T cell receptor (TCR) interaction with haptens: metal ions as non-classical haptens

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Abstract

Haptens are classified as low molecular chemicals with an intrinsic potential to covalently modify proteins, and many of them are strong inducers of contact hypersensitivity (CHS). CHS is T cell mediated, and hapten-specific T cells have been shown to interact with hapten-modified, MHC-associated peptides. However, the most common contact sensitizer in the industrialized world is nickel. In contrast to classical haptens, nickel ions do not form covalent bonds to proteins, but rather become caught in reversible coordination complexes. We here review work demonstrating that some T cells, indeed, may react to such Ni complexes on the MHC/peptide-surface absolutely comparable to other haptens. In other cases, Ni ions unlike classical haptens, may activate T cells by crosslinking their receptors to MHC molecules, independent of the nature of the associated peptide. Moreover, Ni-interacting proteins appear to make use of the reversibility of Ni-binding, and to mediate the transfer of Ni-ions to the receptor-MHC interphase. We have demonstrated such properties for human serum albumin (HSA) as well as for transferrin and identified numerous new Ni-binding proteins in human B-cell lines or dendritic cells by affinity purification and mass spectroscopy. These proteins include a notable number of known heat shock proteins and chaperones, implying that Ni may functionally interfere with these stress proteins.
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1. Introduction

Karl Landsteiner (Landsteiner and Jacobs, 1935) introduced the term hapten for low molecular chemicals, which upon covalent coupling to proteins give rise to the production of hapten-specific antibodies in experimental animals. Decades later, Shearer

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(1974) and consequently many others (Pohlit et al., 1979) demonstrated that not only B cells, but also T cells might mount specific responses to cell- or protein-bound haptens. Following the unveiling of the structural basis of MHC-restricted antigen recognition by T cells in the 1980s (Buus et al., 1987; Hannum et al., 1984), it was a study by Ortmann et al. (1992) which first demonstrated that hapten recognition by T cells required covalent hapten-attachment to MHC-associated peptides. Subsequently, these findings spurred interactions between allergology and basic immunology since haptenic chemicals and drugs represent major sources of allergens in the human population (Weltzien and Padovan, 1998; Weltzien et al., 2004). On the other hand, it was apparent that also non-reactive chemicals and drugs could give rise to B and T cell mediated allergic hyperreactivities. The term pro-hapten was created to describe molecules, which only upon cellular metabolism were transformed into reactive metabolites, able to modify MHC-binding self-peptides. One of the best-studied compounds in that sense was urushiol, the allergenic essence of the North American poison ivy (Kalish et al., 1994).

A third group of low molecular allergens is composed of transition metals such as chromium, beryllium or nickel, the latter one representing the most prevalent contact allergen in the industrialized world (Nielsen et al., 2002). Nickel ions have been shown to dissolve in sweat (Menne, 1996; Nestle et al., 2002; Nielsen et al., 2002), and upon penetration through the skin to induce metal-specific hyperreactivities via an activation of HLA-restricted, nickel-specific T cells (Budinger and Hertl, 2000; Sinigaglia, 1994; Weltzien et al., 1996). In contrast to classical haptens, metal ions do not form stable covalent protein modifications. They are rather known to produce geometrically highly defined coordination complexes with four or six electron-donators such as nitrogen or oxygen in amino acid side chains of appropriate proteins or peptides (Zhang and Wilcox, 2002; Fausto da Silva and Williams, 2001). By definition, such complexes are reversible and allow the exchange of the allergenic metal ions between different acceptor sites. It has, thus, been exceedingly difficult to define allergenic metal epitopes, and the question arose whether the term hapten would in its original sense actually apply to metal ions. During recent years our studies on nickel contact hypersensitivity have, therefore, concentrated on both: the structural definition of antigenic epitopes as well as the identification of nickel-binding proteins in immunologically relevant cells.

2. Nickel epitopes for T cells

2.1. Nickel-epitopes resembling classical hapten-determinants

We have previously shown that 50–60% of all Ni-reactive T cell clones isolated from peripheral blood cells of Ni-allergic individuals could be activated by autologous APC, pulsed with $NiSO_4$ and subsequently washed free of surplus nickel. In many cases this even worked with glutaraldehyde-fixed APC, indicating that antigen processing was not required (Moulon et al., 1995). In addition, Ni-reactive $CD4^+$ T cells of exceptionally strongly sensitized donors over-represented the TCR Vβ17 element (Budinger et al., 2001; Vollmer et al., 1997). Such clones, thus, appeared to recognize HLA-associated determinants that included Ni-ions as structural part of the epitope. For one of these clones, i.e. the DR52c-restricted clone ANi-2.3, recent data by Lu et al. (2003), indeed, support the recognition of Ni^{2+} complexed to His81 on the HLA-DR52c β-chain and probably two other coordination sites on a limited selection of DR52c-associated peptides. Hence, in this instance Ni^{2+} ions appear to behave much like a classical hapten, forming a rather stable determinant by coordinative binding to amino acid residues of MHC as well as the associated peptide. On the side of the TCR of ANi-2.3 an Arg-Glu motif in the β-chain's CDR3 sequence was defined before as one of the complementary contact sites (Vollmer et al., 1999).

2.2. Nickel-epitopes differing from classical hapten-determinants

Taking into account that only about half of all Ni-reactive T cells are activated by Ni-pulsed APC (Moulon et al., 1995), the above scenario clearly represents only one of probably many different ways in which Ni^{2+} ions may activate specific T cells. Gamerdinger et al. (2003) have studied Ni contact sites in MHC and TCR for another clone (SE9) which also is activated in the presence of fixed APC, but unlike clone ANi-2.3 requires the permanent presence of surplus

Ni-salts in the medium for activation. In contrast to clone ANi-2.3, the Ni-activation of clone SE9 turned out to be totally independent of the kind of peptide associated to the restricting HLA-DR molecule. However, it also crucially depended on the conserved His81 in the DR β-chain. Antigen contacts of the SE9 TCR appeared to localize exclusively to its α-chain, two of them being identified as Tyr29 in the CDR1 and Tyr94 in the CDR3 loop. The model resulting from these studies implied that an appropriate Ni-coordination site is provided only upon the pre-formation of a low-affinity TCR–MHC complex, which subsequently may be stabilized by the insertion of Ni^{2+}.

The scenario for Ni-activation of clone SE9, thus, is reminiscent of T cell activation by superantigens which bridge TCR containing suitable Vβ elements with MHC molecules, independent of the kind of associated peptide and of the specificity of the TCR. However, Ni-recognition by SE9 requires a contact site within the N-region-determined third hypervariable region of the TCR α-chain that, in contrast to superantigens, limits specificity to an extremely small selection of receptors.

The comparison of the two clones reveals that many different molecular interactions between Ni^{2+}, TCR and MHC have to be considered. Moreover, our earlier finding that about 40% of all Ni-specific clones could not be activated with fixed APC indicates the necessity of protein processing in those situations. A proportion of those clones may, in fact, be reactive to Ni-free cryptic self-peptides resulting from Ni-induced alterations of protein processing (Griem et al., 1998).

3. Proteins as carriers and potential mediators in nickel allergy

3.1. Nickel-binding to serum albumins

In vivo, and most likely also in our serum-containing media in vitro, Ni^{2+} most likely never occurs as free ion but rather complexed to serum- or cell-derived proteins. Binding of Ni^{2+} to MHC-peptide or TCR–MHC defined coordination sites, therefore, requires Ni^{2+} transfer from as yet unknown carrier proteins to TCR–MHC contact sites. In addition such Ni-binding proteins may, or actually must, be involved in transporting the metal ions from the outer surface of the skin to the Langerhans cells as the professional antigen

presenters. One well-defined Ni-binding protein in man is human serum albumin (HSA) whose N-terminal four amino acids Asp-Ala-His-Lys form an intensively studied binding site for Cu and Ni (Zhang and Wilcox, 2002). The role of this binding is often seen as a mechanism of detoxification. However, since HSA also represents a major protein constituent of interstitial fluids in the skin, we considered its possible role as a carrier of Ni^{2+} in the process of contact hypersensitivity.

In a recent publication (Thierse et al., 2004) we demonstrated that HSA–Ni complexes, indeed, bear the potential to stimulate Ni-reactive T cells in the presence of appropriate APC. Unlike for trinitrophenylated proteins (Weltzien et al., 1996, 2004), however, complexes of Ni with HSA-derived peptides do not appear to contribute to the antigenic determinant (Thierse et al., 2004). In addition, experiments with fluorescently labeled HSA as well as with Ni-detecting dyes revealed that HSA–Ni complexes are rapidly internalized by APC and T cells with no detectable amount of Ni^{2+} remaining at the cell surface (Thierse et al., 2004).

3.2. A model for HSA–Ni as a potential mediator in T cell activation

From the above findings we concluded that Ni^{2+} needed to be transferred from its binding site on HSA either directly or indirectly to the contact zone between TCR and MHC. This, in turn, implies a regular formation of short-lived, non-stimulatory contacts between peripheral T cells and self-MHC/peptide complexes in the absence of Ni. In fact, others have postulated such contacts as furnishing survival signals for peripheral T cells (Ernst et al., 1999). Model experiments revealed that Ni^{2+} was transferable from HSA only to peptides of comparable or higher affinity for the metal ion (Thierse et al., 2004). Thus, addition to HSA–Ni of the N-terminal HSA peptide or a histidyl dipeptide removed Ni^{2+} from HSA–Ni, whereas the Cu-binding peptide Gly-Gly-His had no such effect (Fig. 1 and (Thierse et al., 2004)). We, therefore, envisage the role of HSA as a shuttle for Ni^{2+}. As schematically indicated in Fig. 2A, HSA–Ni may not transfer Ni^{2+} to an isolated MHC–peptide complex. However, a non-productive TCR–MHC contact site (Fig. 2B) may well serve as an acceptor, if this complex provides a perfect coordination site for Ni^{2+}. This, in turn should confer
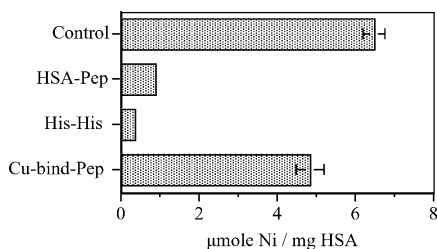
Fig. 1. Exchange of nickel between HSA and peptides. HSA–Ni (120 µM) was treated for 6 h at 37 °C without (control) or with different peptides (500 µM). Peptides used were the N-terminus of HSA Asp-Ala-His-Lys (HSA-Pep), the dipeptide His-His, or the copper-binding peptide Gly-Gly-His (Cu-bind-Pep). Solutions were then dialyzed and protein-bound Ni determined by atomic absorption spectroscopy (Thierse et al., 2004).

sufficient additional strength to the TCR/MHC contact to raise the overall avidity to a level allowing for the activation of the engaged T cell.

In a situation where the coordination sites for Ni^{2+} involve defined amino acids within the MHC-associated peptides, the number of epitopes on any
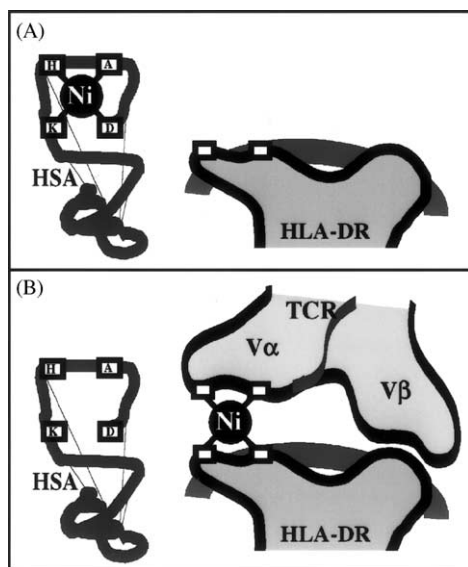

Fig. 2. Proposed nickel exchange between HSA and TCR/MHC complexes. Ni^{2+} complexed to the N-terminal Asp-Ala-His-Lys sequence of HSA may not be released to bind to incomplete coordination sites on MHC/peptide-surfaces (A). However, optimal Ni-acceptor sites formed transiently by appropriate TCR–MHC–complexes may attract Ni^{2+} from HSA–Ni (B). The metal crosslink will supply enough avidity to ensure T cell activation.

APC is expected as low as for any peptide-specific T cell. However, clones such as SE9, which may use the restricting MHC protein as a complementary Ni-binder, independent of the associated peptide, will encounter significantly higher epitope densities on APC. They would be expected to react more sensitive to Ni then the majority of T cells and, hence, to represent a particularly critical proportion of the Ni-reactive T cell repertoire.

3.3. Other nickel–protein complexes as T cell activators

The fact that we demonstrated HSA–Ni as a stimulating entity for Ni-reactive T cells in vitro does not necessarily imply that it also in vivo acts as a or *the* major shuttle for Ni^{2+} ions. Even though it represents a perfect model to study such processes, other proteins may be even more effective in that sense in vivo. In fact, even in vitro we could show that completely unphysiological Ni-carriers such as Ni^{2+} chelated to NTA-modified horseradish peroxidase (Ni-POD), designed to detect His-tagged recombinant proteins, effectively stimulated Ni-reactive T cells in an MHC-restricted fashion (Fig. 3). The about five-fold greater efficacy of Ni-POD versus HSA–Ni relates to a 10 to 20-fold higher Ni-load per mole of POD (data not shown).

Most interestingly, we also were able to load Ni^{2+} onto apo-transferrin, resulting in a complex resembling a molar Ni/protein ratio of maximally 8/1. This complex, in the presence of appropriate APC stimulated
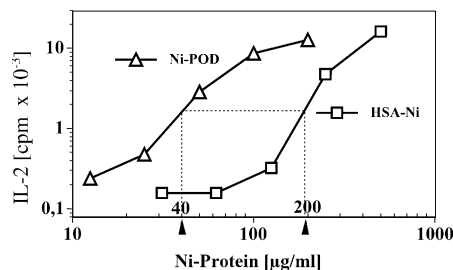

Fig. 3. T cell activation with Ni-NTA-coupled horseradish peroxidase (Ni-POD). The T cell transfectoma T23 (Lu et al., 2003; Vollmer et al., 1999) was incubated with increasing concentrations of either HSA–Ni (squares) or Ni-POD (triangles) in the presence of autologous APC. Secreted IL-2 in the supernatant was determined by proliferation (^{3}H-thymidine incorporation) of an IL-2 dependent T cell line (Vollmer et al., 1999). The molar Ni-content of Ni-POD was at least 10-fold higher than that of HSA–Ni.

Ni-specific T cells about 10 times more effectively than HSA–Ni (data not shown). Hence, HSA despite its general availability in skin may not necessarily be the only, or the most efficient protein in transferring Ni^{2+} to the TCR/MHC interface. Moreover, Ni-binding proteins on or in APC and/or T cells might play a role as intermediates in such processes.

3.4. A sub-proteomic approach to define nickel-binding proteins in cells

Consequently we set out to identify Ni-binding proteins, in immunologically relevant cells such as human B and T cell lines, or in vitro generated human dendritic cells. To that end we lysed cells in a mild Triton buffer and affinity purified proteins attaching to paramagnetic NTA-Ni beads. Upon elution of these proteins from the beads with imidazole (2–5% of the proteins in total lysate), the mixtures were separated by isoelectric focussing in the first and SDS-polyacrylamide gel electrophoresis in the second dimension. Fig. 4 shows silver-stained gels for the human B-cell line Raji (A) and immature human dendritic cells (B). The latter were differentiated from CD14^{+} blood monocytes in IL-4 and GM-CSF according to Jonuleit et al. (1997).

Parallel gels were stained with Coomassie Blue as described by Jungblut et al. (1993), individual spots picked, trypsinized, and analyzed mass-spectroscopically. In these studies more than 25 potentially Ni-interacting proteins could be identified (Heiss et al., 2005). Some of them, such as bovine serum albumin (BSA) from our media as well as actin or

Table 1
Some affinity-purified nickel-interacting proteins from human B cells

No	Protein
Chaperone-/HSP-family proteins	
1	DnaK-type molecular chaperone (HSP-70)
2	HSPA1L (Hsp70-HOM)
3	BiP (Grp78)
4	HSC70
5	Heat shock protein 54
6	T-complex protein 1, epsilon subunit (TCP/CCT)
Other proteins	
7	Actin gamma
8	Tubulin alpha-1 chain
9	Tubulin beta chain
10	Tubulin alpha-6
11	Endoplasmic reticulum ATPase
12	Bovine serum albumin (BSA)

Shown is a collection of proteins repeatedly identified in imidazole eluates from Ni-NTA-beads loaded with Triton X-100 lysates of Raji and WT47 cells. Proteins were isolated from Coomassie stained 2D-gels (see Fig. 4), trypsinized and subjected to mass spectrometry.

tubulin were expected and could even serve as positive controls (see numbers 7–12 in Table 1). Among the other proteins, however, we were surprised to repeatedly find a variety of known chaperones and heatshock proteins, such as HSP-70, BiP, HSC-70, HSP-54 or TCP1/CCT (see numbers 1–6 in Table 1). An essential feature of these stress proteins is their association with other proteins to ensure their correct folding or to protect them from degradation. Their enrichment via Ni-beads, thus, not necessarily indicates their direct interaction with the metal ions, but might be brought about via association with other Ni-binding molecules. For HSP-70 we have controlled for this with recombinant protein, demonstrating direct binding of Ni by atomic absorption spectroscopy (data not shown).

4. Conclusions

Available data on the molecular basis of T cell activation by nickel imply that metal ions in comparison to classical haptens are capable of activating specific T cells by a large variety of molecular mechanisms. Some T cells respond to Ni–MHC–peptide complexes quite comparably to determinants formed by hapten-peptides in an MHC binding groove (Lu et al., 2003). In other cases, however, the metal ion appears to serve as
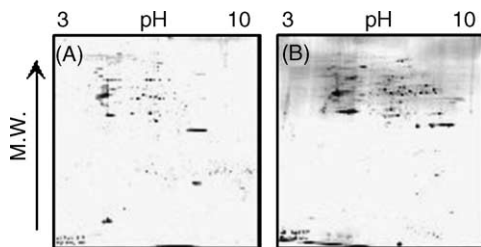

Fig. 4. Two-dimensional gel patterns of Ni-bound cellular proteins. Human Raji cells (A) or immature dendritic cells (B) were lysed in 0.1% Triton X-100 and the lysates treated with Ni-NTA magnetic beads. After washing, bound proteins were eluted with imidazole, separated by isoelectric focussing in the first, and reducing SDS polyacrylamide gel electrophoresis in the second dimension. Staining with silver.

a direct and peptide-independent linker between TCR and MHC, bearing certain similarities to superantigen-mediated activation (Gamerdinger et al., 2003). A third possibility is the interference of transition metals with the processing of self-proteins and the exposure of cryptic self-peptides (Griem et al., 1998).

Furthermore, we presented evidence that nickel, unlike chemically reactive haptens, may employ particular carrier proteins to ensure its transport through epidermis and dermis as well as to allow for its specific transfer to short-lived, high-affinity coordination sites created within certain TCR–MHC contact zones. This fluctuation between different protein-attachments clearly differentiates nickel ions from classical haptens. Finally, their affinity for numerous stress proteins implies that nickel ions in addition to forming allergenic epitopes may functionally interfere with metabolically important chaperones. Thus, Ni has been shown to induce HSP-70 in keratinocytes (Carroll and Wood, 2000). Future studies will have to decide whether or not this may contribute to the exceptional allergenicity of nickel.

Acknowledgements

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