



Published in final edited form as:

Immunol Res. 2013 March ; 55(0): 83–90. doi:10.1007/s12026-012-8351-1.

Structural basis of metal hypersensitivity

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Abstract

Metal hypersensitivity is a common immune disorder. Human immune systems mount the allergic attacks on metal ions through skin contacts, lung inhalation and metal-containing artificial body implants. The consequences can be simple annoyances to life-threatening systemic illness. Allergic hyper-reactivities to nickel (Ni) and beryllium (Be) are the best-studied human metal hypersensitivities. Ni-contact dermatitis affects 10 % of the human population, whereas Be compounds are the culprits of chronic Be disease (CBD). $\alpha\beta$ T cells (T cells) play a crucial role in these hypersensitivity reactions. Metal ions work as haptens and bind to the surface of major histocompatibility complex (MHC) and peptide complex. This modifies the binding surface of MHC and triggers the immune response of T cells. Metal-specific $\alpha\beta$ T cell receptors (TCRs) are usually MHC restricted, especially MHC class II (MHCII) restricted. Numerous models have been proposed, yet the mechanisms and molecular basis of metal hypersensitivity remain elusive. Recently, we determined the crystal structures of the Ni and Be presenting human MHCII molecules, HLA-DR52c (DRA*0101, DRB3*0301) and HLA-DP2 (DPA1*0103, DPB1*0201). These structures revealed unusual features of MHCII molecules and shed light on how metal ions are recognized by T cells.

Keywords

MHC; TCR; Hypersensitivity; Metal; Structure

Metal and immune systems

Metal ions are essential nutrients in all forms of life. From zinc to magnesium, from iron to manganese, different metals play different roles, from DNA replication to enzymatic catalysis, from electron transfer to signal transduction. Despite their important roles, metals can also be toxic and elicit different kinds of immune responses, especially in humans. Inhalation of Be and cobalt dusts can cause CBD or hard metal disease in lungs. Metal-induced allergic contact dermatitis is expressed in a wide range of cutaneous reactions following dermal and systemic exposure. Apart from the well-known significance of Ni in developing contact dermatitis, other metals such as aluminum, Be, chromium, cobalt, copper, gold (Au), iridium, mercury, palladium, platinum, rhodium and titanium represented

emerging causes of skin hypersensitivity [1]. Heavy metals, such as, Au, cadmium and mercury, can induce human autoimmune diseases [2]. Exposure to mercury vapors can result in elevated IgE levels and increased T lymphocyte numbers in humans [3, 4].

Metals are also directly involved in antigen presentation. The involvement of zinc in superantigen activation of T cells has been well established [5]. A zinc-binding site was revealed close to the MHC-binding site on superantigen staphylococcal enterotoxin A (SEA) and several other superantigens [6, 7]. This site interacts with the MHCII molecule via the exposed β His81 of the β 1 domain. This arrangement implies that SEA cross-links two or more MHC molecules on the surface of the antigen-presenting cell (APC) that greatly enhances SEA's binding, "pre-clustering" and more efficient T cell activation. Recent studies showed that zinc may function as an ionic signaling messenger to regulate T cell activation [8, 9].

Metal ions have also been observed to inhibit antigen presentation to T cells. For example, Griem et al. [65] showed that Au^+ interferes with antigen processing by the formation of Au^+ -peptide chelate complexes. $CD4^+$ T cell hybridomas specific for different antigenic peptides were tested in the presence of disodium aurothiomalate (Au^+ salts). In these studies, T cell recognition of peptides possessing two or more cysteine residues could be inhibited by the addition of Au^+ salts. The requirement of two or more cysteine residues for the formation of stable Au^+ complexes was confirmed by the substitution of cysteine by serine in an immunodominant peptide: The inhibitory effect of Au^+ on peptide-specific T cell proliferation was completely eliminated.

Metal hypersensitivity

Allergic reactions to metals are on the rise [10]. Contact hypersensitivity to metal afflicts around of 10–15 % of the human population [11, 12]. Ni represents the most common occupational as well as public contact allergen. In 2008, Ni also named as "Allergen of the Year" by The American Contact Dermatitis Society, because of its rise as a cause of significant contact dermatitis in the North America, particularly among children [13, 14]. Ni-induced contact dermatitis is a skin inflammation resulting in swollen, reddened and itchy skin due to direct contact with an allergen. In most cases, the resulting rash occurs only at the site of contact, though it may be found on other parts of the body as well. Ni sensitivity is acquired through intimate and prolonged skin exposure to Ni metal or Ni salts. Once this sensitization has occurred, exposure to sufficient amounts of Ni ions may result in an allergic response. In addition to Ni, Au, Be and cobalt contacts may also result in immune-mediated contact hypersensitivity. Orthodontic appliances, dental casting alloys and knee arthroplasties are also common sources of Ni and other metal hypersensitivity [15, 16]. Although both $CD4^+$ and $CD8^+$ T cells are major effector cells in Ni hypersensitivity [17], there has been no particular MHC allele or isotype associated with sensitivity to Ni.

A number of groups have isolated T cell clones from patients sensitive to Ni in vitro [18–20]. One of the TCRs (ANi-2.3) was isolated from a Ni-reactive T cell originally isolated from a human patient with Ni-contact hypersensitivity [21]. This TCR (V α 1/V β 17) recognizes Ni bound to the human MHCII molecule, HLA-DR52c. Previous experiments

showed that recognition by this TCR requires the combination of Ni, a particular MHC molecule, HLA-DR52c, and an unknown specific MHC-bound peptide produced in B cells [22]. The paraformaldehyde-fixed APCs could present Ni to ANi-2.3 cells indicating that antigen processing was not required and suggesting that the Ni was presented by a preformed MHC/peptide complex [22]. Ni and the fixed APC interaction assays showed that Ni is reversibly bound to the fixed APC surface via a pH sensitive (pH 3.5–5.0) interaction consistent with coordination by amino acid side chains from His and/or acidic residues.

Metal hypersensitivity in human lungs causes chronic CBD, cobalt-induced hard metal lung disease. CBD is a granulomatous disorder that predominantly affects the lung, although the lymphatic system, skin, liver and spleen may also be affected [23, 24]. This disorder is estimated to occur in up to 18 % of exposed workers, depending on the nature of the exposure and the individual's genetic susceptibility [25–28]. Be exposure in the form of metal, metal oxide, or metal alloy continues to be a major public health concern with approximately 1,000,000 individuals currently at risk for disease development [29, 30]. Most evidence indicates that CD4⁺ T cells are critically involved in the immunopathogenesis of CBD [18, 31–33]. Genetic susceptibility to CBD is associated with certain alleles of the MHCII molecule, HLA-DP, especially HLA-DPB1*0201 and other alleles that contain a glutamic acid residue at position 69 of the β -chain (β Glu69). After more than a decade of research, the reason for the genetic linkage between CBD and particular HLA-DP alleles has been a mystery [34].

The three-dimensional structures of HLA-DR52c and HLA-DP2

In spite of extensive studies on metal hypersensitivity, the structural basis for major MHC-restricted metal recognition is still unclear [35]. We determined the structure of HLA-DR52c bound to a self-peptide derived from the Tu elongation factor (pTu) [36]. Although HLA-DR52c/Tu peptide could not present Ni [22], a structure of HLA-DR52c would be important to understand the HLA-DR52c-restricted Ni presentation and to design the peptide screening libraries. HLA-DR52c has unique peptide-binding pockets. The nature of P4 pocket explains the strong selection for Asn (or Asp) among peptides known to bind to HLA-DR52c [36]. Asn is coordinated by an intricate hydrogen bond network involving three water molecules and Gln α 9, Glu β 28, Lys β 72 and Gln β 74 of HLA-DR52c [37]. These hydrogen bonds lock the position of the pTu peptide Asn side chain. A Pro at P6 sits in a pocket with both hydrophilic and hydrophobic properties that could accept virtually any aliphatic or short polar amino acid side chain. The Ile at P9 sits at the top of a deep mostly hydrophobic tunnel at the bottom of which lies Glu β 9. This pocket is large enough to accept virtually any hydrophobic amino acid, such as Phe or Tyr. In a manner similar to the mouse MHCII molecule, IE^k, this pocket might also accept a Lys with its ϵ -amino group salt bridged to the carboxylate of Glu β 9 [38].

The potential Ni-binding site was not apparent on the TCR interacting surface of HLA-DR52c, because the natural Ni-presenting peptide was still unknown [37]. Ni is normally coordinated by at least four ligands. The common Ni ligands are His and Glu (or Asp), although Cys, Ser and Gln (or Asn) ligands were also occasionally found [39]. Since HLA-DR52c and specific peptide are involved in Ni binding and the types of Ni-binding amino

acids are limited, we could identify four possible HLA-DR52c residues for the coordination of the Ni, based on the distance between HLA-DR52c surface residues and the peptide (Fig. 1a). It was suggested that the β His81 of the HLA-DR52c and/or its bound peptide could coordinate Ni for the TCR recognition [22]. Based on HLA-DR52c structure, β Gln70, β Gln74 and α Asp55 could also potentially coordinate the Ni. However, none of these residues alone are sufficient to keep the Ni. This is in agreement with previous data that a specific peptide is needed to form the Ni/HLA-DR52c/peptide complex [22]. The HLA-DR52c structure was determined at 1.8 Å. At this resolution, we can use B-factor to define the flexible regions in the molecule with confidence (Fig. 2). The higher B-factor between β 62 and β 73 of HLA-DR52c shows this region is less rigid. Both molecular dynamics simulations' modeling and conformationally sensitive monoclonal antibody probing showed this region (residues β 53–67) may be flexible [40, 41]. Normally, the Ni ion-binding sites are in flexible parts of proteins. Thus, it is tempting to speculate the Ni binding around β Gln70 and β Gln74 of HLA-DR52c β 1 domain.

The HLA-DP2 structure is critical for the understanding of Be presentation in CBD. Genetic susceptibility to CBD has been associated with certain alleles of the MHCII molecule, HLA-DP, especially HLA-DPB1*0201 and other alleles that contain a glutamic acid residue at position 69 of the β -chain (β Glu69) [34]. The structure of Be presenting HLA-DP2 with a bound self-peptide derived from the HLA-DR α chain (pDRA) was solved to a resolution of 3.25 Å [42]. Properties of the HLA-DP2 peptide side chain-binding pockets are different from other HLA alleles [43]. The very hydrophobic P1 pocket is similar to that found in a number of MHCII molecules, such as HLA-DR1 and HLA-DR4, that have a Gly at β 86 [44]. This deep and very hydrophobic pocket can accommodate virtually all hydrophobic residues including large aromatic amino acids. It is occupied by a Phe of pDRA. In many other MHCII molecules, including most alleles of HLA-DR and HLA-DQ, the P6 peptide-binding pocket heavily selects for small, often charged amino acids. However, the P6 pocket of HLA-DP2 is large and very deep. It is lined with hydrophobic and polar residues: α Tyr11, α Ala14, α Phe25, α Asn65, α Leu69, β Phe9, β Gly11, β Gln13 and β Tyr30. Its deepness is due to the amino acids α Ala14 and β Gly11, whose small side chains open up the bottom of the pocket. The P6 pocket is occupied by a Phe in the HLA-DP2-pDRA structure. The P1 and P6 positions of peptides appear to be the main peptide anchor residues for binding to DP2. The structure is in agreement with previous analysis of peptides naturally found bound to DP2 and mutational studies [42, 45]. The unique binding groove of the HLA-DP2 suggested the Be-binding peptides might have an unusual binding motif.

The unusual feature of the DP2 is the position of the β -chain α -helix in relation to the peptide and the rest of the molecule [43]. The P4-peptide-binding pocket of DP2 is large enough to accept positively charged amino acids or hydrophobic amino acids. A Leu is located at p4 position of pDRA. However, the backbone of pDRA has risen up in the binding groove at the P4 position, and the side chain of p4Leu of pDRA has moved up to the surface of the DP2-pDRA complex. The other unique property of the DP2 structure is the exceptionally wide peptide binding groove compared to other MHCII molecules, mostly due to the movement of the DP2 β -chain α -helix away from the peptide in the region between β 69Glu of the DP2 β -chain and the p5Pro of the peptide [43]. The distance between the

peptide P5 $C\alpha$ atom and the DP2 $\beta 69$ $C\alpha$ atom is 11.2 Å, the widest gap seen in any MHCII structure so far [43]. As a result of this structural change, an acidic pocket opposite the P5 amino acid is formed, between the pDRA p4Leu and p7Leu (Fig. 3a). The acidity of this pocket is due to the presence of three DP2 β -chain amino acids: $\beta 68$ Glu and $\beta 69$ Glu from the β -chain α -helix and $\beta 26$ Glu from the floor of the peptide binding groove (Fig. 3b). In the crystal structure, the guanidinium group of an Arg from symmetry-related molecules filled into this pocket [43]. This positively charged moiety forms an extensive H-bond/salt bridge network with the three glutamic acids, offering a possible model for how Be compounds might occupy this site.

The fact that $\beta 69$ Glu is strongly associated with genetic susceptibility to CBD is in agreement that solved structures of other proteins associated with Be compounds generally show coordination of the compound by acidic amino acids [46] and also suggest that this acidic pocket is the site of binding of Be compounds to HLA-DP2. $\beta 26$ Glu and $\beta 68$ Glu are invariant among DP alleles. The model suggests that the Be antigen-presentation complex consists of MHCII HLA-DP with Be bound to carboxylate groups in the acidic pocket that is formed by a select group of peptides that give Be an appropriate amount of room to enter the acidic pocket. Mutation analysis of the acidic amino acids in the binding groove show that the β Glu69 position is necessary and sufficient to bind Be in a manner that allows T cell activation. Interestingly, there are several more acidic residues around the pocket, for example, Glu 67, Asp26. The roles of these acidic residues were not tested. Probably, they serve as charge attractants to help Be to find the pocket.

How the T cells recognize metal ions complexes with the MHC/peptide

TCRs commonly recognize linear peptides of antigenic proteins bound to MHC molecules. However, MHC molecules can also present components other than, or in addition to, peptides to T cells. Small chemical haptens presented by MHCI and MHCII to T cells have long been known [47]. Organic haptens such as dinitrophenol, trinitrophenol and fluorescein have long been used experimentally to study T cell responses and hypersensitivity [48–50]. T cell-mediated hypersensitivity to natural chemically active haptens, such as the catechols of poison ivy and poison oak, is common. T cells are also involved in allergic reactions to drugs, such as, β -lactam antibiotics and sulphamethoxazole [51]. Ni, Be, Au, cobalt and other metals ions form a unique group of chemicals for which T cell hypersensitivity has been demonstrated two decades ago [20, 34, 52]. While hypersensitivity to Be has been associated with particular alleles of HLA-MHCII alleles, especially HLA-DP2 [53, 54], there has been no particular MHC allele or isotype associated with sensitivity to Ni or Au [55, 56]. However, most often these clones require autologous APC for response to the metal ion, suggesting a role for either a specific peptide or particular MHC polymorphic amino acids in either the metal binding or the interaction with the TCR [57].

Conventionally, the TCRs dock diagonally on top of the surface of the MHC/peptide. The 6 CDR loops contact MHC/peptide, usually with CDR1 α and CDR2 α over the β helix of MHCII or the $\alpha 2$ helix of MHCI, and CDR1 β and 2 β over the $\alpha 1$ helix of MHCII or of MHCI. CDR3 α and CDR3 β are usually focused on amino acids of the peptide [58, 59]. Recent ternary complex structures of the mouse V $\beta 8$ TCR and MHC/peptide demonstrate

that some amino acids in the CDR1 and CDR2 regions of TCR $V\alpha$'s and $V\beta$'s may be evolutionarily conserved for interaction with particular conserved sites on MHC molecules and contribute to the bias of TCRs toward MHC ligands [60–62]. This heavy germline bias of the TCR for MHC may account for the TCR diagonal docking mode on MHC [60, 61].

How metal ions influence the docking orientations of TCRs on MHCs is unknown. There are no structural data for how metal ions become an essential part of the TCR ligand. It has been proposed that metals may bind on the surface of MHC molecules with or without a contribution from the bound peptide, such that they become part of the interface during TCR engagement [52]. Depending on what degree these metals dominate the TCR/MHC interface, TCR interaction with the MHC molecule and its bound peptide may also contribute to the overall specificity of the interaction. A study of effects of noble metal complexes on MHCII peptide binding has raised the possibility of another way that metals may create new epitopes [63]. In these experiments, some platinum, palladium and Au^{3+} complexes with square planar coordination were shown to destabilize peptide binding to MHCII creating stable molecules with the properties of “empty” MHCII, perhaps by binding to the MHCII in such a way as to stabilize an open conformation. Although not tested in the study, one could envision that these empty MHCII molecules could be recognized by T cells as neoantigens, accounting for hypersensitivity in some cases. Based on current biochemical data, hypothetically the metal ions can incorporate into MHC and become the ligands of T cell at least in four different ways (Fig. 4).

Ni-reactive ANi-2.3 T cell requires DR52c, not other HLA alleles, as the Ni-presenting molecule [22]. ANi-2.3 TCR seems to lie on a diagonal above the face of HLA-DR52c/peptide. Vollmer and his works showed that the specific combinations of α and β chains were required for Ni recognition [17, 21]. More strikingly, an Arg95 and Asp96 dyad of CDR β s are absolutely required for the activation of ANi-2.3 T cells. This implied the dyad may be directly ligated to Ni ion or contact HLA-DR52c surface residues. Our recent structure of ANi-2.3 and DR52c/mimotope complex confirmed ANi-2.3 TCR docks on DR52c/peptide in a conventional way (unpublished data). Gamerdinger et al. reported that Ni forms a bridge between HLA-DR molecules and the TCR of a human Ni-reactive T cell clone, SE9 ($V\alpha 22/V\beta 17$), without the requirement for a specific MHC-bound peptide [57]. TCR SE9 is also MHC promiscuous and can be activated by multiple alleles of HLA-DRs [64]. TCR SE9 β chain is not related for TCR SE9 reactivity. Ni activation of these T cells only requires the specific α chain. The His81 of $\beta 1$ domain of DR molecule and Tyr29 and Tyr94 of $V\alpha$ domain of SE9 TCR may coordinate the Ni ion [57]. This suggested TCR SE9 has unusual binding topology. The TCR SE9 may greatly tilt toward the $\beta 1$ domain HLA-DR molecule, causing no interactions between its α chain and the $\alpha 1$ domain of HLA-DR molecule. There are several ways of Ni presentation. One cannot rule out the possibility that metals may change the processing of self-peptide and trigger T cells activation with cryptic self-peptides [57, 65].

Several HLA-DP2-restricted, Be-reactive T cell clones have been identified by Fontenot's group [66]. A thorough alanine scanning study of both DP2 and TCR surface residues was performed. The results suggested that, like Ni-reactive SE9 T cells, these TCRs bind Be, DP2 and peptide complex in an unexpected docking orientation [66]. None of the alanine

mutations in the CDR1 α and CDR2 α of these TCRs had any effect on Be reactivity. In contrast, the mutational analysis of the TCR β -chains resulted in either reduced or abolished T cell hybridoma responses compared with the wild-type TCR. Overall, these data show that the V β domain of TCRs plays the major role in Be recognition, with no contribution from the germline-encoded parts of α chain. However, the Tyr at position 95 of the CDR3 α is important for Be presentation. The binding topology of the TCR-peptide-MHC complex can modulate TCR signaling [67]. Until now, the unusual TCR footprints on MHC and peptide have been observed only on autoimmune TCRs [68–70]. These abnormal TCR binding modes may allow a subset of autoreactive T cells to escape thymic selection, causing the autoimmune disease [71, 72]. Likewise, metal ions may induce the unusual docking of TCR on MHC/peptide, triggering hypersensitivity.

Although the structural studies of metal presenting MHC molecules are essential to understand the mechanisms of T cell-mediated metal hypersensitivity, a complete understanding of metal recognition will come from ternary structures of the complete complexes: TCR, MHCII, peptide and metal. The crystallization and structure determination of the complexes of metal ion, MHC, peptide and TCR will be the definitive proof of the role of metal and relative contribution of the MHC molecule, peptide and TCR.

Acknowledgments

This work is supported by NIH KL2 TR000156 and the Boettcher Foundation.

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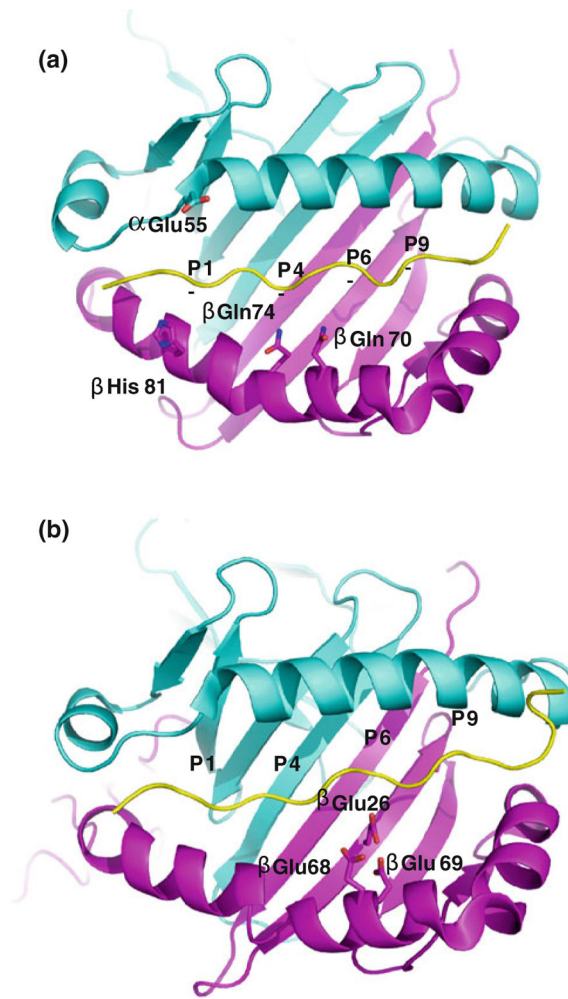


Fig. 1. Ribbon representations of the MHCII/peptide complexes. View is from the top looking down the $\alpha 1$ helix and $\beta 1$ helix of MHCII (the potential metal-binding residues are shown with coloring: oxygen, red; nitrogen, blue). The $\alpha 1$ domain is shown in cyan and the $\beta 1$ domain in magenta. The peptide is colored in yellow. The figures are prepared with program, Pymol. The amino acid names and the peptide anchoring residues are labeled. The four peptide anchoring pockets are labeled with P1, P4, P6 and P9 in all figures **a** The DR52c/Tu peptide complex, **b** The DP2/DRA peptide complex

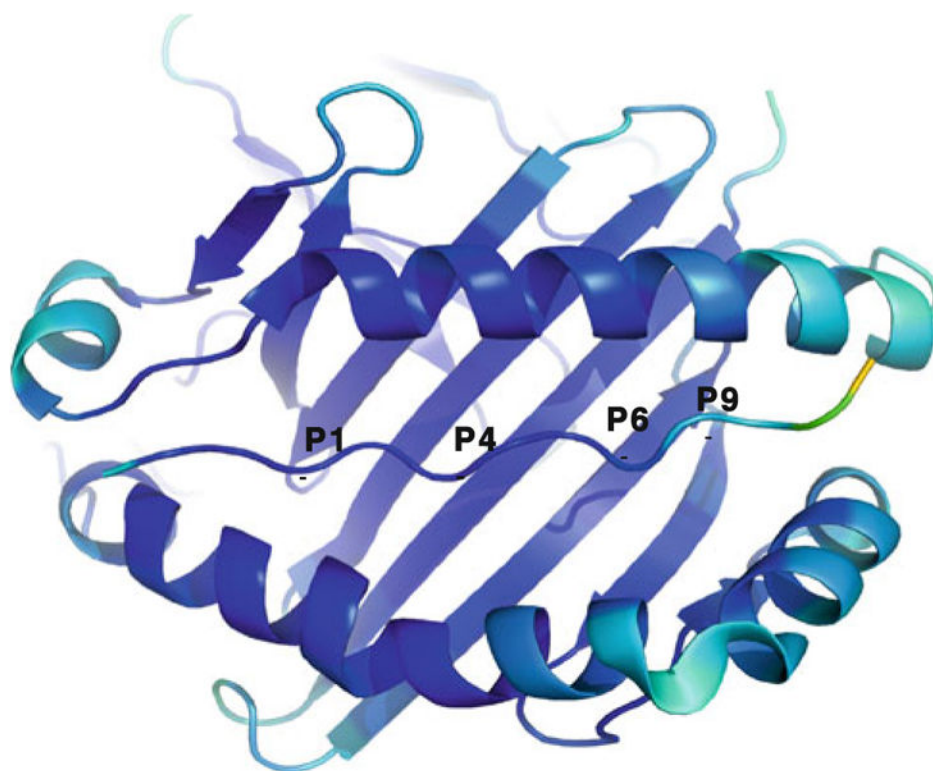


Fig. 2. Ribbon representation of the HLA-DR52c/Tu peptide is viewed as Fig. 1. The model is color coded by *B*-factor. *Blue* indicates a low *B*-factor through to *yellow* indicating a high *B*-factor

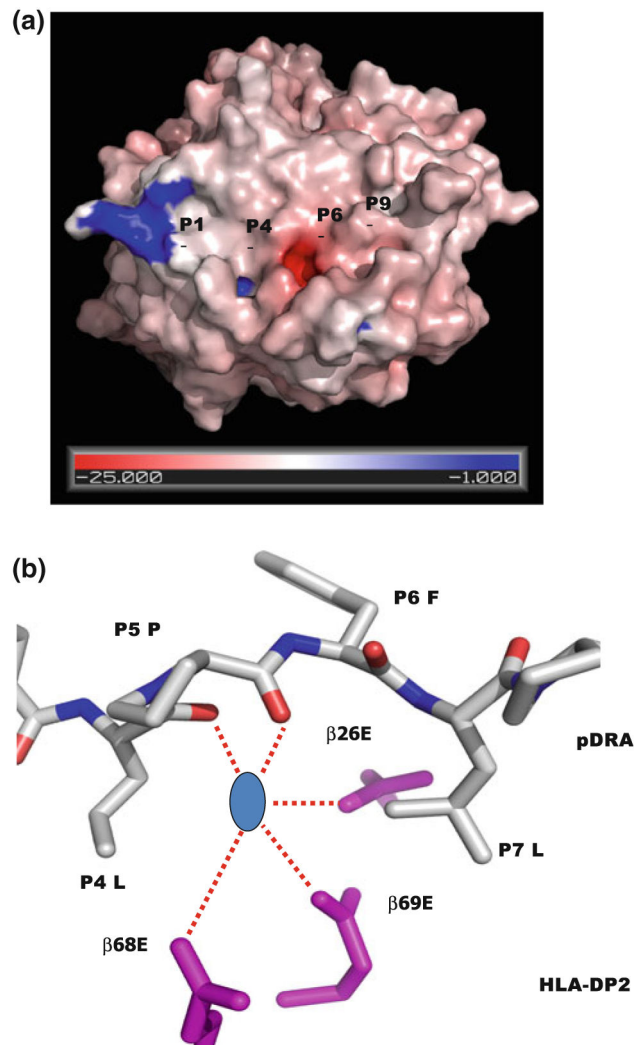


Fig. 3.
 The unusual features of HLA-DP2 **a** The DP2/DRa peptide complex is viewed as Fig. 1. The water accessible surface of the DP2 molecule (without bound pDRA) is shown. The HLA-DP2 β -chain α -helix colored by the relative charge of the surface atoms (*red*, negative and *blue*, positive). The acidic pocket is located in the area between p5Pro and the DP2 β -chain α -helix. **b** A hypothetic Be compound is modeled in the acidic pocket. View of the acidic pocket looking down the peptide binding groove from the top. Wireframe representations of the side chains of β 26Glu, β 68Glu and β 69Glu are colored in magenta. A wireframe representation of P4 to P6 of pDRA is shown with *white* carbon, *red* oxygen and *blue* nitrogen. Also, shown is a *blue circle* as a hypothetic Be compound

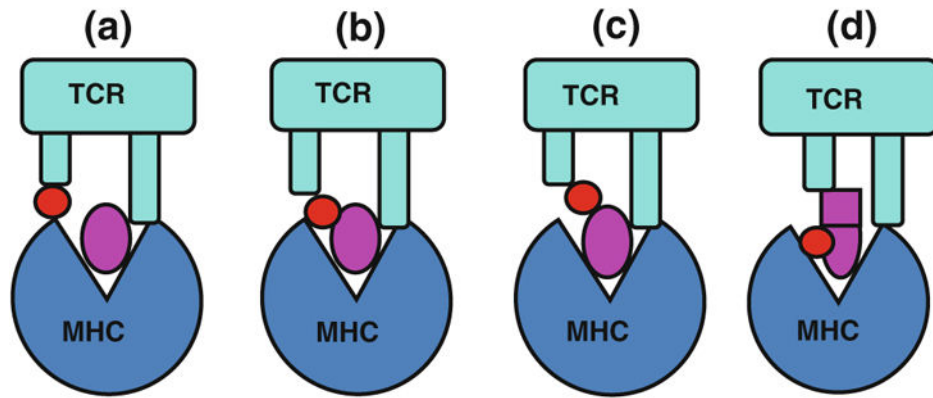


Fig. 4. Models of metal presentation to $CD4^+$ T cells in the context of MHCII molecules. Four models of metal presentation have been hypothesized. The schematic drawings of TCR and MHCII are colored as *cyan* (top) and *blue* (bottom). A metal ion is showed as a *red circle*. A peptide is showed as a *magenta ellipse*. The conformationally changed peptide is showed as a *square* and a *ellipse*, **a** shows the schematic model for the direct binding of a metal to the MHCII molecule only, **b** depicts the model of interaction where the metal binds to the antigenic peptide and the MHC molecule, **c** shows that the metal only interacts with peptide, **d** shows metal induced the change of peptide or MHC as neoantigens