

T-cell receptor (TCR) interaction with peptides that mimic nickel offers insight into nickel contact allergy

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T cell-mediated allergy to Ni⁺⁺ is one of the most common forms of allergic contact dermatitis, but how the T-cell receptor (TCR) recognizes Ni⁺⁺ is unknown. We studied a TCR from an allergic patient that recognizes Ni⁺⁺ bound to the MHCII molecule DR52c containing an unknown self-peptide. We identified mimotope peptides that can replace both the self-peptide and Ni⁺⁺ in this ligand. They share a p7 lysine whose εNH₂ group is surface-exposed when bound to DR52c. Whereas the TCR uses germ-line complementary-determining region (CDR)1/2 amino acids to dock in the conventional diagonal mode on the mimotope–DR52c complex, the interface is dominated by the TCR Vβ CDR3 interaction with the p7 lysine. Mutations in the TCR CDR loops have similar effects on the T-cell response to either the mimotope or Ni⁺⁺ ligand. We suggest that the mimotope p7 lysine mimics Ni⁺⁺ in the natural TCR ligand and that MHCII β-chain flexibility in the area around the peptide p7 position forms a common site for cation binding in metal allergies.

antigen presentation | hypersensitivity | peptide display library | crystal structure | metal recognition

T-cell receptors (TCRs) made up of α- and β-chains have a very large number of ligands, including self- and foreign peptides, superantigens, lipids, small organic haptens, and metal ions. These ligands usually react with TCRs when they are bound to major histocompatibility complex (MHC) proteins. TCRs usually bind MHC/peptide combinations in similar orientations such that the TCR is aligned diagonally across the MHC/peptide, with the Vβ region of the TCR placed over the α1 α-helix of the MHCI heavy chain or of the MHCII α-chain and the Vα region placed over the α2 α-helix of the MHCI heavy chain or the β1 α-helix of the MHCII β-chain (1, 2). We (1, 3–5) and others (6–8) have suggested that germ line-encoded residues in the complementary-determining region (CDR)1 and CDR2 loops of the TCR variable regions are evolutionarily conserved for interaction with the MHC. These residues are also involved when natural killer T (NKT) cells recognize glycolipids bound to the non-classical MHCI protein CD1d, and even when T cells recognize the non-MHC protein CD155 (9).

It is noteworthy, however, that the presence of these evolutionarily conserved amino acids does not assure the conventional diagonal docking mode for TCRs on MHC molecules. For example, the NKT TCRs have a unique docking mode on CD1d (10, 11): Protein superantigens bridge TCRs to MHCII in a manner that prevents conventional TCR–MHCII interaction (12), and TCRs often dock to autoantigen peptides bound to MHCII in unusual orientations (13, 14). Thus, in cases in which the structural details of the interaction between the TCR and its target are not known, we cannot be certain that the interaction will occur with the TCR in its usual orientation on MHC or not. Such is the case for the recognition of metal ions by TCRs (15). Although it is assumed that ions such as Be⁺⁺, Ni⁺⁺, Co⁺⁺, Cu⁺⁺, Cr⁺⁺⁺, and Au⁺⁺⁺ are recognized by TCRs in combination with MHC bound to a particular self-peptide, very little is known about the structures of the TCR/MHC/metal ion combinations. This applies, for example, to the T cells that mediate allergy to Ni⁺⁺, a common contact allergen affecting up to 20% of humans (16).

To begin to understand TCR interaction with metal ions, we decided to study the TCR of one particular CD4⁺, Ni⁺⁺-reactive human T-cell ANi2.3. ANi2.3 belongs to a group of TCRs that contain human Vβ17 that others have found are overrepresented among Ni⁺⁺-specific CD4⁺ TCRs of patients suffering from particularly severe Ni⁺⁺ contact hypersensitivity (17). We have previously reported that ANi2.3 reacts with the human MHCII protein DR52c (HLA-DRA, HLA-DRB3*0301) and Ni⁺⁺ when a particular unknown peptide(s) is bound to DR52c (18). Here, using a display library method we have previously described (19, 20), we screened libraries of DR52c bound to partially randomized peptides in attempts to identify the specific peptide recognized by ANi2.3 in the presence of Ni⁺⁺. Instead, we isolated many peptide mimotopes that, when bound to DR52c, engaged this TCR and activated the ANi2.3 T cell in the absence of Ni⁺⁺. The C-terminal halves of these mimotopes had highly related amino acid sequences that included an almost invariant lysine at the p7 position, which we considered might mimic Ni⁺⁺.

We solved the structures of two of these mimotope peptides bound to DR52c and the structure of the ANi2.3 TCR bound to one of the mimotope complexes. In each of the three structures, the peptide p7 lysine assumes a conformation that puts its εNH₂ group on the surface of the molecule. Interactions between the TCR CDR1/2 loops and the DR52c helices help dock it in the conventional diagonal mode, but the area of strongest TCR contact surrounds and involves the mimotope p7 lysine εNH₂. Mutagenesis of the TCR CDR loops shows that the TCR engages the natural Ni⁺⁺ and mimotope ligands very similarly. We discuss the likelihood that the positive charge of this lysine side chain mimics Ni⁺⁺ in the interaction with the ANi2.3 TCR. Our structure also provides insight into how the area between the MHC-bound peptide and the arched helix of the MHCII β1 helix might be a common site for cation binding in metal allergies.

Results

Common Motif Among Peptide Mimotopes for the ANi2.3 T Cell.

In previous studies, we and others have shown that the human T-cell clone ANi2.3, isolated from a patient with nickel hypersensitivity, reacts with Ni⁺⁺ presented by the human MHCII protein DR52c bearing an unknown peptide (17, 18). Our efforts to find this peptide among the peptides naturally processed and bound to DR52c failed, suggesting that it may be present in very low abundance. Therefore, we turned to the use of baculovirus-expressed MHC–peptide display libraries as described previously

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Data deposition: The atomic coordinates and structure factors reported in this paper have been deposited in the Protein Data Bank, www.pdb.org (PDB ID codes 4H26, 4H25, and 4H11).

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(19, 20). We produced two types of libraries (Fig. 1A). In both, based on the crystal structure of DR52c bound to the Tu peptide (21), we fixed the anchor amino acids at p1, p4, p6, and p9 to those of the Tu peptide (I, N, P, and I). In the first library, six potential TCR contact amino acids (p1, p2, p3, p5, p7, and p8) were randomized via an NNS codon mixture. The second library was a group of four pooled sublibraries, prepared like the first library, except that, in each, either p1, p2, p3, or p5 was fixed to H as well in order to test the idea that a histidine somewhere in the N-terminal half of the peptide might cooperate with DR52c β 81H in binding Ni (18).

The two libraries were separately screened. In each case, SF9 insect cells infected with the library were sorted by flow cytometry for simultaneous binding of a multimeric, fluorescent version of the ANi2.3 TCR in the presence of Ni^{++} and of a fluorescent anti-DR mAb. Within four rounds of sorting, in both cases a population blossomed out that bound both the TCR and anti-DR reagent strongly (Fig. 1B, Lower).

SF9 cells infected with a single virus were sorted for sequencing. Despite the presence of Ni^{++} during the screening, both libraries produced only related peptides that stimulated ANi2.3 in the absence of Ni^{++} . A list of the properties of the 10 best ANi2.3-stimulating mimotopes is shown in Fig. 1C. The most striking feature of these mimotopes was a very strong selection of I, K, or R at p5, p7, and p8, respectively, in both

libraries, with substitutions of L, R, and K occasionally allowed at these positions. There appeared to be no selection of a particular amino acid at p1, p2, or p3. We performed the screen again after predepleting the libraries of Ni^{++} -independent mimotopes for three cycles, as in Fig. 1B, but in the absence of Ni^{++} , before rescreening again in the presence of Ni^{++} . This experiment also did not yield Ni^{++} -dependent mimotopes, but did, however, yield a few Ni^{++} -independent mimotopes similar in sequence to those listed in Fig. 1C (Table S1).

Therefore, we decided to examine examples of the related Ni^{++} -independent mimotopes in Fig. 1 to see whether they might offer some clues as to how the natural self-peptide in normal DR52c⁺ antigen-presenting cells presents Ni^{++} to ANi2.3 T cells. First, in baculovirus, we made a series of mutations in the best-stimulating mimotope, HIRCNIIPKRI (pHIR), such that individual amino acids at p1, p2, p3, p5, p7, and p8 were changed to alanine. ICAM/B7.1⁺ SF9 cells infected with mutant viruses were tested for their ability to stimulate ANi2.3 (Fig. 1D). As predicted from the variability of the N-terminal sequences of the enriched peptides (Fig. 1C), substitution of A at p1, p2, and p3 had no effect on the response of the ANi2.3 T cell. However, substitution of A for the amino acids at p5, p7, or p8 dramatically reduced or eliminated the response. Thus, this alanine scan and the heavy selection of p5I, p7K, and p8R in the libraries indicated that the ANi2.3 T cell focuses on the C-terminal end of the peptide mimotopes.

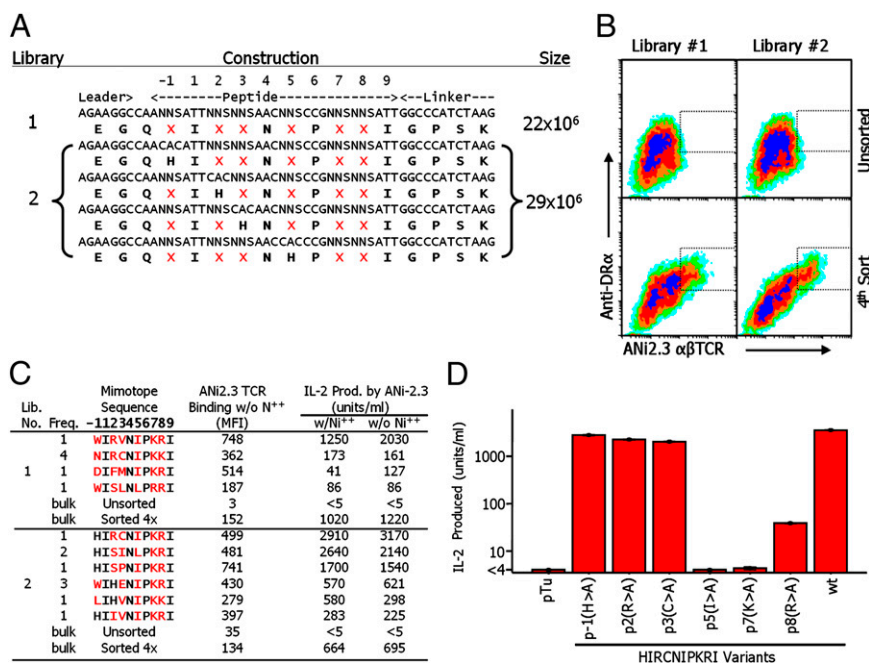


Fig. 1. Identification of ANi2.3-stimulating, Ni^{++} -independent peptide mimotopes in baculovirus DR52c-peptide libraries. (A) The designs and sizes of DR52c-peptide libraries are shown. Positions randomized to all 20 amino acids in the libraries are marked with a red X and were encoded by NNS, where N = A, C, T, or G and S = G or C. The preparation and characterization of library 1 have been previously described (19, 20). The four libraries that were pooled to form library 2 were produced and characterized identically, except that in each an additional position was fixed to histidine. (B) SF9 cells infected with the libraries were analyzed and sorted by flow cytometry at day 3 after infection in the presence of 100 μM NiCl_2 using the combination of a phycoerythrin-labeled anti-DR mAb (20LC.11) and an Alexa 647-labeled multivalent fluorescent version of soluble ANi2.3 TCR. The dotted boxes show the gate used to sort DR⁺/TCR⁺-infected cells from which expanded viral stocks were prepared for the next cycle of analysis and/or sorting. (C) Single infected SF9 cells were sorted based on high DR expression and ANi2.3 TCR binding and then used to prepare expanded viral stocks. Each stock was used to prepare infected SF9 cells, which were retested for DR expression and TCR binding and whose viral DNA was used to determine the encoded peptide. The stocks were also used to infect ICAM/B7.1⁺ SF9 cells, which were tested for stimulation of IL-2 production by ANi2.3 T cells in the presence or absence of Ni^{++} . The figure shows the sequences of the best five stimulating mimotopes from each library (red amino acids were selected at the randomized positions) and the number of times that the sequence was found among the total sequences. The mean fluorescence intensity (MFI) of binding with the multivalent ANi2.3 reagent is shown for cells gated to express equally high staining with the anti-DR mAb. Also shown is the response of ANi2.3 T cells to each peptide with or without Ni^{++} . For comparison, TCR binding and IL-2 production are shown for SF9 cells infected with the bulk unsorted or four times-enriched libraries. (D) The figure shows the effect of mutating six positions of the pHIR (HIRCNIIPKRI) mimotope to alanine on the response of the ANi2.3 T cell. The responses to the control Tu peptide and the unmutated (wt) peptide are also shown. Results are the average of three separate experiments with SEM.

We performed surface plasmon resonance binding studies with the ANi2.3 TCR and soluble versions of two of the DR52c-mimotope complexes produced with baculovirus. The TCR was immobilized in the flow cell of a Biacore sensor chip. Various concentrations of the pHIR or WIRVNIPKRI (pWIR)-containing DR52c complexes (Fig. 2 *A* and *B*, respectively) were injected and the binding kinetics were recorded. Both complexes bound the ANi2.3 TCR with modest on rates and very fast off rates, resulting in *k*_{ds} (dissociation rates) in the micromolar range typical of TCR interactions with MHCII ligands.

Surface Exposure of the p7 Lysine ϵ NH₂ Group of the Mimotopes and Potential Ni⁺⁺ Binding Site. The heavy selection of lysine, and to a lesser extent arginine, at p7 in the mimotopes and the loss of activity when this position was mutated to alanine suggested to us that the positive charge of these amino acids might mimic Ni⁺⁺ in the interaction with the ANi2.3 TCR, thus accounting for the lack of their dependence on Ni⁺⁺ for ANi2.3. In TCR-MHCII structures reported thus far, p7 amino acids with short side chains most often point into the wall of the MHCII β -chain helix; however, p7 amino acids with large/long side chains most often assume rotamers that expose at least part of the side chain on the surface (Table S2). In order to see whether the p7K side chain can be surface-exposed well, we solved the structures of the pHIR and pWIR complexes to resolutions of 2.2 and 2.5 Å, respectively (Table S3). The structures of the two mimotopes bound to DR52c were very similar (Fig. 3). Importantly, the side chain of p7K, as well as those of p5I and p8R, was exposed on the surface of the DR52c protein and was, therefore, well-positioned to interact with the ANi2.3 TCR, lending support to the idea that the ϵ NH₂ group of p7K could mimic the Ni⁺⁺ cation.

The analysis of the two structures presented here and a previous structure of DR52c bound to a Tu-derived peptide show that the width of the space between the DR52c α 1 helix and the DR52c β 1 helix (measured as the distance between the α 65 and β 67 α -carbons flanking the p7 position of the peptide) varies from 15.34 to 15.81 Å (average 15.56 Å), putting it among the widest seen in various mouse and human MHCII (Table S4). This wide space between the peptide and the β 1 helix may allow

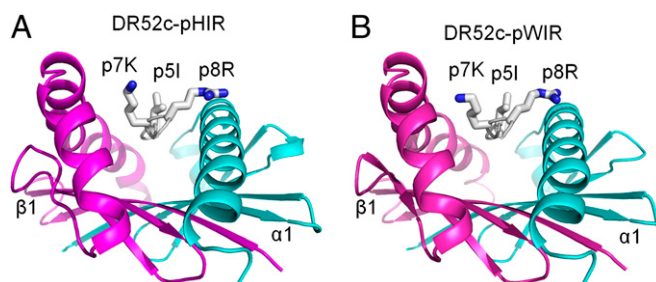


Fig. 3. The side chains of critical mimotope amino acids p5I, p7K, and p8R are surface-exposed on the mimotope-DR52c complexes. The structures of two of the mimotopes in Fig. 1C, (A) pHIR and (B) pWIR, bound to DR52c were solved to resolutions of 2.2 and 2.5 Å, respectively. The peptide binding grooves are viewed from the C terminus of the peptides. Ribbon representations of the α 1 (cyan) and β 1 (magenta) DR52c helices and the peptide backbone (white) are shown with wire-frame representations of surface-exposed side chains of p5I, p7K, and p8R [CPK (Corey, Pauling, Koltun) coloring].

Ni⁺⁺ to bind in this region when DR52c contains the natural peptide that presents Ni⁺⁺.

Extensive Interaction of the ANi2.3 TCR with p7K and Surrounding Area of the DR52c-pHIR Complex. For crystallography, we produced a single-chain version of the ANi2.3 TCR (Fig. S1) containing only the V α and V β domains connected by a glycine-rich linker (scFv) (22). A complex of this TCR with DR52c-pHIR was crystallized and the structure was solved to a resolution of 3.3 Å (Table S3). The TCR engages the MHCII-peptide complex in the usual diagonal mode, with the CDR3 regions of V α and V β centered over the peptide and the V α CDR1 and CDR2 loops poised over the DR52c β 1 helix and the V β CDR1 and CDR2 loops over the α 1 helix (Fig. 4A and B). An overlay of the free versus ANi2.3 TCR-bound DR52c-pHIR shows relatively little adjustment of the MHCII or peptide amino acid side chains during the interaction (Fig. 4C).

A complete analysis of the contacts between ANi2.3 and the DR52c-pHIR complex is shown in Table S5, with a summary shown in Table 1. The TCR V β CDR3 contributes half of the total contacts with the ligand and almost two-thirds of the contacts with the peptide, nearly all of which are with p5I, p7K, and p8R. These results are consistent with those of the mutational analysis of pHIR (Fig. 1D) and the finding that the ANi2.3 TCR heavily selected these three amino acids in the mimotopes whereas tolerated many different amino acids at p1, p2, and p3 (Fig. 1C). The TCR V β CDR3 loop reaches deeply into the peptide binding groove (Fig. 4A) to contact the side chains of all three of these amino acids (Fig. 4B). Most importantly, the carboxylate of V β 95D at the tip of the CDR3 loop makes a salt bridge to the ϵ NH₂ group of p7K, which also forms hydrogen bonds to the peptide backbone at p8 and to Q64 on the DR52c β 1 helix (Fig. 4D). Despite the 3.3-Å resolution of the structure, electron density for these amino acid side chains is clear in the map (Fig. S2).

Similarities Between ANi2.3 TCR Interaction with the Mimotope and Ni⁺⁺. If the binding of ANi2.3 to DR52c-pHIR mimics the way in which ANi2.3 reacts with Ni⁺⁺ presented by DR52c plus the natural unknown peptide, then the TCR amino acids important in the interaction with the two ligands should be similar, especially in the vicinity of the p7 position. To test this idea, we used retroviral transduction (23, 24) to create a series of T-cell transfectomas bearing human CD4 and either the wild-type ANi2.3 TCR or a version with an alanine substitution at one of 29 positions in the V α and V β CDR loops of the TCR. Each transduced T-cell population was sorted in bulk for equally high TCR and CD4 expression, and each was compared with

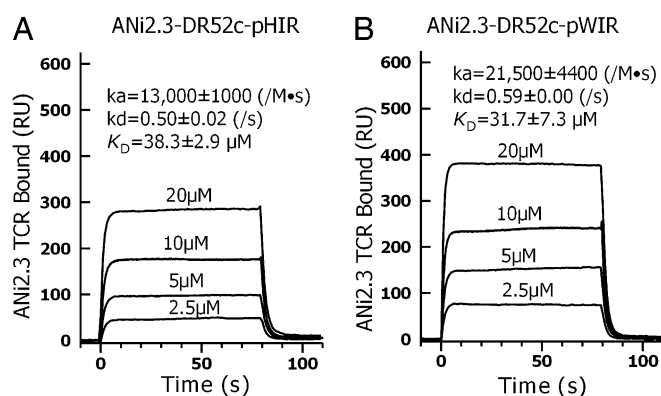


Fig. 2. ANi2.3 TCR binds to mimotope-DR52c complexes with typical TCR kinetics. The results of surface plasmon resonance studies are shown for the binding of soluble (A) DR52c-pHIR and (B) DR52c-pWIR complexes to immobilized ANi2.3 TCR. The binding kinetics were recorded in resonance unit (RU) when four different concentrations of DR52c-mimotope complexes were injected for 80 s through a flow cell of a Biacore CM5 biosensor chip containing immobilized ANi2.3 TCR. Identical injections in a second flow cell with an immobilized control TCR (Yae62) were used to correct for the fluid phase signal. Association rates (*k*_{as}), dissociation rates (*k*_{ds}), and overall (*K*_D) (dissociation constant) with SEM were calculated from the kinetic data using instrument software.

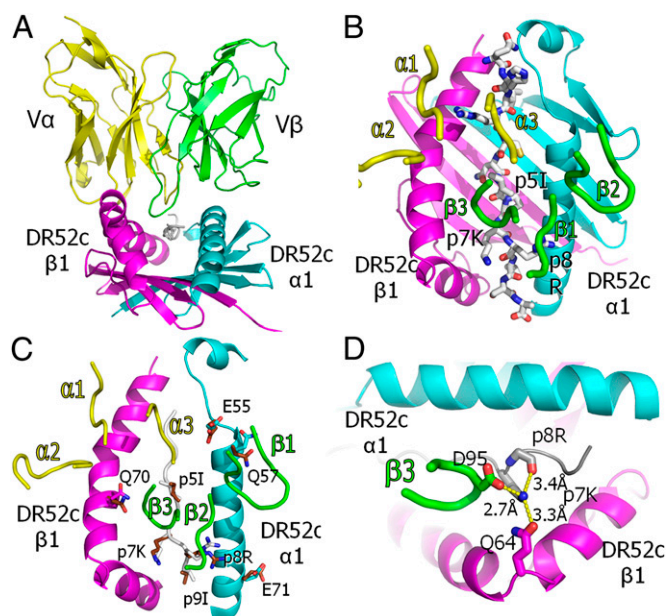


Fig. 4. Conventional orientation of the ANi2.3 TCR on pHIR-DR52c and V β CDR3 coordination of the highly selected p7K. (A) Ribbon representations of the V α (yellow) and V β (green) domains of the ANi2.3 TCR bound to the DR52c-pHIR complex: DR52c α 1, cyan; β 1, magenta; pHIR, white. The view is from the peptide C terminus down the peptide binding groove. (B) Ribbon representations of DR52c α 1 (cyan) and β 1 (magenta) domains, and a wire-frame representation of the pHIR peptide (O, red; N, blue; C, white). The ANi2.3 TCR CDR loops are presented as tubes (V α , yellow; V β , green). (C) DR52c and the ANi2.3 CDR3 loops are shown as in B, and the pHIR backbone is shown as a white tube. Side chains of the eight amino acids of the ligand that change their conformation upon ANi2.3 binding are shown in wire-frame representation (O, red; N, blue; α 1: C, cyan; β 1: C, magenta; peptide: C, white). Superimposed are the same side chains in the unbound DR52c-pHIR structure (O, red; N, blue; C, brown). (D) Top view of salt bridge/H bond coordination of the ϵ NH₂ of pHIR p7K with the carboxylate of ANi2.3 V β CDR3 95D, the carbonyl oxygen of DR52c β 1 Q64, and the backbone oxygen of p8R.

the wild-type T cell for its ability to respond to either the DR52c⁺ human lymphoblastoid B-cell line HO301 in the presence of optimal Ni²⁺ or to the chicken B-cell line DT40 transduced with retrovirus encoding DR52c with the HIR mimotope covalently attached. The results are shown in Fig. 5*A* and schematically mapped onto the pHIR-DR52 surface along with the ANi2.3 TCR footprint in Fig. 5*B*.

The effects of the mutations on the responses to the two ligands were very similar, especially in the area interacting with p7K of pHIR. Many of the mutations that dramatically reduced both responses clustered in this region. Most dramatically, mutation of any of the four amino acids (R94, D95, G96, and Y97) at the tip of V β CDR3 eliminated both responses. In the structure of the complex, these amino acids are not only in extensive contact with the critical pHIR amino acids p5I, p7K, and p8R, as mentioned above, but they also interact with the adjacent DR52c α 1 and β 1 helices (Table 1). In a previous study of another Ni²⁺-reactive TCR whose V α and V β CDRs were nearly identical to those of ANi2.3 and that also had RDGY at the tip of V β CDR3, mutation of the D to A was also shown to eliminate the Ni²⁺ response, but so did the very conservative mutation of D to E (25, 26). Therefore, we also assessed the effect of this V β D95-to-E mutation on the ANi2.3 T cell (Fig. 5*A*). The ANi2.3 response both to the mimotope and to Ni²⁺ was eliminated by this mutation, suggesting that a very precise geometry is required for both responses, supplied by the D but not by the E, rather than the simple presence of a negatively charged amino acid at this position. In the case of Ni²⁺, this would be consistent with the

Table 1. Summary of contacts between ANi2.3 TCR and DR52c-pHIR

CDR3 loop	Amino acid	Atom-to-atom contacts to DR52c-pHIR			
		Total	To DR52c α 1	To pHIR	To DR52c β 1
V α CDR1	A28	3	—	2	1
	T29	32	—	7	25
	P30	1	—	1	—
	Y31	18	—	3	15
V α CDR2	F50	21	—	—	21
	S51	4	—	—	4
V α CDR3	S95	5	—	5	—
	G96	16	—	16	—
	N97	3	—	3	—
	T98	1	1	—	—
V β CDR1	D28	5	—	5	—
V β CDR2	Y46	3	3	—	—
	Q48	7	7	—	—
	I49	9	9	—	—
	N51	1	1	—	—
	D52	14	14	—	—
	Q54	20	20	—	—
	R94	31	18	13	—
V β CDR3	D95	45	—	44	1
	G96	24	—	13	11
	Y97	53	—	5	48
	T98	2	—	2	—
Total		318	73	119	126

participation of this D in the precise coordination of the Ni²⁺ cation in this location.

H27 and D28 of the V β CDR1 loop are two other important amino acids in this cluster, because the mutation of either one affects both responses (Fig. 5*A* and *B*). In the ANi2.3-pHIR structure, D28 interacts with the heavily selected p8R of pHIR (Table 1 and Table S5), but H27 makes no contact with the DR52c or pHIR. Rather, its side chain points up toward the center of the V β CDR1 loop and interacts closely with the V β β -strand leading up to CDR3 and with the J β β -strand leading away from CDR3. Its mutation to A could potentially indirectly affect binding to DR52c-pHIR by altering the V β CDR1 shape or the shape and position of the critical V β CDR3 loop.

No mutation in V α CDR3 had any effect on either response (Fig. 5*A* and *B*), despite considerable contact with p1H and p2R of pHIR in the DR52c-mimotope structure (Table 1 and Table S5). Likewise, mutation of V α CDR1 amino acids Y26 and T29 had no effect on the response of ANi2.3 to the mimotope, despite considerable contact of T29 with both p2R of pHIR and the DR52c β 2 helix (Table 1 and Table S5). In contrast, the mutation of either Y26 or T29 eliminated the response to Ni²⁺, the most striking difference in the effects of the mutations on the two responses.

Taken together, these results support the conclusion that the ANi2.3 TCR recognizes the mimotopes and Ni²⁺ bound to the unknown self-peptide similarly in the conventional diagonal binding mode but that the p7K of the mimotopes is likely a mimic of Ni²⁺, both of which dominate the TCR interaction via V β CDR3.

Discussion

The more than 20 y of published MHC and TCR-MHC structures have taught us a great deal about how peptides bind to classical MHCI and MHCII and how TCRs recognize this complex in the response to foreign peptide antigens presented by self-MHC or to self-peptides bound to foreign MHC alleles. However, we understand little about the nature of the T-cell ligands that form when small chemical moieties, such as plant urushiols or penicillin (27, 28) or metal cations (15), attach to self-peptide-MHC complexes to

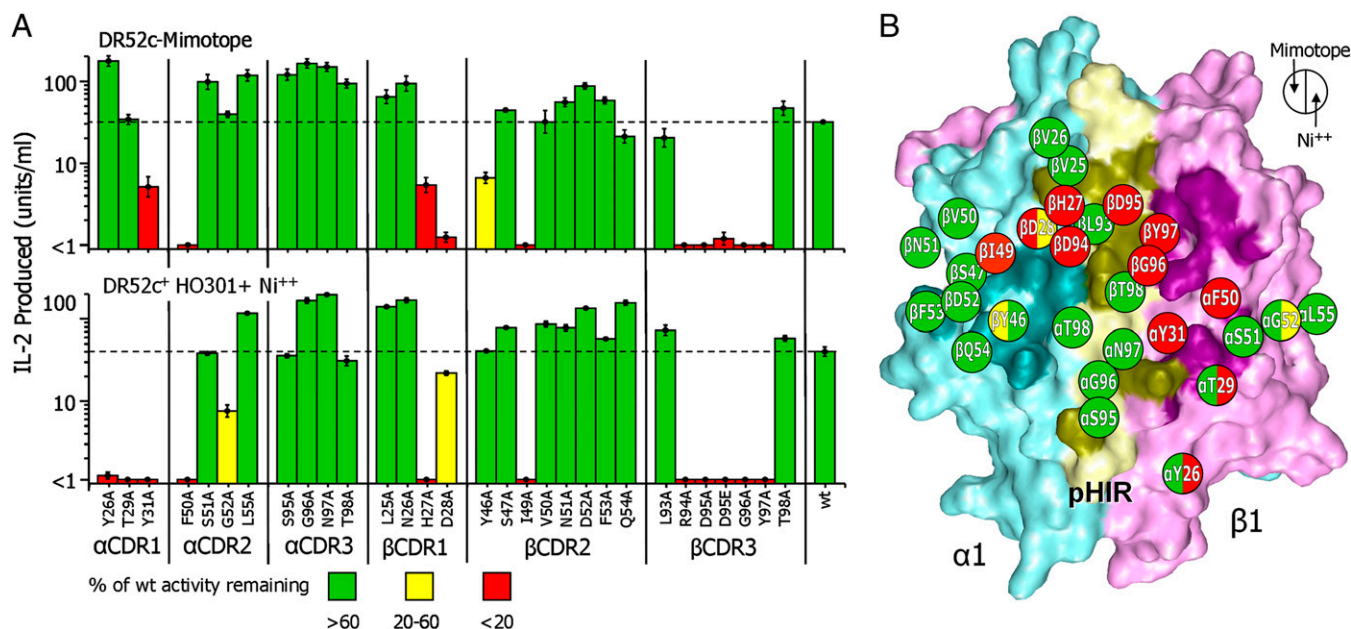


Fig. 5. Similar pattern of ANi2.3 TCR interaction with the pHIR mimotope and Ni⁺⁺. (A) The bar graph shows the sensitivity of the response of ANi2.3 to mutation of 29 amino acids of its six CDR3 loops to alanine or mutation of V β D95 to E. T cells bearing the wild-type or each mutant TCR were tested for response to DT40 chicken cells bearing DR52c covalently attached to the pHIR mimotope (*Upper*) or to HO301 DR52c⁺ lymphoblastoid cells plus 100 μ M Ni⁺⁺ (*Lower*) as measured by IL-2 secretion. The average $\pm$ SEM of three experiments is shown. The bars for responses that were less than 20% of the wild-type response are colored red, yellow (between 20% and 60%), and green (greater than 60%). (B) The solvent-accessible surface of the DR52 α 1 (cyan) and β 1 (magenta) and pHIR (yellow) is shown with the footprint of the ANi2.3 TCR in darker versions of the same colors. The 29 mutated residues of the ANi2.3 TCR are schematically mapped onto the pHIR-DR52 surface with their side-chain positions labeled within a circle. The left half of each circle is colored the same as the bars in A, *Upper* and the right half is colored the same as the bars in A, *Lower*.

form a hapten-like target for T cells. For metal cations, these range from Ni^{++} , mediating the widespread hypersensitivity to nickel-containing jewelry (16), to Be^{++} , which causes the rarer, but much more serious, fulminating lung inflammation seen in chronic beryllium disease (CBD) (29). At present, we do not know the structure of the MHC-peptide-cation complex ligand involved in any of these diseases. The structures we present here offer insight into how Ni^{++} becomes part of such a ligand.

We have found a series of peptide mimotopes that, when bound to DR52c, activate the Ni⁺⁺-reactive ANi2.3 T cell in the absence of Ni⁺⁺. A common feature of these mimotopes was a lysine at the p7 position whose side chain assumed a rotamer that brought its eNH₂ group to the surface of the DR52c-peptide complex to form a salt bridge to an aspartic acid in the ANi2.3 TCR V β CDR3 loop. This TCR aspartic acid was essential for recognition of both the mimotope and the natural Ni⁺⁺ epitope. This and other similarities between the interaction of the mimotope and the natural Ni⁺⁺ ligand with the ANi2.3 TCR have led us to propose that the positively charged lysine occupies the same position in the mimotope complex as the Ni⁺⁺ cation does in the natural complex.

The footprint of the ANi2.3 TCR on the mimotope–DR52c complex was dominated by the V β CDR3 loop, which adopts an extended conformation to reach into the peptide binding groove while contacting the p7 lysine. Mutation of any of the amino acids at the end of this loop resulted in complete loss of the ANi2.3 response to both Ni⁺⁺ and the mimotope peptide. For the mimotope, this interaction was so dominant that the ANi2.3 response survived mutation of a number of other TCR amino acids contacting DR52c and the peptide at p1 and p2. This could explain the variety of amino acids that were tolerated at the p1 and p2 positions among the various mimotopes we found for this TCR. On the other hand, the ANi2.3 response to Ni⁺⁺ was much more sensitive to some mutations in the V α CDR1 loop than was the response to the mimotope. This could indicate an important

role for ANi2.3 contact with C-terminal end of the DR52c β 1 helix and/or particular amino acids at the p1 or p2 position of the natural peptide(s) that presents Ni^{++} . This may have contributed to our failure to find Ni^{++} -dependent mimotopes in our libraries, because the dominant, more frequent lysine-containing mimotopes might have won out over the rarer Ni^{++} -dependent mimotopes in the competition for the soluble TCR reagent used to screen our libraries.

Placing the Ni^{++} cation in a location between the peptide and the arch of the MHCII $\beta 1$ helix in the vicinity of p7 bears a striking resemblance to the situation with Be^{++} presentation to T cells in CBD. Genetic susceptibility to CBD is closely linked to MHCII alleles that bear a glutamic acid at position 69 of the β -chain, especially the HLA-DP2 allele (29, 30). We recently solved the structure of DP2, which revealed an acidic pocket on the surface of the molecule that contained the carboxylates of $\beta 69\text{Glu}$ as well as two other DP2 acidic amino acids (31). Mutation of any of these amino acids eliminated Be^{++} presentation to Be^{++} -reactive human T cells. Similar to the position of p7K of pHIR bound to DR52c, this pocket lay in a wide space between the arch of the $\beta 1$ helix and the side chains of p4 and p7 of the peptide. We have argued that this is the functional site of Be^{++} binding to DP2. Also, an analysis of over three dozen published MHCII-peptide structures (31) showed that despite the enormous conservation of structure among the many MHCII isotypes and alleles from human and mouse, the space between the peptide and the $\beta 1$ helix in this region can vary by more than 3 Å. It is tempting to suggest that this flexibility, combined with particular MHCII polymorphisms in the region and particular amino acids at the peptide p4 and p7 positions, can create the ideal site for cation binding in a variety of metal-mediated immune diseases.

In summary, our results show that the ANi2.3 TCR uses a conventional docking mode on the DR52c-pHIR complex and docks very similarly on DR52c containing the natural Ni⁺⁺-self-

peptide ligand. The data also strongly suggest that the ϵNH_2 group of the mimotope p7K mimics the Ni^{++} in the natural ligand. The focused interaction of its V β CDR3 loop with pHIR-p7K or Ni^{++} in the natural ligand modulates the importance of these MHC contacts in the overall affinity of the TCR for the two ligands.

Materials and Methods

Baculovirus DR52c–Peptide Libraries. The baculovirus libraries described in Fig. 1 were produced by direct cloning of PCR fragments into baculovirus DNA (19, 20) as described in *SI Materials and Methods*.

Protein Expression and Purification. DR52c (extracellular domains) with pHIR or pWIR covalently attached were cloned into a single baculovirus as previously described (21). V regions of the ANi2.3 TCR were fused to mouse C regions and expressed in baculoviruses as previously described (15, 32, 33). For crystallography, the V α and V β portions of the ANi2.3 TCR were fused by

GS linker (V α -linker-V β) and were expressed in the periplasmic space of the Rosetta strain of *Escherichia coli* (22). Details are described in *SI Materials and Methods*.

Other Materials and Methods. Other materials and methods are described in *SI Materials and Methods*.

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Supporting Information

Yin et al. 10.1073/pnas.1215928109

SI Materials and Methods

Baculovirus DR52c-Peptide Libraries. The baculovirus libraries described in Fig. 1 were produced by direct cloning of PCR fragments into baculovirus DNA (1, 2). The baculovirus DNA was digested with homing nucleases *SceI* and *CeuI*, leaving 3' overhanging ends. The PCR fragment encoding the randomized peptide library was digested with homing nucleases *BstXI* and purified. The mixture of digested baculovirus DNA and the digested PCR fragment was ligated with a high concentration of T4 ligase (10^5 units/mL) for 1 h at room temperature. The ligation mixture was heated, inactivated, and directly used for transfecting SF9 insect cells using the standard calcium phosphate method. The transfected SF9 insect cells were then sorted by Alexa Fluor647-labeled ANi2.3 T-cell receptor (TCR) multimers 3 d later.

T Cells and T-Cell Assays. The activation of ANi2.3 T-cell transfectoma (3, 4) or its mutational variants was assayed by IL-2 secretion as previously described (5). Mutant variants of ANi2.3 were made by retroviral transduction of the TCR⁺ T-cell hybridoma 5KC-9c6 (6, 7). Three types of antigen-presenting cells were used: (i) ICAM/B7.1⁺ SF9 insect cells (1) infected with baculovirus encoding a surface-expressed version of DR52c bearing a linked DR52c binding peptide (2); (ii) DR52c⁺ HO301 lymphoblastoid B cells (8); and (iii) DT40 chicken leukemia B cells (9) transduced with retroviruses encoding DR52c linked to the pHIR mimotope.

TCR Mutational Analysis. Wild-type and the indicated alanine-substitution mutants of ANi2.3 V α and V β were cloned in murine stem cell virus (MSCV)-based retroviral plasmids with an internal ribosomal entry site plus green fluorescent protein as a reporter. TCR chains were expressed in TCR-deficient human CD4-transduced hybridoma cell line 5KC-9c6 (6) by retroviral transduction as described previously (10, 11). Cells expressing equivalent levels of TCR and CD4 were isolated by flow cytometry cell sorting. Ten thousand cells of each mutant transfectoma were used for the T-cell assays.

Protein Expression and Purification. DNA encoding DR52c (extracellular domains) and DR52c β -chain covalently attached to pHIR or pWIR was cloned into a single baculovirus as previously described (12). The soluble DR52c-pHIR and DR52c-pWIR complexes in the supernatants of virus-infected HighFive insect cells were purified by immunoaffinity chromatography using the anti-DR mAb LB-3.1, followed by size-exclusion chromatography using Superdex 200. For Biacore surface plasmon resonance experiments, V regions of the ANi2.3 TCR were fused to mouse C regions and expressed in baculoviruses as previously described (13–15). For crystallography, the V α and V β portions of the ANi2.3 TCR were fused by GS linker (V α -linker-V β) in a modified (Fig. S1) version of the Mopac16 vector (16). The soluble single-chain ANi2.3 TCR was expressed in the periplasmic space of the Rosetta strain of *Escherichia coli* and purified by an Ni-NTA affinity column and a Superdex 200 gel-filtration column.

Crystal Production and Data Collection. DR52c-pHIR and DR52c-pWIR were crystallized by mixing 0.5 μ L of each protein solution at a concentration of 15 mg/mL with an equal volume of reservoir solution. DR52c-pHIR was crystallized in 16% PEG 4000, 100 mM Tris-HCl (pH 8.0) with space group C2. DR52c-pWIR was crystallized in 17% PEG 4000, 100 mM HEPES (pH 7.5), 10% isopropanol with space group P2₁. An equimolar mixture of ANi2.3 TCR and DR52c-pHIR was crystallized by mixing 0.5 μ L of complex solution at a concentration of 15 mg/mL with an equal volume of reservoir solution. The complex was crystallized in 12% PEG 3350, 100 mM ammonium tartrate (pH 7.0) in space group P6₃.

Data Collection. X-ray diffraction data were collected at the Advanced Light Source on beamline 8.2.2 under liquid-nitrogen cryoconditions at 100 K. All crystals were flash-cooled in liquid nitrogen after a flash-soak in a cryoprotection solution consisting of the reservoir solution with an additional concentration of glycerol (18–25%). The data were indexed, integrated, scaled, and merged using HKL2000 (17).

Structure Determination. The structures of DR52c-pHIR and DR52c-pWIR were determined by molecular replacement using the CCP4 program AMoRe (18) with the DR52c-pTu structure [Protein Data Bank (PDB) ID code 3C5J] without a bound peptide as the search model. In the initial $F_o - F_c$ maps, we could see a clear positive density within the peptide binding groove of DR52c. Peptides were modeled into this positive density. Models were manually adjusted using the program O (19).

The structures of the ANi2.3-DR52c-pHIR complex were determined by molecular replacement using Phaser (20) with the V α of HA1.7 TCR (PDB ID code 1FYT), V β of JM22 TCR (PDB ID code 1OGA), and DR52c-pTu (PDB ID code 3C5J) as search models, respectively. After an initial round of rigid-body refinement, the models were inspected and manually fitted with the program Coot (<http://lmb.bioch.ox.ac.uk/coot>). The models were then subjected to several rounds of alternating simulated annealing/positional refinement in PHENIX (21) followed by B-factor refinement in PHENIX. Model building was performed using the program Coot from the CCP4 suite. Simulated annealing omit maps were routinely used to remove the model bias. All models have good stereochemistry, as determined by the program PROCHECK (22).

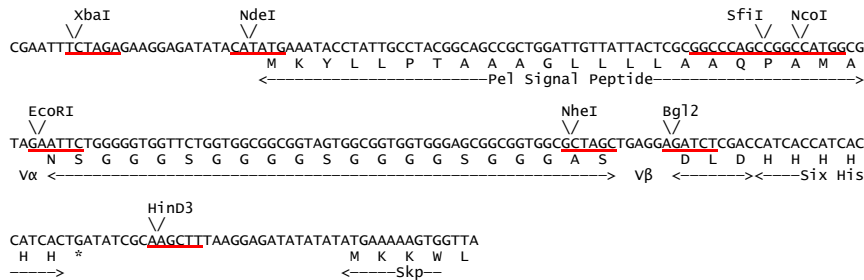
Structure Analysis. Buried molecular surface areas were calculated using GRASP (23). NCONT in CCP4 (18) was used to analyze the contacts between the TCRs and their ligands. Atoms within 4.5 Å of each other were considered part of the interface. Contacts involving potential electron donors and acceptors (O or N) within 3.5 Å were considered potential hydrogen bonds or salt bridges. Other contacts were considered van der Waals contacts. Molecular superimpositions and figures were created with PyMOL (Schrödinger) and Swiss-PDB Viewer (24).

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Modified portion of Mopac54:



ANi2.3 scFV in modified Mopac16

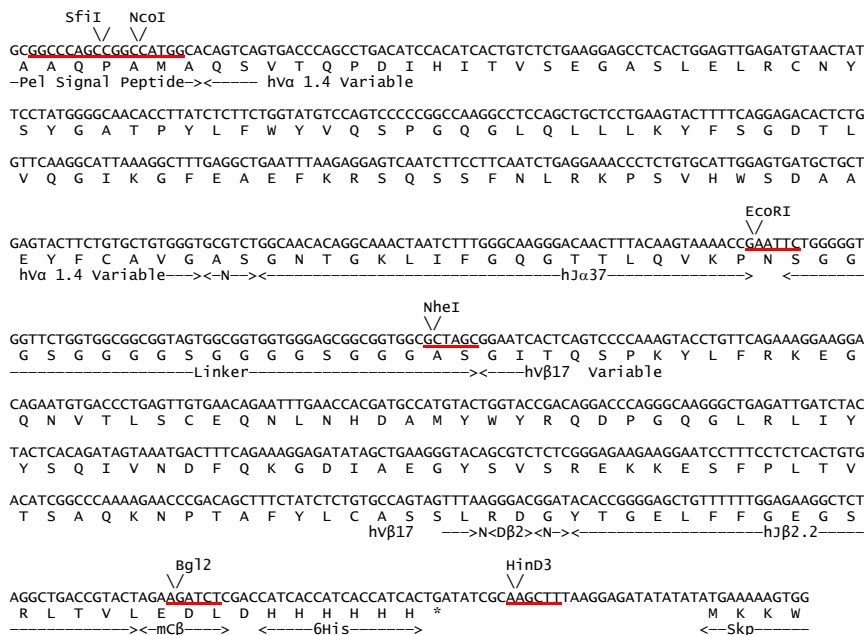


Fig. S1. Modification of pMopac54 and cloning of the ANi2.3 TCR as a single-chain Fv (scFV). The original Mopac54 plasmid was modified to introduce cloning sites for TCR Vα (NcoI and EcoRI) before and Vβ (NheI and BglII) after the GS linker. Also, the sequence encoding human Cκ and residual *E. coli* phage sequence (~500 bp) was removed between the scFV and Skp gene, the original 12-His tag was reduced to a 6-His tag, and an out-of-frame ATG in the Pel signal peptide was removed by changing codon 3 from TGC to TAC. DNA fragments encoding the Vα and Vβ domains of ANi2.3 flanked by the appropriate restrictions sites were synthesized by PCR and cloned into the modified vector.

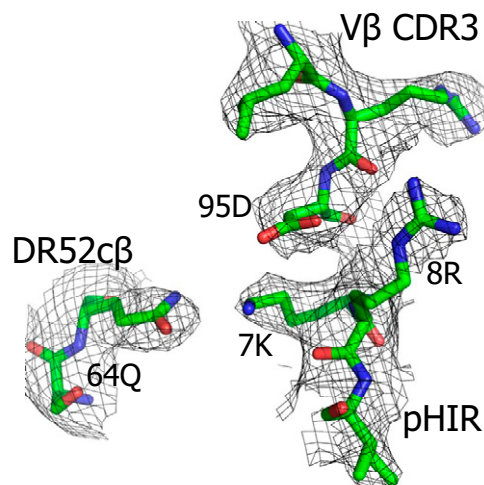


Fig. S2. Electron density at the interface among DR52c, TCR Vβ CDR3, and pHIR. A section of the ANi2.3 TCR Vβ CDR3 loop, the alpha helix of DR52c β1 domain, and the C-terminal end of the pHIR mimotope are shown with electron density based on the final model (2Fo-Fc, 1.5σ). The side chains of the critical TCR βCDR3β D95, DR52c β1 Q64, and pHIR 7K and 8R are labeled.

Other Supporting Information Files

- [Table S1 \(DOC\)](#)
- [Table S2 \(DOC\)](#)
- [Table S3 \(DOC\)](#)
- [Table S4 \(DOC\)](#)
- [Table S5 \(DOC\)](#)

Table S1. Ni⁺⁺ independent mimotopes are still present in Library #1 after 3 cycles of depletion with the ANi2.3 TCR.

Lib. No.	Sequence Freq	Mimotope Sequence -1123456789	ANi2.3 Binding (MFI)		IL-2 Produced (Units/ml)	
			w /Ni ⁺⁺	w/o Ni ⁺⁺	w/ Ni ⁺⁺	w/o Ni ⁺⁺
1	2	MINLNLPKYI	62.5	59.5	6030	5950
	7	VISINLPKFI	28.8	25.6	4240	4160
	2	AIALNLPKRI	46.1	38.5	3400	3290
	1	DIALNLPRI	18.7	15.9	376	385
	Bulk	Negative Sort	3.0	3.0	<2	<2
	Bulk	Positive Sort	5.2	5.2	2890	3900

Baculovirus DR52c-peptide library #1 was depleted of ANi2.3 TCR binding peptides for 3 cycles in the absence of Ni⁺⁺. The depleted library was then enriched for 3 additional cycles for ANi2.3 binding peptides in the presence of 3mM Ni⁺⁺. Single ANi2.3 TCR binding infected insect cells were sorted and characterized as described in the main paper.

Table S2. Orientation and contacts with TCR of the side chain of the p7 residue of the MHC II bound peptide

MHCII	Peptide	TCR	PDB ID code	P7	P7 side chain orientation	No. of TCR contacts with P7 side chain
IE ^k	MCC88-104	2B4	3QIB	A	horizontal	5
IE ^k	MCC88-104	226	3QIU	A	horizontal	4
IE ^k	MCC88-104p5E	226	3QIW	A	horizontal	4
DR1	TPI	E8	2IAN	A	horizontal	2
IA ^b	p3K	B3K506	3C5Z	N	horizontal	9
IA ^b	P3K	2W20	3C6L	N	horizontal	1
IA ^b	p3K	YAE62	3C60	N	horizontal	0
IA ^b	P3K	J809.B5	3RDT	N	horizontal	0
DR1	HA	HA1.7	1FYT	L	horizontal	1
DR2	MBP85-99	OB.1A12	1YMM	I	horizontal	0
DR2	ENGA	OB.1A12	2WBJ	L	horizontal	0
DR4	HA	HA1.7	1J8H	L	horizontal	2
DQ1	MBP85-99	Hy.1B11	3PL6	I	horizontal	2
IA ^k	ConAlb	D10	1D9K	W	up	5
IA ^{g7}	HEL	21.3	3MBE	Y	up	6
IA ^u	MBP1-11	172.1	1U3H	R	up	7
IA ^u	MBP1-11	1934	2Pxy	R	up	5
IA ^u	MBP1-11	CI19	2Z31	R	up	6
DR4	MBP114-126	MS2-3C8	3O6F	R	up	25
DR52c	pHIR	Ani2.3	4H1L	K	up	17

The CCP4 Program Ncont was used to analyze the atom-to-atom contacts between the TCR and the side chain of the peptide p7 amino acid in the structures contained in the indicated PDB files. Total contacts included potential H-bond and salt bridges (≤ 3.5 Å) and van der Waals contacts (≤ 4.5 Å).

Table S3. Diffraction data collection and refinement statistics

	DR52c-pWIR 4H26	DR52c-pHIR 4H25	ANi2.3-DR52c-pHIR 4H1L
Space group	P21	C2	P63
<u>Data Collection</u>			
Cell dimensions a, b, c (Å)	38.82, 129.20, 97.47	154.52, 97.18, 67.10	186.72, 186.72, 166.70
Resolution (Å)	50-2.5(2.59-2.50) ^a	50-2.2(2.28-2.20)	20-3.3 (3.42-3.30)
Rmerge (%)	12.1(44.5)	8.2(28.1)	14.5 (55.2)
I/s (I)	12.4(2.0)	20.9(3.3)	6.3 (2.0)
Completeness (%)	93.3(69.3)	93.6(72.0)	91.9 (60.2)
Redundancy	3.5(2.3)	6.4(3.8)	4.6 (3.0)
<u>Refinement</u>			
Resolution (Å)	50-2.5(2.66-2.50)	50-2.2(2.34-2.20)	20-3.28 (3.36-3.28)
No. reflections	30552(3657)	43194(5713)	44267 (1703)
Complexes per A.U.	1	1	2
Rwork/Rfree(%)	22.5/27.0(32.9/36.5)	20.1/24.6(27.1/32.4)	26.3/28.5(44.1/43.1)
No. of atoms			
Protein	6248	6351	9630
Water	174	375	0
R.m.s deviations			
Bond lengths (Å)	0.007	0.006	0.013
Bond angles (°)	1.3	1.278	1.502
Ramachandran plot			
favored region (%)	87.9	89.1	89.8
allowed region (%)	11.9	10.9	9.9
outlier region (%)	0.2	0	0.3

^aData for outer shell shown in parentheses

Table S4. Width of the peptide binding groove for published MHCII-peptide complexes.

MHCII	Peptide	PDB ID code	Groove width α 65-CA to β 67/69-CA (Å)
IA ^b	P3K	1LNU	13.63
	CLIP	1MUJ	13.53
IA ^k	HEL	1IAK	15.19
	ConAlb	1D9K	14.30
IA ^{g7}	HEL	1F3J	13.66
	GAD	1ES0	14.29
IA ^d	OVA	1IAO	12.95
	HA	2IAD	13.12
IA ^u	MBP	1K2D	14.58
IE ^k	MCC	1KT2	15.27
	HB	1IEA	15.01
	Hsp70	1IEB	15.37
	PCC	1KTD	15.17
DR1	HA	1DLH	15.83
	pA2	1AQD	15.11
	HIV	1SJE	14.87
DR2	MBP	1BX2	15.63
	ENGA	2WBJ	15.63
DR3	CLIP	1A6A	14.79
DR4	HA	1J8H	15.98
	COL	2SEB	14.07
DR51	EBV	1H15	18.23
	MBP	1FV1	16.16
DR52a	IB3	2Q6W	15.49
DR52c	TU	3C5J	15.34
DQ2	GLI	1S9V	15.95
DQ6	HCRT	1UVQ	13.68
DQ8	INS	1JK8	15.09
DP2	DRA	3LQZ	16.15
Average			14.97
Maximum			18.23
Minimum			12.95
DR52c	pHIR		15.54
DR52c	pWIR		15.81
DR52c(w/ ANi TCR)	pHIR		15.95

Table S5. Full contact list between ANi2.3 TCR and pHIR-DR52c

Numbers of Atom-to-atom contacts between ANi2.3 TCR and pHIR-DR52c											
TCR V	TCR aa	TCR atom	MHC-P aa	MHC-P atom	Dist (Å)	H-bond salt brid.	VDW C to C	VDW other	MHCα	PEP	MHCβ
Va	1GLN	OE1	p-1HIS	CE1	3.95			1		1	
Va	28ALA	O	p2ARG	NH1	3.82			1		1	
Va	28ALA	O	p2ARG	NH2	4.5			1		1	
Va	28ALA	O	B81HIS	ND1	4.5			1			1
Va	29THR	CA	B77ASN	OD1	4.47			1			1
Va	29THR	CB	B77ASN	CG	3.91		1				1
Va	29THR	CB	B77ASN	N	4.37			1			1
Va	29THR	CB	B77ASN	CA	4.11		1				1
Va	29THR	CB	B77ASN	OD1	3.36			1			1
Va	29THR	OG1	B77ASN	CB	3.73			1			1
Va	29THR	OG1	B77ASN	CG	2.76			1			1
Va	29THR	OG1	B77ASN	ND2	3.41	1					1
Va	29THR	OG1	B76ASP	CB	4.41			1			1
Va	29THR	OG1	B76ASP	C	4.41			1			1
Va	29THR	OG1	B77ASN	N	3.8			1			1
Va	29THR	OG1	B77ASN	CA	3.66			1			1
Va	29THR	OG1	B77ASN	OD1	2.05	1					1
Va	29THR	CG2	p2ARG	CZ	3.98		1			1	
Va	29THR	CG2	p2ARG	NH1	3.09			1		1	
Va	29THR	CG2	B77ASN	CB	4.11		1				1
Va	29THR	CG2	B77ASN	CG	3.97		1				1
Va	29THR	CG2	p2ARG	NH2	4.13			1		1	
Va	29THR	CG2	B76ASP	C	4.45		1				1
Va	29THR	CG2	B76ASP	O	4.31			1			1
Va	29THR	CG2	B77ASN	N	4.13			1			1
Va	29THR	CG2	B77ASN	CA	3.48		1				1
Va	29THR	CG2	B77ASN	OD1	3.81			1			1
Va	29THR	CG2	B77ASN	C	4.34		1				1
Va	29THR	CG2	B77ASN	O	4.28			1			1
Va	29THR	C	p2ARG	NH2	4.11			1		1	
Va	29THR	O	p2ARG	CZ	4.04			1		1	
Va	29THR	O	p2ARG	NH1	4.03			1		1	
Va	29THR	O	B77ASN	CG	4.12			1			1
Va	29THR	O	B77ASN	ND2	3.97			1			1
Va	29THR	O	p2ARG	NH2	3.31	1				1	
Va	29THR	O	B77ASN	OD1	4.26			1			1
Va	30PRO	CA	p2ARG	NH2	4.14			1		1	
Va	31TYR	CD1	B77ASN	ND2	4.28			1			1
Va	31TYR	CD1	p2ARG	NH2	4.1			1		1	

Numbers of Atom-to-atom contacts between ANi2.3 TCR and pHIR-DR52c

TCR V	TCR aa	TCR atom	MHC-P aa	MHC-P atom	Dist (Å)	H-bond salt brid.	VDW C to C	VDW other	MHCα	PEP	MHCβ
Va	31TYR	CE1	p2ARG	CZ	4.44		1			1	
Va	31TYR	CE1	B77ASN	CG	4.35		1				1
Va	31TYR	CE1	B77ASN	ND2	3.37			1			1
Va	31TYR	CE1	p2ARG	NH2	3.84			1		1	
Va	31TYR	CZ	B77ASN	CG	4.26		1				1
Va	31TYR	CZ	B77ASN	ND2	3.13			1			1
Va	31TYR	CZ	B70GLN	OE1	4.12			1			1
Va	31TYR	OH	B74GLN	OE1	4.06			1			1
Va	31TYR	OH	B77ASN	CB	3.76			1			1
Va	31TYR	OH	B77ASN	CG	3.83			1			1
Va	31TYR	OH	B77ASN	ND2	2.95	1					1
Va	31TYR	OH	B70GLN	OE1	4.09			1			1
Va	31TYR	CE2	B77ASN	ND2	3.88			1			1
Va	31TYR	CE2	B70GLN	CD	4.42		1				1
Va	31TYR	CE2	B70GLN	OE1	3.19			1			1
Va	31TYR	CD2	B70GLN	OE1	4			1			1
Va	50PHE	CB	B70GLN	OE1	4.12			1			1
Va	50PHE	CG	B70GLN	CG	4.13		1				1
Va	50PHE	CG	B70GLN	OE1	4.35			1			1
Va	50PHE	CE1	B69GLU	CB	4.33		1				1
Va	50PHE	CE1	B69GLU	OE2	3.75			1			1
Va	50PHE	CZ	B70GLN	CG	4.41		1				1
Va	50PHE	CZ	B69GLU	CB	4.01		1				1
Va	50PHE	CZ	B69GLU	CD	4.29		1				1
Va	50PHE	CZ	B69GLU	OE2	3.2			1			1
Va	50PHE	CE2	B70GLN	CB	4.4		1				1
Va	50PHE	CE2	B70GLN	CG	3.36		1				1
Va	50PHE	CE2	B70GLN	CD	4.34		1				1
Va	50PHE	CE2	B69GLU	OE2	4.27			1			1
Va	50PHE	CD2	B70GLN	CB	4.39		1				1
Va	50PHE	CD2	B70GLN	CG	3.17		1				1
Va	50PHE	CD2	B70GLN	CD	3.8		1				1
Va	50PHE	CD2	B70GLN	OE1	3.72			1			1
Va	50PHE	C	B77ASN	ND2	4.42			1			1
Va	50PHE	O	B77ASN	CG	4.47			1			1
Va	50PHE	O	B77ASN	ND2	3.81			1			1
Va	50PHE	O	B77ASN	OD1	4.23			1			1
Va	51SER	CB	B77ASN	OD1	3.99			1			1
Va	51SER	CB	B73GLY	CA	4.49		1				1
Va	51SER	OG	B77ASN	OD1	4.06			1			1
Va	51SER	OG	B73GLY	CA	4.17			1			1
Va	95SER	CB	p-1HIS	ND1	4.45			1		1	

Numbers of Atom-to-atom contacts between ANi2.3 TCR and pHIR-DR52c

TCR V	TCR aa	TCR atom	MHC-P aa	MHC-P atom	Dist (Å)	H-bond salt brid.	VDW C to C	VDW other	MHCα	PEP	MHCβ
Va	95SER	OG	p-1HIS	ND1	4.16			1		1	
Va	95SER	OG	p-1HIS	CE1	3.94			1		1	
Va	95SER	OG	p-1HIS	NE2	4.33			1		1	
Va	95SER	O	p-1HIS	CG	4.45			1		1	
Va	96GLY	N	p2ARG	CG	4.32			1		1	
Va	96GLY	N	p2ARG	CD	4.34			1		1	
Va	96GLY	N	p2ARG	NE	3.74			1		1	
Va	96GLY	N	p2ARG	CZ	4.34			1		1	
Va	96GLY	N	p2ARG	NH2	4.43			1		1	
Va	96GLY	CA	p-1HIS	CB	4.43		1			1	
Va	96GLY	CA	p2ARG	CB	4.4		1			1	
Va	96GLY	CA	p2ARG	CG	3.15		1			1	
Va	96GLY	CA	p2ARG	CD	3.58		1			1	
Va	96GLY	CA	p2ARG	NE	3.36			1		1	
Va	96GLY	CA	p2ARG	CZ	4.34		1			1	
Va	96GLY	C	p-1HIS	CB	4.45		1			1	
Va	96GLY	C	p2ARG	CG	3.78		1			1	
Va	96GLY	O	p-1HIS	CB	3.71			1		1	
Va	96GLY	O	p-1HIS	CG	4.14			1		1	
Va	96GLY	O	p-1HIS	CD2	4.47			1		1	
Va	97ASN	N	p2ARG	CG	3.93			1		1	
Va	97ASN	CB	p2ARG	CG	4.26		1			1	
Va	97ASN	CB	p2ARG	NE	3.9			1		1	
Va	98THR	OG1	A55GLU	OE2	4.32			1	1		
Vb	28ASP	CG	p8ARG	NH2	3.52			1		1	
Vb	28ASP	OD1	p8ARG	NH1	4.46			1		1	
Vb	28ASP	OD1	p8ARG	NH2	2.92	1				1	
Vb	28ASP	OD1	p8ARG	CZ	4.07			1		1	
Vb	28ASP	OD2	p8ARG	NH2	3.46	1				1	
Vb	46TYR	CZ	A55GLU	OE2	4.37			1	1		
Vb	46TYR	OH	A55GLU	CD	4.34			1	1		
Vb	46TYR	OH	A55GLU	OE2	3.16	1			1		
Vb	48GLN	CD	A57GLN	CB	4.2		1		1		
Vb	48GLN	OE1	A58GLY	N	4.05			1	1		
Vb	48GLN	OE1	A58GLY	CA	3.97			1	1		
Vb	48GLN	NE2	A55GLU	CG	4.05			1	1		
Vb	48GLN	NE2	A57GLN	CB	3.67			1	1		
Vb	48GLN	NE2	A55GLU	CD	4.22			1	1		
Vb	48GLN	NE2	A55GLU	OE2	3.83			1	1		
Vb	49ILE	CG1	A60LEU	CB	4.49		1		1		
Vb	49ILE	CG1	A61ALA	N	4.43			1	1		

Numbers of Atom-to-atom contacts between ANi2.3 TCR and pHIR-DR52c

TCR V	TCR aa	TCR atom	MHC-P aa	MHC-P atom	Dist (Å)	H-bond salt brid.	VDW C to C	VDW other	MHCα	PEP	MHCβ
Vb	49ILE	CD1	A60LEU	CG	4.08		1		1		
Vb	49ILE	CD1	A60LEU	CD1	3.59		1		1		
Vb	49ILE	CD1	A60LEU	CA	4.42		1		1		
Vb	49ILE	CD1	A60LEU	CB	3.3		1		1		
Vb	49ILE	CD1	A60LEU	C	4.3		1		1		
Vb	49ILE	CD1	A61ALA	N	4.02			1	1		
Vb	49ILE	CG2	A61ALA	CA	4.41		1		1		
Vb	51ASN	O	A39LYS	NZ	4.4			1	1		
Vb	52ASP	CB	A39LYS	NZ	4.26			1	1		
Vb	52ASP	CG	A39LYS	CD	3.99		1		1		
Vb	52ASP	CG	A39LYS	CE	4.32		1		1		
Vb	52ASP	CG	A39LYS	NZ	4.01			1	1		
Vb	52ASP	CG	A57GLN	NE2	3.98			1	1		
Vb	52ASP	CG	A57GLN	CG	4.08		1		1		
Vb	52ASP	OD1	A57GLN	NE2	3.05	1			1		
Vb	52ASP	OD1	A57GLN	CD	3.5			1	1		
Vb	52ASP	OD1	A57GLN	CB	4.39			1	1		
Vb	52ASP	OD1	A57GLN	CG	2.99			1	1		
Vb	52ASP	OD2	A39LYS	CD	3.28			1	1		
Vb	52ASP	OD2	A39LYS	CE	3.63			1	1		
Vb	52ASP	OD2	A39LYS	NZ	3.11	1			1		
Vb	52ASP	OD2	A57GLN	NE2	4.17			1	1		
Vb	54GLN	CD	A57GLN	NE2	4.17			1	1		
Vb	54GLN	CD	A57GLN	CD	3.63		1		1		
Vb	54GLN	CD	A57GLN	OE1	3.75			1	1		
Vb	54GLN	CD	A57GLN	CB	4.21		1		1		
Vb	54GLN	CD	A57GLN	CG	3.68		1		1		
Vb	54GLN	CD	A55GLU	OE2	4.21			1	1		
Vb	54GLN	OE1	A57GLN	NE2	4.25			1	1		
Vb	54GLN	OE1	A57GLN	CD	3.5			1	1		
Vb	54GLN	OE1	A57GLN	OE1	3.79			1	1		
Vb	54GLN	OE1	A57GLN	CB	3.38			1	1		
Vb	54GLN	OE1	A57GLN	CG	3.05			1	1		
Vb	54GLN	OE1	A55GLU	OE2	4.45			1	1		
Vb	54GLN	NE2	A57GLN	NE2	3.95			1	1		
Vb	54GLN	NE2	A57GLN	CD	3.35			1	1		
Vb	54GLN	NE2	A57GLN	OE1	3.07	1			1		
Vb	54GLN	NE2	A57GLN	CB	4.32			1	1		
Vb	54GLN	NE2	A57GLN	CG	3.87			1	1		
Vb	54GLN	NE2	A55GLU	CD	3.63			1	1		
Vb	54GLN	NE2	A55GLU	OE1	3.47	1			1		

Numbers of Atom-to-atom contacts between ANi2.3 TCR and pHIR-DR52c

TCR V	TCR aa	TCR atom	MHC-P aa	MHC-P atom	Dist (Å)	H-bond salt brid.	VDW C to C	VDW other	MHCα	PEP	MHCβ
Vb	54GLN	NE2	A55GLU	OE2	3.51			1	1		
Vb	94ARG	CB	p5ILE	CG2	3.82		1			1	
Vb	94ARG	CD	A61ALA	CB	3.61		1		1		
Vb	94ARG	CD	A61ALA	O	4.44			1	1		
Vb	94ARG	CD	p5ILE	CG2	4.41		1			1	
Vb	94ARG	NE	A61ALA	CB	3.96			1	1		
Vb	94ARG	NE	p5ILE	CG2	4.04			1		1	
Vb	94ARG	CZ	A58GLY	O	4.33			1	1		
Vb	94ARG	CZ	A61ALA	CB	3.96		1		1		
Vb	94ARG	CZ	p5ILE	CG2	3.5		1			1	
Vb	94ARG	CZ	A62ASN	OD1	3.56			1	1		
Vb	94ARG	CZ	p5ILE	CG1	4.04		1			1	
Vb	94ARG	CZ	p5ILE	CB	4.38		1			1	
Vb	94ARG	NH1	A58GLY	O	4.29			1	1		
Vb	94ARG	NH1	p5ILE	CG2	3.9			1		1	
Vb	94ARG	NH1	A62ASN	OD1	3.66			1	1		
Vb	94ARG	NH1	p5ILE	CG1	3.61			1		1	
Vb	94ARG	NH1	p5ILE	CB	4.35			1		1	
Vb	94ARG	NH2	A58GLY	O	3.88			1	1		
Vb	94ARG	NH2	A61ALA	CA	4.25			1	1		
Vb	94ARG	NH2	A62ASN	N	3.76			1	1		
Vb	94ARG	NH2	A61ALA	CB	3.57			1	1		
Vb	94ARG	NH2	A61ALA	C	3.68			1	1		
Vb	94ARG	NH2	A61ALA	O	3.77			1	1		
Vb	94ARG	NH2	A62ASN	CA	4.02			1	1		
Vb	94ARG	NH2	p5ILE	CG2	3.23			1		1	
Vb	94ARG	NH2	A62ASN	CB	4.38			1	1		
Vb	94ARG	NH2	A62ASN	CG	3.72			1	1		
Vb	94ARG	NH2	A62ASN	OD1	2.62	1			1		
Vb	94ARG	NH2	p5ILE	CG1	4.37			1		1	
Vb	94ARG	NH2	p5ILE	CB	4.3			1		1	
Vb	94ARG	O	p8ARG	NH2	4.25			1		1	
Vb	95ASP	CA	p8ARG	N	4.24			1		1	
Vb	95ASP	CA	p7LYS	CD	4.29		1			1	
Vb	95ASP	CA	p6PRO	O	4.12			1		1	
Vb	95ASP	CA	p7LYS	CA	4.1		1			1	
Vb	95ASP	CB	p8ARG	N	4.31			1		1	
Vb	95ASP	CB	p7LYS	CD	3.49		1			1	
Vb	95ASP	CB	p7LYS	CE	4.36		1			1	
Vb	95ASP	CB	p7LYS	NZ	4.46			1		1	
Vb	95ASP	CB	B67LEU	CD2	4.4		1				1
Vb	95ASP	CB	p7LYS	CA	4.43		1			1	

Numbers of Atom-to-atom contacts between ANi2.3 TCR and pHIR-DR52c

TCR V	TCR aa	TCR atom	MHC-P aa	MHC-P atom	Dist (Å)	H-bond salt brid.	VDW C to C	VDW other	MHCα	PEP	MHCβ
Vb	95ASP	CG	p8ARG	N	3.93			1		1	
Vb	95ASP	CG	p8ARG	CB	4.06		1			1	
Vb	95ASP	CG	p7LYS	CD	3.47		1			1	
Vb	95ASP	CG	p7LYS	CE	4.11		1			1	
Vb	95ASP	CG	p7LYS	NZ	3.8			1		1	
Vb	95ASP	OD1	p8ARG	N	3.86			1		1	
Vb	95ASP	OD1	p8ARG	CA	4.32			1		1	
Vb	95ASP	OD1	p8ARG	CB	3.52			1		1	
Vb	95ASP	OD1	p8ARG	NE	4.28			1		1	
Vb	95ASP	OD1	p7LYS	CD	4.3			1		1	
Vb	95ASP	OD2	p8ARG	N	4.26			1		1	
Vb	95ASP	OD2	p8ARG	CB	4.19			1		1	
Vb	95ASP	OD2	p8ARG	O	4.44			1		1	
Vb	95ASP	OD2	p7LYS	CD	3.19			1		1	
Vb	95ASP	OD2	p7LYS	CE	3.36			1		1	
Vb	95ASP	OD2	p7LYS	NZ	2.73	1				1	
Vb	95ASP	C	p5ILE	CG2	4.47		1			1	
Vb	95ASP	C	p5ILE	O	4.36			1		1	
Vb	95ASP	C	p6PRO	C	4.18		1			1	
Vb	95ASP	C	p6PRO	O	3.73			1		1	
Vb	95ASP	C	p7LYS	N	4.17			1		1	
Vb	95ASP	C	p7LYS	CA	3.72		1			1	
Vb	95ASP	O	p5ILE	CG2	3.57			1		1	
Vb	95ASP	O	p5ILE	CA	4.38			1		1	
Vb	95ASP	O	p6PRO	N	4.1			1		1	
Vb	95ASP	O	p7LYS	CB	4.42			1		1	
Vb	95ASP	O	p7LYS	C	4.44			1		1	
Vb	95ASP	O	p5ILE	CB	3.67			1		1	
Vb	95ASP	O	p5ILE	C	3.66			1		1	
Vb	95ASP	O	p5ILE	O	3.24	1				1	
Vb	95ASP	O	p6PRO	CA	4.22			1		1	
Vb	95ASP	O	p6PRO	C	3.2			1		1	
Vb	95ASP	O	p6PRO	O	2.78	1				1	
Vb	95ASP	O	p7LYS	N	3.45	1				1	
Vb	95ASP	O	p7LYS	CA	3.42			1		1	
Vb	96GLY	N	B67LEU	CD2	3.72			1			1
Vb	96GLY	N	p7LYS	CA	4.41			1		1	
Vb	96GLY	CA	B67LEU	CD2	3.98		1				1
Vb	96GLY	CA	B71LYS	CE	3.51		1				1
Vb	96GLY	CA	B71LYS	NZ	3.68			1			1
Vb	96GLY	CA	p5ILE	O	4.18			1		1	
Vb	96GLY	CA	B67LEU	CD1	4.11		1				1

Numbers of Atom-to-atom contacts between ANi2.3 TCR and pHIR-DR52c

TCR V	TCR aa	TCR atom	MHC-P aa	MHC-P atom	Dist (Å)	H-bond salt brid.	VDW C to C	VDW other	MHCα	PEP	MHCβ
Vb	96GLY	C	B70GLN	NE2	3.79			1			1
Vb	96GLY	C	B71LYS	CE	4.14		1				1
Vb	96GLY	C	B71LYS	NZ	3.8			1			1
Vb	96GLY	C	p5ILE	CB	4.26		1			1	
Vb	96GLY	C	p5ILE	O	4.36			1		1	
Vb	96GLY	C	p5ILE	CD1	4.27		1			1	
Vb	96GLY	O	p5ILE	CG2	4.08			1		1	
Vb	96GLY	O	B70GLN	NE2	4.35			1			1
Vb	96GLY	O	p5ILE	N	4.46			1		1	
Vb	96GLY	O	p5ILE	CA	4.22			1		1	
Vb	96GLY	O	p5ILE	CG1	3.44			1		1	
Vb	96GLY	O	B71LYS	CE	4.05			1			1
Vb	96GLY	O	B71LYS	NZ	3.29	1					1
Vb	96GLY	O	p5ILE	CB	3.21			1		1	
Vb	96GLY	O	p5ILE	C	4.28			1		1	
Vb	96GLY	O	p5ILE	O	3.64			1		1	
Vb	96GLY	O	p5ILE	CD1	3.08			1		1	
Vb	97TYR	N	B70GLN	NE2	2.91	1					1
Vb	97TYR	N	B70GLN	CD	4.21			1			1
Vb	97TYR	CA	B70GLN	NE2	2.81			1			1
Vb	97TYR	CA	B70GLN	CD	3.98		1				1
Vb	97TYR	CA	B70GLN	OE1	4.41			1			1
Vb	97TYR	CB	B70GLN	NE2	2.65			1			1
Vb	97TYR	CB	B70GLN	CD	3.66		1				1
Vb	97TYR	CB	B70GLN	OE1	4.18			1			1
Vb	97TYR	CG	B70GLN	NE2	2.75			1			1
Vb	97TYR	CG	B70GLN	CG	4.31		1				1
Vb	97TYR	CG	B70GLN	CD	3.76		1				1
Vb	97TYR	CD1	B70GLN	NE2	2.34			1			1
Vb	97TYR	CD1	B70GLN	CB	3.82		1				1
Vb	97TYR	CD1	B70GLN	CG	3.58		1				1
Vb	97TYR	CD1	B70GLN	CD	3.34		1				1
Vb	97TYR	CD1	B70GLN	OE1	4.45			1			1
Vb	97TYR	CE1	B67LEU	CD2	4.29		1				1
Vb	97TYR	CE1	B67LEU	N	3.65			1			1
Vb	97TYR	CE1	B70GLN	NE2	3.45			1			1
Vb	97TYR	CE1	B66ASP	C	3.88		1				1
Vb	97TYR	CE1	B67LEU	CA	3.49		1				1
Vb	97TYR	CE1	B67LEU	CB	4.42		1				1
Vb	97TYR	CE1	B66ASP	O	3.92			1			1
Vb	97TYR	CE1	B67LEU	C	4.49		1				1
Vb	97TYR	CE1	B70GLN	CB	3.98		1				1

Numbers of Atom-to-atom contacts between ANi2.3 TCR and pHIR-DR52c

TCR V	TCR aa	TCR atom	MHC-P aa	MHC-P atom	Dist (Å)	H-bond salt brid.	VDW C to C	VDW other	MHCα	PEP	MHCβ
Vb	97TYR	CE1	B70GLN	CG	3.96		1				1
Vb	97TYR	CE1	B70GLN	CD	4.2		1				1
Vb	97TYR	CZ	B66ASP	OD2	3.51			1			1
Vb	97TYR	CZ	B67LEU	N	3.77			1			1
Vb	97TYR	CZ	B66ASP	CG	4.1		1				1
Vb	97TYR	CZ	B66ASP	CB	3.77		1				1
Vb	97TYR	CZ	B66ASP	C	3.95		1				1
Vb	97TYR	CZ	B67LEU	CA	4.06		1				1
Vb	97TYR	CZ	B66ASP	O	4.3			1			1
Vb	97TYR	OH	B66ASP	OD2	2.22	1					1
Vb	97TYR	OH	B67LEU	N	2.99	1					1
Vb	97TYR	OH	B66ASP	CG	2.8			1			1
Vb	97TYR	OH	B66ASP	OD1	4			1			1
Vb	97TYR	OH	B66ASP	N	4.49			1			1
Vb	97TYR	OH	B66ASP	CA	3.57			1			1
Vb	97TYR	OH	B66ASP	CB	2.73			1			1
Vb	97TYR	OH	B66ASP	C	3.27			1			1
Vb	97TYR	OH	B67LEU	CA	3.65			1			1
Vb	97TYR	OH	B67LEU	CB	4.27			1			1
Vb	97TYR	OH	B66ASP	O	3.98			1			1
Vb	97TYR	CE2	B66ASP	OD2	4.09			1			1
Vb	97TYR	CD2	B70GLN	NE2	4.05			1			1
Vb	97TYR	C	B70GLN	NE2	4.33			1			1
Vb	97TYR	C	p5ILE	CG1	4.48		1			1	
Vb	97TYR	O	p5ILE	CG2	4.1			1		1	
Vb	97TYR	O	p5ILE	CG1	4.14			1		1	
Vb	97TYR	O	p5ILE	CB	4.15			1		1	
Vb	97TYR	O	p5ILE	CD1	4.48			1		1	
Vb	98THR	OG1	p5ILE	CG1	4.17			1		1	
Vb	98THR	OG1	p5ILE	CD1	3.71			1		1	
Total						20	91	208	73	120	126

The CCP4 Program Ncont was used to analyze the atom-to-atom contacts between the ANi2.3 TCR and pHIR-DR52c. Potential H-bond or salt bridge contacts were assigned when acceptors and donors were within 3.5 Å without regard to geometry. Van der Waals contacts were defined between atoms within 4.5Å or each other.