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MECHANISMS OF IMMUNOSENSITIZATION TO METALS

(IUPAC Technical Report)

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Mechanisms of immunosensitization to metals

(IUPAC Technical Report)

Abstract: A project is underway within IUPAC to evaluate and harmonize the use of various biomarkers for immunosensitization to metals. This present review summarizes our knowledge of the mechanisms by which certain trace elements evoke allergenicity. Some physiological electrolytes (e.g., Na^+, K^+) and macronutrients (e.g., Ca^{2+}, Fe^{3+}) are immunologically inactive. However, some trace elements essential for cell function (e.g., Co^{2+}, Cu^{2+}, Cr^{3+}), as well as nonessential elements generally considered toxic (e.g., Hg species) or in use as therapeutic agents (e.g., some species of Pt and Au), can give rise to adverse immune reactions. Specific immunological responses to Ni, Co, Cr, Hg, Be, Cu, Pt, Pd, Ir, In, and Au are discussed. In general, these elements can activate T or B cells by specific receptor interactions, resulting in clonal expansion of a metal-specific lymphocyte and an immune response (typically dermatitis) upon re-exposure. Compelling evidence points to the primary role of the T cell in responding to the metal. T-cell activation occurs when a protein of the major histocompatibility complex (MHC) binds to a T-cell receptor in the presence of an MHC-binding peptide. Many antigenic substances result in presentation of MHC-bound antigenic peptides to the T-cell receptor; metal ions appear to act as haptens that directly or indirectly cause structural changes in MHC molecule-peptide complexes that result in recognition of these complexes by specific T-cell subsets. Nickel and gold are particularly instructive in understanding mechanisms, and are used to discuss models in which the metal may bind to the antigenic peptide (i) before or (ii) after its association with the MHC/T-cell receptor complex, (iii) may bind to the MHC/receptor complex prior to recruitment of the antigenic peptide, or (iv) may bind to the formed peptide/MHC/receptor complex through ligands contributed by one or more of the components.

1. INTRODUCTION

Many metal ions produce immunosensitization. While metals common in the body, such as Na, K, Ca, Mg, and Fe, are immunologically inactive, some trace elements are strong immunosensitzers. Often, exposure to high local concentrations of a metal in the lung or on the skin initiates the immunological process and leads to acute or chronic disease. Exposures to such metals in the workplace, in connection with drug therapy, or in everyday life have considerable health consequences for society. A goal of the present IUPAC project is to evaluate and harmonize the use of specific biomarkers for metal sensitization. As background to this initiative, the focus in this review is on metal-specific, immune-mediated responses leading typically to anaphylactic (Type I) or contact (Type IV) allergic reactions, or autoimmune disease. A better understanding of the mechanisms of metal sensitization should help to avoid or treat metal-related diseases.

Metal ions may modulate immune function in two general ways [1]. They may activate T or B cells by specific receptor interactions, with resultant expansion of the metal-specific lymphocyte causing an immune response upon re-exposure. Alternatively, metals may be toxic to the immune system in nonspecific ways, for example, causing generalized immunosuppression, or enhanced activation of the immune system [2]. A distinction between specific sensitization and general effects can be made based

on whether metal-specific lymphocytes occur and respond to subsequent exposure. Contact dermatitis is one of the most common manifestations of metal allergy, and delayed-type hypersensitivity caused by Ni^{2+} is present in up to 10 % of the Caucasian population, mainly women [3,4]. Other ions, such as Co^{2+}, Cu^{2+}, Cr^{3+}, and Pd, Pt, Hg, and Au species [1,5,6] can also cause contact dermatitis, but a considerable literature has dealt with understanding Ni^{2+} as a hapten [6], and has produced strong evidence for T-cell involvement in the pathogenesis [6–8]. $CD4^+$ T cells in particular play an important role in metal-specific responses [1,9,10]. The frequent clinical sensitization to multiple metals [11,12] also should be accounted for by any proposed mechanism. Here we will deal with the major sensitizing metals individually, beginning with some general principles in the context of Ni allergy, before discussing some general mechanisms.

2. SURVEY OF SENSITIZING METALS

2.1 Nickel

Occupational exposures to Ni are widespread and occur in mining and refining of Ni ores, and production of alloys. Electroplating with Ni, stainless steel welding, and use of Ni alloys in the electronics industry are also important sources of exposure. Further exposures occur in nickel-cadmium battery manufacture, the chemical, pigment, and ceramics industries, and manufacture of Ni-based catalysts for industrial hydrogenation reactions. Nonoccupational exposures arise from handling metallic objects such as jewelry and coins. A high rate of Ni allergy is associated with ear piercing and subsequent wearing of Ni alloy jewelry [13]. Interestingly, comparing the lower incidence of Ni allergy in those who wore Ni-releasing dental braces before ear piercing, with the higher incidence in those who wore them only after or not at all, provided evidence for the development of tolerance to Ni [14,15]. Dietary intake of Ni is at least 100 μg/day [16], and diets naturally high in Ni can exacerbate dermatitis in Ni-sensitized individuals [17]. Quantitative aspects of skin exposures have been reviewed, and threshold values leading to sensitization have been suggested to be 0.1–1 $\mu g/cm^2$ on occluded skin and 15 $\mu g/cm^2$ when nonoccluded [18,19]. Dermal exposure to Ni is primarily related to contact dermatitis that begins as an erythematous lesion, usually on the hands and forearms, eventually becoming eczematous [20]. Asthma, conjunctivitis, and local inflammation associated with prosthetic devices also occur [20].

The role of cytokines in Ni sensitization has been studied, and cytokine profiles in Ni-specific T cells may have potential use as biomarkers of Ni allergy. In general, epidermal Langerhans cells or dermal dendritic cells capture hapten and migrate to regional lymph nodes where they prime hapten-specific T cells [21]. On re-exposure, $CD4^+$ T cells expand and migrate to the skin to release inflammatory cytokines such as IL-2, IFN-γ, and TNF-α. These cytokines are products of the T helper (Th) cell subpopulation Th1 and are generally involved in cell-mediated immunity. Another population, Th2, secrete interleukins such as IL-4, IL-5, and IL-10, that mediate humoral immunity and tend to inhibit the delayed-type hypersensitivity reaction [22]. However, both classes of cytokines may be involved in the pathogenesis of Ni-contact dermatitis. Reverse transcription-PCR of skin biopsies taken from individuals 24 h after application of a $NiSO_4$ patch showed increased mRNA for IFN-γ, IL-2, and IL-4 in both atopic individuals and nonatopic subjects with previous Ni allergy. IL-10 mRNA was increased in the nonatopic group only [22]. Higher IFN-γ and lower IL-10 were also noted by Cavani et al. [23] in Ni^{2+}-specific $CD4^+$ cells of Ni-allergic patients. On the other hand, Borg et al. found that Ni-stimulated lymphocytes from Ni-allergic individuals secreted increased IL-4 and IL-5 but not IFN-γ or TNF-α [24], suggesting that IL-4 and IL-5 might be the most useful indicators of Ni sensitivity. Cavani et al. [23] also noted the presence of Ni^{2+}-specific $CD8^+$ T cells in peripheral blood samples that may modulate the cytokine profile.

Keratinocytes are a target for IFN-γ and TNF-α, which stimulate expression of ICAM-1 and other adhesion molecules on the keratinocyte cell surface [25], which are important for keratinocyte attachment and lysis [26]. Nickel-specific T lymphocytes have been shown to secrete IL-17, which modulates

the effects of IFN-γ and TNF-α on keratinocytes. In particular, IL-17 augments IFN-γ-induced ICAM expression [25]. This is independent of the direct toxic effect of Ni and Co increasing the expression of ICAM and other adhesion molecules, e.g., on endothelial cells, through a redox-dependent NF-κB pathway [27]. Both Ni-specific Th1 and Th2 clones secrete IL-17 [28].

Numerous lines of evidence demonstrate that these effects are not due to direct effects of Ni on the cells, but are part of an antigenic response. Lisby et al. [29] noted no direct effect of $NiSO_4$ on isolated T cells, but preincubation of macrophages with $NiSO_4$ led to subsequent activation of both naive and memory T cells from patch test-negative individuals. The activated cells were mainly of the $CD4^+$ subset, as expected. Importantly, the response was blocked by antibodies to MHC class II molecules, supporting the notion of their role in the response to hapten. Possible mechanisms are discussed below.

Cross-reactivity is an important issue in Ni allergy, as Ni exposure often occurs in the context of multiple metal exposures, and positive patch tests to other metals (e.g., Co and Cr) are common. However, some Ni-specific T-cell clones cross-reacted in vitro with Cu and Pd, but not with Co, Cr, or Pt [30], indicating that true cross-reactivity can occur at the clonal level. Another study [31] confirmed clonal cross-reactivity with either Cu or Pd, but not Co or Cr, in $CD4^+$ clones from eight patients. The authors noted the similar binding ability of Ni, Cu, and Pd to histidine residues of peptides that might be found in the pocket of MHC class II molecules (see Section 3). Nickel and Pd were confirmed to be the most common concomitant sensitivities in a series of 1000 consecutive patch tests [32]. That sulfates of other metals inhibit a positive $NiSO_4$ patch test in Pd-insensitive, but not Pd-sensitive subjects suggests that in the latter case Ni and Pd share a common protein-binding site to achieve molecular mimicry.

Evidence that the chemical reactivity of Ni species toward protein is an important determinant of sensitization comes from the work of Artik et al. [33]. Noting that mice are difficult to sensitize against Ni(II), but that sensitization is easier in inflamed tissue, they suggested that production of the more reactive Ni(III) and Ni(IV) by H_2O_2 and HOCl, respectively, released from activated macrophages could be responsible. Indeed, they found that Ni(III) and Ni(IV), but not Ni(II), were able to sensitize naive mouse T cells.

2.2 Cobalt

Occupational exposures to metallic cobalt and its alloys are also widespread, and have been summarized elsewhere [34]. They include metal production, chemical production, rubber, paint, cement, and varnish industries, pottery finishing, and diamond polishing. Hard metal production and use in drilling and machining are important sources of cobalt exposure as mixed metal carbide dusts. Respiratory disease and skin sensitivity are associated with chronic exposure.

Respiratory pathology in hard metal workers may take the form of obstructive disease (occupational asthma or work-related wheezing) or interstitial lung disease (fibrosing alveolitis). A cross-sectional study of more than 1000 tungsten carbide workers at 22 sites identified work-related wheezing in 10.9 % and a profusion of opacities on chest radiograms in 2.6 % [35]. The relative odds of work-related wheeze was 2.1 when those with current workplace Co exposure exceeding 50 $\mu g/m^3$ were compared with those exposed to lower ambient concentrations. Studies by Lison and Lauwerys [36–38] cast some doubt on the exclusive role of Co, suggesting that synergy between Co and tungsten carbide dust is responsible for disease. However, diamond polishers exposed to Co have developed a pattern of occupational asthma and fibrosing alveolitis termed "cobalt lung" [39,40]. These similarities have strengthened the argument for Co as an etiologic agent in hard metal dust.

That a small percentage of exposed workers develop interstitial lung disease and true occupational asthma has focused debate on the distinction between the sensitizing effects of Co and its direct cytotoxicity. Evidence for the importance of sensitization has come from Japan: eight cases of confirmed occupational asthma related to hard metal dust were positive in inhalational provocation tests with neb-

ulized aqueous $CoCl_2$ [41]. Specific IgE antibodies to a complex of cobalt with human serum albumin were found in serum samples from four of these patients, but not in samples from 60 unexposed asthmatics or 25 nonasthmatic coworkers [41]. Furthermore, ^{57}Co was selectively bound to protein component(s) in serum from seven of the eight. Type I hypersensitivity mediated by IgE is suggested. More recently, 2.0 % of 700 workers producing hard metal tools had cobalt-specific IgE antibodies and symptoms of asthma [42]. However, the same investigators subjected eight patients with hard metal asthma to bronchial provocation challenge with $NiSO_4$ [43]. Seven responded with a drop >20 % in forced expiratory volume (FEV_1, an indicator of lung function), and antibodies to Ni-conjugated albumin were present in four, suggesting that Ni may also have played a role in their asthma. Alternative nonimmune mechanisms may involve susceptibility of macrophages to activation by Co or reactivity of target cells to macrophage-derived mediators [44]. Clearly, the full mechanisms remain to be elucidated.

Consistent with immunologic modes of action, allergic dermatitis has been reported in workers in numerous cobalt-related industries, including those in hard metal and Co alloy, paint, cement, and rubber industries [45,46]. An allergic dermatitis has been observed in Finnish potters handling Co clay [45]. The dermatitis is of an erythematous maculopapular type [47] and is strongly associated with a history of eczema [48]. Many patients with Ni^{2+} sensitivity also have Co^{2+} sensitivity, but this may reflect concomitant sensitization rather than cross-reactivity. As noted above, cross-reactivity of Ni^{2+}-reactive T-cell clones is seen with Cu^{2+} and Pd^{2+}, but not with Co^{2+} [30,31].

2.3 Chromium

Chromium mainly is found as metallic Cr or in the trivalent (3+) and hexavalent (6+) states. Chromium exposures occur in industries using anodizing and metal plating, etching, photography and lithography, Portland cement, stainless steel, and television picture tube manufacture, to list only a few [49]. Trivalent Cr is poorly absorbed through the skin, but Cr^{6+} is readily absorbed and acts as both an irritant, causing burns or ulceration, and a sensitizer. When sensitization to chromates and dichromates occurs, symptoms are generally long-lived [50,51] and may be exacerbated by contact with chromates in textiles, pigments, and tanned leather products [49].

Exposure to cement is an important cause of Cr sensitization in society. In some countries, iron(II) sulfate is being added to cement to reduce chromate to the nonsensitizing Cr(III). In Britain, where this is not done, the incidence of positive patch tests has remained the same (about 2 % of females and 4 % of males) during the 1980s and 1990s [52]. About 40 % of positive subjects were co-sensitized to Co^{2+}, a crude indication of sensitization via cement.

2.4 Mercury

The toxicity of both inorganic and organic (methylated) species of mercury are well known [53,54], and steps in past decades to curtail human exposure to environmental mercury have surely decreased incidents of acute toxicity. The major source of exposure to inorganic Hg today is probably through its use in dental amalgams [55], although it is still used in preservatives such as thiomersal (e.g., for preservation of protein solutions). Whether the occurrence in amalgams is of any serious toxicological consequence remains very controversial; whether it can lead to sensitization is only somewhat less so [55]. Contact with Hg salts such as the chloride or ammonium chloride commonly causes hypersensitivity [56], though occupational contact dermatitis is considered rare [1]. It has been noted that positive patch tests to mercurials infrequently have a clinical manifestation [56]. Skin eruptions have been noted in the areas of tattoos with mercury-based red pigment [56].

Mercury exposure can elicit IgG and IgE formation. Glomerular deposition of IgG complexes is a common feature of glomerulopathy, and may contribute to the nephrotoxicity of Hg [57]. A specific issue is whether the immune syndromes seen following Hg exposure are related directly to activation of the immune system by Hg, or are secondary to Hg-induced tissue damage. The autoimmune profile

of increased antinuclear antibodies observed after treating mice with mercuric salts [58] would support the latter, and the possibility of systemic autoimmunity following Hg exposure has been discussed [1]. Most of the autoantibodies react with the nuclear ribonucleoprotein fibrillarin, which may also be a target in scleroderma patients [59]. Antilaminin and antinucleolar antibodies have also been reported [1]. Evidence is that $CD4^+$ Th2 cells are preferentially activated that utilize IL-4, IL-5, IL-6, and IL-10 to stimulate IgE production [10,60]. When IL-4 action was suppressed with anti-IL-4 antibodies, $HgCl_2$ failed to increase serum IgE levels in mice.

Whether the autoimmune response to Hg^{2+} is a secondary toxicity or not, the evidence is that Hg^{2+} is a bona fide sensitizer. A mouse model of $CD4^+$ T cell-dependent $HgCl_2$ autoimmunity has been used to study the specificity of the T-cell response [59]. Donor T cells from mice treated for one week with subcutaneous injections of $HgCl_2$ were used in a popliteal lymph node assay. Cells mounted a response to $HgCl_2$ and to splenic proteins isolated from $HgCl_2$-treated mice. The Hg content of the splenic proteins from the treated mice was 1.4 pg/mg protein. On the other hand, after eight weeks of $HgCl_2$ treatment, donor T cells reacted poorly with $HgCl_2$ 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 [59].

Thus, the pattern of Hg immunosensitization differs from that of Ni or Co, in that Hg is a weaker sensitizer yet shows more prominent systemic effects. Nevertheless, it fits with the model of peptide modification sensed by the antigen-presenting cell (see Section 3) [1]. Interestingly, a high incidence of positive patch test reactions to $HgCl_2$ in patients with Au dermatitis has been noted. Guinea pigs contact-sensitized to mercurochrome developed positive patch tests to both Hg compounds and Au salts [61], supporting the idea of cross-reactivity between Au and Hg.

2.5 Beryllium

Exposure to Be occurs in the electronics, aerospace, and ceramics industries, as well as in primary metal industries and the manufacture of nuclear weapons and fluorescent lamps [62]. The National Institute for Occupational Safety and Health estimates that up to 800 000 individuals are exposed to Be at the workplace in the United States alone [63]. Exposure to relatively high concentrations of soluble Be compounds, such as the sulfate or fluoride, leads to an acute dermatitis, as well as inflammation of the nasal mucous membranes, pharynx, trachea, and bronchi. This acute Be disease usually resolves on cessation of exposure. The long-known chronic Be disease (CBD; beryllosis) [62,64], on the other hand, includes loss of pulmonary function, chest pain, fatigue, weight loss, and anorexia. Sensitization is caused by local deposits of Be-containing particles in the airways. Skin, lymph nodes, liver, and spleen may be involved, and interstitial inflammation and fibrosis may lead to cor pulmonale and death. While Be in beryls (aquamarine and emeralds of formula $Al_2O_3{\cdot}3BeO{\cdot}6SiO_2$) 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 [65]. In only one of 57 beryl cutters was a positive Be lymphocyte proliferation test (BeLPT) result found; that in a subject with measurable urinary Be. There appears to be no clinical significance to this form of exposure.

CBD is a noncaseating granulomatous disorder clinically indistinguishable from pulmonary sarcoidosis. Despite the lack of evidence for a direct dose–response relationship to Be exposure [66], the observation of a mean latency period of 20 years to develop symptoms [67], and the granulomatous lesion typical of an irritant response, the characteristic picture of CBD supports a specific immunosensitization to Be. Indeed, the primary distinguishing features between sarcoidosis and CBD should be a history of exposure and immunosensitivity to Be. The BeLPT test has been developed in part to distinguish specific Be allergy from other causes of pulmonary granulomatous disease such as sarcoidosis [68,69].

2.6 Copper

Copper is widely used in electrical wiring, gas and water pipes, coinage, printing, electroplating, and more. The Cu-based contraceptive intrauterine device exposed many women to Cu through a mucosal surface. But, though Cu sensitization is widely recognized, it has not been thoroughly investigated. Allergy to Cu is generally assumed to be rare, but Cu allergy could be instructive in distinguishing immune mechanisms of sensitization from other responses to metals acting just as irritants. Studies addressing specifically this point have not been undertaken. In an early study [70], 6 of 354 subjects reacted to a Cu patch test; all were women with hand eczema, but none had apparent occupational exposure to Cu, and none wore a contraceptive intrauterine device. Cross-reactivity with Cu, of Ni-selective T-cell clones, is noteworthy [31]. Common protein-binding motifs for Ni, Pd, and Cu, such as the Asp-Ala-His sequence at the *N*-terminal of albumin, may be important.

2.7 Platinum

A major source of occupational exposure to Pt is in the manufacture and recycling of automobile catalytic converters. Here, the exposure is predominantly to the chloroplatinic acid catalysts. Among Pt refinery and Pt catalyst plant workers, occupational asthma persists in a high percentage of subjects even after removal from exposure, and deteriorates with continued exposure, even at low levels [71]. Exposure to Pt salts in refinery workers is associated with elevated IgE levels [72]. "Platinosis" may develop in over 50 % of exposed workers [1], with immediate-type reactions (rhinitis, conjunctivitis, bronchial asthma) upon provocation with chlorplatinates. Delayed-type hypersensitivity may also occur, but has not been proven by large-scale patch testing [1]. A case of contact dermatitis from wearing a Pt ring has been reported [56], and contact urticaria has been observed following occupational exposure to the antineoplastic agent cisplatin (*cis*-$[NH_2]_2PtCl_2$) [73].

Mice chronically treated with Pt complexes do not develop autoimmune disease such as seen with Hg^{2+} treatment, and do not have elevated IgE [1]. Although elevated IgE levels have been observed in some Pt-exposed refinery workers [74,75], Pt-specific antibodies or T cell activation have not been demonstrated in humans [1]. In mice, subcutaneous injections of chloroplatinates induces a primary immune response in a popliteal lymph node assay, that is mediated by $CD4^+$ T cells [76]. The response is absent in athymic mice. Topical application of high doses of $PtCl_6^{2-}$ to susceptible mice produced both primary and secondary local immune responses [1].

2.8 Palladium

Palladium is mainly used in industrial catalysts, and specifically in catalytic converters in automobiles, in electronics, in jewelry, and as a major component of some dental prostheses [56]. Sensitization rates of up to about 10 % have been observed by patch testing in several studies (reviewed in [1]). Perhaps the most interesting aspect of Pd^{2+} sensitization is, as noted above, its frequent, specific cross-sensitization with Ni^{2+} [30–32]. The similarities in chemistry of Ni^{2+} and Pd^{2+} supports the idea of a similar mechanism involving common protein binding sites and conformational alterations [77]. While $PdCl_2$ and chloropalladates such as $PdCl_4^{2-}$ and $(NH_4)_2PdCl_6$ elicit a potent immune response in rodents, with occurrence of Pd-specific T cells [1], the incidence of sensitization within the human population probably reflects sensitization to Ni^{2+}.

2.9 Iridium and indium

Iridium and indium are platinum group metals used industrially for catalytic and chemical resistance properties, and in dental alloys. Cases of sensitization are known but rare. A worker exposed to aerosols of platinum group metal salts in HCl developed contact urticaria and respiratory symptoms [78]. Four

solutions from the work place were tested on the forearm in a scratch test. Two solutions produced strong weal and flare reactions. These two solutions were found to contain mainly H_2IrCl_6; Ir was absent from the other two solutions. The patient did not react on testing with Pt. Six patients testing positive to In and Ir with exposure from dental alloys were also allergic to Co, Ni, Au, and Pd [79]. In one series of Pt refinery workers, positive prick tests to Ir, Ru, Rh, and Pd were observed, but all these workers also tested positive to Pt [80], and cross-reactivity is suggested.

2.10 Gold

An important aspect of the immunology of gold is the development of allergy after therapeutic use of gold compounds in rheumatoid arthritis (chrysotherapy) [81–85]. Salts of Au(I) such as sodium aurothiomalate or aurothiosulfate are used to delay progression of inflammatory arthritides such as rheumatoid arthritis, and less frequently skin diseases such as pemphigus and psoriatic arthritis. The mechanisms underlying immunosensitization with Au and its therapeutic effect in autoimmune diseases remain elusive, but may well be related; Au salts inhibited peptide-specific responses of Au-specific T cells in patients treated with Au compounds [82]. Allergic reactions necessitate discontinuing therapy in up to one-third of rheumatoid arthritis patients within a few months. Patients develop dermatitis, stomatitis, and eosinophilia, and less commonly immune-complex glomerulonephritis, lymphadenopathy, antinuclear antibody, increased serum IgE, hypogammaglobulinemia, 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 [86]. Until recently, Au allergy was considered rare in the absence of its medical use. During the 1990s, increasing numbers of reports of Au sensitivity have appeared [87,88].

Case reports of contact allergy to Au have been reported from jewelry, dental fixtures, orbital implants, electroplating solutions, etc. [84,88]. Various studies have reported positive patch test frequencies of between less than 1 % and greater than 10 %, but it has been proposed that many cases of type IV hypersensitivity to Au are asymptomatic, and the standard protocol of patch testing with gold sodium thiomalate may produce false negatives because a prolonged exposure may be necessary; a positive reaction may require three weeks or more [86]. Women account for the majority of positive subjects in most studies [84,88]. The lymphocyte proliferation test has been shown to be of value in diagnosis of Au allergy in rheumatoid arthritis patients [89]. Rats that develop increased serum IgE levels in response to Au compounds have identifiable markers in the chromosomal MHC and IL-4 regions [90], suggesting multiple mechanisms for Au allergy.

The clinical significance of Au allergy remains somewhat unclear. Most of those testing positive do not have clinical symptoms. Bruze and Andersen have highlighted this controversy and pointed out the need to demonstrate exposure and causation or aggravation of dermatitis in addition to showing allergy [87]. They point out the need for further research into the role of jewelry and dental work in sensitization, and investigation of the release of Au from various alloys into sweat and saliva.

3. MECHANISMS

Central to an understanding of the immune response to metals is the mechanism whereby T cells can recognize a metal ion or complex. While metalloenzymes have highly specific metal binding sites, peptides in general present a number of residues that can contribute to metal chelation or binding with varying degrees of specificity and affinity. In general, "soft" metal ions such as Hg^{2+} and Cd^{2+} will bind to protein sulfhydryl groups through bonds with higher covalent character, whereas "hard" ions like Ca^{2+} will prefer carboxylate groups. Ions of intermediate character, such as Ni^{2+} and Zn^{2+}, will additionally involve nitrogen through amino and histidine (imidazole) groups.

Whereas antibodies bind over specific epitopes on the surface of a protein antigen, T-cell receptors recognize only short peptides presented on the surfaces of antigen-presenting cells (APCs) bound

to a major histocompatibility complex (MHC) class I or class II molecule. Structural aspects of the molecular interactions have been recently reviewed [91]. In general, initiation of the immune response involves activation of $CD4^+$ T lymphocytes upon recognition of the antigenic peptide bound to the so-called antigen-presenting groove present in MHC molecules on the APC surface [92]. The antigen-presenting groove is specialized for protein binding; there is no evidence for other compounds, and in particular for metal ions, binding at this site. However, other entities that may be referred to as haptens also elicit a T-cell receptor-mediated response, with trinitrophenyl being a classical example [93,94] and various metal ions falling into the same category. This has suggested a model wherein metal ion haptens, directly or indirectly, cause defined structural changes in MHC-peptide complexes that, in turn, result in recognition by specific T-cell subsets [9].

The evidence that recognition of metals by cells of the immune system is restricted to T cell involvement is convincing. The MHC consists of the antigenic peptide bound to a human leukocyte antigen (HLA) class I (HLA-A, HLA-B, HLA-C) or class II (HLA-DR, HLA-DQ, HLA-DP) molecule complexed to the T-cell receptor. Nickel- or gold-specific $CD4^+$ T-cell clones were isolated from patients with nickel or gold contact dermatitis [7,9,95], respectively. The T cells were tested against a series of APCs expressing different HLA isotypes. The T cells responded to Ni^{2+} only when it was presented by cells expressing HLA-DRw11, and to Au in association with HLA-DR4. These strict associations argue that the recognition is dependent upon the HLA isotype, and therefore likely to depend upon the MHC. Further evidence for this conclusion was obtained with Be-specific T cells from patients with chronic beryllium disease [96]. The cells were $CD4^+$, and specifically anti-HLA class II antibodies but not anti-HLA class I antibodies inhibited proliferation of the T cells in response to Be.

Metal ions such as Ni^{2+} require specific spatial arrangements of amino acids to form stable complexes. Therefore, they may be more restricted in interactions with proteins than a chemically reactive hapten such as trinitrophenyl [97]. Nevertheless, metals may still interact with many proteins, and several possibilities must be considered [97]. The ion might bind to soluble or cell surface proteins, which subsequently are taken up and processed by the APC. It might also enter the cell and modify a cytosolic protein, which is subsequently processed and presented on MHC molecules. Alternatively, the hapten could bind directly with preformed peptide-MHC complexes on the cell surface. Some T-cell clones react to Ni presented by glutaraldehyde-fixed APC [30], suggesting that Ni is part of the antigenic epitope and binds to peptides already associated with MHC molecules. T-cell clones have also been identified that recognize glutaraldehyde-fixed B cells treated with Au(I) [82].

The idea that an antigenic metal acts as a hapten and is MHC class-restricted suggests four possibilities (Fig. 1). The metal might bind to the antigenic peptide (i) before or (ii) after its association with the HLA component, in either case changing the conformation or presentation of the peptide. It might also (iii) interact with the MHC class II molecule before peptide binding, to influence subsequent peptide binding and presentation, or (iv) interact with the MHC protein component only in the intact complex (perhaps requiring ligands from both peptide and MHC protein), again changing the way the complex is seen by the T-cell receptor. To begin to distinguish these mechanisms, Sinigaglia and coworkers [9,98] used two DRw11-restricted T-cell clones recognizing two different peptides. Proliferation of one of the clones in response to the appropriate peptide was inhibited by Ni^{2+}. To determine whether this was due to inhibition of peptide binding or, for instance, to an effect on the intact complex, the effect of treating the APC with Ni^{2+} before the peptide, or vice versa, was determined. Treatment with Ni^{2+} before exposure to the peptide in the absence of Ni^{2+} was without effect. However, when the peptide was first allowed to bind to the APC and then the complex was exposed to Ni^{2+}, proliferation of the Th cell was inhibited. This suggested that interaction of Ni^{2+} with the bound peptide was responsible for blocking recognition by T-cell receptor. In an elegant experiment, a putative Ni-binding histidine residue in the peptide was replaced by Lys [98]. The Lys variant peptide was still able to stimulate T-cell proliferation, but now the stimulation was not inhibited by Ni^{2+}. Furthermore, NMR spectra showed that Ni^{2+} did indeed interact with the histidine-containing peptide, but not the ly-

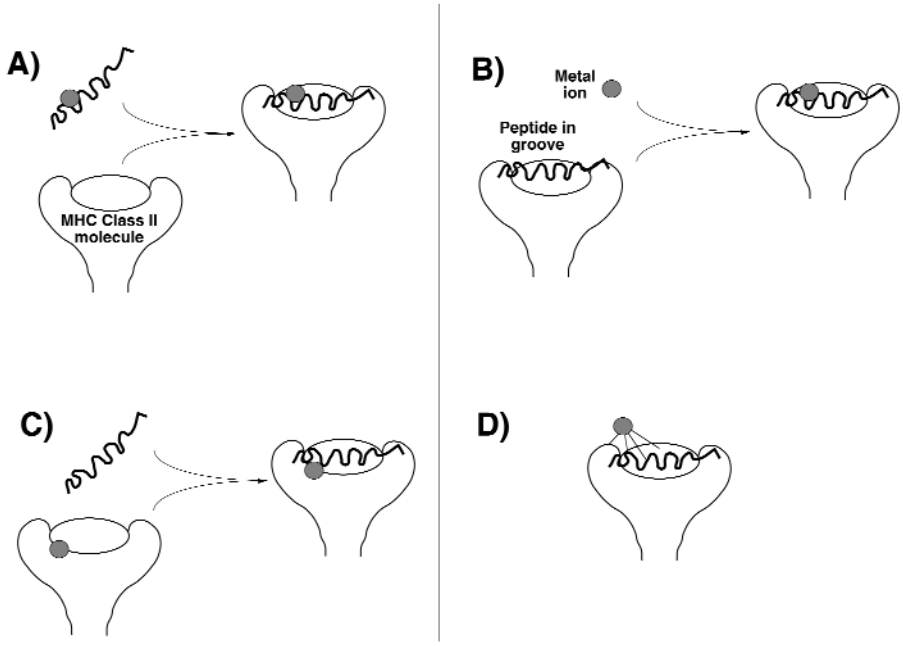

Fig. 1 Possible scenarios for presentation of an antigenic metal hapten. (A) Metal ion bound to antigenic peptide before association with the MHC. (B) Antigenic peptide associates with HLA class II molecule before binding the metal. (C) Metal ion binds with the HLA class II molecule before recruitment of the antigenic peptide to the MHC. (D) Metal ion binds to the intact complex, perhaps requiring ligands from multiple sources. See text for further discussion.

sine-containing variant. Thus, Ni^{2+} binding by the peptide in the complex is strongly suggested to account for inhibition of T-cell proliferation.

The above experiments present an example of a metal ion interacting with an MHC-bound peptide to influence Th cell recognition. The effect is inhibitory, and does not directly address the issue of metal-dependent T-cell activation. However, it does establish the principle of metal hapten-peptide binding influencing T-cell recognition. Multiple peptides are available for binding to a given MHC molecule, depending on the presence of an arrangement of anchoring residues on the peptide [99,100]. One can imagine that in some cases metal binding would inhibit, and in other cases enhance T-cell recognition. Presentation of Ni^{2+} to HLA-DR4-restricted Ni^{2+}-sensitive T cells with carefully mapped HLA-DR4 cells supported the idea that some of the DR subtypes stimulated the T cells by binding to endogenous peptides in the antigen-presenting groove [101]. In this model, the preponderance of IL-4/IL-5-secreting Ni^{2+}-specific T cells in sensitized individuals can be explained by a low density of enhancing Ni^{2+}-binding ligands on the APC: a low density of ligands favors stimulation of Th2 over Th1 cells [102,103].

Gold also inhibits T-cell proliferation [9] in an assay similar to that described above for Ni^{2+}. However, in contrast to Ni^{2+}, pretreatment of the APC with Au before exposure to the peptide still inhibited proliferation, suggesting direct binding of Au to the MHC molecule, rather than to the peptide as is the case with Ni^{2+}. 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 [9]. 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 [104]. This may underlie the therapeutic effect of the Au compounds. Thus, Au may block autoreactive T cells in rheumatoid arthritis patients but also enhance T-cell reactivity in other circumstances, through

direct interactions with MHC molecules. Beryllium may share a common mechanism; a particular glutamate residue in an allele of an HLA-DP member of the MHC II family has been found to be a strongly associated with susceptibility to chronic Be lung disease [105].

While the above considerations focus on haptenic determinants on the APC, Ni^{2+} is not in general preferentially presented in association with distinct HLA-DP, -DQ, or DR alleles in sensitized individuals [6,9,106]. Therefore, recognition determinants on the T-cell receptor must also be important. Supporting this view, the Vβ region of the T-cell receptor appears to be involved, with a strong association between the Vβ phenotype and Ni^{2+} sensitization; TCR-Vβ17 correlates with the extent of the T-cell response in vitro [77] and with the clinical severity of Ni^{2+}-induced dermatitis [107]. Vollmer et al. (107) have suggested that Ni^{2+} may bridge the complementarity-determining region-1 of the T-cell receptor Vβ17 element and the MHC/peptide complex, enhancing otherwise weak receptor–peptide contacts. This region contains a His-Asp-Ala motif reminiscent of the Ni-binding sites in several proteins. The same group also identified Arg^{95} and Asp^{96} in the complementarity-determining region-3 as important for Ni recognition [108].

In addition to the fairly clear evidence of binding Ni(II), Pd(II), and Au(I) salts to the MHC-peptide complex, other mechanisms, less well substantiated, are nevertheless quite feasible. These include denaturation of proteins by oxidizing elements such as Au(III), Pd(IV), and Pt(IV); altered protein unfolding upon formation of stable coordination complexes with species such as Hg(II), Pt(II), and Au(I); and metal-catalyzed peptide hydrolysis [1,109]. Each mechanism could lead to altered peptide presentation or presentation of cryptic self-peptides by APC. The importance and detail of these mechanisms remain to be elucidated, but as has been noted [1], the potential for metal-dependent "sensitization to cryptic self-peptides blurs the distinction between metal-induced allergy and autoimmunity".

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