Allergic contact dermatitis, metals, epidemiology, prevention, human health.

INTRODUCTION

Allergic contact dermatitis (ACD) is one of the most common environmental and occupational skin diseases. In fact, it has been recognized that of all the dermatological disorders the ACD manifested is about 10% [1] and represented about 50% of all occupational dermatosis, depending on industries, geographical areas, age and sex distribution of patients, etc. [2]. ACD is defined with an inflammatory process of the skin caused by contact with exogenous substances, generally having a low molecular weight [3]. These substances are naturally occurring in the environment or can be synthetics and skin contact may occur at workplace or at home.

The ACD represented the most prevalent manifestation of immunotoxicity in humans and it develops in two stages. The first is the induction or sensitization phase, where the skin is sensitized following topical exposure to a concentration of the allergen sufficient to induce the immune response. This condition produces a rapid and more aggressive secondary immune response in case of an additional re-exposure to the same allergen. In the second or elicitation phase, the response is triggered and the T-cells are the key mediators of the reaction. Once activated, the cytokines, chemokines, and cytotoxins released from the T-cells stimulate the local blood vessels with recruitment of macrophages and eosinophils, leading to an amplification of the reaction. The time necessary to observe elicitation of ACD is approximately 24-96 hours [4, 5]. More details regarding the sensitization and the elicitation phases are shown in Figs. (**1**) and (**2**), respectively. The dermal inflammatory acute responses are also called eczematous dermatitis and the morphology of eczema goes from erythema and edema in the mildest form to vesicles in the severe form and these symptoms begin to disappear when the allergen is no longer in contact with the skin [6]. The immune response can be mediated by humoral antibodies or by sensitized lymphocytes and can be classified in four types. In particular, type I is mediated by the release of IgE from the mast cells after elicitation; type II is mediated by the production of IgG or IgM after cytotoxic reactions; type III is mediated by the deposition of the complex antibody-antigen in tissues; and type IV is due to T-cells-mediated reactions [7].

The importance of this kind of disease is not only related to the high number of affected people worldwide, but also to economical (increase expenses of each national health service) and psycological (worsening of the quality of life of patients) issues. In fact, considering the losses in productivity and the cost for treating the disease, more than 1 billion of dollar are spent annually in the United States (US) [8, 9]. In this context, people with ACD of the face or subjects who are obliged to change job reported the worst quality of life [10].

Among the spectrum of substances that act as allergens, metals represent an important class. Metals are ubiquitous in the environment because they are normally present in the Earth's crusts, in food and water. Nowadays, metals are involved in several fields such as in industrial productions and in consumer products (jewellery, cosmetics, paints, leather, dental/body implants, household products, dyes, personal adornments, pharmaceuticals, etc) where they can be present as main components or as impurities. It is for their extreme use that metals represent a risk for developing ACD. In this context, nickel (Ni), chromium (Cr) and cobalt (Co) as ions and compounds, are well recognized skin sensitizers. In particular, in Europe, the Ni, Cr and Co ACD prevalences were of about 20%, 4% and 7%, respectively (data from the

*Address correspondence to this author at the Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy; E-mail: giovanni.forte@iss.it

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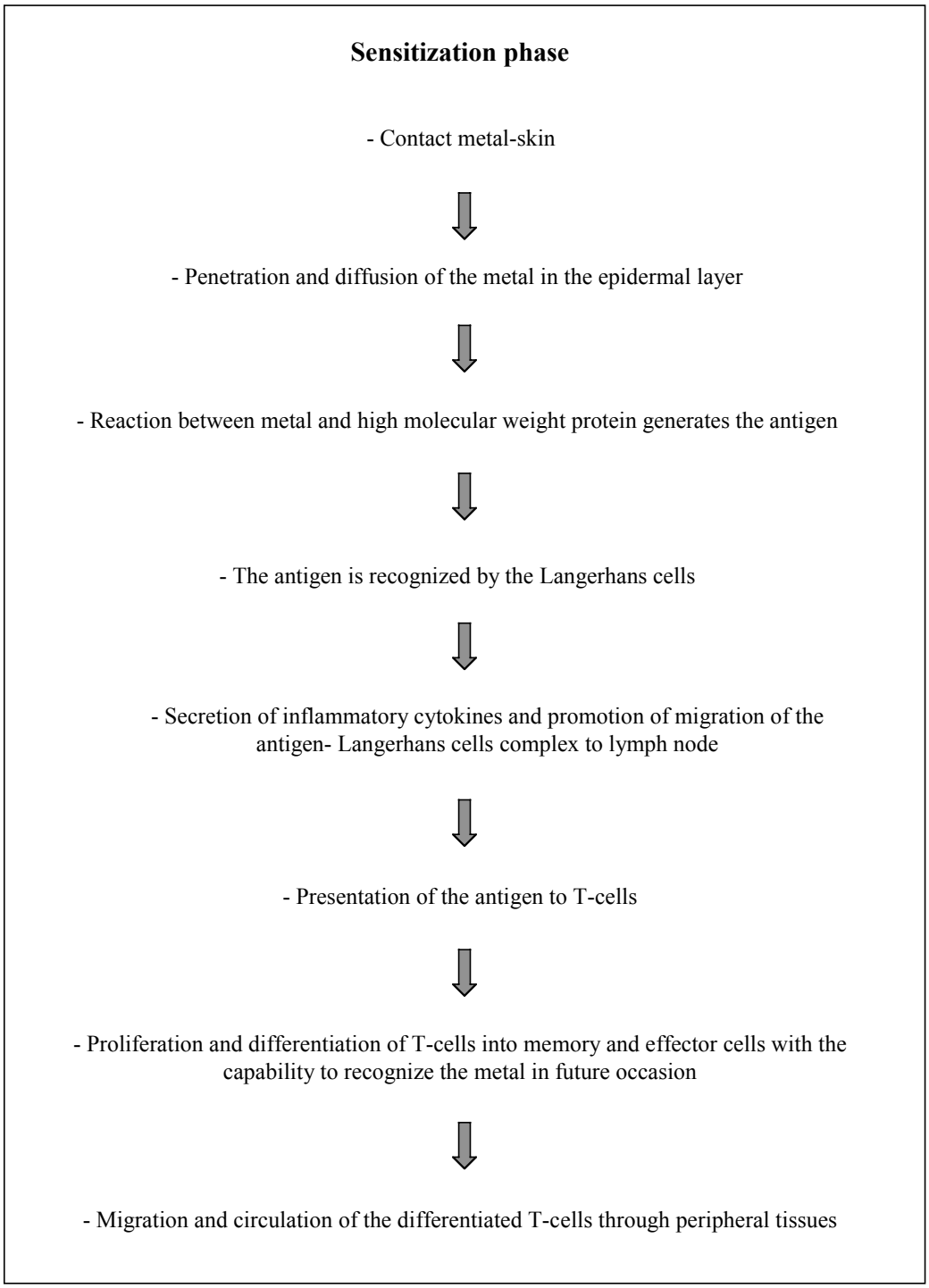

Fig. (1). Flow-chart showing the sensitization phase in metal induced ACD.

European Surveillance System of Contact Allergies, ESSCA) [11]. These data are similar to those evidenced in the US with a prevalence of about 14% for Ni, 4 % for Cr and 9% for Co [12, 13]. In addition, females are affected by Ni and Co ACD more than males due to ear piercings and jewellery; while Cr ACD affects mainly males because of occupational activities [14]. Moreover, it has been demonstrated that the rate of Ni and Co ACD is higher at younger age, while the prevalence of Cr ACD remained high for the whole life [15].

Recently, other elements such as aluminium (Al), beryllium (Be), copper (Cu), gold (Au), iridium (Ir), mercury (Hg), palladium (Pd), platinum (Pt), rhodium (Rh) and titanium (Ti) are of growing concern amongst dermatologists for their capability under favorable circumstances to act as allergens, even if the reason why some metals are able to create sensitization more than others is not cleared as well as the pattern of multiple metal reactivity, cross reactivity and multiple sensitizations are almost unknown [14].

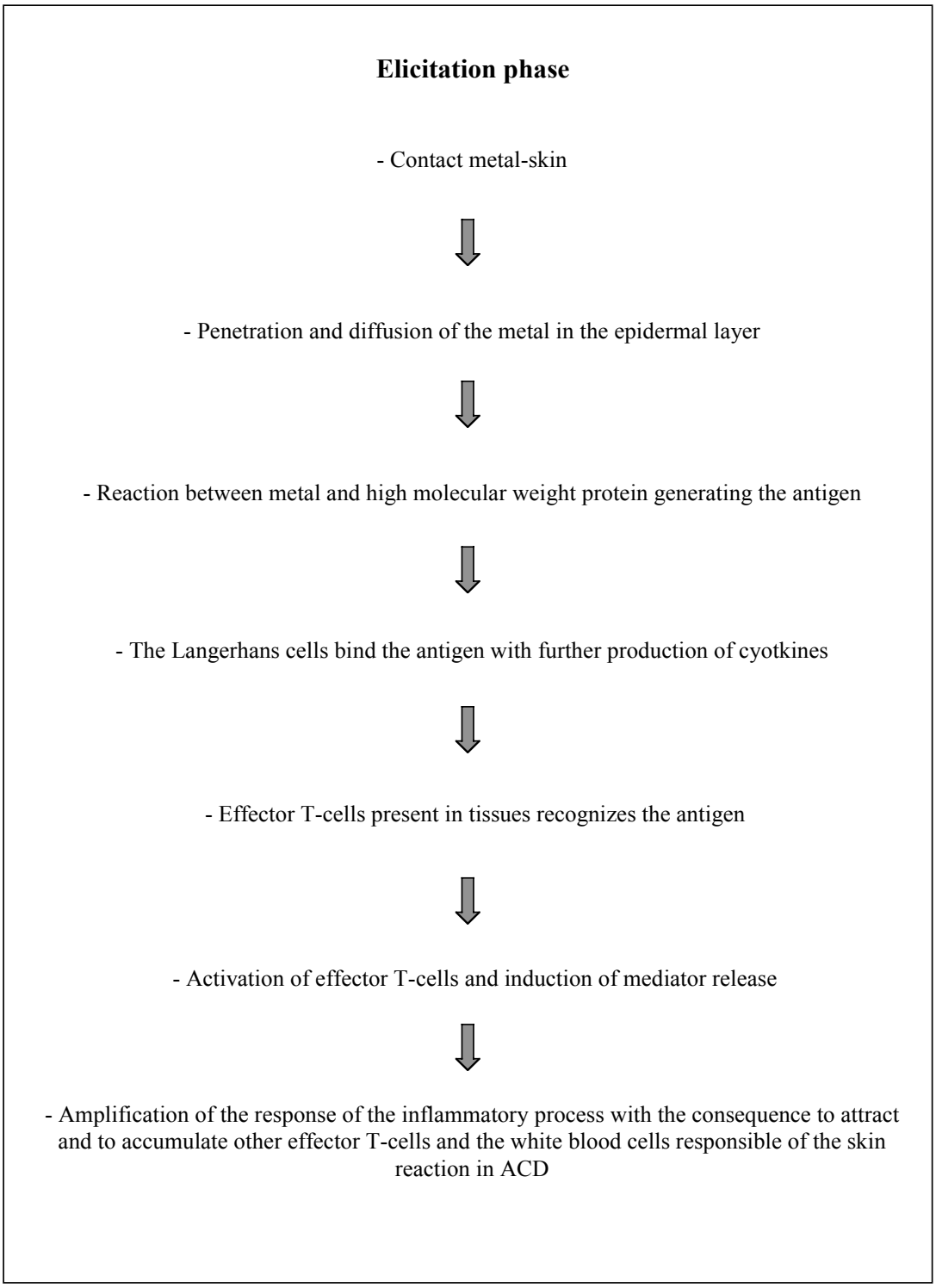

Fig. (2). Flow-chart showing the elicitation phase in metal induced ACD.

Efforts have been done for the reduction and prevention of metal ACD. The management of the risk can be achieved by understanding the potency and prevalency of sensitizers, developing and optimizing diagnostic tests, restricting the skin contact by regulatory limits and informing about skincare strategies such as hygiene, gloves and protective creams [16]. At present, in the EU are existing regulations for limiting metals in products destined for skin contact. In particular, the Council Directive 94/27/EC limited the total Ni content in alloys and its released rate in artificial sweat, the Council Directive 2003/53/EC fixed the presence of the water soluble Cr(VI) in cement, and the Council Directive 76/768/EEC (implemented by the Commission Directive 2004/93/EC) banned some metals in cosmetic formulations [17-20].

This paper highlights the worldwide state-of-the-art on the sensitization and contact dermatitis provoked by Al, Au, Be, Co, Cr, Cu, Hg, Ir, Pd, Pt, Rh and Ti in terms of

epidemiology, immunotoxicology and strategies for the diagnosis and limitation of the disease.

ALUMINIUM (Al)

The more typical sensitization to Al is *via* the absorption of Al through hyposensitization injections and vaccines [21, 22]. Hyposensitization injections are used as treatment for IgE-mediated allergies, and the most commonly used extracts in these solutions are Al-contacting antigens. Additionally, Al compounds have been widely used as adjuvants in prophylactic and therapeutic vaccines because they prolong the period of adsorption and increase the immune response [23, 24]. The two main clinical features of Al sensitization are represented by persistent granulomas and recurrent eczema [25, 26]. Aluminium allergy seems to be more common in pediatric patients than in adults. Children with Al sensitivity have been reported to develop persistent subcutaneous nodules at the sites of injection or excoriated papules at the sites of hyposensitization therapy [27, 28].

The second route of sensitization to Al is the prolonged application of Al-containing antiperspirants and topical medications and clinical manifestations are axillary rashes and hand dermatitis [29]. A patient in Sweden who regularly used an aluminium chloride roll-on antiperspirant developed an itchy dermatitis in the axillae and patch tests with aluminium chloride were positive [30]. Another case of axillary eczema was observed in a 16-year-old girl; the use test with the deodorant containing aluminium chloride hexahydrate resulted to be positive [31]. In addition, cutaneous granuloma and skin sensitivity appeared when Al is complexed with zirconium (Zr) and glycine in antiperspirants [32]. Two cases of contact allergy to Al after use of topical medications containing aluminium acetotartrate have also been reported [33]. Pruritus due to allergic conditions was seen after the usage of a toothpaste containing 30-40% of aluminium oxide. When the toothpaste was replaced with a brand not containing Al, pruritus resolved in 1 month [34].

Even if Al is extensively used in several industries, only a small number of cases of skin sensitization have been reported; one dealt with aircraft workers and another with an hospital attendant [35, 36]. A study described a man who had a compressed air pistol in his right hand to blow fillings out of newly milled narrow Al threads; particles of Al penetrated the skin and erythema, hyperkeratosis and partial desquamation appeared in his right hand [37].

Only one case of contact urticaria to Al has been documented because of the presence of Al in coins as a contaminant with a maximum concentration of 0.01%. A simple test with a Norwegian coin was performed on the patient's forearm and back; erythema and itching developed after 5 min; vesicular infiltration appeared after 8 min, and 2 days later, there were large crusts [38].

Researchers have proposed that tattoo pigments containing Al can induce granulomatous reactions. In fact, in the 87% of 30 tattoo inks studied, the most commonly identified element was Al [39]. A case study of a 21-year-old man with delayed hypersensitivity granuloma formation in a tattoo is reported. Four weeks after tattooing, three separate tumorous areas appeared in the violet areas of the tattoo. Intermittently pruritic lesions had existed for 5 months from the first examination. With the use of scanning electron microscopy and energy dispersive X-ray (SEM-EDX) microanalysis, Al particles were found in the involved skin sections with infiltration of pigment particles at extracellular and intracellular levels [40]. Another report described a case of a woman who underwent blepharopigmentation with aluminium silicate and in whom a delayed hypersensitivity granulomatous reaction appeared [41].

BERYLLIUM (Be)

Occupational exposure to Be occurs in aerospace, nuclear, military, automotive, electronics, telecommunications industries and alloy applications, such as tubing for oil and gas drilling. Recycling of electronics, computers, and scrap alloy to recover Cu also results in Be exposure. The National Institute for Occupational Safety and Health estimates that up to 800,000 individuals are exposed to Be at the workplace in the US alone [42]. The general population is mainly exposed to airborne Be from the combustion of fossil fuel at levels that are usually low. Where Be-containing casting alloys are used for dental prostheses, skin and oral contact with Be can not be disregarded [43].

Skin exposure to Be salts, such as fluoride, chloride, nitrate and sulphate, is known to result in local toxicity responses that can include 5 groups of cutaneous disease: ACD, irritant contact dermatitis, chemical ulcers, ulcerating granulomas and allergic dermal granulomas [44]. Also poorly soluble Be particles could penetrate the skin and provide an immunologic route to Be sensitization. Tinkle *et al*. demonstrated that 0.5- and 1.0-μm Be particles penetrated the stratum corneum of human skin and reached the epidermis and, occasionally, the dermis [45]. Another study indicates that relatively insoluble particles ≤ 1 μm in diameter may be transported through the skin and around hair follicles [46].

In 1951, Curtis was the first to diagnose Be contact allergy in workers at two Be plants by patch testing with different soluble Be compounds, e.g., fluoride and sulphate [47]. Beryllium present in alloys has been reported to cause allergic contact reactions of the oral mucosa [48]. Incorporation of Be into the base metal alloy formulation facilitates castability and increases the porcelain metal bond strength. The dissolution of Be from dental alloys that contain Ni and Be has been proved to be several orders of magnitude greater than expected [49]. After incubation of pieces of dental alloys in human saliva for 120 days at 37 °C, the saliva contained Be between 0.3 and 3.48 mg/l at pH 6 and between 12.4 and 43.0 mg/l at pH 2. A study describes 2 patients who developed gingivitis (gum disease) adjacent to a Be containing alloy (Rexillium III) in dental prostheses and patch testing showed positive reactions to beryllium sulphate (1% in petrolatum) while none of the 30 controls reacted to this preparation [50]. In Spain, 3 patients with dental prostheses exhibited sensitization to beryllium chloride (l% petrolatum) while 150 controls were negative [51]. Another case reported a 29-year-old man with a popular eruption on his arms, left thigh and right knee. He had been employed at a factory for the past 3.5 years where he operated a Be-alloy production furnace that melted Be, Cu, Co, Ni and Zr. Treatment with a 2-week course of systemic corticosteroids and mid-potency topical steroids had been successful [52]. While Be in beryls (aquamarine and emeralds) is generally thought to be in a

biologically unavailable silicate form, one interesting study found a correlation between measurable Be in urine of beryl cutters and positive Be stimulation indices [53].

Contact dermatitis following exposure to Be compounds is of the delayed form and likely to be due to T-cell mediated hypersensitivity. The availability of the Be ion determines the intensity of skin hypersensitivity. In the study of Marx and Burrell, skin reactions developed 6–8 hours after the subsequent patch test challenge and lasted up to 3 weeks [54]. The severity of the skin reaction was greater when a more soluble salt was used for the challenge (fluoride > sulphate > oxide). Krivanek and Reeves found that Be-sensitized guinea pigs with beryllium sulphate elicited different skin reactions depending on the Be compound used. The beryllium albuminate produced the greatest hypersensitivity, followed by beryllium sulphate, whereas beryllium hydrogencitrate and beryllium aurintricarboxylate produced essentially negative reactions due to the fact that the Be was strongly bound to the anion and therefore unavailable for interaction with the skin [55]. Moreover, a delayed skin hypersensitivity reaction in 30% to 60% of pre-sensitized guinea pigs in response to challenge with Cu-Be and Al-Be alloys was observed [56]. Hypergammaglobulinemia, due principally to an increase in IgG levels, was frequently found in patients with acute berylliosis, Be dermatitis and in Be workers with no evidence of disease [57]. Patients with Be dermatitis may in addition develop a granuloma at the test site. Subcutaneous granuloma may also develop following patch testing in chronic Be disease [58]. Both lymphocyte transformation and leukocyte migration inhibition have been demonstrated in Be sensitive subjects and in animal experiments [59, 60]. In a study designed to assess the potential sensitizing and granulomagenic capacities of Be salts, rabbits were inoculated intradermally with beryllium sulphate. The salt resulted to be highly toxic for isolated alveolar macrophages and also depressed lymphocyte stimulation in sensitized animals, which demonstrated delayed skin reactivity and macrophage migration inhibition [61]. Another experiment reported that topical application of Be to susceptible mice generated Be-specific sensitization documented by peripheral blood and lymph node Be lymphocyte proliferation tests (BeLPT) and by changes in lymph node T-cell activation markers, increased expression of CD44, and decreased CD62L [45].

For the diagnosis of Be sensitization, positive results were obtained when dermal patch tests were applied to patients sensitized to Be [62], but one work indicated that the patch test itself may be sensitizing and may promote the condition of those already sensitized [63]. In fact, patch testing experiments with 1% of beryllium fluoride sensitized approximately 90% of a small number of volunteers. Testing at a lower concentration (0.1%) resulted in sensitization of less than 1% of test subjects [64]. On the other hand, the BeLPT has found widespread application in screening for Be sensitization in populations of exposed workers. Recent studies have linked markers such as HLA-DRAArg74 (HLA-DR3) to sensitization to Be. The marker might be linked to low interferon gamma (IFN-γ) production. In addition, sensitization to Be is with a gene for the cytokine tumor necrosis factor alpha (TNF-α), the TNF-α-308*2 marker [65]. Susceptibility to Be-hypersensitivity has also been associated with a mutation of the gene for the human leukocyte antigen HLA-DPB1, carrying a glutamate at position 69 [66].

Skin Be eruptions should be treated with avoidance of Be exposure, mid-potency to high-potency topical corticosteroids, compresses, and antibiotics to prevent secondary infection. When Be nodules are present, surgical excision is the definitive treatment [67]. A study demonstrates that, even with the implementation of control measures to reduce skin contact with Be as part of a comprehensive workplace protection program, measurable levels of Be continue to reach the skin of workers in production and production support areas. Based on the Authors current understanding of the multiple exposure pathways that may lead to sensitization, they support prudent control practices such as the use of protective gloves to minimize skin exposure to Be salts and fine particles [68].

CHROMIUM (Cr)

Skin contact with Cr and Cr-compounds occurs by alloys, cement, leather tanning, chemicals, anticorrosives, ceramic, wood preservatives, paints and varnishes, textile mordants and dyes, batteries, magnetic tapes, detergents and bleaches, electroplating and so on [14]. Variation in toxicity is associated with Cr(III) and Cr(VI); the former has a percutaneous permeability poorer than that of Cr(VI) resulting, thus, less able to elicit ACD [69].

In the European general population, the Cr allergy rate was approximately 4.5% in 2004. Such evidence was reported by the ESSCA working group that collected data from 31 dermatological departments in 11 European Countries (Austria, Denmark, Germany, Italy, Lithuania, Poland, Spain, Switzerland, Sweden, The Netherlands and United Kingdom). Both the lowest and the highest values were recorded in United Kingdom with 1.3% in Sheffield and 9.1% in Liverpool, respectively [11]. In Singapore and Turkey, the rate was similar (i.e., 5%) where the main sources of exposure were cement and tanned leather [70, 71]. Allergy in India has reached 10% and the cause was referable to the use of shoes without socks [72]. In most cases, the Cr allergy was more frequent in males than females. For example, in Czech Republic, percentages equal to 5.93% in males *vs* 2.81% in females were found and in Hong Kong 7.1% *vs* 2.3%. Again, in Turkey, the males were affected by Cr ACD 2.3 times more than women, and in US, this ratio was about 2 times in favor of males. The causes were related to the occupational activities in construction and leather sectors, and those involving machine operation or repair and in these last cases, Cr(VI) was present in anticorrosion coatings or Cr-plating [70,73-75].

Cement has long been known as a cause of Cr ACD. In fact, the raw material used for cement production contains Cr and, in the high temperature production process, Cr(III) is oxidized into water soluble Cr(VI) to be able to penetrate the skin barrier and thereby create sensitization. More than 20 years ago in Denmark, it was found that with the addition of iron sulphate to cement, the Cr(VI) could be reduced at less than 2 μg/g [76] and it was the basis for the risk reduction of ACD in construction workers [77, 78]. In 2003, the European Union (EU) has adopted this concentration as a safe limit in the Council Directive 2003/53/EC on marketing and use of cement [18]. Outside the EU, in the period 2000-2005,

57% of 86 Brasilian construction workers resulted positive to Cr [79]. Similarly, in Taiwan, the 12% of 153 cement workers was affected by Cr ACD. This percentage reflected the fact that the addition of iron sulphate in cement is not a common practice. In addition, the Taiwanese Authors reported that among the considered workers exposed to cement, those with TNF-α promoter-308 heterozygous genotype or GST-T1 null genotype had increased risk of chromate sensitization [80].

Cr(III), used in leather tanning process to stabilize proteins and give them resistance to degradation, may be converted in Cr(VI) by light or heat in the presence of oxidized fats or high pH in leather. Cr(VI) is responsible for leather-induced dermatitis. In this regards, a Danish investigation on the content of Cr(VI) in 15 tanned leathers evidenced a concentration in the range 4.1-16.9 mg/kg and 5 patients had positive skin reactions after leather contact. Considering that no correlation between eczema and Cr(VI) or Cr(III) alone in leather was observed, it was suspected that skin responses were the result of a combined Cr(III) and Cr(VI) allergy [81]. In India, there were 155 cases of footwear dermatitis where the contribution to the frequency of positive patch tests to chromate was the 45.8% [82]. It has also been suggested that a treatment to convert Cr(VI) in Cr(III) by soaking the tanned leather in 5% Vitamin C solution might prevent or minimize contact dermatitis [83].

Chromium contained in detergents and bleaches can increase the risk of ACD on the hand and forearm of women. In Italy, 8.4% of 65 cases resulted to be sensitized to total Cr contained in detergents at a mean concentration of 4.12 μg/g [84]. Household products marketed in Israel had very high total Cr concentration; in particular, above 5 μg/g in 56% of products; between 1 and 5 μg/g in 32%, and less than 1 μg/g in only the 12%. The labeling of the consumer products with regard to active ingredients was insufficient in most cases [85]. Iyer *et al*. found that the form under which Cr is present in detergents sold in India was Cr(III) and not Cr(VI). No reaction to the detergent bar with 40–50 μg/g of Cr(III) was observed in any of the Cr-sensitized volunteers and this finding confirmed the general opinion that Cr(III) did not elicit ACD. It is also recommended that, wherever possible, Cr(VI) should be replaced with Cr(III) in consumer products [86]. Basketter *et al*., on the basis of patch test dose-responses, repeated open application test (ROAT) responses in Cr allergic volunteers and finger immersion test results, recommended that household products should contain Cr(VI) < 5 μg/g or for a better protection < 1 μg/g. This last level makes the elicitation of Cr ACD highly improbable [87].

New causes of Cr allergy are related to daily activities as the use of cellular phone and playing the guitar. In the first case, patients showed erythema and papule in the hemilateral and preauricular region due to the handling of the phone and resulted positive to patch testing with chromate at different concentrations. This problem was caused by the chromate present in the plating procedure of the phone [88]. The second case referred to two musicians, which revealed a strong reaction to Cr contained in the guitar string [89].

Cases of Cr ACD have been provoked by orthopedic metal implants. Normally, the alloys used in implants are stainless steel (mainly Cr and Ni and trace of manganese and molybdenum) or vitellium (mainly Cr and Co and small amounts of Ni). In patients suffering from poor implants tolerance, skin eruptions in the vicinity of the prosthesis were observed and patch test demonstrated that 6 people out 14 were sensitized to Cr. The change of the prosthesis contributed to solve skin eruptions [90]. Again, Menezes *et al*. reported that 8 people showed a positive reaction to Cr before and other 2, after the placement of the orthodontic appliances and this positivity was observed most in males than in females [91].

Moreover, despite the EU has banned the use of Cr(III) salts in cosmetic products because being contaminated by Cr(VI) [19], cases of skin contact with Cr from cosmetics do exist. Sainio *et al*. determined total Cr (0.4-5470 μg/g) and water soluble Cr (< 0.25-318 ng/g) in 88 different eyeshadows and 9 products contained soluble Cr above 2 μg/g [92]. Moreover, in 11 body creams sold as "Ni-tested", the amount of Cr was ≤ 65 ng/g in 9 of them and 150 ng/g and 300 ng/g in 2, but these levels were well below the threshold for sensitization [93]. Also, cheap earrings available on the Italian market released Cr in artificial sweat, with the highest value equal to 0.253 $\mu g/cm^2$/week [94].

The immunotoxicological Cr(VI) form, after penetrating the cell membrane, is reduced to Cr(III) by the sulfhydrylic groups present in the cysteine or methionine. Once this complex has reached the lymph nodes, the memory of the T-cells is stimulated. In consequence of a new exposure to Cr, the T-cells are activated leading to lymphokines mediated ACD (type IV reaction) [95]. In addition, an vitro study on keratinocytes of healthy and sensitized volunteers evidenced that Cr(VI) was significantly cytotoxic, able to highly bound to keratinocytes, and to induce a powerful pro-inflammatory reaction with dose dependant release of interleukyn (IL)-1α [96]. Again, Burastero *et al*. demonstrated that exposure of dendritic cells (DCs) to different amounts of Cr(VI) increased the expression of membrane markers as CD86, CD80 and major histocompatibility complex (MHC) class II, suggesting that these variations can help in determining the immunotoxicity of this metal [97].

To evaluate the elicitation to Cr(VI), patch test is routinely used and the adoption of the 0.5% of potassium dichromate in petrolatum is recommended. Notwithstanding this, Cr patch test has some limitations. One is the pH value of the exposure medium; it has been reported that varying the pH value from 6.8 to 10 the penetration of Cr(VI) through full thickness human abdominal skin *in vitro* increased 100-fold [98]. Another is the time of application and the type of vehicle used to dissolve the allergen. In this context, shortened patch tests resulted in fewer reproducible positive reaction in subjects. It was observed that half of patients did not react to Cr(VI) in water after 6 hours of application and that absorption by the skin continued for up to 72 hours suggesting that more time is needed to favor skin elicitation [99]. As a complement to patch testing, *in vitro* tests seemed to be able to detect the activity of Cr. In particular, the Enzyme-Linked Immunosorbent Assay (ELISA) and Enzyme-Linked Immunosorbent spot (ELIspot) test demonstrated that Th1- and Th2 cytokines (especially IL-2 and IL-13) production were enhanced in the peripheral blood mononuclear cells (PBMCs) stimulated with Cr salts from patch test positive patients [100]. Fowler *et al*. determined the elicitation threshold for Cr(VI) by the immersion test.

Twenty-six patients already Cr(VI) sensitized were exposed to Cr(VI) by immersion of one forearm for 30 minutes per day on 3 consecutive days in a solution containing 25 μg/ml of potassium dichromate at pH 9.5, while the other arm was immersed in the alkaline buffer only. Ten subjects developed symptoms related to the Cr(VI) allergy on the arm immersed in the chromate solution [101]. Nielsen *et al*. used the test of the immersion of one finger in a solution of 10 μg/g of Cr(VI) for 10 minutes/day for 1 week to demonstrate that low levels of Cr(VI) are able to elicit dermatitis in sensitized subjects [102].

The repeated open application test (ROAT), where the allergen is applied for brief discontinuous periods, the application site is not occluded and lower and more realistic concentrations of the allergen are adopted, proving to be another valuable diagnostic test [69]. The ROAT was used to examine 17 Cr allergic individuals to determine their threshold value. The test was performed in two phases; in the first, solutions of 5 and 10 μg/ml of potassium dichromate containing 1.0% sodium lauryl sulphate were applied to the antecubital fossa of subjects 2 times per day with an interval of 6–8 hours for 1 week. In patients who did not show skin responses after a 1-month rest period, concentrations of 20 and 50 μg/ml of chromate were applied in the second phase using the same method of application. In particular, 8/14 individuals failed to react to 50 μg/ml, whilst 3/15 reacted to 5 μg/ml of Cr(VI). This found limit overlapped that of 5 μg/ml recommended for household products [103].

COBALT (Co)

Cobalt is largely present in the environment because of its application in different fields such as metallurgical and electronic industries, magnetic alloys production and building construction sector. Sources of Co also include ceramics, enamels, paints as drying agent, catalysts, dental prosthesis, jewellery, particular adhesives, household products, hair dyes, fertilizers and feeding for animal [14]. Considering the wide spread appliances of Co, cases of Co-induced ACD are not rare. In 2004, the ESSCA working group reports positive responses to Co in the 6.74% of the 10,000 patch tested subjects and Co is addressed as the third most important allergen. The lowest percentage of Co allergy is found in Denmark (1.1%) and the highest in Italy (17.6%) [11]. These rates are similar to those of other countries for the general population (i.e., the range reported is 5-10%) [70-73, 75,104-106] and Co dermatitis was mainly prevalent in females than in males due to the wearing of jewels or personal adornments [70, 75, 105,107]. Patient's age did not significantly change the distribution of Co positive reactions [107].

Cobalt is a well recognized cause of occupational ACD, which has been described in hard metal workers, construction workers, employees in the rubber, in pottery factory and glass-fibre-reinforced plastics industries, and printers [108,109].

Cobalt sensitivity may also be caused by exposure to domestic detergents, jewellery, ear piercing and dyes. Cobalt is found to be responsible for hand eczema in domestic work due to its presence in household products. Of isolated Co sensitive patients, 68% were housewives [110]. For this reason, in 1993, it was recommended that the amount of Co in household products should not exceed 5 μg/g to avoid elicitation; in 2003, the limit was revisited and lowered to 1 μg/g [87]. A recent survey of 95 detergent products by the Dutch authorities showed that approximately 90% contained < 1 μg/g of Co, and all were well below 5 μg/g. In those products, the highest level of Co was 0.28 μg/g [111]. The release of Co in artificial sweat from a necklace caused the development of vesicular eczema; the chain released a concentration of cobalt 40,000 times higher than the minimal elicitation concentration dose. On normal skin, the minimum eliciting concentration was 2.26 μg/ml [112]. Cobalt contained in the alloy replaced Ni with the aim of being in compliance with statutory requirements of the Directive 94/27/EC. Even so, the modification of the alloy resulted to be unsafe [113]. Moreover, a Co-containing alloy for jewels was developed and tested on Co allergic patients. 18% of them were found to be positive after 7-8 days of exposure, but the skin responses were less important than those produced by 1% cobalt chloride patch testing. This tolerance was because Co is compactly bound in the alloy by Pt [114]. Bocca *et al*. reported a release rate of Co ions in the range 0.013-0.188 $\mu g/cm^2$/week from the 40% of cheap earrings tested. These amounts are not likely to pose a risk for skin sensitization [94].

The practice of ear piercing and tattooing has increased the incidence of Co-induced ACD among young people. A Swedish study performed on 520 young men demonstrated that the 1% of them had Co ACD related to ear piercing and there was a higher prevalence of sensitization in patients with pierced earlobes [115]. In Japan, 9 out 106 pierced subjects had eczema and resulted to be positive to Co patch test, even if they did not significantly differ from non-pierced Co allergic patients [116]. Skin hypersensitivity caused by the presence of Co in the blue ink used for tattoo was observed. In particular, the tattooed patient suffered of urticaria on the tattooed right deltoid [117]. Kang *et al*. found Co in 4 different henna dyes at a concentration of about 3 mg/kg and, in their opinion, this amount was able to provoke sensitization but not contract dermatitis [118].

In addition, Co was determined in 88 colors of different brand of eye shadows. The Co concentrations levels were in the range < 0.5-41.2 μg/g and approximately 75% of the products contained more than the safe limit of 1 μg/g of Co. Although these amounts were low when systemic toxicological effects were considered, the Author's opinion was that the risk of acquire allergy in unsensitized subjects due to the use of these products cannot be excluded [92]. In a series of 11 body cream labelled as "Ni tested", Bocca *et al*. quantified Co; in 9 of them it was below 5 ng/g, while in 2 cases, Co increased up to 200 ng/g [93]. In both the two latter studies, the Authors pointed out the importance of declaring metals as impurities in the list of ingredients of a cosmetic in a framework of a higher consumer's protection.

Literature also reported cases of Co-based clothing dermatitis. In particular, a nurse with pruritic rush on the inner thighs and posterior calves resulted to be positive to Co. The metal was contained in the dyes used for manufacturing the blue trousers of the nursery uniform [119]. Again, another nurse working in an intensive care unit reported itchy dermatitis on the dorsum of both feet and toes due to Co contained in the green plastic shoes [120]. In both cases, symptoms disappeared on avoidance of trousers and shoes. Moreover,

an Indian study reported that the incidence of footwear dermatitis was 24.2% (155 patients out 640) and the occurrence for Co sensitization was the 38.1%. Authors traced back these findings to the habit to wear shoes without socks [82].

Dental treatment involves the use of various materials able to sensitize people creating clinical manifestations in the oral mucosa. In this context, in Israel, 6 people out of 121 (5%) reported Co oral ACD with symptoms as cheilitis and perioral dermatitis, burning mouth syndrome (BMS) and orofacial granulomatosis [121]. In addition, for the 5.2% of a US population of 307 patients the cause of oral lesions was attributable to Co in dental devices. In particular, 60% of the patients with positive reactions to Co had perioral dermatitis [122]. A single case of lichenoid on buccal mucosa and tongue was reported. The symptoms were in areas of contact with the fixation clasps and lingual bar of the denture. The disease was related to the Co/Cr content of the dental prosthesis and withdrawal of the device allowed remission of the lesion [123].

Another cause of non-occupationally ACD is related to the presence of Co in polyester resins or in ABS plastic used for PC mouse manufacture. The cobalt naphthenate is adopted as catalyst in such plastic production posing a risk for skin sensitisation [124]. Similarly, a patient reported hand eczema due to latex gloves. In this case, the Co ACD was due to the presence of the cobalt octoate in plastic, which is used as accelerator in the polyester resin production. The treatment of ACD included cotton lined PVC gloves to protect the hand [125].

Cobalt positive reactions are associated with nickel sulphate and/or potassium dichromate sensitivity [75,107]. In 2594 subjects, Co sensitivity was seen in association with positive reactions to Ni and Cr in 95.2% of cases [107]. Patients tested to Co, Cr and Ni, sensitized to any one of the metals had significantly higher odds of sensitization to an additional metal [75].

The main mechanism with which Co induces ACD is a T-cell mediated reaction (type IV reaction) with production and release of various cytokines, as also demonstrated by an *in vitro* study of Minang *et al*. In addition, some patients that reacted with Co *in vitro* also reacted with Ni, and patients patch tested positive to Co were *in vitro* negative for Co but positive for Ni. This fact corroborates the evidence that processes of co-induction between metals are more frequent than isolated reactions to Co [100]. Hypersensitivity of type I has also been reported for Co. In this regard, in a farm where hard metal tools were produced, 7 employers had asthmatic symptoms significantly associated with sensitization to Co. In fact, the specific IgE antibody against Co conjugated to serum albumin of patients was evidenced by the radioallergosorbent test [126].

The diagnostic patch test used in the European standard series depicted 1% cobalt chloride in petrolatum. This concentration may however elicit non-allergic porous reactions. Cobalt chloride was used in the human and guinea pig maximization tests (GPMT), proving to be an allergen of grade 3 and 5, respectively (on a scale with the highest grade equal to 5) [127,128]. A diagnostic *in vitro* test was performed by Moed *et al*. on PBMCs of allergic patients and healthy volunteers in the presence and absence of Co. The addition of type 1 (IL-7 and IL-12) and type 2 (IL-7 and IL-4) stimulating cytokines allowed the significant IFN-γ and the IL-5 secretions in the presence of the allergen. These results showed increased proliferative capacity and cytokine production by allergen-specific T-cells from allergic patients, but not in healthy individuals [129].

No regulation that limits Co in consumer products to prevent contact dermatitis has been released, as was done for Ni. Considering that Co is a potent skin sensitizer, the replacement of Ni with Co in the various product could create the risk for an increment of ACD due to this metal. The best way to prevent the flare-up of ACD in a sensitized individual is to avoid direct skin contact with the allergen. When this is not possible, the prevention can be obtained through the use of particular creams that contain chelating agents. In this regard, the preventive effect of 10% diethylenetriaminepentaacetic acid (DTPA) in an oil-in-water emulsion in Co-sensitized patients has been demonstrated [130].

COPPER (Cu)

Copper finds large use in coins, personal adornments (clasps, pins, belt, necklaces, buttons, hooks, etc.), jewellery, dental restorations (oral prosthesis, bridges, band, wires or cements), pipes and contraceptive objects as intrauterine devices (IUDs). In addition, organic and inorganic Cu salts are also used in agriculture as algicides and fungicides [14].

Copper has a low sensitizing potential and, thus, it is considered to be a rare cause of ACD. For this reason, the low number of cases of Cu allergy did not allow to calculate the prevalence among the general population in terms of percentage [131].

The most reported clinical symptoms of ACD are related to the use of Cu-containing IUDs and dental prosthesis. A woman user of an IUD reported skin eruption some day before menstrual cycle and the severity improved with the onset of the bleeding [132]. In another case, a patient showed diffused urticaria, angioedema of the eyelids and the labia major and minore [133]. In both cases, the IUD users positively reacted to copper sulphate and removal of the IUD led to complete remission of the symptoms. With reference to dental devices, Wöhrl *et al*. suggested that a high percentage (15.2%) of children sensitized to Cu was due to the increased use of this metal in dental amalgam [134]. In the same way, a woman developed Cu ACD of the oral mucosa caused by the long-term exposure to Cu enriched dental amalgam fillings [135]. Houger *et al*. observed a relationship between intraoral metal ACD (i.e., mucositis) and pathogenesis of squamous cell carcinoma. Because of this high prevalence, Cu was considered an additional risk factor in the evolution of cancer [136]. Additionally, a case of a woman with lesions of oral lichen planus due to the Cu contained in her prosthesis has been reported. The change of the prosthesis made the lesions improved [137].

The Cu contained in objects in contact with the uterine and oral environment is oxidized and free Cu ions are released. The ions, through the blood stream and the lymphatic circulation, reach the skin and mucosa where the T-cells recognize the allergen creating the basis for developing systemic ACD. In case of dental materials, reactions can be immunologic contact stomatitis (type I reaction) or delayed contact stomatitis (type II reaction). Free Cu ions release

from IUDs can react with the proteins resulting in a complete antigen able to activate both the IgE antibody production (type I reaction) and the cellular immune reactions (type IV reaction) [131]. The above reported Cu oxidation process has been demonstrated by an *in vivo* study of Hostynek *et al*. on healthy volunteers. It has been shown that Cu may penetrate the stratum corneum of the skin after its oxidization and further become complex with the skin exudates. The rate of the process depends on the time of contact and the amount of oxygen present [131,138]. Moreover, it has been suggested that Cu has an epidermal permeability coefficient (max 10^{-4} cm/h) even higher than that of Ni (max $10^{-5}/10^{-6}$ cm/h) and this is why immune reactions to Cu seem to proceed at a higher speed in comparison to those to Ni; in addition, application of copper oleate to the human skin resulted in a significant increase of urinary Cu levels [139].

Other cases of induced Cu ACD are rare; in this context, a single case of ACD developed on fingertips, upper eyelids and the outer canthi of a bingo-hall worker's caused by the Cu present in the 2-euro coins has been reported [140]. Additionally, a case of a woman affected by ACD placed on the right upper arm due to the Cu present in the composition of a microphone used in an ambulatory was reported [141]. In the study of Nakada *et al*. performed on 107 subjects having their ear pierced, 9 of them were found positive to Cu patch testing [116]. Most of the common Au-alloys used for jewellery contain silver and Cu as less noble compounds. In two surveys, all alloys were found to release considerable amounts of free Cu ions into synthetic sweat [142,143]. Finally, pool swimmers presented greenish discoloration of their natural colored hair. This particular symptom was related to the Cu pipes used to build swimming pool [144].

There was a high incidence of Ni sensitization in Cu sensitive subjects. The statistical association of Cu and Ni hypersensitivity was extremely strong. In light of the possible Cu-Ni cross-sensitization, it is unsafe to suggest to cover Ni goods with a layer of Cu to protect individuals allergic to Ni [134]. In 30 patients known to be contact sensitive to Ni but patch-test negative to Cu, the severity of patch test reaction to a Cu/Ni mixture was greater ($p < 0.001$) than to Ni alone, suggesting that ions enhanced the sensitivity reaction to Ni. The authors proposed that the presence of Cu ions facilitated the formation of Ni protein complexes in the skin even though the precise mechanism is unclear [145].

A few studies observed that occupational exposure to Cu can cause dermal problems; for example, indurated erythematous areas of the face, neck, chest and forearms, periungual telangiectasia and nail changes were noted in a group of female laborers occupationally exposed to fertilizers, weedkillers and a copper sulphate-containing fungicide [146]. Contact dermatitis was reported in 10 furniture polishers using commercial spirit (ethyl/methyl alcohol) colored blue with copper sulphate. All patients developed erythema, itching and vesiculopustular areas on the skin of the hands. The symptoms improved avoiding contact with spirit [147].

At present, the threshold-inducing sensitization concentration to be used in patch tests and the concentration able to create elicitation in sensitized subjects are still not defined. Anyway, it is copper sulphate is generally used at the level of 2% in petrolatum. With this concentration, 1% of positive reactions were observed in Sweden and 3.5% in Austria, but in both cases, these results were considered of low clinical importance [134, 148]. In addition, a concentration of 5% in petrolatum of copper sulphate was indicated as adequate for the patch testing of Cu hypersensitivity even if the test reproducibility was modest [134]. Also metallic Cu foil was found to be a valuable tool for the diagnosis of Cu hypersensitivity in 2 of 26 patients [149]. Intradermal tests to confirm positive patch tests and individuate false-negative reactions have been used. For example, intradermal test is capable to distinguish the irritant from allergic nature of a patch test. The concentration injected was equal to 0.1% or 0.01% of copper sulphate and readings were done after 48 hours from the injection [150]. In order to evaluate the sensitizing potential of Cu two predictive immunological tests have been taken into account. They are the GPMT and the local lymph node assay (LLNA). In the first case, Cu resulted to be a weak sensitizer, while, in the second case, an increased lymph node cells proliferation in the presence of Cu salts was observed, resulting in a positive test response [131].

No regulations reporting limits for this metal in products are existing, and in order to prevent Cu, ACD creams containing chelating agents can be adopted by sensitized patients. This is the case of 10% DTPA, which has revealed significant capability to abrogate positive patch test reactions to Cu [130].

GOLD (A**u**)

Allergic contact dermatitis to Au was felt to be rare in the past, but from the beginning of 1990s when Au has been added to the standard screening series in many countries around the world, an increasing prevalence of positive patch test reactions has been documented. In the US, Au was patch tested as gold sodium thiosulfate (GST) (0.5% in petrolatum) and, in 1998–2000, it ranked as the sixth most frequent cause of positive patch test reactions [151]. Similar prevalence was observed in Europe and Japan. In a large Swedish study, 8.6% of 832 patients with suspected contact allergy on routine patch testing gave a positive response with GST. Other patients with contact allergy to GST also gave positive reactions to potassium dicyanoaurate, but were negative to gold sodium thiomalate (GSTM) and metallic Au [152]. These findings were confirmed by another group of investigators, who found that 4.6% of 278 patients in United Kingdom had positive reactions to GST on routine testing [153]. All of these patients were females, with a mean age of 37 years and the most frequent site of eczema was the head and neck. In Japan, 8.4% of 653 patients tested from 1990 to 2001 showed a positive reaction to gold chloride, and also in this work significantly more women (10.2%) than men (0.8%) reacted [154]. A study by Bruze *et al*. reported that a large percentage of the patch tests was long lasting, and 35% developed late reactions [155]. In a number of cases, positive test sites were seen to remain negative after 3 days, but to turn positive by day 7. These findings emphasize the necessity of a second patch test reading at a distance of 1 week, at least [156, 157].

Gold salt therapy, restorative materials in dentistry, orthopedic appliances and jewellery are the most accepted causes for Au ACD. Medical practitioners have long recognized the adverse effects, including ACD, in the risk-benefit balance of the usage of Au in anti-inflammatory therapy. In

particular, an increasing incidence of delayed skin reactions has been noted since the introduction of GST and GSTM in the treatment of rheumatoid arthritis. Allergy to Au was seen in more than 50% of patients so treated, as indicated by patch testing with GSTM [158]. Patients developed dermatitis, stomatitis, and eosinophilia, and less commonly immune-complex glomerulonephritis, lymphadenopathy, antinuclear antibody, increased serum IgE and other blood disorders. Although this has led to a decline in the therapeutic use of Au salts in recent years, their history in the treatment of arthritis has given rise to a growing recognition of the importance of Au as a sensitizer [159].

Gold-based dental restoration appeared to be an important risk factor for Au ACD. Several Authors have found that a positive patch test to Au is significantly correlated with Au dental restorations [160,161]. The saliva may slowly dissolve Au and transport it through the mucous membranes into the bloodstream [159] and the amount of dental Au has been found to be correlated qualitatively and quantitatively to the blood level of Au [162,163]. Oral lichenoid mucositis, clinically and histologically similar to oral lichen planus, were observed at sites directly adjacent to Au dental restorations. A study of Yiannias *et al*. retrospectively reviewed 46 patients with oral lichenoid lesions who had also been patch tested; 2 patients who were sensitized only to Au showed marked clinical improvement with removal of their dental Au restorations [164]. Hypersensitivity to Au has been reported in students involved in the manufacture of prosthetic materials in a dental clinic in Japan, and 3 of 12 individuals tested had positive reactions to sodium thiosulfatoaurate [165]. Moreover, implanting a Au-plated stent seemed to represent a risk of sensitizing the patients to Au. In the stent group, 45.5% of patients had a contact dermatitis to Au while in the control group, 20.0% of subjects reacted and this difference was significant [166].

More recently, it has been realized that more mundane uses of Au in the form of the diverse alloys used in jewellery bring with them the risk of sensitization. The risk is greatest when Au-containing alloys are introduced and left in permanent contact with live tissue, as occurs in the practice of piercing the skin of the ears and other parts of the body for decorative purposes. Dissolution of metallic Au is notoriously difficult, but the process is facilitated by the presence of other metals in the alloy or in the neighborhood [167,168]. Evidence that ear piercing increases the risk of Au sensitization is that there were significantly more positive reactions to 0.2% gold chloride in the patients with pierced than in patients without pierced ears [116]. Gold allergy often presents as dermatitis at the site of jewellery contact, i.e., earlobes and fingers, but it also may present solely as eyelid dermatitis [169]. In 1988, Fowler reported 2 women with eyelid dermatitis and positive patch tests to Au whose eruptions cleared with avoidance of Au jewellery. It was postulated that the allergen was being transferred from the hands to the eyelids as is commonly seen with allergic reactions to tosylamide formaldehyde resin [170]. In Portugal, contact allergy to GST and to potassium dicyanoaurate was found in 23 patients, all the reactors were women and had their ears pierced with Au earrings [171]. Ehrlich and Belsito found that 7 of 15 Au-allergic patients cleared their dermatitis by not wearing Au jewellery [172]. In Spain it was described that a lady presented Au ACD in the proximal root of a finger due to her wedding ring [173].

The presence of Au in metallic form has been visualized in human skin biopsies taken from areas of prolonged contact with the metal such as rings and jewellery, confirming absorption of the solubilized metal even through the intact stratum corneum [167]. In some cases, hypersensitivity to Au was associated with the formation of intracutaneous nodules in the earlobes at the sites of piercing. The nodules at pierced sites were described as lymphocytoma cutis, indicating the formation of a benign lymphocytic infiltrate, which is distinguishable from malignant lymphoma. When this did not resolve over time, nodules had to be removed surgically [174,175].

The immunomodulatory mechanism of action of Au is still largely unknown, but Au accumulates in lysosomes of macrophages [176] and inhibits lymphocyte maturation, differentiation, and function *in vitro*. It could also be established histochemically that Au is selectively taken up by Langerhans cells in whole viable human epidermis [177]. Furthermore, GSTM inhibits expression of IL-1b [178] and TNF-α [179] by inhibiting transcription factors, such as activator protein-1 or nuclear factor kB, as well as by inhibiting specific caspases needed for post-translational modification of IL-1b and IL-18 [178]. Gold also inhibits T-cell proliferation in an assay similar to that described for Ni [180]. However, in contrast to Ni, pre-treatment of the antigen-presenting cells (APCs) with Au before exposure to the peptide still inhibited proliferation, suggesting direct binding of Au to the major histocompatibility complex (MHC) molecule, rather than to the peptide as is the case with Ni. Further support for this interpretation comes from the observation that radiolabeled Au bound to a panel of MHC class II-positive, but not class II-negative, cell lines [180]. On the other hand, there is evidence that Au(I) (as disodium aurothiomalate) forms complexes with MHC-binding peptides containing two or more cysteine residues and inhibits T-cell receptor binding [181].

The recommended diagnostic procedure for testing Au allergy is the skin patch test, but the test reading has to be performed also after 1 week because of the late-appearing positive reactions. As a valuable complement to patch testing in the diagnosis of Au allergy, some studies have proposed the lymphocyte transformation test (LTT) [182]. Lymphocyte proliferation *in vitro* shows good correlation to allergic epicutaneous test reactions to Au [183, 184]. The use of cytokine fingerprinting has also been evaluated for their possible diagnostic allergenic properties [185]. Several cytokines, IL-1 receptor antagonist (ra) and TNF-α in particular, are instrumental in the pathogenesis of the Au ACD, in the primarily elicited eczematous reaction as well as in the endogenous flare up after systemic provocation [186]. The TNF-α as well as the IL-1ra are released in blood when GSTM is given by intramuscular injection to a subject with contact allergy to GST [187]. The release has been shown to be specific and activated only by the proper contact allergen [188].

MERCURY (Hg)

The inorganic and organic (methylated) species of Hg are well recognized toxicants [189,190], and during the past decades, the steps to limit the environmental exposure to Hg

have successfully reduced the cases of acute poisoning. Nowadays, the primary source of exposure to inorganic Hg is probably through its use in dental amalgams [191], even if Hg is still a component in some preservatives such as thiomersal. Symptoms of an amalgam allergy include skin rashes in the oral, head and neck area, itching, swollen lips, localized eczema-like lesions in the oral cavity. These clinical signs usually require no treatment and will disappear on their own within a few days of exposure. However, in some instances, an amalgam filling will have to be removed and replaced with a filling made of another restorative material, such as resin or porcelain even if these substances are more expensive and less pliable than amalgam. In patients showing positive patch test reactions with Hg-compounds, the placement or removal of amalgam fillings has led to significant improvements [192].

Contact allergy to Hg-compounds is important in the pathogenesis of oral lichen planus, especially in case of close contact with amalgam fillings and in the absence of concomitant cutaneous lichen planus. Thirteen patients with symptomatic oral lichen planus had been shown by patch testing to be allergic to ammoniated mercuric (AM) chloride. In some cases, the resolution of symptoms was dramatic following the replacement of one or two fillings [193]. Moreover, a strong association between orofacial granulomatosis and contact allergy to Hg-containing dental fillings has been reported [121]. Mercury is thought to be an allergen implicated in BMS as well as in the systemic reactivation of ACD. Patch testing with dental series has a greater sensitivity in BMS patients [194].

The 1998–2000 North American Contact Dermatitis Group (NACDG) data base reported thiomersal to have a definite or probable relevance in 2.9% of the patients with a positive test. Thiomersal may be found in topical medications, especially ophthalmic and nasal preparations, cosmetics, and as a preservative in vaccines and contains organic Hg and thiosalicylate [195]. Positive patch test reactions to one or both the constituents of thiomersal have been frequently encountered. Thiomersal resulted to be the fifth most common allergen in patients with a positive patch test and it was found to be "possibly relevant" in 7.8% of those patients tested, with a single patient having "probable relevance" [195]. In a group of patients with increasing strengths of thiomersal by intramuscular injection of 100 μg/ml solution, 9% of them highlighted a mild local reaction consisting of induration and micropapules [196].

Systemic contact dermatitis induced by Hg is provoked by Hg-containing creams and contact lens solutions, inhalation of Hg vapor and broken thermometers [197]. Systemic contact dermatitis is the result of a systematically administered allergen reaching the skin from the circulatory system and producing a generalized rash. Symptoms as nausea, vomiting, headache, malaise, arthralgia, and diarrhea can be related to systemic contact dermatitis [198]. The use of skin whitening creams in developing countries is a recognized cause of chronic Hg poisoning. In Indonesia, a 34-year-old woman with membranous nephropathy used regularly a skin-whitening cream containing Hg; her blood and urinary Hg levels were elevated and symptoms improved after she stopped using the cream [199]. In Taiwan where skin-lightening creams are widely used, a total number of 507 individuals reported facial dermatitis and 308 had eczema confined to the face. The two most frequent allergens were found to be Ni and AM, and the majority of AM-sensitive cases resulted from cosmetics [200]. A case report involving a 25-year-old woman presented with an itchy erythematous bullous dermatitis, restricted to the region around the eyes and mouth, which was the area of application of a Taiwanese whitening cream. The Hg concentration in cosmetic resulted to be the 7.2% w/w, and patch testing was positive to mercury chloride and AM [201]. Among 314 cream users (99% women), the majority had increased urine or blood Hg concentrations, while the symptomatic Hg poisoning from dermal application appeared at a concentration of Hg higher than 57,000 μg/g [202]. A 42-year-old woman presented facial Hg pigmentation, raised levels of Hg in the blood and urine and possible neuropsychiatric toxicity after the topical application of a cream containing 17.5% of AM chloride. Health workers, particularly pharmacists and medical practitioners, should be aware that over-the-counter Hg-containing creams may raise the level of Hg in the body to potentially toxic levels. A warning on the package should be considered and use of the cream restricted [203]. Cases of symmetric flexural exanthema 'baboon syndrome', which is considered as a systemic contact dermatitis have been induced from Hg exposure. For example, inhalation of Hg vapor from a thermometer in sensitive patients accounts for most instances of this condition [204]. Another case of Hg exanthema with its vesiculobullous clinical presentation and late onset of lesions after Hg exposure to a broken thermometer has been reported. Serum and urine Hg levels were both increased with respect to normal and a remission was observed after a period of oral corticosteroids [205].

New hidden sources of Hg in consumer goods, as piercing, tattoos and polyvinyl chloride (PVC) boots, may represent a potential source of danger for the future if the use is not more strictly regulated. The number of positive reactions was significantly greater among patients with pierced ear lobes (29/107) than among those who did not have pierced ears (29/270) [206]. Patients with baboon syndrome and Au dermatitis due to ear-lobe piercing were tested with 0.05% mercuric chloride applied for 2 days; 5 of 5 patients with baboon syndrome were patch-test positive, 21 out 35 of those had pierced ears [116]. Allergic reactions to metal salts used in tattooing are surprisingly frequent. Mercury together with Cr and Co have been reported as contact sensitizers with various type of skin reactions in tattooed areas [207]. In particular, the red tattoo pigments (cinnabar and vermilion) are known to include Hg, and are able to produce a delayed hypersensitivity reaction [208]. A 5-year-old child, with previous skin intolerance to mercurochrome, developed a severe ACD of both feet when wearing new PVC boots. Within a few days, he developed a Hg exanthema in legs, groins and lateral parts of the trunk. He had strong patch test reactions to both organic and inorganic Hg-compounds and in particular to mercury chloride (in 0.01% petrolatum), which was identified by atomic absorption spectrometry and polarography in the boots worn [209]. A 24-year-old woman, with a previous history of contact sensitization to mercurochrome, attended an erythemotous vescicular dermatitis on both ring fingers that corresponded to the areas in contact with her wedding ring. Interestingly, the X-ray fluorescence analysis

revealed the presence of Hg on the ring surface just in the place of the developed dermatological disease [201].

Doses of mercury chloride can elicit an increase in the IgG and IgM formation from spleen cells and in the IgM, IgG and IgE production in serum of Balb/c mice. These results suggested that Hg was able to develop histopathological changes in lymphoid tissues [210]. A specific issue is whether the immune syndromes following Hg exposure are related directly to the immune system activation by Hg, or are secondary to Hg-induced tissue damage. The autoimmune profile of elevated antinuclear antibodies observed after treating mice with Hg salts would support the latter hypothesis [211]. The main target of autoantibodies is the ribonucleoprotein fibrillarin, which may also be a target in scleroderma patients [212]. Donor T-cells from mice treated for 1 week with subcutaneous injections of mercury chloride were used in a popliteal lymph node assay. Cells mounted a response to mercury chloride and to splenic proteins isolated from mercury chloride-treated mice. The Hg content of the splenic proteins from the treated mice was 1.4 pg/mg protein. On the other hand, after 8 weeks of mercury chloride treatment, donor T-cells reacted poorly with mercury chloride and Hg-containing splenic proteins, but reacted strongly to nuclei and fibrillarin, whether isolated from Hg-treated or untreated animals. It was suggested that activation of T-cells by Hg-altered nuclear proteins may eventually result in the activation of T-cells specific for the unaltered self protein [212].

In a study, 18 patients with oral lichen planus, adjacent to amalgam fillings, were tested *in vitro* with an optimized lymphocyte proliferation test, memory lymphocyte immunostimulation assay (MELISA), and with a patch test. Twenty subjects with amalgam fillings but without oral discomfort and 12 amalgam-free subjects served as controls. The results show that patients with lichen planus have significantly higher lymphocyte reactivity to inorganic Hg compared to control groups. Removal of amalgam fillings resulted in the disappearance of oral mucosal changes, thus indicating a causal relationship. Positive responses to phenylmercury, a bactericidal agent in root fillings and in pharmaceutical preparations, were also noted in the oral lichen group but not in the control groups. Thus, low-grade chronic exposure to Hg may induce a state of systemic sensitization as verified by Hg-specific lymphocyte reactivity *in vitro* [213].

PLATINUM GROUP ELEMENTS (PGEs)

The platinum group elements (PGEs) – platinum (Pt), palladium (Pd), rhodium (Rh) and iridium (Ir) – are rare in the earth's crust in comparison with other elements. In contrast, their specific physical and chemical properties have led to the development of some highly sophisticated technical applications, especially in the field of catalysis. They are also used in making jewellery and in dentistry. The general population may come into contact with PGEs mainly through mucosal contact with dental restorations and jewellery containing PGEs, and possibly *via* emissions from automobile catalysts.

Platinum is a highly reactive transition metal that is most likely to be sensitizing as a chlorinated soluble compound. A study shows that Pt salts have *in vitro* immune effects and their potency is ranked in the following order: diammonium hexachloroplatinate > diammonium tetrachloroplatinate > sodium hexaiodoplatinate and cisplatinum > platinum tetrachloride > platinum dichloride. Certain Pt salts also affect lymphocyte proliferation and cytokine release (TNF-α, IFN-γ, and IL-5) [214]. "Platinosis" refers to type 1 reactions to Pt and may develop in over 50% of exposed workers, with rhinitis, conjunctivitis, bronchial asthma upon provocation with chloroplatinates. Type 4 hypersensitivity reactions to Pt may also occur, but has not been proven by large-scale patch testing [215]. A major source of occupational exposure to Pt is in the manufacture and recycling of automobile catalytic converters, where the exposure is predominantly to the chloroplatinic acid catalysts [216]. Elevated IgE levels have been observed in some Pt-exposed refinery workers [217]. A case of contact dermatitis from wearing a Pt ring has been reported [207], and contact urticaria has been observed following occupational exposure to the antineoplastic agent cisplatinum [218].

Palladium is increasingly used in industry, jewellery and dentistry since the European Directive restricted the use of Ni in all products placed in direct and prolonged contact with the skin. For this reason, during a 10-year period, the trend of sensitization to Pd in a clinic population increased to a maximum of 9.7% in the year 2000, with a higher percentage in females than in males. In the majority of cases, subjects were polysensitized (92.8%), but 7.2% of subjects were positive to Pd alone. Of Pd-sensitized patients, 40.5% complained of hand dermatitis, 47.4% complained of body dermatitis, and 1.7% complained of BMS [219]. As observed for Pt, the immune capacity of Pd depends on speciation. The diammonium hexachloropalladiate showed stronger dose-related inhibitory effects than the diammonium tetrachloropalladiate and palladium dichloride. It has also been demonstrated that the *in vitro* activity of Pd compounds is higher than that of Pt and Rh salts [220]. There are several reports on Pd sensitivity associated with exposure to Pd-containing dental restorations [221-225]. Symptoms observed included signs of contact dermatitis, stomatitis and mucositis, and oral lichen planus. General symptoms like swelling of the lips and cheeks, dizziness, asthma, chronic urticaria, and other symptoms have also been reported. In some case reports, complaints disappeared after replacement with Pd-free (or metal-free) constructions. Perhaps the most interesting aspect of Pd^{2+} sensitization is its frequent specific cross-sensitization with Ni^{2+} [226-228]. The similarities in chemistry of Ni^{2+} and Pd^{2+} support the idea of a similar mechanism involving common protein binding sites and conformational alterations [229]. A study with > 10,000 of participants tested with about 25 allergens, confirmed that of all patients 5.4% reacted to palladium dichloride alone, whereas all other patients also had a positive reaction to nickel sulphate [230]. Very few reports are available on non-dermatological populations; in the study of Kanerva *et al*. comprising 700 schoolchildren, 7% had an allergic patch test reaction to palladium chloride (11% in girls and 1% in boys) [231]. Among the case reports, two cases of sarcoidal-type allergic contact granuloma due to Pd in ear piercing have been presented, the first to Pd only, and the second to Pd in combination to other metals [232]. Moreover, a case of developed dermatitis at contact sites of metallic spectacle frames which were declared as 99.7% Ti but with Au-plating

using Au (90%), Cu (3%) and Pd (7%) has been observed [233].

Rhodium and Ir are sometimes reported as sensitizers in the form of salts, though not as metals, in subjects employed in precious metals or jewellery industries [234, 235] or with dental amalgams or prostheses [236, 237]. It was observed that the activity on the PBMCs proliferation and the cytokine release of diammonium hexachlororhodate was slightly higher than that of rhodium trichloride [220]. During 2001-2002, 720 consecutive informed eczematous patients were patch tested with 1% rhodium chloride and 1% iridium chloride, both in water. None of the 720 patch tested subjects showed positive or irritant reactions to iridium chloride, but 2 were found to have a positive patch test to rhodium chloride as well as other metals. These study results suggested that Rh and, above all, Ir are allergologically safe even in patients sensitized to metals [238]. In one series of Pt refinery workers, positive prick tests to other metals as Ir, Rh and Pd were observed, but all these workers also tested positive to Pt, and cross-reactivity had been proposed [239].

As regards prevention strategies, since PGEs-containing dental or jewellery alloys have been identified as a possible source of sensitization, protection of the public from related adverse effects may be achieved either by limiting the use of certain alloys or by the use of alloys with high corrosion stability and thus minimal release of PGEs. It is also recommended that dentists world-wide should be informed of the composition of alloys and of possible sensitization effects of PGEs. Further, patients should be informed about the composition of dental alloys and those patients who have an allergy to Ni should be informed about the effects of PGEs-containing dental materials. In industry, personal protective equipment should be used to prevent skin contact with PGE compounds.

TITANIUM (Ti)

Titanium and its alloys are used for medical appliances like osteosynthesis, arthroplasty, pacemaker encasing, teeth- and arch-wires, or in daily-use articles like body piercing and spectacle frames. This broadened spectrum of Ti applications depends on the unique property of nitinol - which is an alloy based on 50% Ni and 50% Ti - of having shape memory effect, i.e., the material can undergo substantial plastic deformation and be triggered into returning to its original shape by heating. There are also Ti-Al-vanadium (V) alloys (β-titanium) and Ti-Co alloys on the market today and other alloys under an assortment of trade names. Also "pure Ti" may be used in implant materials and spectacle frames, even if products marketed in this way contained Ni traces as a result of the production process.

The existence of ACD to Ti is still under discussion due to incomplete allergological work up and insufficient patch test preparations. However, reports on suspected delayed-type hypersensitivity reactions to Ti do exist. Titanium is firstly reported as an allergen of pacemaker system contact dermatitis. A patient with implanted cardiac pacemakers presented redness, swelling and pruritus of the skin overlying the pacemaker several weeks after insertion. These reactions were interpreted as contact sensitivity to pure Ti encasing of the pacemaker because of a ++ patch test reaction to a thin square of metallic Ti applied with artificial sweat [240]. Granulomatous dermatitis after implantation of a Ti-containing pacemaker was also observed both by Brun and Hunziker and Viraben *et al*., even they were unable to detect positive reactions to neither titanium dioxide nor to a square of the metallic pacemaker base [241, 242]. Yamauchi *et al*. utilized a different approach to evaluate Ti dermatitis induced by a pacemaker. They prepared eluates from Ti encasing coincubating it with the serum of patients. An intracutaneous test with the eluate gave a positive reaction at the second day together with an *in vitro* lymphocyte stimulation [243].

With regard to orthopedic implants, Lalor *et al*. described sensitivity to Ti in patients with failed Ti-based total hip replacement, in whom periimplantar tissue showed lymphohistiocytic inflammation. Patients showed a positive skin test to an ointment containing 20% titanium dioxide, 5% titanium peroxide, 3% titanium salicylate and 0.1% titanium tannate in a silicone-paraffin base, but they did not react to the Ti salts administered alone. Moreover, EDX microanalysis of tissues from all the cases demonstrated that the particulate debris in the macrophages and the surrounding matrix was Ti [244]. Another case of impaired fracture healing and eczema localized to the perioperative area upon Ti-based osteosynthesis has been observed. During patch testing, no reactions to Ti developed, but when the LTT was applied, the patient's lymphocytes showed markedly enhanced *in vitro* proliferation to Ti. After removal of the Ti material, fracture healing was obtained, the eczema cleared, and also the *in vitro* hyperactivity to Ti disappeared [245].

Moreover, an episode of skin irritation around percutaneous implants for hearing aids has been described but allergological testing was negative in this patient [246]. In addition, gingival hyperplasia adjacent to intraoral Ti implants has been reported and following substitution of the Ti abutments with custom-fabricated Au abutments, the epithelial condition returned to normal [247].

Concerning Ti alloy used in body piercing, lymphocytoma cutis has been reported in two cases of women wearing Au-pierced earrings; zinc was detected by SEM-EDX microanalysis from the specimen of case 1 and Au and Ti from case 2. This study demonstrates the existence of metal fragments in the lesion, which may suggest the permanence of metal for 20 years [248]. Moreover, a 68-year-old man who had pierced his ears approximately 10 years earlier developed nodules at the sites of piercings. Microscopic examination demonstrated epithelialized tracts surrounded by a granulomatous infiltrate of macrophages, lymphocytes, and plasma cells. Closer examination revealed minute brown-black particles within macrophages and SEM-EDX microanalysis demonstrated the particles to be composed of Ti, Al and V [249].

Contact dermatitis from topical exposure to Ti compounds is rare. In one report, patients presented an adverse reaction to titanium lactate used in a deodorant [250]; another paper observed generalized eczema in a patient working with melted Ti in a confined space [251]. Nanoparticles of titanium dioxide are added to various paints and tattoo pigments as a brightening agent, and is also a common ingredient in sunscreens as a physical blocker of ultraviolet light. In a recent study, a commercially available blue ink was revealed to contain a high concentration of Ti (36.82%)

by quantitative EDX microanalysis [252]. In another work, it is speculated that titanium dioxide contained in cosmetics and sunscreens may adsorb Au particles in jewellery that occasionally contacts facial skin and causes contact dermatitis on this area despite the absence of dermatitis under Au jewellery worn on the hands [253].

Because standard Ti alloys (TiAl6Nb7, TiAl6V4) and pure Ti discs were shown to contain up to 0.034% Ni as impurities, this metal may further act as elicitor of hypersensitivity in cases where reactions are falsely attributed to Ti material itself [254]. Furthermore, in Ti spectacle frames it was Pd which acted as the alternative allergy elicitor [255], whilst Ni, Co and Pd were responsible for allergic reactions in frames erroneously declared as being made of Ti [256].

All these different case reports reflect the difficulty in evaluating suspected Ti hypersensitivity also in consideration of the fact that no standardized valid patch test preparation exists for this. A Japanese study has suggested that patch testing with the 0.1% and 0.2% titanium sulphate solutions and 0.1% and 0.2% titanium chloride were successful reagents for Ti skin-patch tests and can be a valuable alternative to the patch testing with titanium oxide [257]. On the other hand, using LTT, the sensitization to Ti might be revealed with a higher sensitivity [258]. Recently, it has been proposed that the optimized version of LLT, i.e., MELISA, had a greater potentiality in diagnosing hypersensitivity to Ti. In a recent study, 56 patients chronically exposed to Ti *via* dental or endoprosthetic implants presented clinical symptoms and were subjected to the MELISA test against 10 metals including Ti. Of the 56 patients tested, 21 (37.5%) were positive to Ti. On the contrary, when patients were patch-tested, all resulted to be negative to Ti. Following removal of the implants, patients showed remarkable clinical improvement [259].

To explain the sensitivity to Ti, several hypotheses have been proposed. Under favorable conditions (acidic pH, mechanical friction), Ti implants may corrode and release ions; for example, exposing the surface of nitinol to an acidic environment, a substantial leaching of Ti and Ni was observed [260]. This mechanism has been suggested to play a role in the loosening of implants. Furthermore, Ti has a high affinity to proteins; Ti-bound cell membrane proteins (neo-antigens) might induce autoimmune reactions, whereas Ti-bound intracellular proteins might disrupt normal cell physiology [261]. Finally, Ti has been reported to activate macrophages, either directly or subsequent to phagocytosis. Such activated macrophages may secrete both pro- and anti-inflammatory cytokines [262].

CONCLUSION

The ACD is a skin disease that today affects millions of people worldwide. Allergens in contact with the skin can develop different immunological responses and in many cases be so severe to create inability to work or, in consideration of the site of the skin eruption, to affect negatively the quality of life of patients.

Due to their large appliances, metals are considered a major risk factor in ACD development. Among them, Ni, Co and Cr represented those with the highest allergizing prevalence, while others such as Al, Au, Be, Cu, Hg, PGEs and Ti are new emerging allergens.

Daily people aome in contact with metals because they are present in several objects and products such as coins, personal adornments (clasps, belts, pins, buttons), jewellery, ear piercings, dental restorations, body prosthesis, ceramics, catalysts, inks and tattoos, household products, hair dyes, cement, and leather tanning.

It is well recognized that to prevent the development of metal ACD in sensitized people, contact with the allergen should be avoided. Whenever this is not possible, personal care by the use of cotton gloves or active and protective creams has been suggested. Other possible ways to prevent ACD might be the industrial modification of composition of alloys or plating and labeling of consumer products with adequate warnings.

Despite the regulations released in the EU with the aim to protect the health of people, a high number of subjects is still affected by metal induced-ACD. More efforts in the identification of the sources of human exposure to metal sensitizer, characterization of metal allergological potency, development of *in vivo* and *in vitro* tests as diagnostic tools should be done in order to create a base of knowledge about this health problem and adopt adequate prevention programs.

REFERENCES

[1] Rycroft, R.J.G.; Menné, T., Frosch, P.J.; Lepoittevin, J.-P. *Textbook of Contact Dermatitis*, Springer-Verlag: Berlin, **2001**.
[2] Belsito, D.V. *J. Am. Acad. Dermatol*., **2005**, *53*, 303.
[3] Pontoppidan Thyssen, J.; Linneberg, A.; Menné, T.; Johansen, J.D. *Contact Dermatitis,* **2007**, *57*, 287.
[4] Fyhrquist-Vanni, N.; Alenius, H.; Lauerma, A. *Dermatol. Clin*., **2007**, *25*, 613.
[5] Sharpe, A.H.; Abbas, A.K. *N. Engl. J. Med.,* **2006**, *355*, 973.
[6] Fischer, A.A. *Contact Dermatitis*, Lea & Febiger: Philadelphia, **1986**.
[7] Hultmann, P. In *Handbook on the Toxicology of Metals*; Nordberg, G.F.; Fowler, B.A.; Nordberg, M.; Friberg, L.T., Eds.; Academic Press: San Diego, **2007**, pp. 197-211.
[8] Andersen, K.E.; Benezra, C.; Burrows, D.; Camarasa, J.; Dooms-Goossens, A.; Ducombs, G.; Frosch, P.; Lachapelle, J.M.; Lahti, A.; Menné, T.; Rycroft, R.; Scheper, R.; White, I.;Wlikinson, J. *Contact Dermatitis,* **1987**, *16*, 55.
[9] Mathias, C.G.T. *J. Am. Dermatol.,* **1989**, *20*, 842.
[10] Kadyk, D.L.; McCarter, K.; Achen, F.; Belsito, D.V. *J. Am. Acad. Dermatol*., **2003**, *49*, 1037.
[11] The ESSCA Writing Group. *JEADV,* **2008**, *22*, 174.
[12] Krob, H.A.; Fleischer Jr, A.B.; D'Agostino Jr, R.; Haverstock, C.L.; Feldman, S. *J. Am. Acad. Dermatol.,* **2004**, *51*, 349.
[13] Nguyen, S.H.; Dang, T.P.; Macpherson, C.; Maibach, H.; Maibach, H.I. *Contact Dermatitis,* **2008**, *58*, 101.
[14] Lidèn, C.; Bruze, M.; Menné, T. In: *Contact Dermatitis,* Frosch, P.J.; Menné, T.; Lepoittevin, J.-P., Eds.; Spinger: Heidelberg, **2006**; pp. 537-568.
[15] Wöhrl, S.; Hemmer, W.; Focke, M.; Götz, M.; Jarisch, R. *Pediat. Dermatol*., **2003**, *20*, 119.
[16] Basketter, D.A. *Br. J. Dermatol*., **2008**, *159*, 267.
[17] Council Directive 94/27/EC of 30 June 1994. Official Journal L 188, 1.
[18] Council. Directive 2003/53/EC of 18 June 2003. Official Journal L 178, 24.
[19] Council Directive 76/768/EEC of 27 July 1976. Official Journal L 262, 169.
[20] Commission Directive 2004/93/EC of 21 September 2004. Official Journal L 300, 13.
[21] Veien, N.K.; Hattel, T.; Justesen, O.; Norholm, A. *Contact Dermatitis,* **1986**, *15*, 295.
[22] Castelain, P.Y., Castelain, M.; Vervloet, D.; Garbe, L.; Mallet, B. *Contact Dermatitis,* **1988**, *19*, 58.
[23] Fiejka, M.; Aleksandrowicz, J. *Rocz. Panstw. Zakl. Hig*., **1993**, *44*, 73.

[24] Lopez, S.; Pelaez, A.; Navarro, L.A, Montesinos, E.; Morales, C.; Carda, C. *Contact Dermatitis,* **1994**, *31*, 37.
[25] Böhler-Sommeregger, K.; Lindemayr, H. *Contact Dermatitis*, **1986**, *15*, 278.
[26] Skowron, F.; Grezard, P.; Berard, F.; Balme, B.; Perrot, H. *Contact Dermatitis*, **1998**, *39*, 135.
[27] Kaaber, K.; Nielsen, A.O.; Veien, N.K. *Contact Dermatitis,* **1992**, *26*, 304.
[28] Bergfors, E.; Björkelund, C.; Trollfors, B. *Eur. J. Pediatr*., **2005**, *164*, 691.
[29] Gallego, H.; Lewis, E.J.; Crutchfield III, C.E. *Cutis*, **1999**, *64*, 65.
[30] Fischer, T.; Rystedt, I. *Contact Dermatitis,* **1982**, *8*, 343.
[31] Hindsén, M. *Contact Dermatitis,* **2005***, 53*, 301.
[32] Montemarano, A.D.; Sau, P.; Johnson, F.B.; James, W.D. *J. Am. Acad. Dermatol.,* **1997**, *37*, 496.
[33] Meding, B.; Augustsson, A.; Hansson, C. *Contact Dermatitis*, **1984**, *10*, 107.
[34] Veien, N.K.; Hattel, T.; Laurberg, G. *Contact Dermatitis*, **1993**, *28*, 199.
[35] Hall, A.F. *JAMA,* **1944**, *125*, 180
[36] Purello-D'Ambrosio, F.; Gangemi, S.; Minciullo, P.L.; Lombardo, G.; Ricciardi, L.; Isola, S.; Merendino, R.A. *Allergol. Immunopathol.,* **2000**, *28*, 74.
[37] Peters, T.; Hani, N.; Kirchberg, K.; Gold, H.; Hunzelmann, N.; Scharffetter-Kochanek, K. *Contact Dermatitis,* **1998**, *39*, 322.
[38] Helgesen, A.L.O.; Austad, J. *Contact Dermatitis,* **1997**, *37*, 303.
[39] Timko, A.L.; Miller, C.H.; Johnson, F.B.; Victor Ross, E. *Arch. Dermatol*., **2001**, *137*, 143.
[40] McFadden, N.; Lyberg, T.; Hensten-Pettersen, A. *J. Am. Acad. Dermatol*., **1989**, *20*, 903.
[41] Schwarze, H.P.; Giordano-Labadie, F.; Loche, F.; Gorguet, M.B.; Bazex, J. *J. Am. Acad. Dermatol*., **2000**, *42*, 888.
[42] NIOSH. National occupational hazard survey. U.S. Government Printing Office, Washington, DC **1978**.
[43] IPCS Environmental Health Criteria 106. *Beryllium*. World Health Organization, Geneva; **1990**.
[44] Epstein, W.L. In: *Beryllium Biomedical and Environmental Aspects;* Rossman, M.D.; Preuss, O.P.; Powers, M.B., Eds.; Williams & Wilkins: Baltimore, **1991**; pp113-117.
[45] Tinkle, S.S., Antonini, J.M.; Rich, B.A.; Roberts, J.R.; Salmen, R., DePree, K.; Adkins, E.J. *Environ. Health Perspect.,* **2003**, *111*, 1202.
[46] Tan, M.H.; Commens, C.A.; Burnett, L; Snitch, P.J. *Aust. J. Dermatol.,* **1996***, 37*, 185.
[47] Curtis, G. *AMA Arch. Dermatol. Syph*., **1951**, *64*, 470.
[48] Vilaplana, J.; Romaguera, C.; Cornellana, F. *Contact Dermatitis,* **1994***, 30*, 80.
[49] Covington, J.S.; McBride, M.A; Slagle W.F.; Disney, A.L. *J. Biomed. Mat. Res*., **1985**, *19*, 747.
[50] Haberman, A.L.; Pratt, M.; Storrs, F.J. *Contact Dermatitis*, **1993**, *29*, 222.
[51] Vilaplana, J.; Romaguera, C.; Grimaldi, F. *Contact Dermatitis*, **1992**, *26*, 295.
[52] Berlin, J.M.; Taylor, J.S.; Sigel, J.E.; Bergfeld, W.F.; Dweik, R.A. *J. Am. Acad. Dermatol*., **2003**, *49*, 939.
[53] Wegner, R.; Heinrich-Ramm, R.; Nowak, D.; Olma, K.; Poschadel, B.; Szadkowski, D. *Occup. Environ. Med.,* **2000**, *57*, 133.
[54] Marx, J.J.; Burrell, R. *J. Immunol.*, **1973**, *111*, 590.
[55] Krivanek, N.; Reeves, A.L. *Am. Ind. Hyg. Assoc. J*., **1972**, *33*, 45.
[56] Zissu, D.; Binet, S.; Cavelier, C. *Contact Dermatitis*, **1996**, *34*, 196.
[57] Resnick, H.; Roche, M.; Morgan, W.K.C. *Am. Rev. Respir. Dis*., **1970**, *101*, 504.
[58] Kazantzis, G. In: *13th International Congress on Occupational Health*, New York, **1960**, p. 290.
[59] Hanifm, J.M.; Epstein, W.L.; Cline, M.J. *J. Invest. Dermatol*., **1970**, *55*, 284.
[60] Henderson, W.R., Fukuyama, K.; Epstein, W.L.; Spitker, L.E. *J. Invest. Dermatol.*, **1972**, *58*, 5.
[61] Kang, K.Y., Bice, D.; Hoffman, E.; D'Amato, R.; Salvaggio, J. *J. Allergy Clin. Immunol*., **1977**, *59*, 425.
[62] Bobka, C.A., Stewart, L.A., Engelken, G.J.; Golitz, L.E., Newman, L.S. *J. Occup. Environ. Med.*, **1997**, *39*, 540.
[63] Cotes, J.E.; Gilson, J.C.; McKerrow, C.B.; Oldham, P.D. *Br. J. Indust. Med*., **1983**, *40*, 13.
[64] Epstein, W.L. *Cleve Clin. Q*., **1983**, *50*, 73.
[65] Saltini, C.; Richeldi, L.; Losi, M.; Amicosante, M.; Voorter, C.; van den Berg-Loonen, E.; Dweik, R.A.; Wiedemann, H.P.; Deubner, D.C.; Tinelli, C. *Eur. Respir. J*., **2001**, *18*, 677.
[66] Amicosante, M.; Berretta, F.; Rossman, M.; Butler, R.H.; Rogliani, P.; van den Berg-Loonen, E.; Saltini, C. *Respir. Res*., **2005**, *6*, 94.
[67] Meyer, K.C. *Chest*, **1994**, *106*, 942.
[68] Day, G.A.; Dufresne, A.; Stefaniak, A.B., Schuler, C.R.; Stanton, M.L.; Miller, W.E., Kent, M.S., Deubner, D.C., Kreiss, K., Hoover, M.D. *Ann. Occup. Hyg*., **2007**, *51*, 67.
[69] Shelnutt, S.R.; Goad, P.; Belsito, D.V. *Crit. Rev. Toxicol*., **2007**, *37*, 375.
[70] Akyol, A.; Boyvat, A.; Peksari, Y.; Gürgey, E. *Contact Dermatitis*, **2005**, *52*, 333.
[71] Goon, A.T.J.; Goh, C.L. *Contact Dermatitis*, **2005**, *52*, 130.
[72] Bajaj, A.K.; Saraswat, A.; Mukhija, G.; Rastogi, S.; Yadav, S. *Indian J. Dermatol. Venereol. Leprol*., **2007**, *73*, 313.
[73] Machovcova, A.; Dastychova, E.; Kostalova, D.; Vojtechovska, A.; Reslova, J.; Smejkalova, D.; Vaneckova, J.; Vocilkova, A. *Contact Dermatitis*, **2005**, *53*, 162.
[74] Lam, W.S.; Chan, L.Y.; Ho, S.C.K.; Chong, L.Y.; So, W.H.; Wong, T.W. *Int. J. Dermatol.*, **2008**, *47*, 128.
[75] Ruff, C.A.; Belsito, D.V. *J. Am. Acad. Dermatol.*, **2006**, *55*, 32.
[76] Thyssen, J.P.; Johansen, J.D., Menné, T. *Contact Dermatitis*, **2007**, *56*, 185,
[77] Avnstorp, C. *Contact Dermatitis,* **1989**, *20*, 365.
[78] Roto, P.; Sainio, H.; Reunala, T.; Laippala, P. *Contact Dermatitis*, **1996**, *34*, 43.
[79] Macedo, M.S., de Oliveira de Avelar Alchorne, A.; Costa, E.B.; Montesano, F.T. *Contact Dermatitis*, **2007**, *56*, 232.
[80] Wang, B.-J., Shiao, J.-S.; Chen, C.J; Lee, Y.-C.; Guo, Y.-L. *Contact Dermatitis*, **2007**, *57*, 309.
[81] Hansen, M.B.; Menné, T.; Johansen, J.D. *Contact Dermatitis*, **2006**, *54*, 278.
[82] Chowdhuri, S.; Ghosh, S. *Indian J. Dermatol. Venereol. Leprol*., **2007**, *73*, 319.
[83] Srinvas, C.R.; Shanmuga Sundaram, V.; Selvaraj, K. *Indian J. Dermatol. Venereol. Leprol.*, **2007**, *73*, 428.
[84] Nava, A.; Campiglio, G.; Caravelli, G.; Galli, D.A.; Gambini, M.A.; Zerbini, R.; Beretta, E. *Med. Lav*., **1987**, *78*, 405.
[85] Ingber, A.; Gammelgaard, B.; David, M. *Contact Dermatitis*, **1998**, *38*, 101.
[86] Iyer, V.J.; Banerjee, G.; Govindram, C.B.; Kamath, V.; Scinde, S.; Gaikwad, A.; Jerajani, H.R.; Raman, G.; Cherian, K.M. *Contact Dermatitis*, **2002**, *47*, 357.
[87] Basketter, D.A.; Angelini, G.; Ingber, A.; Kern, P.S.; Menné, T. *Contact Dermatitis,* **2003**, *49*, 1.
[88] Seishima, M.; Oyama, Z.; Oda, M. *Dermatology*, **2003**, *207*, 48.
[89] Smith, V.H.; Charles-Holmes, R.; Bedlow, A. *Clin. Exp. Dermatol*., **2005**, *31*, 129.
[90] Kręcisz, B.; Kieć-Świerczyńska, M.; Bąkowicz-Mitura, K. *Int. J. Occup. Med. Environ. Health*, **2006**, *19*, 178.
[91] Menezes, L.M.; Campos, L.C.; Quintão, C.C.; Bolognese, A.M. *Am. J. Orthod. Dentofacial. Orthop*. **2004**, *126*, 58.
[92] Sainio, E.L.; Jolanki, R.; Hakala, E.; Kanerva, L. *Contact Dermatitis*, **2000**, *42*, 5,
[93] Bocca, B.; Forte, G.; Petrucci, F.; Cristaudo, A. *J. Pharmaceut. Biomed*., **2007**, *44*, 1197.
[94] Bocca B.; Forte, G.; Senofonte, O.; Violante, N.; Paoletti, L.; De Berardis, B.; Petrucci, F.; Cristaudo, A. *Sci. Total Environ*., **2007**, *388*, 24.
[95] Thomas, P.; Summer, B.; Sander, C.A.; Przybilla, B.; Thomas, M.; Naumann, T. *Allergy*, **2000**, *55*, 969.
[96] Curtis, A.; Morton, J.; Balafa, C.; MacNeil, S.; Gawkrodger, D.J.; Warren, N.D.; Evans, G.S. *Toxicol. Vitro*, **2007**, *21*, 809.
[97] Burastero, S.E.; Paolucci, C.; Breda, D.; Ponti, J.; Munaro, B.; Sabbioni, E. *Int. J. Immunopathol. Pharmacol*., **2006**, *19*, 581.
[98] Gammelgaard, B.; Fullerton, A.; Avnstorp, C.; Menne, T. *Contact Dermatitis,* **1992***, 27*, 302.
[99] Kosann, M.K.; Brancaccio, R.R.; Shupack, J.L.; Franks, A.G.J.; Cohen, D.E. *Am. J. Contact Dermatitis* **1998***, 9*, 92.
[100] Minang, J.T.; Areström, I.; Troye-Blomberg, M.; Lundeberg, L.; Ahlborg, N. *Clin. Exp. Immunol*., **2006**, *146*, 417.
[101] Fowler. J.F.; Kauffman, C.L.; Marks Jr, J.G.; Proctor, D.M.; Fredrick, M.M.; Otani, J.M.; Finley, B.L.; Paustenbach, D.J.; Nethercott, J.R. *J. Occup. Environ. Med*., **1999**, *41*, 150.

[102] Nielsen, N.H., Kristiansen, J.; Borg, L.; Christiensen, J.M.; Poulsen, L.K.; Mennè, T. Contact Dermatitis*, **2000,** 43, 212.*

[103] Basketter, D.; Horev, L.; Slodovnik, D.; Merimes, S.; Trattner, A.; Ingber, A. *Contact Dermatitis,* **2001***, 44*, 70.

[104] Kashani, M.N.; Gorouchi, F.; Behnia, F.; Nazemi, M.J.; Dowlati, Y.; Firooz, A. *Contact Dermatitis*, **2005**, *52*, 154.

[105] Dotterud, L.K.; Smith-Sivertsen, T. *Contact Dermatitis*, **2007**, *56*, 10.

[106] Krob, H.A.; Fleischer Jr, A.B.; D'Agostino Jr, R., Haverstock, C.L.; Feldman, S. *J. Am. Acad. Dermatol*., **2004**, *51*, 349.

[107] Stingeni, L.; Pelliccia, S.; Lisi, P. *Giorn. It. Allergol. Immunol. Clin*., **2003**, *13*, 17.

[108] Bock, M.; Schmidt, A.; Bruckner, T.; Diepgen, T.L. *Br. J. Dermatol*., **2003**, *149*, 1165.

[109] Fischer, T., Rystedt, I. *Contact Dermatitis*, **1983**, *9*, 115.

[110] Vilaplana, J.; Grimalt, F.; Romaguera, C.; Mascaro, J.M. *Contact Dermatitis,* **1987**, *16*, 139.

[111] Gaikema, F.J.; Nakotta, R.A.; Dannen, F. Rapportnummer NDCCP007/01, Keuringsdienst van Waren Noord, **2002**.

[112] Allenby, C.F.; Basketter, D.A. *Contact Dermatitis*, **1989**, *20*, 185.

[113] Hindsén, M.; Persson, L.; Gruvberger, B. *Contact Dermatitis*, 2005, *53*, 350.

[114] Perryman, J.H.; Fowler, Jr., J.F. *Cutis*, 2006, *77*, 77.

[115] Meijer, C.; Bredberg, M.; Fischer, T.; Widström, L. *Contact Dermatitis*, **1995**, *32*, 147.

[116] Nakada, T.; Ijima, M.; Nakayama, H.; Maibach, H.I. *Contact Dermatitis*, **1997**, *36*, 233.

[117] Bagnato, G.F.; De Pasquale, R.; Giacobbe, O.; Chirico, G.; Ricciardi, L.; Gangemi, S.; Purello d'Ambrosio, F. *Allergol et Immunopathol*., **1999**, *27*, 32.

[118] Kang, I.-J.; Lee, M.-H. *Contact Dermatitis*, **2006**, *55*, 26.

[119] Laing, M.E.; Hackett, C.B.; Murphy, G.M. *Contact Dermatitis*, **2005**, *52*, 293.

[120] Goossens, A.; Bedert, R.; Zimerson, E. *Contact Dermatitis*, **2001**, *45*, 172.

[121] Khamaysi, Z.; Bergman, R.; Weltfriend, S. *Contact Dermatitis*, **2006**, *55*, 216.

[122] Torgerson, R.R.; Mark, D.P.; Davis, M.D.P.; Alison, J.; Bruce, A.J.; Farmer, S.A.; Rogers III, R.S. *J. Am. Acad. Dermatol*., **2007**, *57*, 315.

[123] Sockanathan, S.; Setterfield, J.; Wakelin, S. *Contact Dermatitis*, **2003**, *48*, 342.

[124] Kanerva, L.; Kanerva, K.; Jolanki, R.; Estlander, T. *Contact Dermatitis,* **2001**, *45*, 126.

[125] Anavekar, N.S.; Nixon, R. *Australas. J. Dermatol*, **2006**, *47*, 143.

[126] Kusaka, Y.; Iki, M.; Kumagai, S.; Goto, S. *Occup. Environ. Med*., **1996**, *53*, 188.

[127] Kligman, A.M. *J. Invest. Dermatol*., **1966**, *47*, 393.

[128] Wahlberg, J.E.; Boman, A. *Contact Dermatitis*, **1978**, *4*, 128.

[129] Moed, H.; von Blomberg, M.; Bruynzeel, D.P.; Scheper, R.; Gibbs, S.; Rustemeyer, T. *Exp. Dermatol*., **2005**, *14*, 6347.

[130] Wöhrl, S.; Kriechbaumer, N.; Hemmer, W.; Focke, M.; Brannath, W.; Götz, M.; Jarisch, R. *Contact Dermatitis*, **2001**, *44*, 224.

[131] Hostynek, J.J.; Maibach, H.I. *Dermatol. Ther*., **2004**, *17*, 328.

[132] Pujol, R.M.; Randazzo, L.; Miralles, J.; Alomar, A. *Contact Dermatitis,* **1998**, *38*, 288.

[133] Purello D'Ambrosio, F.; Ricciardi, L.; Isola, S.; Gangemi, S.; Cilia, M.; Levanti, C.; Marcazzò, A. *Allergy*, **1996**, *51*, 658.

[134] Wöhrl, S.; Hemmer, W.; Focke, M.; Götz, M.; Jarisch, R. *J. Am. Acad. Dermatol.*, **2001**, *45*, 863.

[135] Gerhardsson, L.; Björkner, B.; Karlsteen, M.; Schütz, A. *Sci. Total Environ*., **2002**, *290*, 41.

[136] Hougeir, F.G.; Yiannias, J.A.; Hinni, M.L.; Hentz, J.G.; el-Azhary, R.A. *Int. J. Dermatol.,* **2006**, *45*, 265.

[137] Vergara, G.; Silvestre, J.F.; Botella, R.; Albares, M.P.; Pascual, J.C. *Contact Dermatitis*, **2004**, *50*, 374.

[138] Hostynek, J.J.; Dreher, F.; Maibach, H.I. *Food Chem. Toxicol*., **2006**, *44*, 1539.

[139] Guy, R.H.; Hostynek, J.J.; Hinz, R.S.; Lorence, C.R. In *Metals and the skin—topical effects and systemic absorption;* Guy, R.H.; Hostynek, J.J.; Hinz, R.S.; Lorence, C.R., Eds.; New York: Marcel Dekker: New York, **1999**; pp. 179-189

[140] Paredes Suárez, C.; Fernández-Redondo, V.; Toribio, J. *Contact Dermatitis*, **2002**, *47*, 182.

[141] Hayashi, S.; Dekio, S.; Kakizoe, E.; Jidoi, J. *Environ. Dermatol*., **1995**, *2*, 283.

[142] Flint, G.N. *Contact Dermatitis*, **1998**, *39*, 213.

[143] Lidén, C.; Nordenadler, M.; Skare, L. *Contact Dermatitis*, **1998**, *39*, 281.

[144] Basler, R.S.; Basler, G.C.; Palmer, A.H.; Garcia, M.A. *J. Am. Acad. Dermatol*., **2000**, *43*, 299.

[145] Santucci, B.; Cannistraci, C.; Cristaudo, A.; Picardo, M. *Contact Dermatitis*, **1993**, *29*, 251.

[146] Narahari, S.R.; Srinivas, C.R.; Kelkar, S.K. *Contact Dermatitis*, **1990**, *22*, 296.

[147] Dhir, G.G.; Rao, D.S.; Mehrotra, M.P. *Ann. Allergy*, **1977**, *39*, 204.

[148] Karlberg, A.-T.; Boman, A.; Wahlberg, J.E. *Contact Dermatitis*, **1983**, *9*, 134.

[149] Boman, A.; Karlberg, A.T.; Einvarsson, Ö.; Wahlberg, J.E. *Contact Dermatitis*, **1983**, *9*, 159.

[150] Herbst, R.; Lauerma, A.; Maibach, H.I. *Contact Dermatitis*, **1993**, *29*, 1.

[151] Garner, L.A. *Dermatol. Ther*., **2004**, *17*, 321.

[152] Bjorkner, B.; Bruze, M.; Moller, H. *Contact Dermatitis*, **1994**, *30*, 144.

[153] McKenna, K.E., Dolan, O.; Walsh, Y.M.; Burrows, D. *Contact Dermatitis*, **1995**, *32*, 143.

[154] Nonaka, H.; Nakada, T.; Iijima, M. *Contact Dermatitis*, **2003**, *48*, 112.

[155] Bruze, M.; Hedman, H.; Björkner, B.; Moller, H. *Contact Dermatitis*, **1995**, *33*, 386.

[156] Aro, T.; Kanerva, L.; Hayrinenimmonen, R.; Silvennoinenkassinen, S. *Contact Dermatitis*, **1993**, *28*, 276.

[157] Möller, H.; Larsson, A.; Björkner, B.; Bruze, M. *Acta Derm.-Venereol*., **1994**, 74, 417.

[158] Fisher, A.A. *Am. J. Contact Derm*., **1992**, *3*, 52.

[159] Hostynek, J.J. *Food Chem. Toxicol*., **1997**, *35*, 839.

[160] Vamnes, J.S.; Morken, T.; Helland, S.; Gjerdet, N.F. *Contact Dermatitis*, **2000**, *42*, 128.

[161] Schaffran, R.M.; Storrs, F.J.; Schalock, P. *Am. J. Contact Derm*., **1999**, *10*, 201.

[162] Möller, H. *Contact Dermatitis*, **2002**, *47*, 63.

[163] Ahnlide, I.; Ahlgren, C.; Bjorkner, B.; Bruze, M.; Lundh, T.; Möller, H.; Nilner, K.; Schutz, A. *Acta Odontol. Scand*., **2002**, *60*, 301.

[164] Yiannias, J.A.; el-Azhary, R.A.; Hand, J.H.; Pakzad, S.Y.; Rogers III, R.S. *J. Am. Acad. Dermatol*., **2000**, *42*, 177.

[165] Kawahara, D.; Oshima, H.; Kosugi, H.; Nakamura, M.; Sugai, T.; Tamaki, T. *Contact Dermatitis*, **1993**, *28*, 114.

[166] Svedman, C.; Tillman, C.; Gustavsson, C.G.; Moller, H.; Frennby, B.; Bruze, M. *Contact Dermatitis*, **2005**, *52*, 192.

[167] Brown, D.H.; Smith, W.E.; Fox, P.; Sturrock, R.D. *Inorg. Chim. Acta*, **1982**, *67*, 27.

[168] Holland, R.I. *Scand. J. Dent. Res*., **1980**, *88*, 269.

[169] Bruze, M.; Bjorkner, B.; Moller, H. *Contact Dermatitis*, **1995**, *32*, 5.

[170] Fowler, J. *Arch. Dermatol*., **1988**, *124*, 181.

[171] Silva, R.; Pereira, F.; Bordalo, O.; Silva, E.; Barros, A.; Gonçalo, M.; Correia, T.; Pessoa, G.; Baptista, A.; Pecegueiro, M. *Contact Dermatitis*, **1997**, *37*, 78.

[172] Ehrlich, A.; Belsito, D.V. *Cutis*, **2000**, *65*, 323.

[173] Camarasa, J.G.; Serra-Baldrich, E. *Med. Cutan. Ibero Lat. Am*., **1989**, *17*, 187.

[174] Kobayashi, Y.; Nanko, H.; Nakamura, J.; Mizoguchi, M. *J. Am. Acad. Dermatol*., **1992**, *27*, 457.

[175] Nakada, T.; Iijima, M.; Fujisawa, R. *Jpn. J. Clin. Dermatol*., **1992**, *46 (Suppl. 5)*, 16.

[176] Littman, B.H.; Hall, R.E. *Arthritis Rheum*., **1985**, *28*, 1384.

[177] Shelley, W.B.; Juhlin, L. *Arch. Dermatol*., **1977**, *113*, 187.

[178] Seitz, M.; Valbracht, J.; Quach, J.; Lotz, M. J. *Clin. Immunol*., **2003**, *23*, 477.

[179] Kumar Mangalam, A.; Aggarwal, A.; Naik, S. *Cell Immunol*., **2002**, *219*, 1.

[180] Sinigaglia, F. *J. Invest. Dermatol*., **1994**, *102*, 398.

[181] Griem, P.; Takahashi, K.; Kalbacher, H.; Gleichmann, E. *J. Immunol*., **1995**, *155*, 1575.

[182] Silvennoinen-Kassinen, S.; Niinimaki, A. *Contact Dermatitis*, **1984**, *11*, 156.

[183] Walzer, R.A.; Feinstein, R.; Shapiro, L.; Einbinder, J. *Arch. Dermatol*., **1972**, *106*, 231.

[184] Cederbrant, K.; Hultman, P.; Marcusson, J.A.; Tibbling, L. *Int. Arch. Allergy Immunol*., **1997**, *112*, 212.

[185] Dearman, R.J.; Warbrick, E.V.; Skinner, R.; Kimber, I. *Food Chem. Toxicol*., **2002**, *40*, 1881.
[186] Möller, H.; Ohlsson, K.; Linder, C.; Björkner, B.; Bruze, M. *Am. J. Contact Dermatitis*, **1998**, *9*, 15.
[187] Larsson, Å.; Möller, H.; Björkner, B.; Bruze, M. *Acta Derm. Venereol*., **1997**, *77*, 474.
[188] Möller, H.; Ohlsson, K.; Linder, C.; Björkner, B.; Bruze, M. *Contact Dermatitis*, **1999**, *40*, 200.
[189] International Programme on Chemical Safety (IPCS). Environmental Health Criteria 101. *Methylmercury*. World Health Organization, Geneva **1990**.
[190] International Programme on Chemical Safety (IPCS). Environmental Health Criteria 118. *Inorganic Mercury*. World Health Organization, Geneva **1991**.
[191] Eneström, S.; Hultman, P. *Int. Arch. Allergy Immunol.,* **1995**, *106*, 180.
[192] Laeijendecker, R.; Dekker, S.K.; Burger, P.M.; Mulder, P.G.; Van Joost, T.; Neumann, M.H. *Arch Dermatol.,* **2004**, *140,* 1434.
[193] Smart, E.R.; Macleod, R.I.; Lawrence, C.M. *Br. Dent. J.*, **1995**, *178,* 108.
[194] Pigatto, P.D.; Guzzi, G.; Persichini, P.; Barbadillo, S. *Dermatitis*, **2004**, *15*, 75.
[195] Rietschel, R.L.; Fowler Jr, J.F. Antiseptics and disinfectants. In *Fisher's Contact Dermatitis;* Rietschel, R.L.; Fowler Jr, J.F., Eds.; Philadelphia: Lippincott Williams & Wilkins, **2001**, pp 149–155.
[196] Audicana, M.T.; Munoz, D.; Del Pozo, M.D.; Fernandez, E.; Gastaminza, G.; DeCorres, L.F. *Am. J. Contact Dermat.,* **2002**, *13,* 3.
[197] Boyd, A.S.; Seger, D.; Vannucci, S.; Langley, M.; Abraham, J.L.; King Jr, L.E. *J. Am. Acad. Dermatol.*, **2000**, *43*, 81.
[198] Rietschel, R.L.; Fowler Jr, J.F. Systemic contact-type dermatitis. In *Fisher's contact dermatitis*; Rietschel, R.L.; Fowler Jr, J.F., Eds.; Lippincott, Williams & Wilkins: Philadelphia **2001**, pp. 89–91.
[199] Soo, Y.O.; Chow, K.M.; Lam, C.W.; Lai, F.M.; Szeto, C.C.; Chan M.H.; Li, P.K. *Am. J. Kidney Dis*., **2003**, *41*, 250.
[200] Sun, C. *Cont. Derm*., **1987**, *17,* 306.
[201] Kawai, K.; Zhang, X.M.; Nakagawa, M.; Kawai, J.; Okada, T.; Kawai, K. *Contact Dermatitis*, **1994**, *31*, 330.
[202] Sin, K.W.; Tsang, M.B. *Hong Kong Med. J*., **2003**, *9*, 329.
[203] Dyall-Smith, D.J.; Scurry, J.P. *Med. J. Aust.*, **1990**, *153,*409
[204] Nakayama, H. Niki, F.; Shono, M.; Hada, S. *Contact Dermatitis*, **1984**, *9*, 411.
[205] Belhadjali, H.; Youssef, M.; Amri, M.; Douki, W.; Zili, J. *Contact Dermatitis,* **2008**, *58*, 110.
[206] Nakada, T.; Higo, N.; Iijima, M.; Nakajama, H.; Maibach, H.I. *Contact Dermatitis*, **1997**, *36,* 237.
[207] Rietschel, R.L.; Fowler Jr, J.F. *Fisher's Contact Dermatitis*, Williams and Wilkins: Baltimore, **1995**.
[208] Rietschel, R.L.; Fowler Jr, J.F. Contact dermatitis and other reactions to metals. In *Fisher's contact dermatitis*; Rietschel, R.L.; Fowler Jr, J.F., Eds.; Lippincott, Williams & Wilkins: Philadelphia, **2001**, pp. 607–608.
[209] Koch, P.; Nickolaus, G. *Contact Dermatitis,* **1996**, *34*, 405.
[210] Kim, N.S.; Koh, D.H.; Kim, C.S.; Lee, J.S.; Kim, N.S; Lee, H.H. *Korean J. Prev. Med*., **1994**, *27*, 11.
[211] Ochel, M.; Vohr, H.W.; Pfeiffer, C.; Gleichmann, E. *J. Immunol*., **1991**, *146*, 3006.
[212] Kubicka-Muranyi, M.; Kremer, J.; Rottmann, N.; Lübben, B.; Albers, R.; Bloksma, N.; Lührmann, R.; Gleichmann, E. *Int. Arch. Allergy Immunol*., **1996**, *109*, 11.
[213] Stejskal, V.D.; Forsbeck, M.; Cederbrant, K.E.; Asteman, O. *J. Clin. Immunol.* **1996**, *16,* 31.
[214] Di Gioacchino, M.; Di Giampaolo, L.; Verna, N.; Reale, M.; Di Sciascio, M.B.; Volpe, A.R.; Carmignani, M.; Ponti, J.; Paganelli, R.; Sabbioni, E.; Boscolo, P. *Ann. Clin. Lab. Sci.*, **2004**, *34,* 195.
[215] Schuppe, H.C.; Rönnau, A.C.; von Schmiedeberg, S.; Ruzicka, T.; Gleichmann, E.; Griem, P. *Clin. Dermatol.,* **1998**, *16*, 149.
[216] Merget, R.; Schulte, A.; Gebler, A.; Breitstadt, R.; Kulzer, R.; Berndt, E.D.; Baur, X.; Schultze-Werninghaus. G. *Int. Arch. Occup. Environ. Health,* **1999**, *72*, 33.
[217] Calverley, A.E.; Rees, D.; Dowdeswell, R.J. *Clin. Exp. Allergy,* **1999***, 29*, 703.
[218] Schena, D.; Barba, A.; Costa, G. *Contact Dermatitis,* **1996**, *34*, 220.
[219] Larese Filon, F.; Uderzo, D.; Bagnato, E. *Am. J. Contact Dermatitis*, **2003**, *14*, 78.
[220] Boscolo, P.; Di Giampaolo, L.; Reale, M.; Castellani, M.L.; Ritavolpe, A.; Carmignani, M.; Ponti, J.; Paganelli, R.; Sabbioni, E.; Conti, P.; Di Gioacchino, M. *Ann. Clin. Lab. Sci.*, **2004**, *34(3),* 299.
[221] Fernandez-Redondo, V.; Gomez-Centeno, P.; Toribio, J. *Contact Dermatitis,* **1998**, *38*, 178.
[222] Hay, C.; Ormerod, A. *Contact Dermatitis*, **1998**, *38*, 216.
[223] Mizoguchi, S.; Setoyama, M.; Kanzaki, T. *Dermatology*, **1998**, *196*, 268.
[224] Katoh, N.; Hirano, S.; Kishimoto, S.; Yasuno, H. *Contact Dermatitis*, **1999**, *40,* 226.
[225] Yoshida, S.; Sakamoto, H.; Mikami, H.M.; Onuma, K.; Shoji, T.; Nakagawa, H.; Hasegawa, H.; Amayasu, H. *J. Allerg. Clin. Immunol*., **1999**, *103,* 1211.
[226] Moulon, C.; Vollmer, J.; Weltzien, H.U. *Eur. J. Immunol.,* **1995**, *25*, 3308.
[227] Pistoor, F.H.; Kapsenberg, M.L.; Bos, J.D.; Meinardi, M.M.; von Blomberg, M.E.; Scheper, R.J. *J. Invest. Dermatol.,* **1995**, *105*, 92.
[228] Santucci, B.; Cannistraci, C.; Cristaudo, A.; Picardo, M. *Contact Dermatitis,* **1996**, *35*, 283.
[229] Büdinger, L.; Neuser, N.; Totzke, U.; Merk, H.F.; Hertl, M. *J. Immunol.,* **2001**, *167*, 6038
[230] Kränke, B.; Binder, M.; Derhaschnig, J.; Komericki, P.: Pirkhammer, D.; Ziegler, V.; Aberer, W. *Wiener klein. Wochens*., **1995**, *107*, 323.
[231] Kanerva, L.; Kerosuo, H.; Kullaa, A.; Kerosuo, E. *Contact Dermatitis,* **1996**, *34,* 39.
[232] Goossens, A.; De Swerdt, A.; De Coninck, K.; Snauwaert, J.E.; Dedeurwaerder, M.; De Bonte, M. *Contact Dermatitis*, **2006**, *55*,338.
[233] Suhonen, R.; Kanerva, L. *Contact Dermatitis,* **2001**, *44*, 257.
[234] Bedello, P.G.; Goitre, M.; Roncarolo, G.; Cane, D. *Contact Dermatitis*, **1987**, *17*, 111.
[235] De La Cuadra, J.; Grau-Massane´s, M. *Contact Dermatitis,* **1991**, *25*, 182.
[236] Vilaplana, J.; Romaguera, C.; Cornellana, F. *Contact Dermatitis*, **1994**, *30*, 80.
[237] Marcusson, J.A.; Cederbrant, K.; Heilborn, J. *Contact Dermatitis*, **1998**, *38,* 297.
[238] Santucci, B.; Valenzano, C.; De Rocco, M.; Cristaudo, A. *Contact Dermatitis,* **2000**, *43*, 333.
[239] Murdoch, R.D.; Pepys, J. *Ann. Allergy,* **1987**, *59*, 464.
[240] Peters, M.S.; Schroeter A.L.; van Hale, H.M.; Broadbent, J.C. *Contact Dermatitis*, **1984**, *11*, 214.
[241] Brun, R.; Hunziker, N. *Contact Dermatitis,* **1980**, *6*, 212.
[242] Viraben, R.; Boulinguez, S.; Alba, C. *Contact Dermatitis,* **1995**, *33,* 437.
[243] Yamauchi, R.; Morita, A.; Tsuji, T. *Contact Dermatitis,* **2000**, *42*, 52.
[244] Lalor, P.A.; Revell, P.; Gray, A.B.; Wright, S.; Railton, G.T.; Freeman, M.A. *J. Bone Joint Surg.,* **1991**, *73*, 26.
[245] Thomas, P.; Bandl, W.; Summer, B.; Przybilla, B. *Contact Dermatitis,* **2006**, *55*, 199.
[246] Holgers, K.M.; Thompson, P.; Tjellstrom, A. *Scand. Reconstr. Hand*., **1994**, *28*, 225.
[247] Mitchell, L.; Synnott, S.A.; VanDercreek, J.A. *Int. J. Oral. Maxillofac. Implants*, **1990**, *5*, 79.
[248] Watanabe, R.; Nanko, H.; Fukuda, S. *J. Cutan. Pathol.*, **2006**, *33 (Suppl 2),* 16.
[249] High, W.A.; Ayers, R.A.; Adams, J.R.; Chang, A.; Fitzpatrick, J.E. *J. Am. Acad. Dermatol.,* **2006**, *55,* 716.
[250] Basketter, D.A.; Whittle, E.; Monk, B. *Contact Dermatitis*, **2000**, *42*, 310.
[251] Castelain, M.; Grob J.J. *Lettre du GERDA,* **2001**, *18,* 6.
[252] Kim, J.-W.; Lee, J.-W.; Won, Y.O.; Kim, J.H.; Lee, S.-C. *Acta Derm. Venereol.* **2006**, *86*, 110.
[253] Nedorost, S.; Wagman, A. *Dermatitis,* **2005**, *16*, 67.

[254] Schuh, A.; Thomas, P.; Kachler, W.; Göske, J.; Wagner, L.; Holzwarth, U.; Forst, R. *Orthopade*, **2005**, *34*, 327.
[255] Suhonen, R.; Kanerva, L. *Contact Dermatitis*, **2001**, *44*, 257.
[256] Bircher, A. J.; Stern, B. *Contact Dermatitis*, **2001**, *45*, 244.
[257] Okamura, T.; Morimoto, M.; Fukushima, D.; Yamane, G. *J. Dent. Res*., **1999**, *78*, 1135.
[258] Basketter, D.A.; Menne, T. *Contact Dermatitis*, **2005**, *53*, 1.
[259] Müller, K.; Valentine-Thon, E. *Neuro Endocrinol. Lett.*, **2006**, *27*, 31.
[260] Huang, H.H.; Chiu, Y.H.; Lee, T.H.; Wu, S.C.; Yang, H.W.; Su, K.H.; Hsu, C.C. *Biomaterials*, **2003**, *24,* 3585.
[261] Stejskal, V.D.; Hudecek, R.; Stejskal, J.; Sterzl, I. *Neuro Endocrinol. Lett.,* **2006**, *27,* 7.
[262] Nakashima, Y.; Sun, D.H.; Trindade, M.; Maloney, W.; Goodman, S.; Schurman, D. *J. Bone Joint Surg*., **1999**, *81*, 603.

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