

# Components of the Ligand for a Ni<sup>2+</sup> Reactive Human T Cell Clone

Linh Lu,<sup>1</sup> Jörg Vollmer,<sup>2</sup> Corinne Moulon,<sup>2</sup> Hans Ulrich Weltzien,<sup>2</sup> Philippa Marrack,<sup>1,3</sup> and John Kappler<sup>1,4</sup>

<sup>1</sup>Howard Hughes Medical Institute, Integrated Department of Immunology, National Jewish Medical and Research Center, Denver, CO 80206

<sup>2</sup>Max-Planck-Institute für Immunbiologie, Stübeweg 51, 79108 Freiburg, Germany

<sup>3</sup>Department of Biochemistry and Molecular Genetics, and <sup>4</sup>Department of Pharmacology and Program in Biomolecular Structure, University of Colorado Health Science Center, Denver, CO 80262

## Abstract

The major histocompatibility complex (MHC) restriction element for a human Ni<sup>2+</sup> reactive T cell, ANi-2.3, was identified as DR52c. A series of experiments established that the functional ligand for this T cell was a preformed complex of Ni<sup>2+</sup> bound to the combination of DR52c and a specific peptide that was generated in human and mouse B cells, but not in fibroblasts nor other antigen processing-deficient cells. In addition, ANi-2.3 recognition of this complex was dependent on His81 of the MHC  $\beta$  chain, suggesting a role for this amino acid in Ni<sup>2+</sup> binding to MHC. We propose a general model for Ni<sup>2+</sup> recognition in which  $\beta$ His81 and two amino acids from the NH<sub>2</sub>-terminal part of the MHC bound peptide coordinate Ni<sup>2+</sup> which then interacts with some portion of the V $\alpha$  CDR1 or CDR2 region.

Key words: hypersensitivity • T cell receptor • antigen presentation • hapten • nickel

## Introduction

Although much of what we know about  $\alpha\beta$ TCR recognition comes from the study of peptide antigens,  $\alpha\beta$ TCRs can recognize ligands that contain moieties other than, or in addition to, peptides. For example, the class Ib MHC molecule CD1 presents lipids and glycolipids to T cells (1). Haptens are another group of chemicals that have been shown to be capable of forming part of the  $\alpha\beta$ TCR receptor ligand. Small organic haptens such as dinitrophenol (DNP), trinitrophenol (TNP), and fluorescein (FL) have long been used experimentally to study T cell responses and hypersensitivity. Also, metals, such as nickel, gold, or beryllium, form another group of chemicals for which T cell hypersensitivity has been demonstrated. Contact sensitivity to nickel in jewelry is quite common (2). Patients undergoing colloidal gold therapy can develop hypersensitivity (3). Sensitivity to beryllium is a serious problem in industries that handle this element (4).

In the case of CD1, the MHC binding groove is very hydrophobic and appears to bind the lipid portion of the antigen, presenting the most hydrophilic portion of the antigen on the MHC surface (5). Studies with TNP have suggested that a hapten-modified MHC bound peptide is most often the relevant antigen (6, 7). However, there are a few cases in which haptens appear to dominate the interaction with the  $\alpha\beta$ TCR, such that the interaction can be measured even in the absence of peptide and MHC (8, 9).

In the case of hypersensitivity to metals, it is most likely that  $\alpha\beta$ TCRs recognize metal ions complexed with the MHC/peptide surface in a manner analogous to the organic haptens, but to date there is no formal demonstration of this complex. In the present study, we have defined the ligand for the  $\alpha\beta$ TCR of a Ni<sup>2+</sup> reactive T cell clone (ANi-2.3) isolated from a patient with Ni<sup>2+</sup> hypersensitivity (10, 11). We show that recognition by this  $\alpha\beta$ TCR requires the combination Ni<sup>2+</sup>, a particular MHC molecule, DR52c (DRA\*0101, DRB3\*0301), and an unknown specific MHC bound peptide produced in B cells, but not fibroblasts nor other nonprofessional APC. These results are consistent with the recognition of Ni<sup>2+</sup> bound to the MHC surface by amino acid side chains from the MHC and/or the MHC bound peptide.

Jörg Vollmer's present address is Coley Pharmaceutical GmbH, Elisabeth-Selbert-Str. 9, D-40764 Langenfeld, Germany.

Corinne Moulon's present address is Dictagene, Chemin de la Vuillette 4, 1000 Lausanne 25, Switzerland.

Address correspondence to John Kappler, Howard Hughes Medical Institute, National Jewish Medical and Research Center, 1400 Jackson St., Denver, CO 80206. Phone: 303-398-1322; Fax: 303-398-1396; E-mail: kapplerj@njc.org

## Materials and Methods

*Oligonucleotides, Peptides, and Superantigen.* Oligonucleotides used in DNA constructions, mutagenesis and sequencing were produced in the Molecular Resources Center at National Jewish Medical and Research Center. The following peptides were also produced at this facility: pTT, amino acids 830–840 of tetanus toxin (12); pTu, amino acids 342–359 of the elongation factor Tu; pDRA, amino acids 110–128 of HLA DR  $\alpha$  chain; p $\lambda$ , amino acids 11–29 of human Ig lambda chain (13). The superantigen, staphylococcal enterotoxin B (SEB),\* was purchased from Sigma-Aldrich.

*Cell Lines.* Production and characterization of the Ni<sup>2+</sup> reactive T cell transfectoma bearing the  $\alpha\beta$ TCR of ANi2.3, has been described previously (14). The T cell transfectoma, AL8.1, was a gift from Dr. U. Blank (Pasteur Institute, Paris, France; reference 12). It is specific for pTT presented by an allele of DR13 (DRB1\*1302, DRA). The EBV transformed B cell line, HO301, was a gift from Dr. J. Hansen (University of Washington, Seattle, WA). The MHC class II alleles expressed in HO301 are DRA\*01012, DRB1\*1302, DRB3\*0301, DQA\*101021, DQB1\*0604, DPA1\*01, and DPB1\*1601 (15). The mouse B cell lymphoma line, M12.C3, was obtained from Dr. Laurie Glimcher (Harvard University, Cambridge, MA). It is a variant of M12 that fails to express MHC class II due to mutations in both the IA<sup>d</sup> and IE<sup>d</sup>  $\beta$  chain genes (16). The mouse fibroblast cell line DAP was a gift from Dr. J. Bill (University of Colorado Health Sciences Center, Denver, CO). The human cell line T2 is a B cell/T cell hybrid (17) that carries a large genomic deletion (18) including the structural genes for all MHC class II molecules and for DM. The MHC class II<sup>-</sup> mouse mastocytoma line, P815 and the DR1<sup>+</sup> (DRB1\*0101, DRA) EBV-transformed human B cell line, LG2, were obtained from the American Type Culture Collection.

*Monoclonal Antibodies.* B cell hybridoma, FK7.3.19.1, which produced a mAb specific for DR52c (19), was a gift from Dr. F. Koning (Leiden University Medical Center, Netherlands). L243 (anti-DRB1) and L227 (anti-DR $\alpha$ ) B cell hybridomas were obtained from the American Type Culture Collection.

*Cell Fixation.* EBV B cells were fixed with paraformaldehyde.  $2 \times 10^6$  cells were incubated with 1 ml of 0.25% paraformaldehyde at 37°C for 40 min and then washed thoroughly with balanced salt solution (BSS).

*IL-2 Production.* IL-2 production by stimulated transfectoma T cells was assayed as previously described (20, 21) using the IL-2-dependent T cell line, HT-2. Briefly, cultures were prepared containing  $10^5$  transfectoma T cells and  $10^5$  antigen-presenting cells. Unless otherwise stated, stimuli were either nothing, 100  $\mu$ M Ni<sup>2+</sup> (as NiCl<sub>2</sub>), or 50  $\mu$ g/ml SEB.

*Preparation of Soluble DR52c.* DR52c was isolated from lysates of HO301 by an adaptation of published methods (22, 23). Briefly, 1–5  $\times 10^9$  HO301 cells were harvested and washed three times with cold BSS. Cells were suspended in lysis buffer (20 mM octanoyl-N-methylglucamide (MEGA-8), 20 mM nonanoyl-N-methylglucamide (MEGA-9), 1 mM PMSF, 50 mM iodoacetamide, 10  $\mu$ g/ml leupeptin, 0.2 mg/ml EDTA, and 0.7  $\mu$ g/ml pepstatin A in PBS, pH 7.4) and incubated on ice for 30 min. The lysate was centrifuged at 100,000 *g* for 1 h at 4°C and passed through a prewashed FK7.3.19.1 coupled Sepharose column. Class II molecules were eluted with pH 11.4, 50 mM 3-[cyclohexylamino]-1-propanesulfonic acid, 150 mM NaCl, 20 mM

MEGA-8, and 20 mM MEGA-9. The eluate was collected into siliconized glass tubes and neutralized with 2 M Tris (pH 6.8). All reagents were purchased from Sigma-Aldrich. To remove the transmembrane domain from natural DR52c, 8 vol of 1.5 mg/ml DR52c were incubated with 3 vol of 0.1 mM dithiothreitol, 0.1 mM EDTA, 1 mM Tris, and 0.1 mg/ml papain solution for 1 h at 37°C. The reaction was stopped with 1 vol of 20 mM iodoacetamide and 100 mM Tris solution, pH 8, incubated on ice for 30 min. This was stored in PBS.

*Extraction of MHC Bound Peptides.* DR52c molecules in 10 mM Tris buffer, pH 7.5, were incubated 2 $\times$  with 2.5 M acetic acid for 30 min at 37°C. This solution was passed twice through Centricon C-10 filters. The pass-through was collected and lyophilized to dryness. The residue was redissolved in water and lyophilized to dryness three more times.

*Vectors, Constructs, and Transduction of Cell Lines.* The genes for the  $\alpha$  and  $\beta$  chains of DR52c were transduced into various cells using an MSCV retroviral system in which green fluorescent protein (GFP) or thy-1.1 served as surrogate markers (24, 25). Bacteria stock carrying the plasmid pBEX WT46 BIII that encoded the DRB3-0301  $\beta$  chain of DR52c, was a gift from Dr. J. Gorski (Milwaukee Blood Center, Milwaukee, WI). cDNA encoding the full length DR52c  $\beta$  chain was cloned into MSCV-GFP between the BglII and NotI restriction sites of the polylinker. cDNA encoding the full length DR $\alpha$  chain gene was cloned into MSCV-thy1.1 between the EcoRI and NotI restriction sites of the polylinker. The plasmids were transfected into a retroviral packaging cell line as described (25). 4 ml of the resultant viral stock was then used to transduce  $5 \times 10^5$  target cells using a spinfection protocol. Transductants were then cloned at limiting dilution. A variant of the DR52c  $\beta$  chain/MSCV-GFP construct was made in which the PCR was used to change the codon for His (CAC) to that of Gln (CAG) at the position encoding amino acid 81 of the  $\beta$  chain.

## Results

*DR $\beta$ 3-0301 Is the Restriction Element for ANi-2.3.* The ANi-2.3 T cell clone was originally isolated from a patient with nickel hypersensitivity (11). The clone and a T cell hybridoma transfectant (14) expressing an  $\alpha\beta$ TCR containing the ANi-2.3 V $\alpha$  and V $\beta$  linked to mouse C $\alpha$  and C $\beta$  respond to autologous antigen-presenting cells pulsed with Ni<sup>2+</sup>. Based on the reactivity of the clone to Ni<sup>2+</sup> presented by a series of APCs of different HLA genotypes and the inhibition of its reactivity with a specific anti-DR $\alpha$  mAb, the restriction element of this clone was thought to be DR13 (DRB1\*1302, DRA\*0101; references 11 and 14). However, in preliminary experiments in which we transfected the DRB1\*1302  $\beta$  chain gene into a number of cells types that contained the DR $\alpha$  gene, we were unable to transfer Ni<sup>2+</sup> presenting ability (data not shown). Therefore, we considered that some other class II MHC molecule in this patient was the Ni<sup>2+</sup> presenting element. As DRB1\*1302 is in very tight linkage disequilibrium with the DR52c  $\beta$  chain gene (26, 27), we turned our attention to this molecule.

Two types of experiments convincingly demonstrated that DR52c is in fact the MHC restriction element for

\*Abbreviations used in this paper: BSS, balanced salt solution; GFP, green fluorescent protein; SEB, staphylococcal enterotoxin B.

$\text{Ni}^{2+}$  presentation to ANi-2.3. In the first, we used the EBV transformed cell line, HO301, which is homozygous for both DRB1\*1302 and DR52c, as an APC for  $\text{Ni}^{2+}$  presentation. Fig. 1 A shows the expression of DR13 and DR52c on HO301 using the  $\beta$  specific mAbs L227 (anti-DRB1) and FK-7.3 (anti-DR52c). Both  $\beta$  chains are well expressed as is the common DR $\alpha$  chain detected with the mAb, L243. Fig. 1 B shows the reactivity of ANi-2.3 to  $\text{Ni}^{2+}$  presented by HO301. As a control we used another T cell transfectoma, AL8.1, which is specific for a tetanus peptide presented by DRB1\*1302 (12). ANi-2.3 responded to  $\text{Ni}^{2+}$  presented by HO301 and AL8.1 responded to the tetanus peptide. The response of ANi-2.3 to  $\text{Ni}^{2+}$  was nearly completely blocked by the DR52c and DR $\alpha$  specific mAbs, but not the DRB1 specific mAb. As expected, the AL8.1 response to the tetanus peptide was inhibited by the DRB1 and DR $\alpha$  specific mAbs, but not by the DR52c specific mAb. These results strongly implicated DR52c as the  $\text{Ni}^{2+}$  presenting MHC restriction element for ANi-2.3.

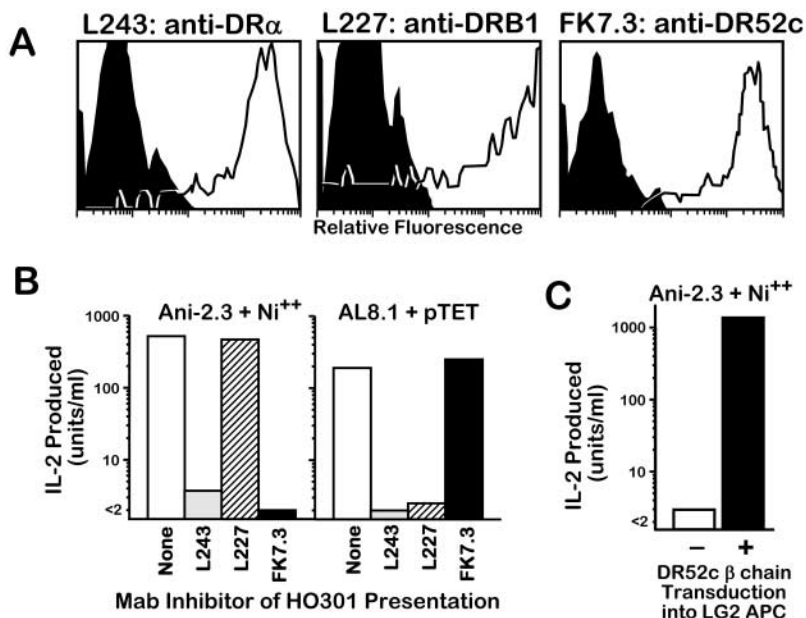
In a second experiment, we used a retroviral vector to transduce the gene for the DR52c  $\beta$  chain into the EBV transformed cell line, LG2. LG2 is homozygous for DRB1\*0101 and does not express DR52c. We then compared LG2 to its DR52c transductant for the ability to present  $\text{Ni}^{2+}$  to ANi-2.3 (Fig. 1 C). The nontransduced LG2 cells did not present  $\text{Ni}^{2+}$  to ANi-2.3, but the transductant presented  $\text{Ni}^{2+}$  very well. Taken together these results confirmed that DR52c was the required MHC restriction element for  $\text{Ni}^{2+}$  presentation to ANi-2.3.

*$\text{Ni}^{2+}$  Presentation Occurs via a Preformed Peptide/MHC Complex.* Previous experiments had shown that fixed APCs could present  $\text{Ni}^{2+}$  to ANi-2.3 cells (10), indicating that antigen processing was not required and suggesting that the  $\text{Ni}^{2+}$  was presented by a preformed MHC/peptide complex. We performed several experiments to confirm

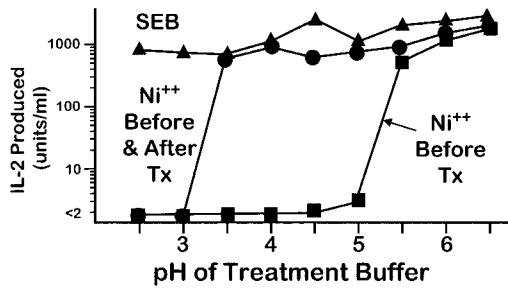
this suggestion. In the first experiment, we showed that  $\text{Ni}^{2+}$  interaction with fixed APC was reversible under conditions consistent with a protein/ $\text{Ni}^{2+}$  complex. Fixed HO301 cells were preincubated with  $\text{Ni}^{2+}$ , extensively washed and then exposed to various pHs in an attempt to remove any bound  $\text{Ni}^{2+}$ . The treated cells were tested for stimulation of ANi-2.3 cells with or without adding back  $\text{Ni}^{2+}$  to the IL-2 production culture. The results are shown in Fig. 2.  $\text{Ni}^{2+}$  pulsed, fixed HO301 cells exposed to neutral pH presented  $\text{Ni}^{2+}$  to ANi-2.3 cells equally well, whether or not additional  $\text{Ni}^{2+}$  was added to the culture, showing that the preincubation with  $\text{Ni}^{2+}$  stably saturated the presenting ability of the fixed cells. As the pH of the treatment was lowered below pH 5.5, there was a precipitous drop of presenting ability that could be restored by adding  $\text{Ni}^{2+}$  back to the culture medium. Treatment below pH3.5 resulted in irreversible loss of  $\text{Ni}^{2+}$  presenting ability.

These results suggest that  $\text{Ni}^{2+}$  is reversibly bound to the fixed APC surface via a pH sensitive (pH 3.5 to 5.0) interaction consistent with coordination by amino acid side chains from histidine (pK  $\sim$ 6) and/or aspartic/glutamic acids (pK  $\sim$ 3.5). These amino acids and cysteine are those most commonly found coordinating transition metal ions in proteins. The irreversible loss of  $\text{Ni}^{2+}$  presenting ability at pH 3 and below suggested loss of DR52c integrity, perhaps by loss of peptide from its binding groove, a possibility we explored further as described below in the following section.

We then tested directly whether or not the MHC/peptide complex for  $\text{Ni}^{2+}$  presentation preexisted on the surface of HO301. In tissue culture wells, we immobilized DR52c that had been immunoaffinity purified from lysates of HO301. This immobilized MHCII was able to present  $\text{Ni}^{2+}$  to ANi-2.3 (Fig. 3). This result indicated that  $\text{Ni}^{2+}$ , DR52c, and a bound peptide were sufficient for engaging the  $\alpha\beta$ TCR of ANi-2.3.



**Figure 1.** Identification of DR52c as the  $\text{Ni}^{2+}$  presenting element for ANi-2.3. (A) Fluorescent staining with mAb's was used to assess the surface expression of DR13 and DR52c on the surface of HO301 cells. Three mAbs were used: L243, specific for the common DR $\alpha$  chain; L227, specific for all DRB1  $\beta$  chains (in this case DRB1\*1302); and FK7.3, specific for DR52c  $\beta$  chain. HO301 cells were incubated with biotinylated versions of the antibodies, which were then detected with phycoerythrin streptavidin (unfilled histograms). The negative controls (filled histograms) were HO301 cells incubated with only phycoerythrin streptavidin. (B) The same set of anti-DR mAb's was used in an attempt to inhibit IL-2 production by either ANi-2.3 stimulated by  $\text{Ni}^{2+}$  or AL-8.1 stimulated by pTT presented by HO301. The antibodies (10  $\mu\text{g}/\text{ml}$ ) were added at the initiation of the IL-2 production cultures. (C) The DRB1\*0101 homozygous EBV transformed B cell line, LG2, was tested before and after transduction with the gene for DR52c  $\beta$  chain for its ability to present  $\text{Ni}^{2+}$  to ANi-2.3.

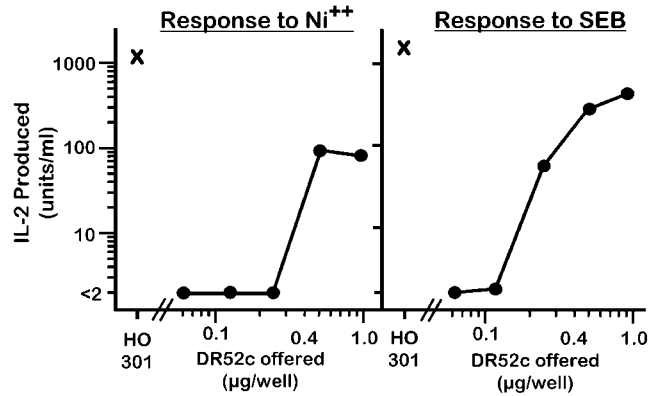


**Figure 2.** pH-dependent, reversible binding of  $\text{Ni}^{2+}$  to fixed HO301 cells. HO301 cells were fixed with paraformaldehyde and then incubated with  $250 \mu\text{M}$   $\text{Ni}^{2+}$  overnight at  $37^\circ\text{C}$ . The cells were washed to remove unbound  $\text{Ni}^{2+}$  and then incubated at various pHs for 40 min at  $37^\circ\text{C}$ . The cells were then centrifuged and washed three times with BSS. The treated cells were used as antigen presenting cells for ANi-2.3, alone (squares), plus  $100 \mu\text{M}$   $\text{Ni}^{2+}$  (circles), or plus  $50 \text{ ng/ml}$  SEB (triangles).

*Ni<sup>2+</sup> Presentation to ANi-2.3 Requires a Specific B Cell-derived Peptide.* While our results thus far established that a preformed DR52c/peptide complex was required for  $\text{Ni}^{2+}$  presentation to ANi-2.3, they did not tell us the function of the peptide in the recognition. It was possible that this peptide functioned only to stabilize the DR52c molecule and that its specific sequence was not important for  $\text{Ni}^{2+}$  presentation. Several experiments have led us to conclude that this is not the case and that a particular bound peptide is required for DR52c presentation of  $\text{Ni}^{2+}$  to ANi-2.3.

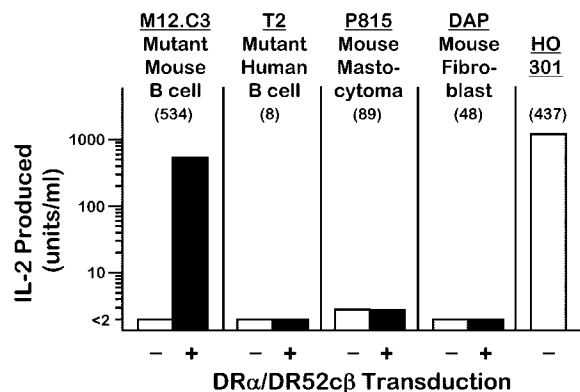
First, in transduction experiments, we expressed the DR52c molecule on the surface of a number of different mouse and human cell types, by cotransducing the DR52c and the DR $\alpha$  gene. The transductants were compared for their ability to present  $\text{Ni}^{2+}$  to ANi-2.3. The results are shown in Fig. 4. As was the case with the human EBV B cell line, LG2, good  $\text{Ni}^{2+}$  presentation was seen with the transduced mouse  $\text{IA}^d_{\beta^-}/\text{IE}^d_{\beta^-}$  BALB/c B lymphoma variant cell line, M12.C3 (16). However, three transduced cell types failed to present  $\text{Ni}^{2+}$ : P815, a MHCII<sup>-</sup>/H2-DM<sup>-</sup> mouse mastocytoma cell line; T2, a human mutant B/T cell hybrid line that lacks the genes for MHCII and HLA-DM; and DAP, a mouse fibroblast line. While these latter three transductants expressed less surface DR52c than did the M12.C3 transductant, the results suggest that the correct  $\text{Ni}^{2+}$  presenting DR52c/peptide complex is generated only in a professional, functional antigen-presenting cell and may only occur in B cells. Two possibilities are that this peptide is derived from a B cell-specific protein or is generated from a ubiquitous protein but for loading into DR52c requires a fully functioning CII vesicle compartment that is lacking in P815, T2, and DAP.

To test this idea of a specific peptide further, we extracted and purified bulk peptides from DR52c immunopurified from H0301 lysates. We preincubated these peptides with two types of APCs that expressed DR52c, but failed to present  $\text{Ni}^{2+}$  to ANi-2.3: DR52c transduced DAP fibroblasts and fixed, pH 3 treated H0301 cells. The peptide preincubated cells were then tested for  $\text{Ni}^{2+}$  presentation to ANi-2.3 (Fig. 5). As above, neither the trans-



**Figure 3.** Natural DR52c purified from HO301 cells can present  $\text{Ni}^{2+}$ . Various amounts of papain treated DR52c, immunopurified from HO301 cells were immobilized by absorption to plastic tissue culture wells. The immobilized MHCII was then used to present either  $\text{Ni}^{2+}$  ( $100 \mu\text{M}$ , left panel) or SEB ( $50 \text{ ng/ml}$ , right panel) to ANi-2.3. As a control, HO301 cells (X) were used for antigen presentation.

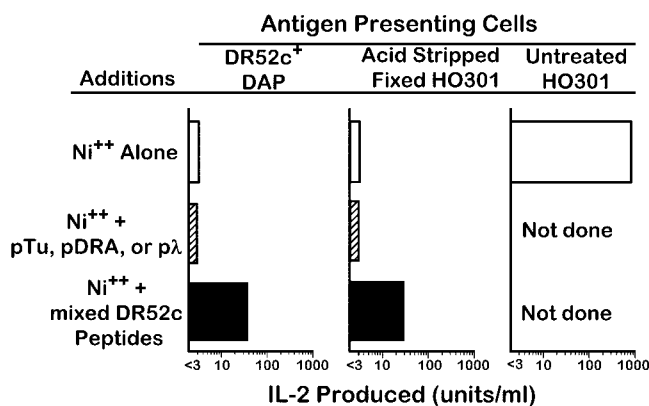
duced DAP cells nor the fixed and acid stripped H0301 cells could present  $\text{Ni}^{2+}$  to ANi-2.3. However, after preincubation with H0301-derived DR52c bound peptides, both APCs presented  $\text{Ni}^{2+}$ . Several peptides dominantly bound to DR52c on an EBV transformed cell line have been identified (13). To see if any peptide strongly bound to DR52c would restore  $\text{Ni}^{2+}$  presentation we tested three of these peptides, pTu, pDRA, and p $\lambda$ , for their ability to confer  $\text{Ni}^{2+}$  presenting activity to these APC. A high concentration of any of these peptides was not able to restore  $\text{Ni}^{2+}$  presentation when added to the DR52c bearing cells. We concluded from these experiments that ANi-2.3 requires  $\text{Ni}^{2+}$ , DR52c, and a particular peptide that is generated in human and mouse B cells (and perhaps other professional APCs), but not in other cell types.



**Figure 4.**  $\text{Ni}^{2+}$  presentation requires DR52c expression in a professional APC. A number of human and murine cell lines lacking DR expression were transduced with the genes for the common DR  $\alpha$  chain and the DR52c  $\beta$  chain. Surface expression of DR52c on the transductants was monitored by staining with FK-7.3 (mean channel fluorescence shown in parentheses). Cells were used before (white bar) and after (black bar) transduction to present  $\text{Ni}^{2+}$  ( $100 \mu\text{M}$ ) to ANi-2.3. Untransduced HO301 cells were used as a control APC.

**Possible Role of DR52c  $\beta$ 81His in  $Ni^{2+}$  Presentation.** Our results suggested that surface amino acids of the DR52c and/or its bound peptide coordinate  $Ni^{2+}$  in a way that presents the cation for recognition by the  $\alpha\beta$ TCR. We had no information about sequence of the functional peptide, but we examined the predicted surface amino acids of the MHC  $\alpha$  or  $\beta$  chains for possible candidates for  $Ni^{2+}$  coordination. As our experiments suggested the involvement of histidine, we looked for a predicted pair of histidines, such as is often found in transition metal ion binding sites. In fact, only one MHC histidine ( $\beta$ 81) is predicted to lie on the surface of DR52c within what has been the  $\alpha\beta$ TCR interaction footprint on MHC seen in structures of  $\alpha\beta$ TCR/MHC complexes (for reviews, see references 28 and 29). This histidine lies on the top of the  $\beta$  chain  $\alpha$  helix near the  $NH_2$ -terminal end of the bound peptide. It is conserved in nearly all MHCII alleles and isotypes of all species. In all crystal structures of MHCII thus far, the side chain of this histidine points toward the peptide making an important H-bond with the peptide backbone. This histidine has in fact been shown to be capable of coordinating  $Zn^{2+}$  as part of the interaction site between MHCII and  $Zn^{2+}$  containing bacterial superantigens (30, 31).

Therefore, we tested the importance of this histidine in  $Ni^{2+}$  presentation to ANi-2.3 by mutating it in the DRB3-\*0301  $\beta$  chain to glutamine. The geometry of the glutamine side chain should allow it to H-bond to the peptide backbone similarly to histidine, but glutamine would not normally be predicted to coordinate  $Ni^{2+}$ . The mutated  $\beta$  chain gene was transduced into LG2. The mutated  $\beta$  chain was well expressed on the surface of the transduced LG2 cells, but had lost its ability to present  $Ni^{2+}$  to ANi-2.3 (Fig. 6). This result is consistent with a role for  $\beta$  histidine



**Figure 5.** Peptides isolated from HO301 produced DR52c can transfer  $Ni^{2+}$  presenting ability to other DR52c bearing cells. Peptides were acid stripped and purified from DR52c immunopurified from HO301 cells. An aliquot of the peptides ( $3 \times 10^8$  cell equivalents) were added to either DR52c expressing DAP cells or pH3-treated fixed HO301 cells. The cells were used either before (white bar) or after (black bar) peptide exposure to present  $Ni^{2+}$  (100  $\mu$ M) to ANi-2.3 cells. As negative controls (hatched bar), peptides (pTu, pDRA, pλ) known to bind well to DR52c were used (100  $\mu$ g/ml). Presentation by untreated HO301 cells served as the positive control.

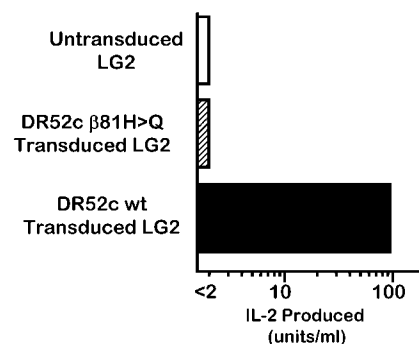
81 in coordinating  $Ni^{2+}$ , but, of course, does not eliminate the alternate interpretation that this amino acid is a contact residue for  $\alpha\beta$ TCR/MHC, rather than  $Ni^{2+}$ /MHC, interaction.

## Discussion

A number of laboratories have isolated  $Ni^{2+}$  reactive T cell clones from patients with  $Ni^{2+}$  hypersensitivity (10, 32, 33). The properties of these clones have varied dramatically. There has been no particular MHCII allele or isotype associated with  $Ni^{2+}$  sensitivity. Some clones, such as the ANi-2.3 clone studied here are self-MHC-restricted in their specificity requiring one of the patient's allelic forms of an MHC molecule as the  $Ni^{2+}$  presenting element. Others have proven to be much more promiscuous, in that they can respond to  $Ni^{2+}$  presented by foreign allelic forms of the MHC molecule. These results contrast to those seen in berylliosis in which there is a very high correlation between Be sensitivity and the presence MHCII DP2 allele (34). Furthermore, in vitro, T cells from berylliosis patients require DP2 bearing antigen presenting cells to respond to Be (35).

The specific role of the MHC bound peptide in  $Ni^{2+}$  presentation has not been extensively examined. The ability of  $Ni^{2+}$  to bind to an MHC bound peptide was demonstrated in one series of experiments, by showing that peptide dependent binding of  $Ni^{2+}$  to an MHC/peptide complex could interfere with conventional T cell recognition of the complex (36). However, there is very little direct data on the role of the MHC bound peptide in  $Ni^{2+}$  presentation.

Our experiments with ANi-2.3 suggest an essential role for a particular MHC bound peptide in  $Ni^{2+}$  presentation whose function is more than simply stabilizing the MHCII molecule. Among the cells we examined, only B cells with an intact antigen processing pathway were able to generate this peptide. However, we did not examine other profes-



**Figure 6.** DR52c  $\beta$ 81His is important for ANi-2.3 recognition of DR52c presented  $Ni^{2+}$ . LG2 cells were transduced with the gene for the DR52c  $\beta$  chain in which the codon for  $\beta$ His81 was changed to that for Gln. Presentation of  $Ni^{2+}$  to ANi-2.3 cells by the LG2 cells transduced with the mutant  $\beta$  chain (hatched bar) was compared with that seen with untransduced LG2 cells (white bar) or LG2 cells transduced with the wild type  $\beta$  chain (black bar).

sional antigen presenting cells such as macrophages and dendritic cells. Furthermore, other peptides with a wider cellular distribution and less stringent processing requirements may be adequate for  $\text{Ni}^{2+}$  presentation to other T cell clones (37). Since in our studies we used a costimulation independent T cell transfectoma to detect  $\text{Ni}^{2+}$  presentation, we cannot say whether in vivo a requirement for costimulation might also limit the type and state of antigen presenting cells driving the response to  $\text{Ni}^{2+}$ .

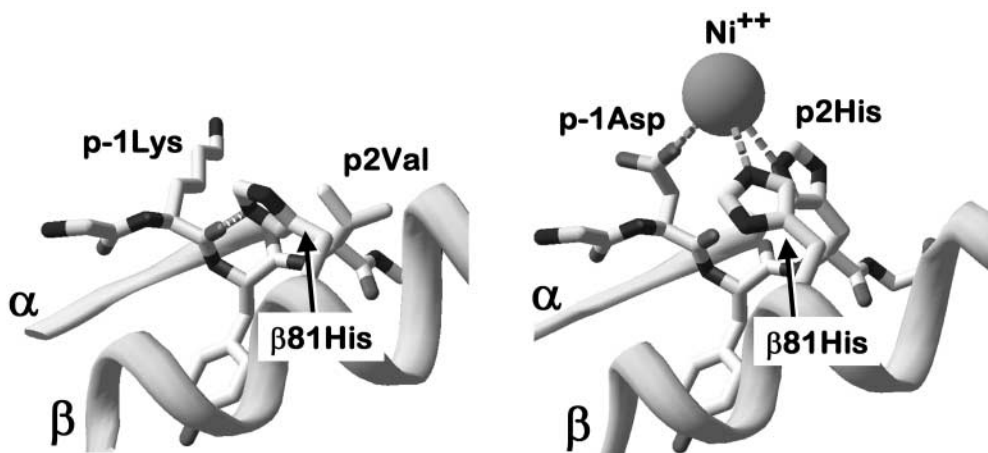
The variety of MHCII alleles capable of  $\text{Ni}^{2+}$  presentation and the MHC promiscuity of some  $\text{Ni}^{2+}$  reactive clones might be taken as evidence that  $\text{Ni}^{2+}$  can be presented to T cells in many different ways. However, our findings that the ANi-2.3 clone requires a specific peptide for  $\text{Ni}^{2+}$  presentation and is dependent on His81 of the MHC  $\beta$  chain suggest the possibility of a common theme in  $\text{Ni}^{2+}$  presentation. As mentioned above,  $\beta\text{His81}$  is conserved in nearly all MHCII molecules regardless of allele or isotype. In crystal structures reported thus far this His forms a hydrogen bond to the peptide backbone. For example, Fig. 7 A shows this area of the DR1 molecule bound to an influenza hemagglutinin peptide (38). In this case  $\beta\text{His81}$  is hydrogen bonded to the backbone carbonyl of a Lys at the p-1 position of the peptide. It is reasonable to assume that the  $\beta\text{His81}$  of DR52c is in a similar configuration.

If the loss of  $\text{Ni}^{2+}$  recognition by ANi-2.3 upon our mutation of DR52c  $\beta\text{His81}$  to Gln, indicates a role for  $\beta\text{His81}$  in  $\text{Ni}^{2+}$  coordination, then we can predict several additional properties of the  $\text{Ni}^{2+}$  presenting MHC/peptide complex. First, there must be a new rotamer for the side chain of  $\beta\text{His81}$ . Second, as there are no other suitable amino acids on the MHC surface in the vicinity to provide additional  $\text{Ni}^{2+}$  coordination, these must be supplied by the peptide. The peptide amino acids in positions to perform this function are located at p-1 and p2. Therefore, in the DR1 structure we modeled an Asp and a His for the influenza peptide amino acids at positions p-1 and p2 respectively (Fig. 7 B). Allowing only preferred rota-

mers for these two substituted amino acids and for  $\beta\text{His81}$ , we could easily place these side chains in positions nearly ideal for tetragonal coordination of  $\text{Ni}^{2+}$ . No accommodations were required of the MHC or peptide backbone or of the rotamers of any other amino acids. Square planar or tetragonal coordination involving aspartic acid and histidine is very common in  $\text{Ni}^{2+}$  ion complexes to proteins (39–41) and would be consistent with our findings of the low pH sensitivity of  $\text{Ni}^{2+}$  binding to fixed antigen presenting cells. While the order of the Asp and His in the peptide theoretically could be reversed, this alternate configuration did not yield ideal tetragonal geometry with only preferred rotamers.

The attraction of this model is that it suggests a conserved peptide dependent mode of  $\text{Ni}^{2+}$  presentation among many different MHCII alleles and isotypes. This hypothesis is also consistent with the finding that T cells reactive to  $\text{Ni}^{2+}$  often cross react with other metal ions. For example, ANi-2.3 also responds to copper and gold cations. These could be expected to be coordinated similarly to  $\text{Ni}^{2+}$ . Furthermore, if sometimes the coordinated  $\text{Ni}^{2+}$  dominates other peptide contributions to the interface with the  $\alpha\beta\text{TCR}$ , then one could also explain the promiscuity of some T cell clones in recognizing  $\text{Ni}^{2+}$  bound to a variety of MHC alleles. As long as the bound peptide provided the appropriate coordination groups, the combination of the bound  $\text{Ni}^{2+}$  and other conserved amino acids on the MHC surface common to many alleles could satisfy the T cell receptor. These ideas can be tested in structural and functional studies.

One consequence of placing  $\text{Ni}^{2+}$  at a particular location on the MHC/peptide surface is a prediction about the portion of the  $\alpha\beta\text{TCR}$  that is likely to be in contact with the  $\text{Ni}^{2+}$ . There are now numerous crystal structures of  $\alpha\beta\text{TCR}$ s bound to MHC/peptide complexes involving both MHCI and MHCII molecules. In all cases, the receptor has a somewhat diagonal orientation on the MHC/peptide surface with the  $\text{V}\alpha$  portion toward the peptide  $\text{NH}_2$  ter-



**Figure 7.** Possible model for  $\text{Ni}^{2+}$  coordination by MHCII  $\beta\text{His81}$  and amino acids at positions p-1 and p2 of the peptide. In the left panel a portion of the structure of the DR1 ( $\text{DRA}^*0101$ ,  $\text{DRB1}^*0101$ ) bound to a peptide from influenza hemagglutinin (reference 38) is shown in the vicinity of  $\beta\text{His81}$ . The conserved hydrogen bond between  $\beta\text{His81}$  and the peptide backbone is shown as a dashed line. In the right panel, using Swiss PDB Viewer, peptide amino acids at position

p-1 and p2 were changed to Asp and His, respectively. These amino acids and  $\beta\text{His81}$  were given rotamers that create the correct geometry for three ligands for potential tetragonal coordination (dashed lines) of a  $\text{Ni}^{2+}$  ion (sphere).

minus, the V $\beta$  region toward the peptide COOH terminus and the CDR3 regions focused on the center of the peptide. Within these constraints there is quite a bit of rotational variation among different  $\alpha\beta$ TCRs. Therefore, while it is not possible to predict precisely the orientation of the ANi-2.3  $\alpha\beta$ TCR on a Ni<sup>2+</sup>/peptide/DR52c complex, the CDR1 or CDR2 of V $\alpha$  is most likely to be oriented over a Ni<sup>2+</sup> coordinated by  $\beta$ His81 and the p-1 and p2 amino acids of the peptide. The V $\alpha$  element used by ANi-2.3 is AV01s4, whose CDR1 sequence is SYGATPY and CDR2 sequence is KYFSGDTLV. The most obvious candidate to provide a fourth group to complete the tetragonal coordination of Ni<sup>2+</sup> is the Asp in the CDR2 region. Interestingly, an Asp or Glu is present in the COOH-terminal half of the CDR2 regions of nearly all of the V $\alpha$  elements of the  $\alpha\beta$ TCRs of Ni<sup>2+</sup> reactive T cell previously reported (11, 14, 33). In fact, a T cell with a highly MHC promiscuous response to Ni<sup>2+</sup> bears a V $\alpha$  element with a pair of Asp in this region (33).

Although direct coordination of Ni<sup>2+</sup> by the side chain of an  $\alpha\beta$ TCR His or acidic amino acid is the most obvious choice, there are other possibilities. For example, in the various solved structures of Ni<sup>2+</sup> ions bound to proteins, one of the ligands for the metal can be an oxygen from water, the protein backbone or a side chain from Ser or Thr (39, 41). Furthermore, we cannot rule out the possibility that the  $\alpha\beta$ TCR recognizes the MHC and peptide amino acid side chains that have changed their configuration in order to accommodate the Ni<sup>2+</sup> rather than the Ni<sup>2+</sup> itself. The definitive answer to these questions will require crystal structures of these complexes. However, in the mean time the model we propose here offers a good starting point for designing structure/function studies.

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