Commentary

Metal-derivatized Major Histocompatibility Complex: Zeroing in on Contact Hypersensitivity

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One of the prices we pay for our love of jewelry is allergic contact hypersensitivity to metal, an autoimmune condition that variably afflicts around 10% of all Caucasians (1). Contact hypersensitivity is a classic type IV DTH (delayed type hypersensitivity) response, involving primed T cells that are specific for metal modified antigens generated at a local site in the body (most commonly the epidermal layer of the skin). The T cells respond by producing proinflammatory mediators that result in local redness, swelling, and itching (2). In many cases nickel is found to be the culprit and unfortunately for sensitive people, nickel is one of the most common metals in the environment, which makes it particularly difficult to avoid. Being a ubiquitous component of metal alloys, nickel is found not only in catheters, needles, dental braces, and many other medical devices, but also in everyday items such as jewelry, watches, coins, and even in some foods. Cases can range in severity from a mild localized swelling, redness, and itchiness to a much more debilitating reaction involving larger areas (e.g., the entire mouth in some patients with hypersensitivity to the metal of their dental braces). Ni\(^{2+}\) reactive T cell clones have been isolated from patients and found to display varying degree of MHC class II restriction (some are more promiscuous than others) and two models have been advanced to explain what might be happening at the molecular level (3). The first model (Fig. 1) proposes that Ni\(^{2+}\) derivatized self-proteins are naturally processed and presented as Ni\(^{2+}\)/peptides by the APC resident in the skin (e.g., Langerhan cells). The second model proposes a processing independent pathway where the metal directly derivatizes MHC peptide complexes on the surface of the APC. While both mechanisms seem feasible, firm evidence for either has been elusive . . . until now. In this issue, Lu et al. (4), present direct evidence for the formation of a preformed HLA-DR/Ni\(^{2+}\) complex that stimulates a Ni\(^{2+}\) reactive human T cell clone called ANi-2.3. ANi-2.3 is a CD4\(^{+}\) T cell with a VB17/Vα1 T cell receptor and is restricted by HLA-DR52c pretreated with soluble Ni\(^{2+}\). Using mutagenesis, they localize the Ni\(^{2+}\) binding site to histidine 81 on top of MHC class II β-chain. His81 is familiar to anyone who has worked with bacterial superantigens because it’s the same residue targeted by a subset of bacterial superantigens using a Zn\(^{2+}\) atom to bind tightly to MHC class II (5). His81 is one of only a few conserved residues on the top of the MHC class II and plays an important role in stabilizing bound peptide through a hydrogen bond to the peptide backbone (6). The important feature of Zn\(^{2+}\) in superantigen binding is the stability it provides the MHC class II/Sag complex, so the analogy between stable superantigen binding and metal-mediated TCR binding is clear and important. The Lu paper reveals for the first time, a possible molecular structure that might explain how normally tolerant, self-reactive T cells are stimulated into action through the addition of a single metal atom on the top of the MHC class II molecule.

How Do Metals Bind Proteins? Every stage I biology student knows that transition metals such as zinc, copper, iron are essential components in many biochemical reactions. Nickel on the other hand, has no known biological role in humans but is used by some microbes; the best known being the two Ni\(^{2+}\) atoms at the active site of bacterial urease (see the PDB structure 2UBP and see Fig. 2). Transition metals form coordination complexes with the imidazole nitrogens of histidine, the carbonyl group of aspartate or glutamate or the free sulfur atom of cysteine amino acids. Like Zn\(^{2+}\) which is arguably the best-studied metal, Ni\(^{2+}\) has a coordinate number of 4. At least 3 amino acid side-chains must be correctly spaced in a trigonal pyramid or planar geometry to tether the metal ion to the protein surface (see Fig. 2). Zn\(^{2+}\) binds via a conserved motif L1 - (X)_n - L2 - X_{(1-2)} - L3 with the critical component being the strictly spaced L2-L3 bidentate ligand separated by either 1 amino acid when present on a β-strand or 2 amino acids apart when on an α-helix (7). Ni\(^{2+}\) binding sites are less well studied and subsequently, no Ni\(^{2+}\) binding motif has been proposed. There are a few crystal structures of Ni\(^{2+}\) protein complexes and these also involve His and Asp in a square planar geometry to Zn\(^{2+}\) (see Fig. 2; references 8 and 9). However Ni\(^{2+}\) and Zn\(^{2+}\) are not interchangeable so any comparison between these two metals sitting on the top of an MHC class II molecule

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must remain speculative but because there are a number of structures which detail how Zn\(^{2+}\) binds to MHC class II at His81, it would seem reasonable to discuss this in light of the Lu paper. In the structure of the streptococcal superantigen SPEC bound to HLA-DR (5), the Zn\(^{2+}\) atom is pre-bound to the superantigen by 3 amino acid side-chains in trigonal pyramid geometry. A water molecule acts as the 4th ligand for Zn\(^{2+}\) and is displaced in favor of His81 to form a stable tetravalent complex buried within the hydrophobic core of the Sag/MHC interface (see Fig. 2). The exclusion of water from the interface provides a stable and long-lasting complex. Only His81 from the MHC class II is involved in the metal coordination complex.

In the Lu paper, the authors propose that the Ni\(^{2+}\) atom is instead prebound to His81 on DR52c and that the other two side-chain ligands come from self-peptide(s). The rationale for this is simply that there are no other His, Asp, or Glu side-chains on the DR52c molecule close enough to contribute. More importantly, ANi2.3 only responds to Ni\(^{2+}\) treated DR52c on human and mouse B cells but not fibroblasts or other antigen presenting deficient cells. Moreover, the authors are able to elute mixed peptides from the APCs and show that these can be used to refurbish a Ni\(^{2+}\) dependent response on acid-stripped, formalin-fixed APCs. The sequence of these peptides is not revealed in the paper and their identification will be critical in confirming this model.

The fact that presentation of Ni\(^{2+}\) only occurs with certain peptides begs two questions. The most obvious is do they have His, Asp, or Glu or even potentially Cys at the NH\(_2\)-terminal end adjacent to His81 and second; do certain self-peptide MHC combinations predispose an individual to Ni\(^{2+}\) hypersensitivity – is this all that’s required to generate the disorder? Obviously not all Ni\(^{2+}\) hypersensitive patients are HLA-DR52c (10% of Caucasians have metal hypersensitivity) but His81 is found in almost all HLA-DR molecules so if this model holds as a predominant mechanism for Ni\(^{2+}\) induced DTH, are there a range of other HLA class II peptide combinations that can mimic the presentation of Ni\(^{2+}\) by DR52c? Direct sequencing of peptides eluted from the purified DR52c would be one option to identify these peptide(s) but this is risky as it assumes the peptides in question are abundant enough to isolate and sequence. A quicker route might be to randomly test candidate synthetic peptide libraries based on a theoretical DR52c motif with introduced His, Asp, and Glu residues at positions adjacent to His81 (these are P1, P2, P3, or P4).

The Role of TCR in Metal Reactivity. One can safely assume that a reactive Ni\(^{2+}\) ion perched on the top of the MHC class II molecule is going to react in some way with TCR - so how would this occur and alter the stability of the complex formed? It is very unlikely that an MHC–Ni\(^{2+}\)–TCR complex works in a similar fashion to Sag activation where the metal atom alone promotes strong binding irrespective of polymorphisms in the surrounding interface. If this were the case, we would expect superantigen-like oligoclonal T cell responses to Ni\(^{2+}\). In fact, the precursor frequency of Ni\(^{2+}\) reactive clones in hypersensitive patients is low implying that the binding energy provided by a Ni\(^{2+}\) complex is not in itself sufficient to circumvent required complementarity between MHC–peptide and TCR surfaces.

![Diagram](image.png)

Figure 1. Two hypothetical pathways for nickel presentation. (1) Processing-dependent pathway. Nickel ions bind to self-proteins which are then processed and presented by MHC on Langerhan cells. (2) Processing-independent pathway. Nickel ions enter through the skin where they bind directly to preformed MHC/peptide complexes on Langerhans cells.
The literature on this aspect of contact hypersensitivity is awash with contradiction and unfortunately the Lu paper, despite its importance, does little to clarify things at this stage. There have been many Ni\textsuperscript{2+} reactive T cells clones studied over the years, but there doesn’t seem to be any real consistency in their modus operandi. They consistently display varying degrees of MHC promiscuity or preference for TCR chains that does not support a consistent model for Ni\textsuperscript{2+} derivatization of MHC class II at His81 as a predominant mechanism of metal hypersensitivity. One thing that does appear consistent, is that they are mostly CD4\textsuperscript{+} T cells and that they respond to Ni\textsuperscript{2+} in the absence of CD4, a sure sign of increased stability of the MHC–TCR complex in the presence of Ni\textsuperscript{2+} (10).

Several studies reveal that Ni\textsuperscript{2+} reactive T cells (including the ANi-2.3 used in the Lu et al. paper) prefer to use TCR VB17 domain (11, 12). BV17 is very interesting because the CDR1 loop has a unique Histidine\textsuperscript{29}-Aspartic acid\textsuperscript{30} that would make an excellent bidentate metal binding site. Unfortunately, all crystal structures of MHC class II:TCR complexes so far show that these residues would be positioned over the MHC α-chain; well away from a Ni\textsuperscript{2+} atom bound to His81. Unless the ANi2.3 TCR binds in a reversed orientation on MHC class II (this would be extremely interesting) a simple model of a metal complex between His81 and two residues in BV17 can be discounted – although the high frequency of BV17 in Ni\textsuperscript{2+} reactive clones needs to be explained somehow.

Previous mutagenesis studies of the ANi2.3 TCR and other Ni\textsuperscript{2+} reactive T cell clones implicate instead the CDR3B region particularly Arg95Asp96 which when mutated, abrogated ANi2.3 reactivity to Ni\textsuperscript{2+} (10). This also seems somewhat contradictory since these residues are also not “over” His81 (remembering that the chelating atom in the residue must be a mere 2 Å away from the metal atom). The residues closest to His81 are in fact in the CDR1α loop. Looking at the ANi2.3 TCR, there is nothing in this region that would immediately suggest a contribution as a 4th ligand for the Ni\textsuperscript{2+} atom.

**Immune Consequences.** Although the kinetics and exact structure of the MHC/peptide-Ni\textsuperscript{2+} interaction remains to be determined, an analogous higher affinity-binding mode would have a number of important consequences. For example, certain MHC alleles have recently been found associated with the sensitivity of an individual to the toxic effects of superantigens (13). Could the same be translated to Ni\textsuperscript{2+} sensitivity? Severe hypersensitivity reactions can be related to overexpression of TCR VB17 but whether this correlates to Ni\textsuperscript{2+} reactivity itself or to the restricting MHC class II molecules is unknown (11). Without question, a DR52c crystal structure with Ni\textsuperscript{2+} bound, will remove much of the guesswork.

The paper by Lu et al. shows for the first time that delayed type hypersensitivity to Ni\textsuperscript{2+} results from the direct derivatization of the MHC class II molecule itself and that unidentified self-peptide(s) are required to support the Ni\textsuperscript{2+}-mediated response by providing additional ligands for the metal complex. What is yet to be revealed is how tightly the Ni\textsuperscript{2+} is bound and whether other metals replace it. What is so special about Ni\textsuperscript{2+} as opposed to other metals? Simultaneous sensitivity to several metals is clinically quite common. In particular Ni\textsuperscript{2+}-reactive T cell clones

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**Figure 2.** Crystal structures of two known metal chelation sites. The first is the arrangement of the superantigen SPEC residues His167, His201, and Asp203 and His81 from HLA-DR1 in a tetrahedral coordination around Zn\textsuperscript{2+} (PDB 1HQR and reference 5). The distance from each atom to the Zn\textsuperscript{2+} is 1.8–2.0 Å. The second is the square planar coordination complex surrounding one of the Ni\textsuperscript{2+} atoms found in the bacterial urease molecule (PDB -2UBP and reference 8). The distance from the center of the Ni\textsuperscript{2+} atom to each of the coordinating atoms is 2.1–2.3 Å so the Ni\textsuperscript{2+} coordination site is slightly larger.
can also respond to Pd$^{2+}$ and Cu$^{2+}$ and a common feature of these metal ions is that they can adopt identical coordination geometry to Ni$^{2+}$ plus they have similar atomic radii. The discovery of the role of His81β in Ni$^{2+}$ coordination certainly explains this metal cross-reactivity but it does not entirely explain why Ni$^{2+}$ and not Cu$^{2+}$ is the more common the culprit. Affinity binding and competition studies might provide a quantitative explanation as to why Ni$^{2+}$ is more effective at causing metal hypersensitivity.

Implications on the Processing-dependent Pathway. While the majority of metal reactive T cell clones such as ANi2.3 are processing independent, there is still a large proportion (>40%) that are strictly processing dependent (2, 14) meaning that Ni$^{2+}$ is not presented by formalin-fixed APCs. Can the model described by Lu et al. explain both pathways? It seems difficult to imagine how Ni$^{2+}$ derivatized exogenous peptides can survive the tortuous route of the exocytic pathway intact to be presented by MHC class II. Perhaps processing is required to maintain an abundance of a bound peptide that can sustain stable Ni$^{2+}$ binding at His81. One would predict that if an exogenous protein can be derivatized before processing, then it could just as easily be (more so in fact) derivatized as a peptide bound to MHC class II. One simple way to test this question would be to attempt to stimulate processing dependent Ni$^{2+}$ reactive clones with MHC class II defective in His81.

Metal hypersensitivity and DTH responses are part of the fabric of clinical immunology yet the underlying mechanisms and molecular basis for this classic immune disorder have remained elusive. The paper of Lu et al. offers the first tangible model for what might be occurring at the interface between MHC class II and TCR. There is much that is tantalizingly absent from this paper that no doubt will reveal itself in the fullness of time. Once the individual components have been isolated, biochemical and biophysical measurements will provide quantitative information about such things as how tightly Ni$^{2+}$ binds and exactly how much the metal coordination complex improves in the binding affinity of the TCR. As we have pointed out, the paper does not resolve many of the ambiguities concerning TCR usage in Ni$^{2+}$ reactivity and in fact creates a few more. What is important about this paper is that for the first time, the metal atom has a definite location on the top of MHC class II. One hopes that in the not too distant future, there will be an all revealing three dimensional structure of Ni$^{2+}$ at the center of a DR52c: ANi2.3 TCR complex.

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