

Review article Series V. The skin as target for IgE-mediated allergic reactions

Immunologic mechanisms in hypersensitivity reactions to metal ions: an overview

Metal ions such as Ni^{2+} , Co^{2+} , Cu^{2+} , or Cr^{3+} are haptens with a high immunogenic potential, as contact dermatitis caused by ionic metals occurs in about 10–15% of the human population. Since alloys containing Ni^{2+} , Co^{2+} , and Cr^{3+} are components of implants in replacement surgery, dentures, orthodontic wires, and various other devices, adverse reactions to metal ions create serious problems in practical medicine as incompatibility reactions to metal-containing biomaterials. On the other hand, contact dermatitis to metal ions such as Ni^{2+} is a well-established model for studying the molecular mechanisms involved in the recognition of haptens by the immune system. Although many investigations have been performed to elucidate the molecular interactions causing contact hypersensitivity in man, many aspects remain to be clarified. This review will focus on the experimental data accumulated so far on the immunologic mechanisms responsible for the recognition of metal ions by T cells and eliciting adverse immune reactions causing contact dermatitis.

L. Büdinger, M. Hertl

Department of Dermatology, Rheinisch-Westfälische Technische Hochschule Aachen, Aachen

L. Büdinger

Interdisciplinary Center of Clinical Research (IZKF)
Biomat, Rheinisch-Westfälische Technische Hochschule Aachen, Aachen, Germany

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Lioba Büdinger,
Hautklinik der RWTH Aachen
Pauwelsstr. 30
D-52074 Aachen
Germany

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Nickel (Ni^{2+}) represents the most common occupational as well as public contact allergen, with up to 10% skin test positivity in the Caucasian population (1). Ni^{2+} -induced allergic contact dermatitis is caused by delayed-type hypersensitivity (2). Other metal ions such as cobalt (Co^{2+}), copper (Cu^{2+}), chromium (Cr^{3+}), palladium (Pd^{2+}), and platinum (Pt^{2+}) can also induce contact dermatitis, and simultaneous contact sensitivity to several metals is a common clinical observation (3–5). These reactions result from skin contact with the metal ions, which bind to cellular and matrix proteins of the skin and induce a cellular immune response. Because *in vitro* stimulation of peripheral blood mononuclear cells (PBMC) by metal ions leads to strong proliferative responses in allergic individuals, there is strong evidence for T-cell involvement in the pathogenesis of metal hypersensitivity (6, 7). In our investigations, we found significant proliferative responses to metal ions upon incubation of PBMC from allergic patients in most instances (Table 1). Nevertheless, different phenotypes and cytokine patterns of the T cells involved are reported.

Ni^{2+} -specific T cells have been shown to express either the CD4 or CD8 surface molecules (7–10) and to produce T helper 1 (Th1) (IL-2, IFN- γ) (11) or

Th2 (IL-4, IL-5) cytokines (12, 13). In addition, several groups have identified Th0-type cells (producing both Th1 and Th2 cytokines) (12). This difference in cytokine production may be due to the microenvironment of the lymphocytes involved, which has been demonstrated to play a critical role in directing T-cell responses versus T helper type 1 or type 2 cytokine secretion. Soluble and membrane-associated molecules expressed by resident cells or antigen-presenting cells (APC), as well as the nature of T-cell receptor–MHC interactions, may be important for the development of T-cell effector functions (14) in different compartments of the organism. Due to their small size, metal ions are incomplete antigens (haptens) which have to bind to (endogenous) peptides to become full antigens. They associate in a trimolecular complex of MHC II molecules, peptide, and T-cell receptor to induce a specific T-cell response. Human T-cell clones specific for metal ions have been shown to recognize haptenated peptides in the context of HLA class II molecules (6, 11, 15). No preferential association with certain HLA-DR, -DQ, and -DP alleles was found in individuals sensitized to Ni^{2+} , Cr^{3+} , and Co^{2+} (16). However, Ni^{2+} -specific T cells from Ni^{2+} -allergic individuals have been demonstrated to express a limited T-cell

Table 1. Results of epicutaneous patch test reactions and *in vitro* proliferative lymphocyte responses to metal ions in metal-allergic patients

Patient	Epicutaneous patch test*				<i>In vitro</i> response (SI**)						
	NiSO ₄	CoCl ₂	PdCl ₂	K ₂ Cr ₃	NiSO ₄	CoCl ₂	CuCl ₂	CrCl ₃	AgNO ₃	PdCl ₂	Background***
M1	++		+		1.1	1.0	1.1				1426
M2	++				7.9	0.9	1.2	1.0		1.4	1660
M3	++	+			10.3	2.5	2.0				1073
M4	++		+		4.7	2.2	1.7				1061
M5	++				1.8	0.9	0.7				1279
M6	+				1.0	1.8	1.7				1415
M7	+				16.6	2.1	1.2				1156
M8	++	+		++	16.5	0.5	0.4	0.8			3153
M9	++	+			1.8	0.8	0.5	1.0		1.1	2034
M10	+				4.6	1.1	0.3	1.0		1.5	940
M11	++	++			0.7	0.4	0.3	0.8		0.7	3385
M12	+++	+++			12.2	6.8	2.2	2.0		2.6	2122
M13	++				1.8	0.7	0.8	1.2		1.0	1819
M14	+				1.6	1.3	0.9	1.2		0.9	2653
M15	++				1.7	0.8			1.7	1.0	936
M16	++				0.2	0.2	0.3			0.2	1775
M17	++				0.3	0.2	0.1				4738
M18					1.9	1.9		1.0			210
M19	++				1.0	1.7			6.0		1546
M20	++				1.5	0.8	0.6		2.0		1256
M21	++				2.0	1.6	2.2		2.2		533
M22	++				1.7	1.4	1.0		1.7		902
M23	+				1.4	1.2	0.6	0.9	0.7	1.1	2884
M24	++				2.8	14.7	0.8				1319
M25	++				19.4	2.4	1.6				515
M26	++				8.3	0.7	0.7	1.1	1.0	1.2	712
M27					0.5	1.1	0.7	1.2	7.1	1.3	1108
M28					0.8	1.5	0.9				422
M29	++				2.1	0.6	2.1				197
M30	++				5.0	1.2	1.4			1.1	998
M31	++				3.8	2.1	1.2		1.7	0.9	1858
M32	++				3.7						1382
M33	+++				40.3	3.7	1.0	1.0	1.1	1.4	746
M34	++				2.5						1366
M35	++				2.7	0.7	1.1		1.0		891
M36	++				2.2	0.7	1.1		0.6	0.5	1000
M37	++				30.3	6.3	1.5		1.3	2.1	439
M38	++				2.0	3.7	2.2	1.1	1.8	3.0	228
M39	++				4.6	1.2	1.0	0.9	1.6	1.5	306
M40	++				8.6	1.6	2.9		1.1	1.4	209
M41	++				8.8	1.7	0.5		1.3	1.2	2551

* +: erythema; ++: papules; +++: papulovesicles.

** SI: mean cpm (counts per min) in cultures with antigen; mean cpm in cultures without antigen; an SI > 2 was considered significant.

*** cpm, ³H-labeled thymidine uptake in cultures without antigen.

receptor V β repertoire (13). In addition, the number of T cells expressing a TCR with distinct V β chains seems to correlate with the clinical severity of Ni²⁺-induced contact dermatitis (17).

Until now, little was known about the structure of the antigenic epitopes created after epicutaneous application of metal salts recognized by the antigen-specific receptors of Ni²⁺-responsive T cells. Processing requirements (Fig. 1) for recognition of Ni²⁺ by T cells have been investigated by Moulon et al. (15), and hapten recognition has been demonstrated to be independent of processing by an APC in most of the studied human T-cell clones.

Furthermore, two independent studies were able to show immunologic cross-reactivity of CD4+ Ni²⁺-specific T-cell clones with other metals (e.g., Cu²⁺ or Pd²⁺) (15, 18). The knowledge of cross-reactive recognition of metal ions by the immune system may be important for the choice of implant materials in order to avoid incompatibility reactions. With regard to metallic implants in the deeper human tissues, little is known about the uptake and evolving immune response to free metal ions. Lacy et al. (19) corroborated the experimental findings that there is a difference in the uptake of Ni²⁺ or other metal salts in different tissues. According to their studies, there is much more uptake of Ni²⁺

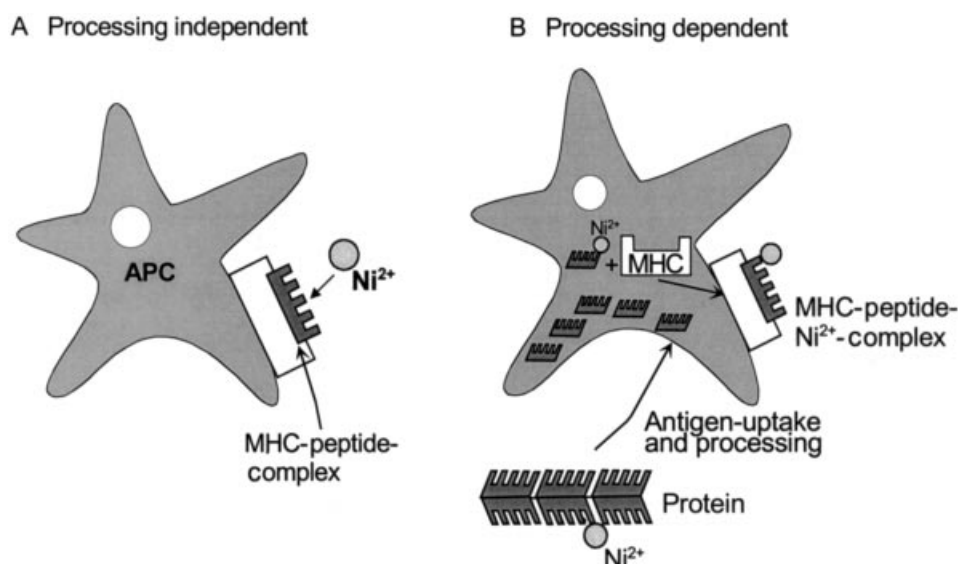


Figure 1. Mechanisms of metal ion (e.g., Ni^{2+}) presentation. Two pathways of metal ion presentation are outlined: **A)** To become a complete antigen, the metal ion binds directly to a MHC-bound peptide (processing-independent presentation). **B)** The metal ions form coordinative bonds to cysteine or histidine residues of soluble or membrane-bound proteins. The modified proteins are taken up by antigen-presenting cells (APC), and are processed and presented to T cells as metal-peptide complexes on MHC molecules.

and Co^{2+} by keratinocytes than by fibroblasts. In addition, Ni^{2+} is excreted much faster in the urine upon intramuscular injection than epicutaneous application. The authors thus concluded that metal salts are retained in the skin for an extended period of time, leading to prolonged antigen processing and recognition in the dermal tissue.

Metal recognition by T cells

The activation of T cells via TCR-ligand-interaction is dependent on the presence of a complex consisting of a specific antigen bound to a MHC molecule on the surface of the APC (20). There is no evidence for the binding of nonpeptidic compounds to MHC class I and class II molecules. Both MHC class I and class II restricted T cells have been shown to recognize nonpeptidic molecules such as trinitrophenyl (TNP) (21, 22) or haptens such as metal ions (6, 11, 23, 24).

Haptens such as metal ions are considered to be incomplete antigens which have to bind to proteins or peptides to become immunogenic. The molecular targets of such hapten-binding processes, i.e., formation of metal complexes, may include soluble or cell-associated proteins, including MHC-associated peptides. Metal complexes are usually formed by a coordination complex consisting of a central metal ion and a set of ligands. These ligands may be ions or small molecules, arranged in geometric forms, such as plane squares or polygons. In the coordination bond, the electron-rich ligands make one or more of these electrons available for

the connection to the positively charged central (metal) ion. Ligands for the coordination complex of a metal ion with a peptide may consist of residues of histidine or cysteine. As the binding of a metal to a MHC-bound peptide or to the MHC molecule itself may result in a conformational change of these proteins, different effects are possible:

- 1) The new structural feature of the metal-MHC/peptide-complex may create new epitopes (neoantigens) which may be recognized by T cells.
- 2) Metal ions may directly interact with endogenous or exogenous peptides.

Romagnoli et al. (24, 25) demonstrated that T-cell clones reactive to a malaria circumsporozoite protein-derived peptide (CS peptide) were no longer reactive to this peptide upon treatment of the peptide-pulsed APC with Ni^{2+} . A histidine residue of the CS peptide was responsible for the Ni^{2+} binding. Substitution of histidine by lysine in the CS peptide resulted in a variant peptide which was still recognized by the CS peptide-specific T-cell clone, but its recognition could not be inhibited by NiSO_4 .

Similar findings have been reported by Griem et al. (26), who showed that Au(I) interferes with antigen processing by the formation of Au(I) -peptide chelate complexes. CD4^+ T-cell hybridomas specific for different antigenic peptides were tested in the presence of disodium aurothiomalate (Au(I)TM). In these studies, T-cell recognition of peptides possessing two or more cysteine residues could be inhibited by the addition of Au(I) salts

(Fig. 2). The requirement of two or more cysteine residues for the formation of stable Au(I) complexes was confirmed by substitution of cysteine by serine in an immunodominant peptide: the inhibitory effect of Au(I) on peptide-specific T-cell proliferation was completely eliminated.

Since Ni^{2+} hypersensitivity in man is not associated with the presence of distinct HLA class I or II alleles (16), some investigators analyzed the T-cell receptor (TCR) repertoire of Ni^{2+} -reactive T cells. The structural diversity of the TCR, which is necessary for the recognition of a broad variety of peptide antigens, is generated during lymphocyte ontogeny by random combinatorial rearrangement of gene elements encoding variable (V), diversity (D) (only for the B chain), and joining (J) segments (27). The interaction between the TCR and MHC-antigen complex is determined by the hypervariable complementarity determining region (CDR) sequences. The CDR3 regions appear to interact with the central part of the peptide (28, 29), whereas the genomically determined CDR1 and CDR2 loops contact either the N- or C-termini of the peptide and/or α -helical segments of the MHC molecule (30, 31). Vollmer et al. (17) examined the TCR repertoire of peripheral T cells from six Ni^{2+} -allergic patients who were stimulated by NiSO_4 *in vitro*. They found a strong overrepresentation of the TCR BV17 element in the three Ni^{2+} -allergic individuals who showed the strongest patch test response to Ni^{2+} among the investigated patients. In addition, TCR sequencing of one of those patients revealed a skewing for AV1 as well as a selection for an N region-encoded arginine at position 95 in the CDR3 of the BV17 element. The authors pointed out that this selection of arginine is driven by contacts with peptide rather than with Ni^{2+} , since arginine is not known to participate in Ni^{2+} complexing. Another finding of this investigation was a unique combination of amino acids – that is, the sequence HDA (histidine/aspartate/alanine) – in the CDR1 of BV17. This amino-acid sequence was similar to known motifs of Ni^{2+} -binding proteins; namely, DHAK (aspartate/histidine/alanine/lysine), a Ni^{2+} -binding site in human serum albumin allowing complex formation with metal ions such as Ni^{2+} or Cu^{2+} . In summary, these data provide evidence for an antigen-driven selection in clonal or polyclonal peripheral T-cell cultures. Another study by Werfel et al. (13) also addressed the heterogeneous usage of the TCR in Ni^{2+} hypersensitivity. The authors investigated the TCR repertoire of a panel of 79 blood- or skin-derived CD4^+ Ni^{2+} -reactive T-cell clones from two Ni^{2+} -allergic donors. Their findings suggested a selection of certain TCR-V β elements of NiSO_4 -responsive T cells in these patients: only 20% of

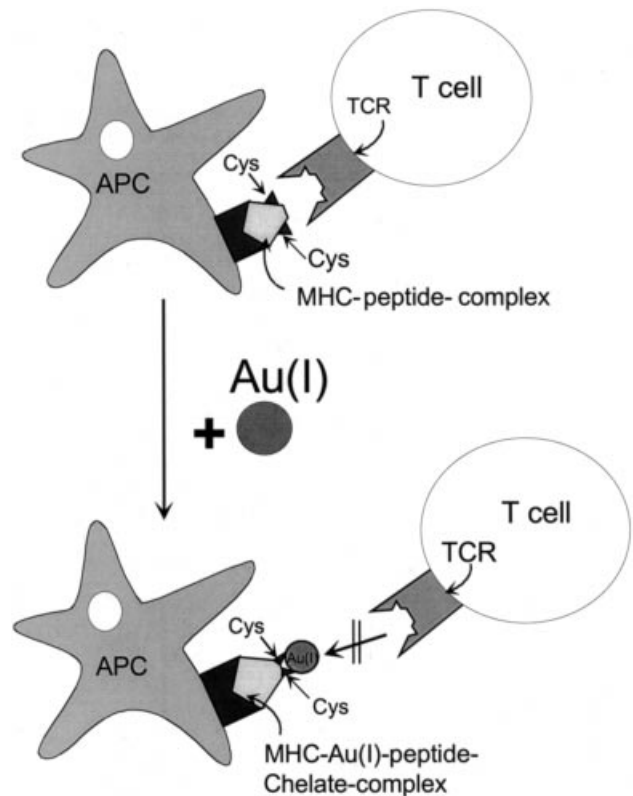


Figure 2. Mechanism of the selective inhibition by metal ions (Au(I)) of antigen recognition by peptide-specific T cells. Here, the metal ion Au(I) forms coordinative bonds with two cysteine (Cys) residues of an antigenic peptide. The formation of Au(I)-peptide chelate complexes selectively inhibits T-cell recognition of antigenic peptides possessing two or more Cys residues. Disodium aurothiomalate (Au(I)TM) is used as an antirheumatic drug. The therapeutic effect of Au(I) in rheumatoid arthritis seems to be due to the mechanism described in this presentation (26).

unstimulated T cells but >40% of Ni^{2+} -stimulated T cells of the same patients expressed the V β elements V β 13.1/13.2, V β 20, V β 2, V β 6.7, or V β 14. There was no significant difference in distribution of these TCR-V β elements between blood- and skin-derived Ni^{2+} -reactive T-cell clones.

Phenotypic and functional diversity of Ni-specific T cells

T cells involved in the recognition of Ni^{2+} seem to represent a very diverse population with regard to their cytokine secretion. Kapsenberg et al. (11) found that blood-derived CD4^+ helper T cells specific for NiSO_4 from allergic and nonallergic individuals produced substantial amounts of the Th1 cytokines IFN- γ and IL-2 and low levels of the Th2 cytokines IL-4 and IL-5. IFN- γ plays an important role in the expression of delayed-type hypersensitivity (DTH) in mice (32) and man (33,

34). The findings of Kapsenberg et al. corroborate the experimental data of Sinigaglia et al. (6), who reported that Ni^{2+} -specific T cells from nickel-allergic donors secreted IFN- γ and IL-2. It is unclear whether the Ni^{2+} -reactive T cells of the latter study also secreted Th2 cytokines. As the differentiation of allergen-specific T-cell subsets into Th1- or Th2-like T cells may depend on the pathway of immunization, CD4+ T cells responsive to contact allergens, which interact with the immune system via the skin, seem to skew the immune response toward a Th1-like pattern (35). However, as Th1-like Ni^{2+} -specific T cells have been found in allergic individuals and a nonallergic donor (11), the elicitation of contact dermatitis to Ni^{2+} cannot be exclusively explained by the presence of T cells secreting Th1 cytokines.

Silvennoinen-Kassinen et al. (36) established 12 Ni^{2+} -specific T-cell clones from the peripheral blood of Ni^{2+} -allergic donors which produced differing cytokine profiles. In this study, all of the T-cell clones produced soluble IL-2R, and 50% of them secreted high amounts of IL-4. Of the T-cell clones, 7/12 secreted IL-6, and 3/12 produced IL-4 as well. The authors suggested that the production of IL-4, which can enhance cytolytic activity, contributed to the tissue damage seen in nickel dermatitis.

Other studies suggested that Th2-like cytokines are involved in DTH reactions to Ni^{2+} . On the RNA level, Szepietowski et al. (37) investigated the expression of different Th1 and Th2 cytokines in the skin of nonatopic and atopic patients with Ni^{2+} allergy after epicutaneous application of Ni^{2+} . After Ni^{2+} challenge, the mRNA expression for IFN- γ , IL-2, and IL-4 was significantly increased in both panels. In contrast, only nonatopic individuals showed increased mRNA expression for IL-10, an immunoregulatory cytokine, which is produced by several cells found in inflamed skin such as T cells, monocytes, keratinocytes, and melanocytes (38, 39). A study by Probst et al. (12) showed that most of the skin-infiltrating T cells responded to Ni^{2+} with the production of high levels of IL-5 and variable amounts of IL-4 and IFN- γ , thus expressing Th2 or Th0 cytokine patterns. Similar findings have been reported by Werfel et al., who established a large panel of blood- and skin-derived Ni^{2+} -reactive T-cell clones from three allergic patients (13). The authors found a significantly higher production of IL-4 by skin-derived Ni^{2+} -specific T-cell clones after stimulation with concanavalin A than by T cells derived from blood. In contrast, T-cell clones generated from peripheral blood secreted significantly higher amounts of IFN- γ . The authors concluded that there was a modulation of cytokine secretion of T cells after immigration into the skin.

In addition, Werfel et al. also investigated the frequency of CD4+ and CD8+ Ni^{2+} -specific T cells. They found that 21% of all established Ni^{2+} -specific T-cell clones carried the CD8 molecule. Interestingly, this ratio varied greatly in the different compartments: 33% of blood-derived T-cell clones, but only 15% of the skin-derived T-cell clones, were CD8+. The type of cytokine produced was independent of the CD4/CD8 phenotype.

So far, the role of hapten-specific CD4+/CD8+ or Th1/Th2 T-cell subsets in the pathogenesis of allergic contact dermatitis in man is largely unknown. As mentioned earlier, both CD4+ and CD8+ T-cell subsets have been detected in skin lesions induced by epicutaneous application of Ni^{2+} . Furthermore, *in vitro* immune responses to Ni^{2+} have also been observed in nonallergic individuals. In a recent study, Cavani et al. compared the characteristics of T-cell responses to Ni^{2+} in allergic patients and healthy, nonallergic individuals (9). The results of this study strongly suggest that CD8+ T cells play an important role in the pathogenesis of DTH to Ni^{2+} , since Ni^{2+} -reactive CD8+ T cells were restricted to individuals with positive patch test reactions to Ni^{2+} and were not detected in nonallergic donors. In contrast, both Ni^{2+} allergics and normals carried CD4+ CLA+ memory T cells responsive to Ni^{2+} . T-cell clones from nonallergic donors secreted cytokines different from those of allergic individuals, in that they produced higher amounts of IL-10 and less IFN- γ (9). These differences in phenotype and cytokine secretion suggest that distinct mechanisms may contribute to the surveillance of clinically inapparent T-cell-driven immune responses to Ni^{2+} . Cavani et al. (9) observed a subset of Ni^{2+} -specific T-cell clones (mainly derived from nonallergic donors) which released high levels of IL-10 and low to undetectable amounts of IFN- γ , IL-4, and IL-2, thus representing a newly identified CD4+ T-cell subset, named T regulatory cells 1 (Tr1). This regulatory T-cell subset has been found to display an immunosuppressive function both *in vitro* and *in vivo* (40).

Another recent study by Moulon et al. identified cytotoxic CD8+ effector T cells in Ni^{2+} contact hypersensitivity (10). All of 15 Ni^{2+} -reactive CD8+ T-cell clones were established from the peripheral blood of three Ni^{2+} -allergic patients. Eleven of these clones produced either cytotoxic T-cell-0- or cytotoxic T-cell-1-type cytokines. All 15 CD8+ T-cell clones were investigated with regard to their effector functions (i.e., cytotoxicity for Ni^{2+} -pulsed EBV-B cells). From these studies, two distinct patterns of T-cell reactivity to Ni^{2+} were identified. Most of the CD8+ T-cell clones lysed only autologous targets in the continuous presence

of Ni^{2+} and thus appeared to be MHC-restricted. In contrast, one CD8+ T-cell clone lysed target cells only in the presence of Ni^{2+} but independently of the expression of HLA class I or II molecules on the APC. The mechanisms of activation of this particular clone could not be elucidated in this study. In summary, this study clearly demonstrated that effector cytotoxic CD8+ T cells are present in Ni^{2+} -allergic individuals and may contribute to Ni^{2+} -induced contact hypersensitivity reactions *in vivo*.

Cross-reactive metal recognition by human T cells

Because simultaneous sensitization to several metals occurs frequently in man, there have been several investigations of whether this *in vivo* observation was due to cross-reactivity on the level of individual T cells. For this purpose, Moulon et al. (15) established a panel of 42 Ni^{2+} -specific T-cell clones from four metal-allergic individuals and studied their cross-reactivity to Pd^{2+} , Co^{2+} , Cu^{3+} , Cr^{2+} , Zn^{2+} , and Pt^{2+} . By their reactivity, these T-cell clones were divided into four different types of reactivity:

- 1) reactivity to Ni^{2+} , but not to the other tested metals
- 2) cross-reactivity to Pd^{2+}
- 3) cross-reactivity to Cu^{2+}
- 4) cross-reactivity to both Pd^{2+} and Cu^{2+} .

These cross-reactive T-cell clones were further investigated with respect to their HLA class II restriction and processing requirements. It was found that metal recognition by cross-reactive T cells worked identically for both cross-reactive metals (i.e., Ni^{2+} and Cu^{2+} , or Ni^{2+} and Pd^{2+}) and was dependent on distinct processing requirements (Fig. 1): T cells which reacted to Ni^{2+} presented by glutaraldehyde-fixed APC (processing-independent) also recognized the cross-reactive metal independently of processing requirements. The same observation was made with T-cell clones which recognized Ni^{2+} upon processing by the APC: these clones also recognized the cross-reacting metal upon presentation by live APC. In addition, the cross-reactive clones recognized their two metal haptens upon presentation on identical HLA class II molecules. The restricting immunogenetic elements were different for $\text{Ni}^{2+}/\text{Cu}^{2+}$ -reactive T-cell clones and $\text{Ni}^{2+}/\text{Pd}^{2+}$ -reactive clones, suggesting that two different structural determinants were involved in the presentation of these metal ions. As there was no observation of cross-reactivity of Ni^{2+} and Co^{2+} on the clonal level, the authors concluded that *in vivo* reactivity to these two metals – which are

present together in most such alloys – may be due to concomitant sensitization (15).

Similar findings were made by Pistoort et al. (18), who investigated 20 Ni^{2+} -specific T-cell clones obtained from eight Ni^{2+} -allergic donors. In their study, most (15/20) Ni^{2+} -specific clones were cross-reactive to either Cu^{2+} or Pd^{2+} . In contrast to Moulon et al., Pistoort et al. did not find differences in the restriction patterns of the two different groups of cross-reactive T-cell clones. The cross-reactivity to Ni^{2+} , Cu^{2+} , and Pd^{2+} is explained by the chemical and structural similarities of these metal ions. It has been shown that Ni^{2+} is able to bind to histidine-containing peptides present in the grooves of MHC class II molecules (25). Because bivalent Cu ions are also known to bind to histidine (41), the cross-reactivity of Ni^{2+} -reactive T cells with Cu^{2+} might be explained by molecular mimicry. The preferential binding site of Pd^{2+} is still unknown, but both Cu and Pd are close to Ni in the periodic table of the elements, a fact which may also contribute to the high frequency of cross-reactivity to Ni^{2+} (18).

Conclusions

An abundant body of literature deals with the identification and characterization of metal-specific T cells in man. Nevertheless, little is known about the relevance of these described T cells *in vivo*, as most of the studies have focused only on *in vitro* investigations of PBMC and PBMC-derived T-cell lines and clones from allergic and nonallergic donors. A particular pattern of secreted cytokines cannot be attributed to distinct effector T-cell populations in Ni^{2+} hypersensitivity, because conflicting cytokine patterns were observed by different investigators. In addition, no clear-cut phenotypic or functional differences between Ni^{2+} -specific T cells were identified in allergic and nonallergic individuals. Even though some investigators suggest that there is a limited TCR V β repertoire involved in T-cell recognition of Ni^{2+} , the molecular mechanisms of T-cell activation by metal ions are not yet elucidated. The experimental data on potential cross-reactivities of metal-specific T cells have been reproduced by two independent groups although the molecular mechanisms of metal recognition are still under investigation.

The presence of Ni^{2+} -reactive T cells in the peripheral blood and skin of Ni^{2+} -allergic individuals strongly suggests that Ni^{2+} -specific T cells are effector cells in Ni^{2+} -induced contact sensitivity. The possibility of *in vivo* investigations is limited for this particular type of DTH reaction, as mice represent a poor model for studying contact

sensitivity to metals (42). With regard to adverse reactions to implanted metallic biomaterials, both *in vitro* and *in vivo* investigations in animal models, as well as in patients with metallic implants, might be helpful for a better understanding of the distinct role of different subtypes of metal-specific T cells

under these conditions *in vivo*. Although many investigations have been carried out to demonstrate the cytotoxic, genotoxic, and allergic effects of metals (43, 44), it still remains to be determined whether adverse reactions to metallic implants are the result of a specific cellular immune response.

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