

Structural Basis of Chronic Beryllium Disease: Linking Allergic Hypersensitivity and Autoimmunity

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<http://dx.doi.org/10.1016/j.cell.2014.04.048>

SUMMARY

T-cell-mediated hypersensitivity to metal cations is common in humans. How the T cell antigen receptor (TCR) recognizes these cations bound to a major histocompatibility complex (MHC) protein and self-peptide is unknown. Individuals carrying the MHCII allele, HLA-DP2, are at risk for chronic beryllium disease (CBD), a debilitating inflammatory lung condition caused by the reaction of CD4 T cells to inhaled beryllium. Here, we show that the T cell ligand is created when a Be²⁺ cation becomes buried in an HLA-DP2/peptide complex, where it is coordinated by both MHC and peptide acidic amino acids. Surprisingly, the TCR does not interact with the Be²⁺ itself, but rather with surface changes induced by the firmly bound Be²⁺ and an accompanying Na⁺ cation. Thus, CBD, by creating a new antigen by indirectly modifying the structure of preexisting self MHC-peptide complex, lies on the border between allergic hypersensitivity and autoimmunity.

INTRODUCTION

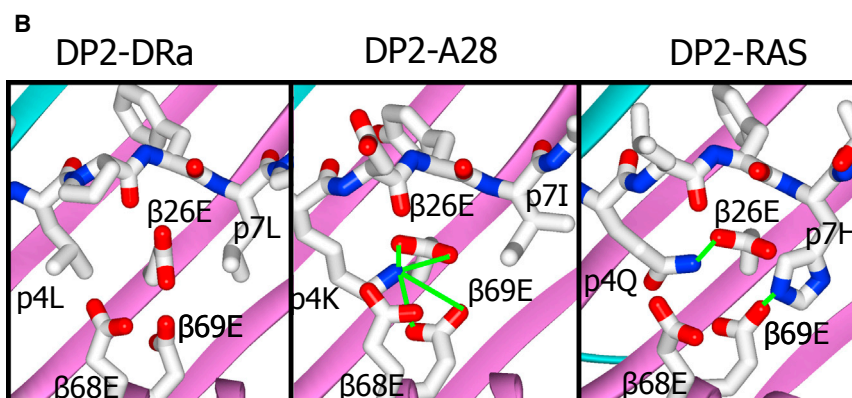
The majority of $\alpha\beta$ T cell antigen receptors (TCRs) recognize ligands in which a classical major histocompatibility complex (MHC) protein presents a peptide secured in its peptide binding groove. To prevent responses to self-peptides presented by self-MHC alleles, an elaborate system of checkpoints has evolved to control self-reactive T cells, either by deleting them while they develop in the thymus (Kappler et al., 1987; Kisielow et al., 1988) or by suppressing their response in peripheral organs (Fontenot and Rudensky, 2004). These mechanisms steer

T cell responses either toward foreign peptides, e.g., those derived from foreign pathogens, bound to self-MHC, or toward peptides bound to foreign MHC alleles, e.g., those expressed on grafted foreign tissues. Experimental work over several decades has created a deep understanding of the nature of these types of MHC-peptide antigens and their recognition by TCRs (Gras et al., 2008; Marrack et al., 2008; Wilson and Garcia, 1997).

However, there are other ligands for T cells that also involve the classical self-MHC molecules, but remain poorly understood. These are often involved in inflammatory immunological diseases. For example, despite the numerous checkpoints, T cells can become activated in some individuals by MHC bound to self-peptides, causing serious tissue-specific autoimmune diseases such as type-1 diabetes, rheumatoid arthritis, and multiple sclerosis. Furthermore, T-cell-mediated allergic hypersensitivities involve a wide variety of nonpeptide environmental chemicals that include metal cations, as well as plant, industrial, and pharmaceutical organic compounds (Fontenot and Amicosante, 2008; Louis-Dit-Sully and Schamel, 2014; Pichler et al., 2006; Thierse et al., 2005). Most evidence indicates that these agents must become associated with a self MHC-peptide complex in order to elicit a T cell response, but how they bind to the complex and how the TCR recognizes their presence is largely unknown. A common hypothesis is that they occupy a position on the surface of the MHCII-peptide complex and are recognized directly by the TCR in a hapten-like manner, but other mechanisms have been proposed (Illing et al., 2013; Pichler et al., 2006).

Beryllium hypersensitivity is an example of this latter type of T cell recognition. Because of its unique chemical properties, industrial importance, and high toxicity, beryllium has been extensively studied by clinicians, biologists, chemists, and physicists for many years. Chronic beryllium disease (CBD) is a life-long CD4 T-cell-mediated lung inflammatory illness, common among certain industrial workers who handle the beryllium metal

A	Peptide Sequence													
	Peptide Name	Source	Predicted DP2 Groove Position											
			1	2	3	4	5	6	7	8	9			
Non-Be ²⁺ Presenting Self Peptides														
	DRa	HLA-DR alpha	R	K	F	H	Y	L	P	F	L	P	S	D
	RAS	Ras/RAB9a	N	K	F	D	T	Q	L	F	H	T	I	T
	A28	HLA-A28	A	Y	D	G	K	D	Y	I	A	L	K	
Be ²⁺ Presenting Mimotope Peptides														
	M2	Peptide Library			F	W	I	D	L	F	E	T	I	G
	M4	"			F	W	I	D	L	F	E	L	I	G
	M18	"			F	W	I	D	L	F	E	F	I	G
Be ²⁺ Presenting Self Peptides														
	Plx4	Plexin A4			F	V	D	D	L	F	E	T	I	F
	Plx2	Plexin A2			F	V	D	D	L	L	E	T	L	G
	Spec	β Spectrin			M	W	A	D	L	L	E	L	I	D



(Dai et al., 2013). It is highly associated with certain human class II MHC (MHCII) alleles, especially HLA-DP2 (DP2), that have a glutamic acid at position 69 of the β chain (β 69E). The active agent in the disease is the Be²⁺ cation. Since the Be²⁺ cation has such a high-charge density, it immediately forms higher-order complexes in biological solutions involving electron donors such as water, hydroxyl ions, carbonate, phosphate, etc. (Everest, 1964; Sutton and Burastero, 2003). It has been suggested that β 69E captures such a Be²⁺ containing complex which is then recognized on the DP2 surface by CD4 T cells, but the structural details of this complex or its recognition are unknown.

Numerous Be²⁺-specific CD4 T cell lines and clones have been isolated from the lungs of CBD patients that express DP2 (Bowerman et al., 2011; Falta et al., 2013; Fontenot et al., 1999; Saltini et al., 1989). In vitro, these T cells respond to Be²⁺, but not other metal cations, presented by autologous or other DP2-expressing antigen presenting cells (APCs). This response is dependent not only on Be²⁺ and DP2, but also on particular DP2 bound self-peptides (Falta et al., 2013). Using positional scanning peptide libraries, we recently identified a

Figure 1. Unique Properties of the DP2 Acidic Pocket

(A) Three groups of DP2-binding peptides are listed. The top group are self-peptides that are commonly found bound to DP2 (Díaz et al., 2005), but cannot present Be²⁺ to AV22. The middle group contains examples from a series of mimotope peptides found in a positional scanning library (Falta et al., 2013) that can present Be²⁺ to AV22. The bottom group contains examples of self-peptides that can present Be²⁺ to AV22, identified by their similarity to the mimotope peptides (Falta et al., 2013).

(B) Details of the acidic pocket of DP2 occupied by each of three peptides at the top of (A), pDRa, pA28, pRAS. Ribbons: DP2 α , cyan; DP2 β , magenta. Wireframe with CPK coloring: peptide and side chains of DP2 β 26E, β 68E, and β 69E. Potential H-bond/salt bridges, green lines. See also Figure S1 and Table S1.

set of peptide mimotopes, and subsequently a set of related natural self-peptides, that can complete the DP2/peptide/Be²⁺ ligand for one of these T cells, AV22, as well as other T cells from the same patient or from nonrelated DP2⁺ patients (Falta et al., 2013). Here, we report the functional, biophysical, and structural properties of the AV22 TCR/DP2/peptide/Be²⁺ quaternary complex and define the changes that occur in the DP2-peptide complex upon Be²⁺ binding that enable TCR recognition. Remarkably, the structures show that the immunogenic form of beryllium is not a beryllium containing compound, but rather the Be²⁺ cation itself, which is buried within the DP2-peptide structure. The TCR does not interact directly with the Be²⁺ ion, but instead recognizes changes in the surface of DP2/peptide complex surface induced by the internally bound Be²⁺.

RESULTS

Common Features of DP2-Peptide Complexes

Listed at the top of Figure 1A are three peptides that are well-represented among those naturally bound to DP2 (Díaz et al., 2005), yet do not present Be²⁺ to the Be²⁺-specific TCR, AV22, or any other Be²⁺ reactive T cell tested so far (Dai et al., 2010; Falta et al., 2013). We previously solved the structure of one of these nonpresenting peptides, DRa, bound to DP2 (Dai et al., 2010). We have now solved the structures of DP2 bound to the other two nonpresenting peptides, A28 and RAS. Although they do not present Be²⁺, the structures of these complexes reveal two common features of DP2 that are relevant to Be²⁺ presentation. First, in all three structures the important β 69E lies in an acidic pocket that includes two other DP2 acidic amino acids, β 26E and β 68E (Figure 1B). We have shown previously that these three

amino acids are important in Be^{2+} presentation (Dai et al., 2010). Second, the wide gap (11.0 Å) between the peptide backbone and the DP2 beta chain alpha helix that we reported previously for the DP2-DRa structure is also present in the DP2-A28 (10.3 Å) and DP2-RAS (10.5 Å) structures (Figure S1 available online). This gap is several angstroms wider than generally seen in the structures of other MHCII alleles (Dai et al., 2010). Consequently, this extra space frees the side chains of the p4 and p7 amino acids that flank the acidic pocket to assume rotamers that can enter the pocket. For example, in the A28 structure the p4K interacts with DP2 β 26E and β 69E. Similarly, in the RAS structure, p4Q and p7H interact with DP2 β 26E and β 69E, respectively. These observations suggested to us that, depending on the peptide sequence, the particular amino acids at p4 and p7 of the peptide might influence the ability of the pocket to capture Be^{2+} from the solvent. We therefore set out to test this idea.

Unique Properties of the Peptides that Allow Be^{2+} Presentation by DP2

Some of the previously identified mimotopes and self-peptides that, when bound to DP2, allow presentation of Be^{2+} to AV22 and related T cells are also listed in Figure 1A in the middle and bottom sections, respectively (Falta et al., 2013). These Be^{2+} presenting peptides all share a p4D and p7E. Given the results described above, we hypothesized that these two acidic amino acids interact with DP2 to capture and coordinate Be^{2+} such that the combination is recognized by Be^{2+} -specific TCRs. Therefore, we investigated their role in the properties of mimotope-2 (M2) bound to DP2 in the presence and absence of Be^{2+} .

We prepared two mutated forms of the DP2-M2 complex: DP2-M2(NQ), in which the p4D and p7E of M2 were changed to the nearly isomorphous amides, N and Q and DP2(β 69K)-M2, in which the DP2 high-risk amino acid for CBD, β 69E, was changed to lysine, the amino acid found at position β 69 in HLA-DP4, a DP allele negatively associated with CBD (Maier et al., 2003). We assessed the ability of the wild-type and mutated complexes to stimulate the AV22 T cell transfectoma with or without Be^{2+} (Figure 2A). As a negative control, we used an irrelevant HLA-DR52c-peptide complex (Yin et al., 2012). None of the complexes stimulated AV22 in the absence of Be^{2+} . In the presence of Be^{2+} , AV22 responded strongly to the DP2-M2 complex, but there was no response to either of the mutant DP2-M2 complexes or the control DR52c complex, indicating the importance of β 69E and the requirement for the carboxylates of the p4D and p7E side chains for Be^{2+} presentation.

We confirmed these results with surface plasmon resonance TCR binding experiments. Equal amounts of the soluble, biotinylated DP2-M2, or the mutant complexes were captured in the flow cells of a BIAcore streptavidin BIA sensor chip and tested for binding of soluble AV22 TCR. In the absence of Be^{2+} the unmutated DP2-M2 complex failed to bind the TCR (Figure 2B, left). As we previously have demonstrated (Falta et al., 2013), in the presence of Be^{2+} the same complex bound AV22 TCR with high affinity (3–6 μM) and first order kinetics (Figures 2B and 2C). In contrast, neither the DP2-M2(NQ) nor the

DP2(β 69K)-M2 mutated versions of the complex, with or without Be^{2+} , bound the AV22 TCR (Figure 2D). Taken together, these results confirm our previous conclusion that the acidic properties of p4D, p7E, and β 69E are essential for Be^{2+} presentation.

Finally, using differential scanning fluorimetry (Niesen et al., 2007), we determined the effect of Be^{2+} on the thermal stability of various MHCII-peptide complexes, using the binding of the fluorescent dye, SYPRO Orange, to follow protein denaturation (Figure 2E). We examined four DP2-peptide complexes, two with peptides, M2 and Plx4, that could present Be^{2+} to AV22 (Figure 1A), one with the self-peptide, A28, that could not present Be^{2+} , and finally the DP2(β 69K)-M2 mutant described above. As a negative control, we used the well-studied mouse MHCII-peptide complex, IA^b-3K (Liu et al., 2002). The stability of each complex was defined by the melting temperature (T_m), i.e., the temperature causing the half maximal increase in SYPRO Orange fluorescence. In the absence of Be^{2+} , the T_m of the control IA^b-3K complex, as well as those of the DP2-A28, DP2-M2, and DP2-Plx4 complexes ranged from 54.4°C–62.5°C. As expected, the addition of Be^{2+} had little effect on the T_m of IA^b-3K or the DP2-A28 complex. However, addition of Be^{2+} raised the T_m of DP2-M2 and DP2-Plx4 complex to more than 70°C, a >10°C increase. Unexpectedly, in the DP2(β 69K)-M2 complex, substitution of lysine for the critical DP2 β 69E led to a similar increase in stability, whether or not Be^{2+} was added. This result suggests that the positively charged ϵ -amino group of β 69K might substitute for Be^{2+} in the stabilization of the complex, but could not create the ligand recognized by AV22 (Figure 2A).

These findings strongly implicated the cluster of acidic amino acids from both DP2 and the presenting peptides as the site of Be^{2+} binding, since both were involved in stabilizing the complex in the presence of Be^{2+} and both were needed to create a Be^{2+} -dependent ligand that could be recognized by the AV22 T cell.

Be^{2+} and an Accompanying Na^+ Cation Bind Internally in the Acidic Pocket of DP2-M2

To understand the nature of the Be^{2+} moiety that binds to DP2-M2, how the DP-M2 structure changes after binding Be^{2+} , and how the AV22 TCR recognizes these changes, we solved the structures of DP2-M2 in the absence of Be^{2+} and of the DP2-M2- Be^{2+} complex bound by the AV22 TCR (relevant methods and statistics for data collection and refinement for all structures reported in the paper are given in the Extended Experimental Procedures and Table S1). We first compare the structure of the acidic pocket in the free DP2-M2 complex to that in the Be^{2+} loaded DP2-M2 bound by the AV22 TCR.

In the DP2-M2 complex, electron density in the acidic pocket clearly shows that the side chains of p4D and p7E have entered the acidic pocket, both prior to (Figure 3A) and after (Figure 3B) Be^{2+} addition. However, their side chain configurations and those of the other acidic amino acids in the pocket have changed in the presence of Be^{2+} , indicating that Be^{2+} has been captured within the pocket. To deduce the position of the Be^{2+} , we relied on previous analyses of numerous small molecule structures containing Be^{2+} coordinated by oxygens, as well as larger protein structures in which an aspartic acid carboxylate has captured BeF_3 . The coordination chemistry of Be^{2+} is well documented in these studies (Everest, 1964; Keizer et al., 2005). The

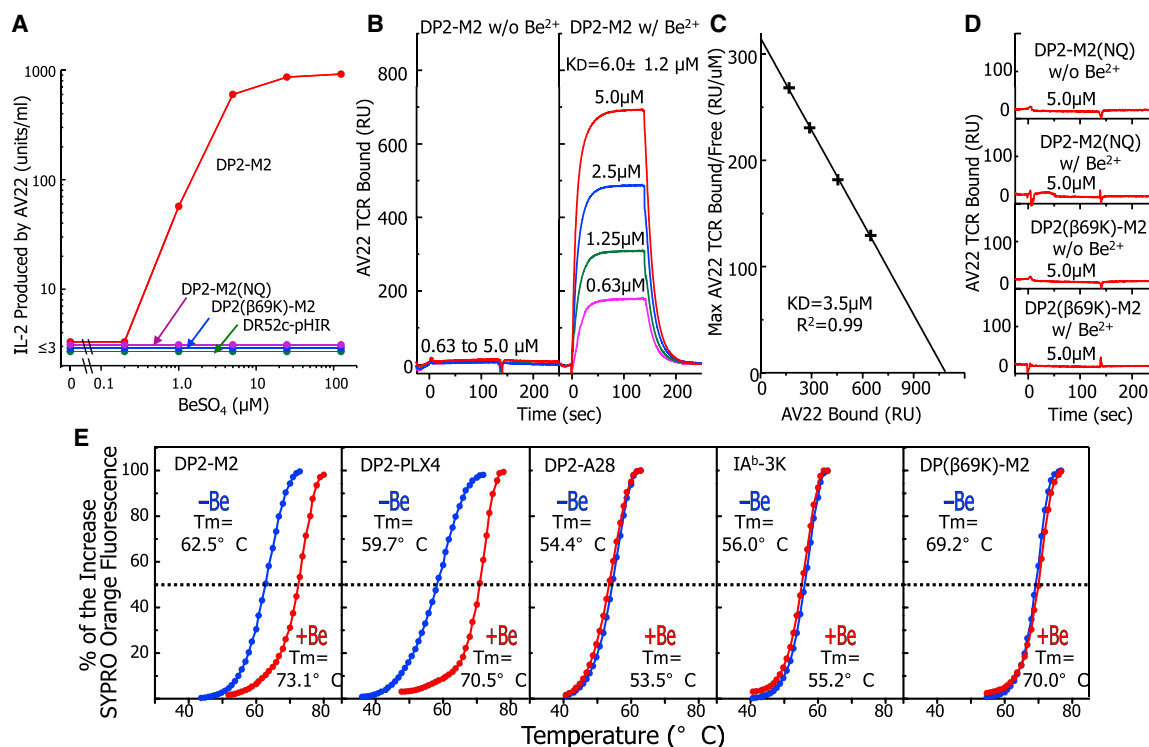


Figure 2. The Acidic Pocket Is Required for the Be^{2+} -Dependent Functional and Biophysical Changes in DP2-Peptide Complexes

(A) Equivalent amounts of biotinylated MHCII-peptide complexes (DP2-M2, DP2-M2(NQ), DP2($\beta 69\text{K}$)-M2, and HLA-DR52c-pHIR) were immobilized in separate culture wells and used to stimulate the AV22 transfectoma T cell with or without various concentrations of BeSO_4 . IL-2 secretion was assessed at 24 hr. The experiment was repeated with equivalent results.

(B) Biotinylated DP2-M2 ($\sim 2,000$ RU) was captured in a flowcell of an SA-biosensor chip in a BIAcore 2000 instrument. Various concentrations of soluble AV22 TCR were injected for 140 s through the flowcell, before and after loading of the immobilized DP2-M2 with Be^{2+} . The surface plasmon resonance signal (RU) was followed both during and after the injection and the binding kinetics analyzed with BIAcore BIAevaluation 4.1 software. The experiment was repeated four times with similar results.

(C) The plateau equilibrium RU data in (B) were used to create a Scatchard plot. The dissociation constant (K_D) and regression coefficient (R) were calculated from the linear least-squares fit to the data.

(D) Same as (B) except that biotinylated DP2-M2(NQ) and DP2($\beta 69\text{K}$)-M2 were immobilized in separate flowcells. Only data for the injection with 5 μM AV22 TCR is shown. The experiment was repeated with similar results.

(E) The thermal stability of the series of soluble MHCII-peptide complexes shown was assessed in the presence or absence of Be^{2+} using differential scanning fluorimetry with the fluorescent dye SYPRO orange. The melting temperature (T_m) was defined as the temperature needed to achieve the half maximal increase in fluorescence. Results are pooled and averaged from six experiments. Each melting curve was performed at least three times among the experiments.

high-charge density of Be^{2+} creates four short, semicovalent bonds (1.5 to 1.7 Å) to its ligands distributed in a tetrahedral arrangement with bond angles of $\sim 110^{\circ}$. Because there are only two electrons in Be^{2+} , in most X-ray structures the position of the Be^{2+} in the complex cannot be deduced from the Be^{2+} electron density alone, but rather from the position where the electron densities of its ligands converge.

Based on the genetic, mutational, functional, and biophysical data, we clearly predicted that a carboxylate oxygen from $\beta 69\text{E}$ would be one of these ligands. In the absence of Be^{2+} , separate densities were seen for each of the five carboxylates in the acidic pocket (Figure 3A), but their oxygens were not in the correct positions to join with $\beta 69\text{E}$ in tetrahedral Be^{2+} ligation. However, in the presence of Be^{2+} , the density for the carboxylates converged (Figure 3B) and carboxylate oxygens from p4D and $\beta 26\text{E}$ were brought into position to ligate an $\beta 69\text{E}$ captured Be^{2+} with the expected Be-O bond lengths of 1.6–1.7 Å and O-Be-O bond

angles of 110° – 115° (Figure 3C). Movement of the p7E carboxylate placed a fourth oxygen at an angle such that it could be the fourth Be^{2+} ligand. However, the 3 Å distance from this oxygen to the Be^{2+} is too far to allow a conventional Be-O bond (Figure 3C). Because there are no published structures of Be^{2+} coordinated solely by protein amino acid carboxylates, we cannot say if this type of Be^{2+} ligation in a protein is unusual, although small molecules containing a Be^{2+} with only three ligands do exist (Diyabalanage et al., 2008), and deviations from ideal ligation have been seen for other cations incorporated into protein structures (Evans, 2007; Harding, 2002). Therefore, this positioning of Be^{2+} among these four oxygens was consistent both with its known coordination chemistry and with our data with mutations of $\beta 26\text{E}$, $\beta 69\text{E}$, p4D, and p7E presented here and previously (Dai et al., 2010; Falta et al., 2013).

The Fo-Fc electron density map of the complex suggested the presence of another larger atom(s) in the acidic pocket in

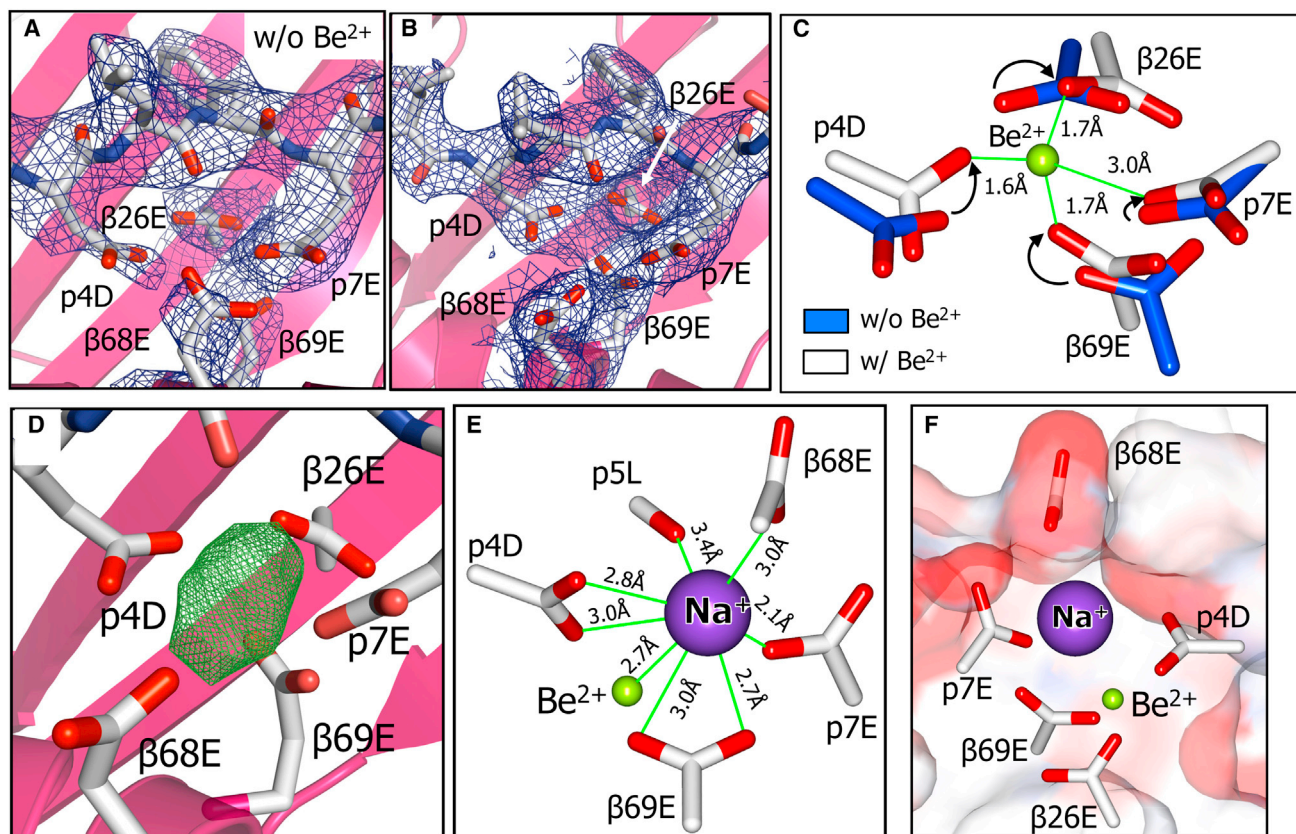


Figure 3. Be^{2+} and an Accompanying Na^+ Cation Captured in the Acidic Pocket Are Shielded from the Solvent

(A and B) View from above looking into the acidic pocket of DP2-M2- Be^{2+} with the side chains of $\beta 26\text{E}$, $\beta 68\text{E}$, $\beta 69\text{E}$, p4D , and p7E shown with 2Fo-Fc (1σ) electron density before (A) and after (B) capture of Be^{2+} .

(C) Overlay of the carboxylates of $\beta 26\text{E}$, $\beta 68\text{E}$, $\beta 69\text{E}$, p4D , and p7E before (carbon, blue) and after (carbon, white) binding of Be^{2+} . Arrows show the Be^{2+} -induced movement of the relevant carboxylate oxygens. Be^{2+} (green) is modeled within the cluster of oxygens based on small molecule structures of Be^{2+} coordinated by carboxylates (Alerghi et al., 1999; Schmidbaur et al., 1991; Schmidt et al., 1997) and the Be-O bond lengths are shown.

(D) Unaccounted for electron density in the Fo-Fc (6σ) map in the acidic pocket of DP2-M2- Be^{2+} .

(E) A Na^+ cation is shown modeled into Fo-Fc density of (D). Distances of the Na^+ from Be^{2+} and from oxygens of the carboxylates of $\beta 68\text{E}$, $\beta 69\text{E}$, p4D , and p7E and the backbone carbonyl oxygen of p5L are shown.

(F) A view looking through the M2 peptide toward the DP2 $\beta 1$ alpha helix showing the water accessible surface of the DP2-M2- Be^{2+} colored by the contributing atoms (oxygen-red, carbon-white). The surface contributed by the DP2 $\alpha 1$ alpha helix has been clipped to reveal the carboxylates of the five acidic amino acids in the pocket along with Be^{2+} and Na^+ among which only $\beta 68\text{E}$, p4D , and p7E are surface accessible.

addition to Be^{2+} (Figure 3D). We considered that this density might be due to an hydroxyl ion acting as a fourth Be^{2+} ligand, but the position and distance of this oxygen in relation to Be^{2+} would be inconsistent with Be^{2+} coordination geometry. It is more likely that this density was due to a second cation, often seen attracted to Be^{2+} complexes because of the net negative charge of the ligated Be^{2+} . We could not determine the nature of this cation from the density alone. However, no ordered cation was seen in the structure of the free DP2-M2 complex in the absence of Be^{2+} (Figure 3A), and the mother liquor used in crystallizing the AV22-DP2-M2- Be^{2+} complex contained 300 mM Na^+ as the only cation. Therefore, by default, we modeled Na^+ into this density (Figure 3E). Placed in this position, the Na^+ is surrounded by oxygens from the carboxylates of p4D , p7E , $\beta 68\text{E}$, and $\beta 69\text{E}$ and from the carbonyl of the p5L backbone, consistent with the analyses of Harding (2002) documenting a

predilection for Na^+ to interact with up to eight oxygens in proteins. In order to accommodate this Na^+ , the carboxylate of $\beta 68\text{E}$ has been pushed up on the surface of the DP2-M2 complex and undergone a 90° rotation, bringing its negatively charged oxygen into alignment with the Na^+ cation.

While our data support the conclusion that the cation captured in this crystal is Na^+ , our structure here does not rule out the possibility that, in vivo or tissue culture, cations other than Na^+ might occupy this position and may even complete the TCR ligand. Mg^{2+} would be a possibility, but so would Ca^{2+} and K^+ , also abundant in biological fluids and tissue culture media.

In summary, upon Be^{2+} binding, the DP2-M2 acidic pocket undergoes a conformational rearrangement in order to capture the Be^{2+} and an accompanying Na^+ cation through a network of interactions via the $\beta 69\text{E}$ carboxylates and other DP2 and M2

Table 1. Contacts between AV22 TCR and DP2-M2-Be²⁺

AV22 TCR Contacts to DP2-M2-Be ²⁺						DP2-M2-Be ²⁺ Contacts to AV22 TCR			
V Domain	CDR Loop	Amino Acid	No. of Atom to Atom Contacts to			Ligand	Amino Acid	No. of Atom to Atom Contacts to	
			DP2 α	M2	DP2 β			V α	V β
V α	1	30Y	–	6	45	DP2 α	55E	7	–
		32S	–	–	3		57Q	4	–
	2	49K	–	–	1	M2	58G	4	–
		51T	–	–	3		65I	–	4
		52K	–	–	2		1F	1	–
	3	95Y	–	59	–		2W	54	–
		97G	3	–	–		3I	24	–
		98A	12	11	–		5L	11	10
		99G	–	14	–		6F	–	4
		101Y	–	–	19		7E	–	6
	V β	1	30R	–	10		–	DP2 β	8T
2		50F	–	1	–	58Y	–		3
		55R	–	3	–	62Q	–		4
3		95L	–	–	12	64D	–		22
		96A	–	–	10	65I	–		4
		97Q	4	23	5	67E	2		–
		98G	–	–	6	68E	–		20
		99G	–	–	1	71A	2		–
		100E	–	–	10	74D	4		–
		101T	–	–	9	75R	24		8
		103Y	–	–	8	78R	15		–
						79H	20	–	
						82E	6	–	
Total			19	127	134	178			102

See also [Tables S1](#) and [S2](#).

oxygen, thus dramatically increasing the thermal stability of the complex. There are two unexpected features of this complex. First, the Be²⁺ moiety captured is the Be²⁺ cation itself, not a larger complex of Be²⁺ already partially ligated by solvent-derived electron donors (water, hydroxyl, carbonate, etc.). This answers a long-standing question in this field regarding the chemical form of Be²⁺ that is captured by DP2. Second, both the Be²⁺ and Na⁺ are not accessible on the surface of the complex, so that the AV22 TCR must recognize some change in the complex surface induced indirectly by the Be²⁺ and Na⁺ ([Figure 3F](#)). Therefore, we analyzed the interface between the AV22 TCR and the DP2-M2-Be²⁺ complex, especially in the region over the inaccessible Be²⁺ and Na⁺. A summary of the atom-to-atom contacts and the complete details of the contacts are listed in [Table 1](#) and [Table S2](#), respectively.

General Features of the AV22 TCR Binding to the DP2-M2-Be²⁺ Ligand

Typically, TCRs bind to MHCII ligands in a diagonal mode that places the CDR3 loops of the V domains over the peptide, while CDR1 and CDR2 loops make multiple interactions with the MHCII helices (see [Marrack et al., 2008](#); [Wilson and Garcia,](#)

[1997](#)). The AV22 TCR also takes this diagonal orientation on the DP2 complex ([Figure 4A](#)), but there are some unusual features of the interface. The TCR is tilted toward the DP2 beta chain helix ([Figure 4B](#)) resulting in extensive contact with the entire length of the M2 peptide and DP2 beta chain helix, at the expense of the DP2 alpha chain helix ([Figure 4C](#); [Table 1](#)). Furthermore, atypically, the hypervariable CDR3 loops of the TCR dominate the binding ([Figure 4D](#); [Table 1](#)), providing almost three quarters of the contacts with the ligand. The CDR1 and CDR2 loops of the TCR contribute little to the interface with the exception of a single TCR V α amino acid, CDR1 α 30Y, which binds extensively to the C-terminal end of DP2 β chain helix ([Table 1](#); [Figure 4C](#)). The focus of the AV22 TCR on the bound peptide and DP2 beta chain helix is consistent with our previous extensive mutational analysis of DP2 and the AV22 TCR ([Bill et al., 2005](#); [Bowerman et al., 2011](#)). Despite this tilted conformation, the footprint of the AV22 TCR on DP2-M2 involves 280 atom-to-atom contacts ([Table 1](#)) and covers 1,288 Å², well within the range typically seen for CD4 T cell TCR contact with MHC-peptide ligands. Moreover, the TCR has a K_D of ~5 μM when binding to the complex ([Falta et al., 2013](#)) ([Figures 2B](#) and [2C](#)), among the highest affinities seen for a TCR from a CD4 T cell.

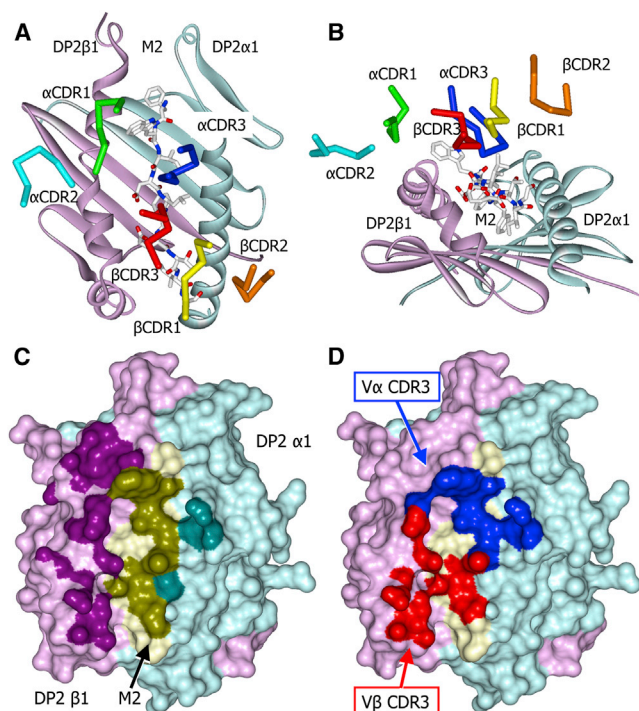


Figure 4. Skewed Docking of the AV22 TCR on the DP2-M2-Be²⁺ Complex

(A) View from above showing the diagonal docking of the six AV22 TCR CDR loops over the DP2-M2-Be²⁺ complex.
 (B) View looking down the peptide binding groove from the peptide C terminus showing the tilt and shift of the AV22 TCR CDR loops toward the DP2 beta chain helix.
 (C) Water accessible surface of the DP2-M2-Be²⁺ complex, DP2α (cyan), DP2β (magenta), and M2 (yellow). The footprint of the AV22 TCR on the complex is shown with darker versions of the same colors. Complex atoms were defined to be part of the footprint if they were within 4.5 Å of a TCR atom.
 (D) The parts of the footprint created by the AV22 TCR CDR3α (blue) and CDR3β (red) are shown.
 See also Tables S1 and S2.

The AV22 TCR Interacts with Changes in DP2-M2 Induced by Be²⁺

As predicted by their internal positions in the ligand structure, the sheltered Be²⁺, Na⁺, β26E, and β69E have no direct TCR contact (Table S2), but there is a large area of TCR contact, especially via Vβ CDR3, with the surface of DP2-M2 that includes the re-positioned β68E carboxylate, as well as surrounding DP2 and M2 amino acids (Figures 4D and 5A; Tables 1 and S2). The repositioned β68E carboxylate bridges the hidden Na⁺ and Vβ CDR3 via an H-bond to the backbone N of Vβ 98G (Figure 5B; Table S2).

In a previous study, we showed that mutation of β68E to A virtually eliminated the Be²⁺ response of a line of lung T cells (containing T cells bearing various TCRs) from the patient that provided the AV22 T cell. In these experiments, the presenting DP2 was on the surface of an APC line and contained the usual array of naturally processed self-peptides (Dai et al., 2010). Therefore, we tested the effect of mutating β68E on the recognition by the AV22 of our soluble DP2, in this case uniformly occu-

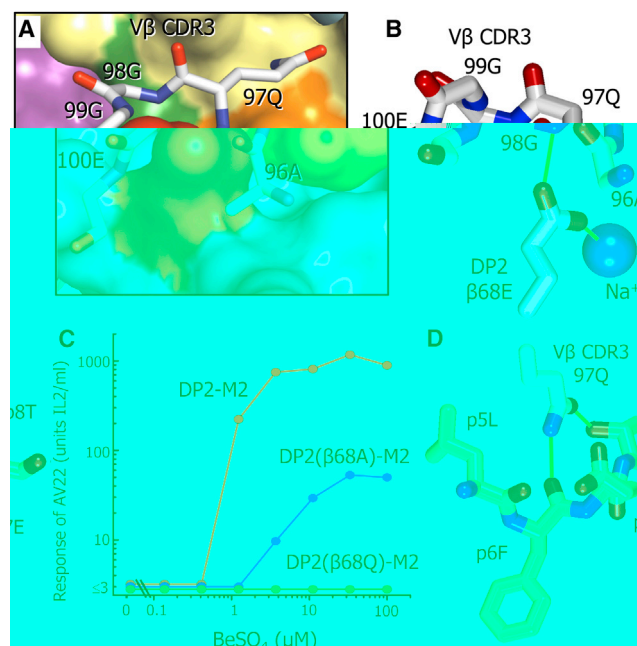


Figure 5. Interaction of AV22 Vβ CDR3 with the Surface of DP2-M2 above the Be²⁺ Binding Site

(A) A wireframe representation is shown of the backbone of the AV22 TCR Vβ CDR3 loop and the side chain of Vβ 97Q interacting with the surface of DP2-M2. Colored as Figure 4C with β68E red, p7E orange, and p4D in green.
 (B) The orientation of the side chain carboxylate of DP2 β68E between the Vβ CDR3 loop of the AV22 TCR and the internal Na cation is shown. Potential H-bond/salt bridges are shown as green lines.
 (C) The response of AV22 without or with various concentrations of BeSO₄ presented by the wild-type DP2-M2 complex or the complex mutated at β68E to glutamine or alanine. The experiment was repeated with similar results.
 (D) Interactions of the carboxamide of Vβ CDR3 97Q with the M2 peptide are shown. Potential H-bonds are shown as green lines.
 See also Figure S2 and Table S2.

pied by M2 and Be²⁺. We tested the mutation of β68E to A (eliminating its side chain) but also to Q (eliminating only the negative charge of its carboxylate). The β68E to Q change completely eliminated the ability of DP2-M2 to present Be²⁺ to AV22 (Figure 5C) or to be bound by the soluble AV22 TCR in the presence of Be²⁺ (Figure S2). However, in this case, mutation of β68E to A only partially reduced the response (Figure 5C). It also decreased by 2-fold the half-life of the AV22 TCR complex with DP2-M2-Be²⁺, but not the overall affinity of the TCR for the ligand (Figure S2). These results suggest that the direct interactions of the Vβ CDR3 with the side chain of β68E contribute to, but are not required for, a response, depending on the specific TCR and peptide presenting the Be²⁺. However, more importantly, when this side chain is present, the Be²⁺, the accompanying Na⁺ and the negative charge of β68E carboxylate may all be required for the proper realignment of the carboxylate. Removing the β68E charge by its mutation to Q must somehow untether the side chain to interfere with TCR binding.

Another area of extensive interaction of the Vβ CDR3 loop is via the carboxamide of the Vβ 97Q side chain, which lies across the peptide making substantial van der Waals' and H-bond

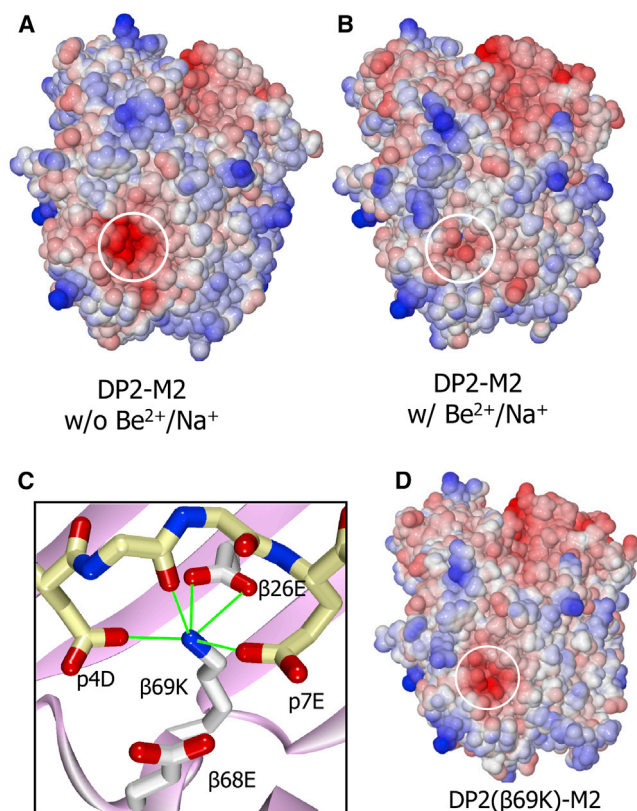


Figure 6. Specific Changes in the Electrostatic Surface Potential of the DP2-M2 Complex Caused by Binding $\text{Be}^{2+}/\text{Na}^{+}$

(A and B) Water accessible surface of the DP2-M2 complex in the absence (A) or presence (B) of Be^{2+} and Na^{+} , colored by the electrostatic surface potential (red, negative; blue, positive). Area overlaying the acidic pocket is circled.

(C) The interaction of the β69K lysine ε-amino group in the DP2(β69K)-M2 mutant structure with amino acids in the acidic pocket is shown. Potential H-bond/salt bridges are shown as green lines.

(D) The electrostatic surface potential of the DP2(β69K)-M2 mutant complex is shown as in (A).

See also Tables S1 and S2.

contacts with the M2 peptide (p5L, p6F p7E, and p8T) (Figure 5D; Tables 1 and S2). These aspects of the interaction agree with our previous mutation analysis of the AV22 TCR, where an alanine scan of the Vβ CDR3 loop showed that the side chain of β97Q was required for Be^{2+} recognition (Bowerman et al., 2011).

In addition to the conformational changes, the most dramatic difference between the DP2-M2 complex before and after Be^{2+} and Na^{+} binding is the change in the predicted electrostatic surface potential (Figures 6A and 6B). In the absence of Be^{2+} and Na^{+} , the surface potential in the entire region around the acidic pocket is very negative, but it becomes substantially neutralized by the addition of the positively charged Be^{2+} and Na^{+} to the acidic pocket. This electrostatic difference would be expected to influence the ability of the Vβ CDR3 loop to approach and interact with this surface. As mentioned above, we can not rule out that, in vivo or tissue culture, the cation accompanying Be^{2+} might be an abundant divalent cation, such as Mg^{2+} or Ca^{2+} , rather than Na^{+} . In this case, we would predict that the

negative electrostatic surface potential would be reduced even further.

The structure of the DP2(β69K) complex supports the importance of these surface changes induced by Be^{2+} and Na^{+} binding. This mutated complex cannot present Be^{2+} to AV22 (Figure 2A), but its thermal stability is equal to that of the wild-type complex in the presence of Be^{2+} (Figure 2E). In the structure, the β69K positively charged ε-amino group has been drawn into the acidic pocket in a position similar to the Be^{2+} in the wild-type structure and is involved in a network of H-bonds and salt bridges to p4D, p7E, DP2 β26E, and the peptide backbone (Figure 6C), accounting for the thermal stability of the complex and predicting that this complex could not bind Be^{2+} . However, there is no evidence of a captured cation and the β68E side chain is aligned similarly to that of the DP2-M2 complex without Be^{2+} . Furthermore, the predicted electrostatic surface potential of the mutant complex is only slightly reduced by the lysine single positive charge (Figure 6D). These differences from the DP2-M2- Be^{2+} complex most likely explain the lack of recognition by AV22.

In summary, after binding to DP2-M2, Be^{2+} and Na^{+} are not exposed for TCR interaction, but rather induce both a reduction in the electrostatic surface potential and a subtle change in surface topology of the DP2-M2 over the cation binding site where the AV22 TCR Vβ CDR3 interacts. Our data indicate that both of these changes probably contribute to creation of the AV22 ligand, but we cannot at present estimate the relative contributions of the two alterations.

DISCUSSION

Dozens of structures of TCRs interacting with MHC-peptide ligands have been published, and there are a number of structures detailing how TCRs bind to nonclassical MHC molecules specialized to present particular nonpeptide or peptide ligands (<http://www.imgt.org/3Dstructure-DB/>). However, T-cell-mediated hypersensitivity to nonpeptide environmental agents such as drugs, plant products, and metal cations are widespread in the human population. There is very little known structurally about how these agents become associated with MHC molecules and how T cells recognize their presence. The work presented here answers a long-standing question about how a metal cation, in this case Be^{2+} , becomes associated with a self MHC-peptide complex to create the ligand for a pathogenic T cell.

The most common hypothesis for how nonpeptide moieties are recognized by T cells has been that they act as haptens, i.e., they become associated with or covalently bound to the surface of an MHC-peptide complex and offer a new site for TCR interaction, in the same way that a mutation in one of the upwardly pointing amino acids of the MHC or bound peptide would create a new antigen. Earlier studies with T cell reactivity to MHC-peptide complexes modified with chemically reactive forms of trinitrophenol and fluorescein or the urushiols produced by plants such as poison ivy were interpreted this way (Kalish, 1990; Martin and Weltzien, 1994).

Our X-ray diffraction data, as well as mutational and biophysical results, show that Be^{2+} is clearly not working in this way,

but, rather, becomes part of the internal structure of the MHC-peptide complex, indirectly causing structural and biophysical changes on the surface of the complex that are now recognized by the T cell. The Pichler group has argued for some years for a concept they term “pharmacological interaction with immune receptors” or the “p-i” concept (Pichler et al., 2006), in which drugs can interact with TCRs or MHC-peptide complexes to promote an interaction without necessarily placing the drug at the interface between the two. Perhaps the most dramatic evidence for this phenomenon is seen in the severe allergic response to the drugs, abacavir and carbamazepine. These reactions are strongly associated with particular MHCII HLA-B alleles (Illing et al., 2013). Recent structural and biochemical data (Illing et al., 2012) indicate that, unlike Be^{2+} , these drugs work by binding inside the MHC peptide binding groove to drastically change the repertoire of self-peptides bound, generating a robust CD8 T cell response similar to an allo-MHC response.

In our studies here, Be^{2+} also binds internally within the peptide binding groove of DP2 without becoming part of the TCR interface, yet it creates the TCR ligand without changing the DP2 bound peptide. Also supporting this conclusion, is our previous study using DP2⁺ antigen presenting cells to present Be^{2+} to CBD T cells. To show that Be^{2+} -dependent loading of new peptides into DP2 was not required, we fixed the antigen presenting cells prior to Be^{2+} exposure without compromising the T cell response to Be^{2+} (Fontenot et al., 2006). Nevertheless, the DP2 complex becomes much more stable in the presence of Be^{2+} and the appropriate mimotope or self-peptide (Figure 2E). Therefore, it is possible that the continuous presence of Be^{2+} in vivo in CBD may promote the loading or stability of peptides that present Be^{2+} in the MHCII endosomal peptide loading compartment, eventually skewing the lung DP2-bound peptide repertoire toward Be^{2+} -presenting peptides.

The nature of the Be^{2+} containing compounds recognized by the immune system has been discussed for many years. Because of its ability to form higher-ordered complexes in aqueous solutions (Everest, 1964; Sutton and Burastero, 2003), it was natural to consider that the Be compound captured by DP2 β 69E might already contain multiple Be ligands, thus creating a surface-exposed hapten-like moiety for T cell recognition. Our data presented here clearly shows that this is not the case. Rather, it is the Be^{2+} cation itself that is captured by the DP2-M2 complex. This explains why individual T cell clones, such as AV22, can be activated by a wide variety of soluble Be-containing compounds (Dai et al., 2010). The Be^{2+} binding acidic pocket in the DP2-peptide complex appears to compete extremely well with other potential ligands for Be^{2+} .

AV22 and related T cells found in the lungs of multiple CBD patients respond to Be^{2+} presented by DP2 containing a variety of related self- or library-generated peptides (Falta et al., 2013) (Figure 1A). The common elements in all of the peptides, besides a DP2 binding motif, are p4D and p7E. However, these peptides do not satisfy all Be^{2+} reactive T cells (Falta et al., 2013). Given the extensive interaction of the AV22 TCR with areas of the DP2-M2 surface away from the DP2 acidic pocket (Table 1), it is likely that these other T cells will require TCR contact with

different peptide amino acids in these other areas. However, we predict that they will have to retain this p4D/p7E or a similar acidic motif in order to effectively capture Be^{2+} .

CBD is a particularly dramatic example of blurring the line between T cell allergic hypersensitivity and autoimmunity. Similar to many of the well-studied autoimmune diseases, CBD risk is tightly associated with certain MHCII alleles, in this case with an existing DP2-self-peptide complex that has been subtly changed by the internally bound Be^{2+} . As structural information begins to accumulate for the actual targets of autoimmune diseases, enzymatic posttranslational modifications (Cordova et al., 2013; Scally et al., 2013; Sollid and Jabri, 2011) and organ-specific peptide processing have been implicated in generating the relevant ligands (Crawford et al., 2011; Mohan and Unanue, 2013; Stadinski et al., 2010). These are often not recognized by the autoimmune T cells directly, but instead affect the way the peptide binds to MHCII. In the same way, Be^{2+} binding could be thought of as a special form of “posttranslational modification” that converts a self-MHCII-peptide complex to which the immune system is tolerant into an immunogenic one. In this light, it is perhaps more useful to think of T cell allergic hypersensitivities versus autoimmunity as involving a continuum of peptides from truly foreign ones through various levels of modifications of self-peptides mediated by both foreign and endogenous mechanisms.

EXPERIMENTAL PROCEDURES

More experimental details are in the [Extended Experimental Procedures](#).

Protein Expression and Purification

All MHC proteins were expressed in baculovirus infected insect cells as soluble proteins as previously described (Crawford et al., 1998; Dai et al., 2010). All contained an antigenic peptide covalently attached via a C-terminal flexible linker to the N terminus of the MHCII beta chain (Kozono et al., 1994). All had a stabilizing C-terminal acid-base leucine zipper (O’Shea et al., 1993) and a peptide tag for enzymatic biotinylation (Crawford et al., 1998), which were removed proteolytically with either papain or thrombin for crystallography and differential scanning fluorimetry.

The V domains of the AV22 TCR were cloned as previously described into a bacterial expression vector in frame with sequence encoding the extracellular portions of human C α or C β (Dai et al., 2008; Kjer-Nielsen et al., 2003). Inclusion bodies containing the separate chains were prepared in *Escherichia coli* BL21 Rosetta cells (EMD Biosciences). The solubilized proteins were mixed and refolded as previously described (Dai et al., 2008; Kjer-Nielsen et al., 2003).

T Cell Stimulation

A T cell transfectoma bearing the AV22 TCR (Bowerman et al., 2011) was stimulated in culture alone or with various concentrations of BeSO_4 . The surface of the culture wells had various biotinylated MHCII-peptide combinations captured via Extravidin (Sigma). The extent of AV22 activation was assessed by IL-2 in the culture supernatants after 24 hr (White et al., 2000).

Surface Plasmon Resonance

The ability of the soluble AV22 TCR to bind to various combinations of MHCII-peptide with or without Be^{2+} was assessed with surface plasmon resonance using a BIAcore 2000 instrument. The biotinylated MHCII-peptide (~2,000 resonance units [RU]) was immobilized in flowcells of a streptavidin BIA sensor chip and various concentrations of the soluble AV22 TCR injected before and after exposure of the MHCII to 50 μM Be^{2+} . Binding kinetics were calculated using instrument software (BIAevaluation 4.1).

Differential Scanning Fluorimetry

The thermal stabilities of various MHCII-peptide complexes were determined by differential scanning fluorimetry using either a Stratagene MX3005p (Agilent) or CFX96 RCT-PCR (Bio-Rad) instrument. Proteins were mixed with the dye SYPRO orange and the fluorescence of the dye bound to the protein was followed as the temperature was increased from 20°C to 100°C. The melting temperature (T_m) was defined as the temperature at which the fluorescence increase was 50% of the maximal increase.

Crystallography

Crystals grown in hanging or sitting drop chambers were cryopreserved in liquid nitrogen. Diffraction data were collected at Brookhaven x25, APS 24 ID-C, and Berkeley 822. Details of crystallization, cryopreservation, data collection, data processing, and model building and refinement are in the [Supplemental Information](#) and [Table S1](#).

Structure Analysis and Representations

Atom-to-atom interactions between the AV22 TCR and DP2-M2-Be²⁺ were calculated with the ncont program in the CCP4 program suite. Electrostatic surface potential was calculated using PDB2PQR and APBS ([Baker et al., 2001](#); [Dolinsky et al., 2004, 2007](#)). Graphical representations of structures were constructed with PyMOL, (PyMOL), WebLab Viewer Pro 4.1 (Accelrys), and Jmol (Jmol).

ACCESSION NUMBERS

The Research Collaboratory for Structural Bioinformatics (RCSB) Protein Database accession numbers for the coordinate and structure factor files for the five new structures reported in this paper are 4P4K, 4P4R, 4P57, 4P5M, and 4P5K.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, two figures, and two tables and can be found with this article online at <http://dx.doi.org/10.1016/j.cell.2014.04.048>.

AUTHOR CONTRIBUTIONS

G.M.C., Y.W., S.D., and J.W.K. designed the studies. G.M.C., F.C., and J.W.K. prepared the expression constructs. G.M.C., F.C., Y.W., S.D., and A.N. produced and purified the proteins. G.M.C., Y.W., and S.D. performed the crystallography and solved the structures. G.M.C., Y.W., S.D., B.T.W., and J.S.K. collected the diffraction data. G.M.C., F.C., and J.W.K. performed the biophysical studies. M.T.F., N.A.B., and A.P.F. provided peptides and mutational data. G.M.C., Y.W., P.C.M., S.D., and J.W.K. wrote the paper with help from the other authors.

ACKNOWLEDGMENTS

We thank Igor Kurinov for data collection assistance at APS Argonne National Laboratory, Beamline 24 ID-C and Corie Ralston at ALS Lawrence Berkeley National Laboratory, Beamline 822. Oliver Smart (Global Phasing) assisted us with beryllium definitions in AUTOBuster, and Mario Santiago made the BIORAD CFX96 RCT-PCR instrument available to us. We also thank Randy Anselment at the Molecular Resource Center, National Jewish Health, for oligonucleotide synthesis and DNA sequencing. This work was supported in part by NIH grant AI17785 (P.C.M.), by NIH grants HL62410, HL92997, and ES011810 (to A.P.F.), by CCTSI KL2TR000156 and KL1TR000155 and the Boettcher Foundation (to S.D.), and NIH grant AI17134 (to J.W.K.).

Received: February 2, 2014

Revised: March 22, 2014

Accepted: April 21, 2014

Published: July 3, 2014

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EXTENDED EXPERIMENTAL PROCEDURES

Differential Scanning Fluorimetry

All proteins were diluted to a final concentration of 200 µg/ml in an assay volume of 25 µl. SYPRO Orange was diluted in DMSO for use at 10x final concentration. Assay buffer unless stated otherwise was 20 mM Tris pH8, 100 mM NaCl with or without 50 µM BeSO₄. Assays were premixed as stocks prior to aliquoting into 0.2 ml nonskirted low profile 96 well white PCR plates (Thermo Scientific AB0700W) then sealed with BIO-RAD optical plates (2239444). After aliquoting, the sample plates were centrifuged for 1 min at 1,000 RPM on Beckman plate carriers in a CS-6R centrifuge at room temperature followed by the assay.

Assays were performed in the Stratagene MX3005p (Agilent) or BIORAD CFX96 RCT-PCR instrument using the dissociation curve option for Sbyr Green and the FFROX filter set (496 nm excitation-610 nm emission) with a temperature gradient of 20 to 100°C at 1 degree/min. Dissociation curve results were exported to Excel for graphical display. Data were normalized setting the starting fluorescence to zero and the maximal fluorescence recorded to 100%. Melting temperature (T_m) of the protein was calculated as the temperature at which the fluorescence was 50%.

Molecular Continuum Electrostatic Surface Potentials

Molecular continuum electrostatic surface potentials were calculated in PDB2PQR (Dolinsky et al., 2007) and the Adaptive Poisson-Boltzmann Solver (Baker et al., 2001) with solvent accessible molecular potential isosurface visualization in Jmol (<http://jmol.sourceforge.net>) using a red-white-blue (negative to positive) gradient, followed by rendering with PovRay (Persistence of Vision Ray-tracer Pty. Ltd). A charge of +2 and radius of 0.3, and a charge of +1 and radius of 1.32 Angstroms were assigned to the Be²⁺ and Na⁺ ions respectively. Surface sugars and waters were removed for the calculations.

Protein Crystallization

DP2-A28 was concentrated to 5 mg/ml in 10 mM Tris pH 8 and crystallized at 20°C using the hanging drop vapor diffusion method with 0.5 µl protein and 0.5 µl of mother liquor (1.3 M Na Citrate, pH 6.5). Crystals were harvested by transfer into a cryo protectant consisting of 25% glycerol and 75% mother liquor followed by rapid transfer into liquid nitrogen.

DP2-RAS was concentrated to 5 mg/ml in 10 mM Tris pH 8. Crystallization experiments were performed at 20°C using the hanging drop vapor diffusion method. Diffraction quality crystals were obtained using mother liquor containing 0.2 M ammonium formate and 25% PEG 4000 in 0.1 M HEPES buffer, pH 6.8. The crystals were cryo protected by transfer into 2–3 ml of the mother liquor plus 20%–25% glycerol for several minutes before plunging into liquid nitrogen.

DP2-M2 was concentrated to 4–6 mg/ml in 10 mM Tris pH 8, 50 mM NaCl. Diffraction quality crystals were grown at 20°C by sitting drop vapor diffusion using the Mosquito LCP robot (ttpLabtech) to aliquot 60 nl of DP2-M2 and 60 nl of a 100 µl mother liquor (0.2 M NH₄tartrate dibasic, 20% PEG-3350). Crystals were harvested by addition of cryo-protectant to the protein drop. Cryo-protectant contained 20% ethylene glycol, 60% mother liquor 20% of a 50% PEG-3350 solution. Crystals were transferred into liquid nitrogen for storage.

DP2(β69K)-M2 was concentrated to 5 mg/ml in 10 mM Tris pH7.5, 50 mM NaCl and crystallized at 20°C by the hanging drop vapor diffusion method using 0.5 µl of protein and 0.5 µl of the mother liquor (200 mM Ca acetate, 10 mM Tris pH 7, 20% PEG-3000). Crystals were harvested by transfer into cryo protectant consisting of 20% ethylene glycol, 60% mother liquor and 20% of a 50% PEG-3000 solution. Crystals were transferred into liquid nitrogen for storage.

For the AV22 TCR complex with DP2-M2-Be²⁺, the TCR and the DP2-M2-Be²⁺ protein were mixed at a ratio of 1:1 (wt:wt) and concentrated to 10 mg/ml in 10 mM Tris pH 7 50 mM NaCl. Diffraction quality crystals were grown at 20°C using the hanging drop vapor diffusion method and 0.5 µl of protein solution with 0.5 µl of well solution containing 2–300 mM sodium cacodylate pH 5.5–6, 20% glycerol and 7% PEG-8000. Crystals were harvested by transfer into cryo protectant containing 20% glycerol, 60% mother liquor and 20% of a 50% PEG8K solution followed by fast transfer into liquid nitrogen for storage.

Data Collection Structure Determination and Refinement

DP2-RAS and DP2-A28 data were collected at beam line 8.2.2 at the Advanced Light Source, Berkley National Laboratory. Data were processed and scaled using HKL2000 (Otwinowski and Minor, 1997). The molecular replacement solution was solved using Phaser (McCoy et al., 2007) with DP2-DRa (PDB-3LQZ) as a search model. The structure was further refined in Refmac (Murshudov et al., 1997) followed by rounds of building in COOT (Emsley et al., 2010) and O (Zou and Jones, 1996). Final refinement used PHENIX (Adams et al., 2010). Structure quality was checked in PROCHECK (Laskowski et al., 1996).

Data for AV22 TCR bound to DP-M2-Be²⁺, DP2-M2 alone and DP2(69K)-M2 were collected at beamlines X25 at Brookhaven National Laboratories and ID-24c at the Advanced Photon Source, Argonne National Laboratory, respectively, using the Pilatus detector and at a wavelength of 1.1 and 0.98. Data were indexed, reduced and scaled in HKL2000 and XDS (Kabsch, 2010). Reduced data were further analyzed in Pointless (Evans, 2006) and PHENIX using the PHENIX Xtriage options. Molecular replacement solutions were found using Phaser with PDB-3LQZ for DP2 complexes and 3C5Z for the AV22 TCR, as initial search models, followed by a round of rigid body refinement in Refmac. Further refinement was carried out in AutoBUSTER (Bricogne et al., 2011) or PHENIX using the PHENIX.refine option followed by rounds of building in COOT. The quality of final models were checked in PHENIX using the Comprehensive Validation option and PROCHECK.

During refinement of the AV22 TCR bound to DP2-M2-Be²⁺ complex, the Fo-Fc map showed an 8 sigma electron density peak inside the acidic hole corresponding to the region mapped by mutational and structural data to be the beryllium binding site. We modeled Be²⁺ at one end of this site based on small molecule X-ray crystal structures of Be²⁺ bound to carboxylate oxygens (CSD-ID, RUNTUP, CUBQF, SOLHEG) (Alerghi et al., 1999; Schmidt et al., 1997; Schmidbaur et al., 1991) our biophysical finds and known physical chemistry of beryllium. The Be²⁺ cation contains only two electrons with no anomalous signal, and its very small radius allows no means for isosteric substitution with another, heavier metal atom. There is also no space for another beryllium ion within the oxygen binding site. This metal site cannot be characterized more completely using metal probing techniques such as Extended X-ray Adsorption Spectroscopy, since Be²⁺ represents only a small percentage of the protein sample, and the Be²⁺ ion is too light for this technique.

Since the crystallization solution contained up to 300 mM NaCl and no other ion was used during purification a Na⁺ ion was modeled as the counter cation. All ions were fixed during refinement. An AutoBuster parameter file was constructed to restrain the Be²⁺ ion within the limits observed for small molecule X-ray crystal structures of Be²⁺ bound to acidic oxygens. B-factors were refined as normal. The presence of the Be²⁺ and Na⁺ ions in the model reduced the 2Fo-Fc electron density peak in the acidic hole to below 3 sigma. It is not discounted that a different counter ion could be present instead of Na⁺ such as a Mg²⁺ ion. A single beryllium ion is not considered to contribute to the electron density in this data.

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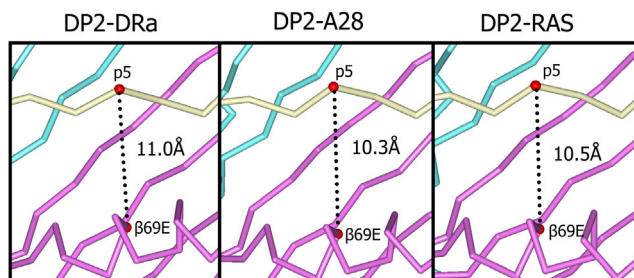


Figure S1. DP2-Peptide Complexes Have a Gap between the Peptide and Beta Chain Alpha Helix, Related to Figure 1B

A portion of the Calpha backbones of DP2 bound to three different peptides are shown with the distances (Å) from the Calpha of p5 to the Calpha of DP2 β69E (dotted line).

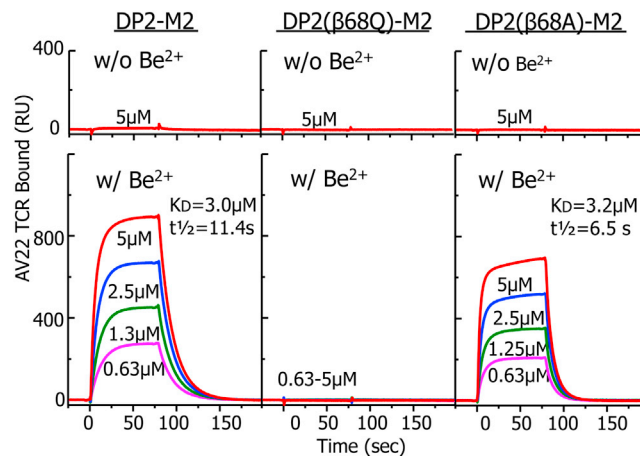


Figure S2. DP2 β68E Plays a Role in Be²⁺-Interaction with the AV22 TCR, Related Figures 5A–5C

Biotinylated β68E, β68Q and β68A versions of DP2-M2 were immobilized in separate flowcells of a BIAcore SA BIA sensor chip. A fourth flowcell contained immobilized DR52c-pHIR as a negative control. Various concentrations of soluble AV22 TCR were injected through the flowcells before and after exposure of the immobilized DP-M2 to 50 μM BeSO₄. The surface plasmon resonance signal (RU) was followed before, during and after TCR injections. The data in the control flowcell was used to correct for the fluid phase resonance signal in the other flowcells. Binding kinetics were analyzed with BIAevaluation 4.1 software using a first order kinetic model. The experiment was repeated with similar results.