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TCR Reactivity in Human Nickel Allergy Indicates Contacts with Complementarity-Determining Region 3 but Excludes Superantigen-Like Recognition

Jörg Vollmer,²* Hans Ulrich Weltzien,³* and Corinne Moulon⁴*¹

Nickel is the most common inducer of contact sensitivity in humans. We previously found that overrepresentation of the TCRBV17 element in Ni-induced CD4⁺ T cell lines of Ni-allergic patients relates to the severity of the disease. Amino acid sequences of these β-chains suggested hypothetical contact points for Ni²⁺ ions in complementarity-determining region (CDR) 1 and CDR3. To specifically address the molecular requirements for Ni recognition by TCR, human TCR α- and β-chains of VB17⁺ Ni-reactive T cell clones were functionally expressed together with the human CD4 coreceptor in a mouse T cell hybridoma. Loss of CD4 revealed complete CD4 independence for one of the TCR studied. Putative TCR/Ni contact points were tested by pairing of TCR chains from different clones, also with different specificity. TCRBV17 chains with different J regions, but similar CDR3 regions, could be functionally exchanged. Larger differences in the CDR3 region were not tolerated. Specific combinations of α- and β-chains were required, excluding a superantigen-like activation by Ni. Mutation of amino acids in CDR1 of TCRBV17 did not affect Ag recognition, superantigen activation, or HLA restriction. In contrast, mutation of Arg⁹⁵ or Asp⁹⁶, conserved in many CDR3B sequences of Ni-specific, VB17⁺ TCR, abrogated Ni recognition. These results define specific amino acids in the CDR3B region of a VB17⁺ TCR to be crucial for human nickel recognition. CD4 independence implies a high affinity of such receptor types for the Ni/MHC complex. This may point to a dominant role of T cells bearing such receptors in the pathology of contact dermatitis.


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Abbreviations used in this paper: CDR, complementarity-determining region; HV, hypervariable; SEB, staphylococcal enterotoxin B; CTL, CTL line; IHW, International Histocompatibility Workshop.

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identify regions involved in Ag contact, α- and β-chains of different TCR with or without specificity for Ni were paired, and individual amino acids within the CDR1B and CDR3B regions were mutated.

Materials and Methods

**Ags, reagents, and media**

Metal salts and other reagents were used at the following concentrations, if not otherwise specified: NiSO$_4 \cdot 5$H$_2$O, 10$^{-4}$ M; CuSO$_4 \cdot 5$H$_2$O, 5 $\times 10^{-4}$ M (both from Sigma, Deisenhofen, Germany); PHA, 1 μg/ml (Murex, Dartford, U.K.); staphylococcal enterotoxin B (SEB), 20 ng/ml (Serva, Heidelberg, Germany); tetanus toxoid peptide TT830-843 (YQIANKSFIGITE), 5 μg/ml; Con A-induced rat spleen supernatant (10%) served as source of IL-2 to maintain CTL line (CTLL) cells. Growth medium for T cell hybridomas (RPMI-FCS) contained RPMI 1640 supplemented with 2 mM L-glutamine, 100 μg/ml kanamycin (all from Life Technologies/BRL, Eggenstein, Germany), and 10% heat-inactivated FCS. Culture of human T cell clones was described previously (25).

**Cell lines and T cell clones**

The Ni-specific T cell clones 4.13, ANi1.3, and ANi2.3 were obtained from the Ni-allergic donor IF and have been previously described (24); Donor IF had been HLA typed (24) as follows: HLA-A1, A26, B35, DRB1*1302, DRB1*0401, DR2, DR53, DQ6 (1), and DQ7 (3). The murine T cell hybridomas 54(17) (26) and To8.1 were a kind gift of Dr. O. Acuto (Institut Pasteur, Paris, France). For APC, we used either autologous EBV-transformed B cells of donor IF or HLA-DR homozygous B cell lines, originating from the International Histo compatibility Workshop (IHW, WT47 (IHW No. 9063) (DRB1*1302, DR52), BSM (IHW No. 9032) (DRB1*0401, DR53), PLH (IHW No. 9047) (DRB1*07, DR53), SVEIG (IHW No. 9037) (DRB1*1101, DR52), and EK (IHW No. 9054) (DRB1*1401, DR52). The EBV-B cell line APD (DRB1*1301) was obtained from Dr. F. Koning, Leiden University Medical Center, Leiden, The Netherlands.

**Proliferation assay**

The T cell clone 4.13 (4 $\times 10^4$ cells) was cocultured in triplicate with 4 $\times 10^4$ irradiated (6000 rad) EBV-B cells of donor IF in 200 μl of complete RPMI 1640 or without NiSO$_4$ (10$^{-4}$ M). After 24 h at 37°C, cultures were incubated with 0.5 μCi $[^{3}H]$thymidine (Amersham Buchler, Braunschweig, Germany), and incorporation of radioactivity was measured in a beta counter (INOTECH, Asbach, Germany) after another 18 h. To assess the requirement of the T cell clone for CD4, the T cell clone was cultured with B cells, 10$^{-6}$ M NiSO$_4$, and either anti-CD4 (13B8.2) (5 μg/ml) or anti-CD8 mAb (B9.11) (5 μg/ml) (both mAb were obtained from Immuno tech, Marseille-Luminy, France).

**IL-2 secretion assay**

Transfectants (5 $\times 10^4$ cells) were cocultured in duplicate or in triplicate in 200 μl RPMI-FCS with 5 $\times 10^4$ irradiated B cells in the presence or absence of Ag. After 20 h at 37°C, 100 μl of the supernatant was used for a CTLL assay as described in Grabstein et al. (27). Stimulation with immobilized purified anti-CD3ε mAb (145-2C11) (PharMingen, San Diego, CA) or anti-VB17 mAb (E17.5F3.15.13, Immunotech) was described previously (28). APC were fixed according to the method of Shimonek et al. (29). Briefly, B cells were resuspended at room temperature in 1 ml of PBS containing 0.05% glutaraldehyde (Life Technologies/BRL). After 45 s, 1 ml of 0.2 M t-lysine (Life Technologies/BRL) was added for an additional 45 s. Cells were then washed. To assess class specificity of HLA restriction, T cells were cultured with B cells, 10$^{-6}$ M NiSO$_4$, and either anti-DR (L243, American Type Culture Collection, Manassas, VA (ATCC)), anti- DP (B7.21, ATCC), or anti-DQ (SPVL3, ATCC) mAb (1:10 diluted culture supernatant). IL-2 secretion was determined as above.

**Abs and flow cytometry**

Hamster anti-murine CD3ε mAb (145-2C11) (30) was used with FITC-conjugated rabbit anti-hamster Ig (Dianova, Hamburg, Germany). Mouse anti-human mAb used included FITC-conjugated and nonconjugated TCRBV17 (E17.5F3.15.13) and FITC-conjugated CD4 (13B8.2) (all from Immunotech). FITC-conjugated mouse IgG1 (MOPC-21) (Sigma) was used as isotype control. For flow cytometric analysis, 2 $\times 10^4$ cells were stained at 4°C in 96-well round-bottom plates either directly with FITC-labeled or with unlabeled mAb, followed by staining with the secondary mAb. Fluorescence was determined in a FACSScan instrument (Becton Dickinson, Mountain View, CA).

**Construction of TCR expression vectors**

Total RNA of human T cell clones 4.13, ANi1.3, and ANi2.3 was extracted from 5 $\times 10^6$ cells using the TRI reagent RNA/DNA/protein isolation reagent (Molecular Research Center, Cincinnati, OH). Transcription into cDNA and analysis of TCR α- and β-chains were done as previously described (24). Nomenclature used for TCR gene segments is according to Arden et al. (31), and CDR3 regions were defined according to Moss and Bell (32). Functionally rearranged human TCR α and β genes were used for construction of mouse-human hybrid TCR expression vectors (consisting of mouse constant regions and human rearranged variable regions) as described in Vollmer et al. (28). Briefly, full-length rearranged TCRV regions of the TCR α- and β-chains of clones 4.13, ANi1.3, and ANi2.3 were amplified from cDNA with the primers listed in Table I. Standard PCR procedures were used, including 5 cycles of 30 s at 95°C, 40 s at 60°C, and 40 s at 72°C, followed by 30 cycles of 30 s at 94°C, 40 s at 57°C, and 40 s at 72°C. The primer pair mut13AV351 sense and mut13AV351 antisense (Table I) was used to eliminate an endogenous BamHI site in the TCRAV351 element without altering the amino acid sequence. The final PCR products were cloned into the pCR-Script vector (Stratagene, Heidelberg, Germany) and sequenced using the Big Dye sequencing kit (Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions. Sequences were read on a 310 Genetic Analyzer (Applied Biosystems).

**Mutations of CDR1B and CDR3B regions**

The rearranged TCRBV17 chain of 4.13 cloned into the pCR Script vector was used as a template for subsequent site-directed mutagenesis. Amino acids in CDR1B (His in position 27) and CDR3B (Arg and Asp in positions 95 and 96, respectively) were mutated to Ala. The asp at position 28 in CDR1B was mutated to Gly (CDR1D-G), as Gly is located at this position in other human TCRBV chains exhibiting high homology to TCRBV17. This should avoid possible alterations of the TCR structure due to the mutations (34, 35). Introduction of point mutations was performed using the QuickChange site-directed mutagenesis kit (Stratagene) according to the manufacturer’s instructions. Primers used are listed in Table I. Mutated TCR β-chains were sequenced as described above and cloned into the TCR β-chain expression vector pV2-15B.

**Transfection of TCR expression vectors into mouse hybridoma cells**

The murine TCR-negative hybridoma T cell line 54(17), expressing a human CD4 molecule, was used as recipient cell for transfection of TCR α- and β-chain expression vectors. Recipient cells (8 $\times 10^6$) were transfected by electroporation as described previously (28). Cultures resistant for G418 (Life Technologies/BRL) were analyzed by FACS for surface expression of TCR, CD3ε, and CD4, and expression of the correct TCR α- or β-chains was confirmed by PCR and/or TCR sequencing. Briefly, total RNA was extracted and transcribed into cDNA. PCR amplifications were performed as above using the primers humanLVβ17, humanVα1, and humanVα3 (for primer sequences see above) together with mouseCoint (TGTCCTCAGTGTCCTCAGT) and pV2-15B (conferring resistance to G418) (33), so that they contained the rearranged human variable parts joined to the complete constant regions of the mouse TCR. Vectors were linearized with ClaI and EcoRI, respectively, and used for transfection.
Table I. Nucleotide sequences of primers used for construction of TCR expression vectors and mutation of TCR α- or β-chains

<table>
<thead>
<tr>
<th>Transfectant</th>
<th>TCR Chain</th>
<th>Primer Sequence (5' → 3')</th>
<th>Orientation</th>
</tr>
</thead>
<tbody>
<tr>
<td>T413</td>
<td>TCR A</td>
<td>ACTCCAGTGCCAGAAGATGCTCTGGAGAAGTTAC   Sense</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GATCGGATCCacttaCGTGTGTATATGTAAG   Antisense</td>
<td></td>
</tr>
<tr>
<td>TCRB</td>
<td>TCR A</td>
<td>ACTCCAGTGCCAGAAGATGCTCTGGAGAAGTTAC   Sense</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GATCGGATCCacttaCGTGTGTATATGTAAG   Antisense</td>
<td></td>
</tr>
<tr>
<td>T23</td>
<td>TCR A</td>
<td>ACTCCAGTGCCAGAAGATGCTCTGGAGAAGTTAC   Sense</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GATCGGATCCacttaCGTGTGTATATGTAAG   Antisense</td>
<td></td>
</tr>
<tr>
<td>T13</td>
<td>TCR A</td>
<td>ACTCCAGTGCCAGAAGATGCTCTGGAGAAGTTAC   Sense</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GATCGGATCCacttaCGTGTGTATATGTAAG   Antisense</td>
<td></td>
</tr>
</tbody>
</table>

Table II. TCRV and TCRJ usage and amino acid sequences of hypervariable TCRα and/or TCRβ regions of T cell clones AL8.1, ANi1.3, ANi2.3, and 4.13

<table>
<thead>
<tr>
<th>T Cell Clone</th>
<th>TCRV</th>
<th>CDR1α</th>
<th>CDR2</th>
<th>CDR3β</th>
<th>TCRJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL8.1</td>
<td>AVS1A</td>
<td>S D S A N Y</td>
<td>D I R S N V G</td>
<td>C A</td>
<td>A E N Y G G S Q G N L I</td>
</tr>
<tr>
<td>ANi1.3</td>
<td>AVS1A</td>
<td>K T S I N N</td>
<td>L I R S N E R E</td>
<td>C A</td>
<td>T A M T P N S K L T</td>
</tr>
<tr>
<td>ANi2.3</td>
<td>AVS1A</td>
<td>S Y G A T P Y</td>
<td>K Y F S G D T L V</td>
<td>C A</td>
<td>V G G S G N T G K L I</td>
</tr>
</tbody>
</table>

Results
Characterization of Ni-specific TCR transfectants and role of the CD4 co-receptor in Ni recognition

In a previous study, we observed an overrepresentation of the TCRBV17 element among CD4+ T cell lines raised from donors with strong hyperreactivity to Ni (24). TCR sequencing of a panel of Ni-specific T cell clones for one of these donors revealed interesting features of these TCRBV17 chains. In many cases, the amino acid Arg is conserved in position 95 of the CDR3B region and is frequently accompanied by an Asp in position 96. We wanted to further examine the role of these specific Ag receptors in Ni recognition, using our previously described method to express Ni-reactive human TCR together with the human CD4 co-receptor in the mouse hybridoma cell line 5417 (28).

Concerning their α-chains, clones 4.13 and ANi2.3 possess identical TCRBV17 alleles and highly similar CDR3B sequences, including an Arg95-Asp96 amino acid motif, but different TCRBJ elements. In contrast, the TCRBV17 chain of ANi1.3 differed from the other two TCR β-chains not only in the TCRBJ region, but also by a different CDR3B sequence and in a slightly different allele of the TCRBV17 segment (Table II).

Expression vectors for the TCR of clones 4.13, ANi2.3, and ANi1.3 were constructed as described in Materials and Methods and transfected into 5417 cells. The resulting TCR transfectants, T413, T23, and T13, respectively, were analyzed for TCR and γ-gation are shown in Table II. All three clones were obtained from the Ni-allergic patient IF. Two of these clones, namely, 4.13 and ANi2.3, contained very similar TCR α-chains (VA1, JA37) exhibiting 99% similarity. In contrast, clone ANi1.3 expressed VA3 and JA56 and exhibited only ~35% similarity to the other two clones. Concerning their β-chains, clones 4.13 and ANi2.3 possess identical TCRBV17 alleles and highly similar CDR3B sequences, including an Arg95-Asp96 amino acid motif, but different TCRBJ elements. In contrast, the TCRBV17 chain of ANi1.3 differed from the other two TCR β-chains not only in the TCRBJ region, but also by a different CDR3B sequence and in a slightly different allele of the TCRBV17 segment (Table II).

![Image of Table II](http://www.jimmunol.org/)

* Restriction sites for BamHI and SalI are in bold print.
* Introduced splice sites are indicated by lowercase letters.
* Mutated nucleotides are underlined.
CD4 cell surface expression by FACS analysis. These data are summarized in Table III and compared with the TCR-negative recipient cell line 54\(\beta\)17. Table III also shows data for several other TCR transfectants, which will be discussed later. The reactivities of the TCR transfectants are shown in Fig. 1. For T413, Fig. 1A reveals that, as for the corresponding T cell clone 4.13 (not shown), the activation by NiSO\(_4\) is inhibited by anti-HLA-DR but not by anti-HLA-DRB1*0401, DRB1*1302, DR52, and DR53. Attempts to activate the TCR transfectant T413 also cross-reacted with Cu (Fig. 1A). This cross-reactivity, which was also observed for T23 and T13 (data not shown), proves that both metal ions are essential components.

The HLA-DR restriction of T413, T23, and T13 was further defined by using HLA-DR homozygous B cell lines matching the HLA-DR haplotype of donor IF. IF was typed as expressing HLA-DRB1*1102 and DRB1*1302 (26), i.e., the same HLA-DR13 allele to which T413 was restricted (Fig. 1B). The resulting transfectant TAL8.1A/413B expressed both TCR and CD4 (not shown). However, TAL8.1A/413B could be stimulated neither by NiSO\(_4\) nor by TT830-843 presented by the HLA-DRB1*1302-positive B cell line WT47, but could be activated by SEB and anti-TCR mAb (not shown). This indicates that Ni does not act in a superantigen-like fashion, but that the structural elements of both chains of the Ni-specific TCR are needed to create a functional Ag recognition site. In a subsequent analysis, we tested the combination of the TCR \(\alpha\)- and \(\beta\)-chains of the two Ni-specific HLA-DR13-restricted T cell clones 4.13 and ANi1.3. The resulting TCR transfectants were designated as T413A/13B (TCR \(\alpha\)-chain of clone 4.13 and \(\beta\)-chain of clone ANi1.3) and T13A/413B (reverse combination). The comparable responses of the two hybrid TCR and of their original TCR in T413 and T13 to mAb and SEB (Fig. 2A) reflect the structural and functional integrity of these Ag receptors. However, neither of the two hybrid TCR was activated in the presence of NiSO\(_4\) (Fig. 2B). The same result was obtained for the cross-reactive Ag Cu SO\(_4\) (not shown). These data confirm, in addition to transfectant TAL8.1A/413B, the contribution of both TCR chains to Ni and Cu specificity and highlight the possible importance of the Arg\(^95\) – Asp\(^96\) motif in the CDR3B region in mediating Ni recognition for the transfectants T413 and T23.

As the two TCR of clones 4.13 and ANi2.3 primarily differ in their CDR3B and CDR3B regions (Table II), we produced hybrid receptors of their TCR \(\alpha\)- and \(\beta\)-chains by transfection into 54\(\beta\)17 cells. The resulting TCR transfectants T413A/23B (TCR \(\alpha\)-chain of clone 4.13 and \(\beta\)-chain of clone ANi2.3) and the reverse T23A/413B expressed TCR and human CD4 (Table III) and compared well with the original TCR transfectants, T23 and T413, in SEB- and anti-CD3\(\varepsilon\) mAb-mediated activation (Fig. 2C). However, unlike the T413/T13 hybrids (Fig. 2B), both T413/T23 hybrids were stimulated by NiSO\(_4\) in a manner comparable with that of the parental TCR (Fig. 2D). This result was also observed for Cu-specific TCR responses (not shown). It implies that a significant part of the CDR3B and the complete CDR3B regions do not interfere with Ag and restriction specificity. This, in turn, puts into focus those amino acids of the CDR3B region that are identical or highly similar between the TCR \(\beta\)-chains of clones 4.13 and ANi2.3 and differ between those and clone ANi1.3. These are the amino acids in positions 93–98 (Table II), and particularly those in positions 95 and 96 (Arg and Asp), which, in several TCR clones, were shown to be most closely in contact with MHC-associated peptide determinants (12, 37).

**Table III. TCR and CD4 surface expression of TCR transfectants**

<table>
<thead>
<tr>
<th>TCR Transfectant</th>
<th>Mean Fluorescence Intensity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>54(\beta)17</td>
<td>4.17</td>
</tr>
<tr>
<td>T413</td>
<td>4.17</td>
</tr>
<tr>
<td>T413CD4(^-)</td>
<td>3.51</td>
</tr>
<tr>
<td>T13</td>
<td>4.53</td>
</tr>
<tr>
<td>T13A/413B</td>
<td>4.53</td>
</tr>
<tr>
<td>T413A/13B</td>
<td>4.53</td>
</tr>
<tr>
<td>T23</td>
<td>4.17</td>
</tr>
<tr>
<td>T23A/413B</td>
<td>4.17</td>
</tr>
<tr>
<td>T413A/23B</td>
<td>4.17</td>
</tr>
<tr>
<td>CDR1HA</td>
<td>3.39</td>
</tr>
<tr>
<td>CDR1DG</td>
<td>3.39</td>
</tr>
<tr>
<td>CDR3RA</td>
<td>3.39</td>
</tr>
<tr>
<td>CDR3DA</td>
<td>3.39</td>
</tr>
</tbody>
</table>

*Mean fluorescence intensity of staining with mAb was determined by flow cytometry on representative transfectants. Controls included stainings with a goat antihamster Ig for the anti-CD3\(\varepsilon\) staining and with isotype control (mouse IgG1) for anti-VB17 and anti-CD4 stainings.

**Shuffling of TCR \(\alpha\)- and \(\beta\)-chains between different DR13-restricted human TCRs**

One possibility for the overrepresentation of VB17+ TCR in NiSO\(_4\)-induced human T cell cultures might be a superantigen-like interaction of Ni\(^{2+}\) ions with amino acids specific for this TCRBV region. In this case, the TCRBV17 sequence alone might dominate the recognition of Ni epitopes and might even be sufficient for Ag recognition. To test this hypothesis, we introduced the TCR \(\beta\)-chain of the Ni-reactive T cell clone 4.13 into a transfectant (Tu8.1) containing the \(\alpha\)-chain of the unrelated human T cell clone AL8.1 (Table II). The TCR of clone AL8.1 has been previously described to react to the tetanus toxoid peptide TT830-843 presented by either HLA-DRB1*1102 or DRB1*1302 (26), i.e., the same HLA-DR13 allele to which T413 was restricted (Fig. 1B).

One peculiarity of the TCRBV17 element is the amino acid sequence His-Asp-Ala in positions 27 to 29 of its CDR1 loop. Although His\(^{27}\) is highly conserved among various TCRBV seg-
ments (30, 38, 39), the combination His-Asp-Ala is rather unique. These amino acids have been identified as participating in the Ni binding sites of several Ni-complexing proteins (40 – 42). Although the above experiments excluded that the motif HDA in the CDR1 region of TCRBV17 itself was sufficient to mediate Ni reactivity, the motif might still participate in the interaction with Ni epitopes. A second possible point of contact between VB17 TCRb-chains and Ni antigenic determinants has been indicated to be represented by the amino acids Arg 95 and Asp 96 in the CDR3B region of the Ni-reactive T cell clones 4.13 and ANi2.3. For further investigations of TCR contacts with Ni, we therefore mutated each of the four amino acids, i.e., His27 and Asp28 in CDR1 and Arg95 and Asp96 in CDR3 of the TCRBV17 chain of clone 4.13 individually into Ala or Gly. The positions of the mutated amino acids in the CDR1B and CDR3B regions are indicated in Table II. The mutated TCRb-chains were transfected together with the nonmutated 4.13a-chain into 54z17 cells, resulting in the transfectants CDR1H-A, CDR1D-G, CDR3R-A, and CDR3D-A. Surface expression of TCR and CD4 on these transfectants is summarized in Table III. All four mutated TCR were effectively activated by anti-TCR mAbs (Fig. 3, A and B) and SEB (Fig. 3, C and D), demonstrating their integrity and capacity to signal.

The reactivity of the CDR1B mutants to SEB is of particular interest, because amino acids in positions 27 and 28 have been shown for several other TCR to be involved in superantigen contact, including SEB (14, 43, 44). When tested for reactivity to Ni on autologous APC, both CDR1 mutants were indistinguishable from the original T413 transfectant (Fig. 3E). The same result was obtained for stimulation with Cu (not shown). Some authors have proposed a role for the conserved His27 and adjacent amino acids in defining MHC restriction (38). Therefore, we tested the ability of the CDR1B mutants to be activated by NiSO4 in the presence of APC expressing nonmatching HLA-DR alleles such as HLA-DRB1*1301, DRB1*1401, or DRB1*1101. Although these HLA-DR molecules exhibit the highest similarities to the restricting HLA-DRB1*1302 molecule and all of them mediated SEB recognition (Fig. 3F), the CDR1B mutants were still able to be activated by NiSO4.
activation, none of them was able to present Ni to T413 or the two CDR1 mutants (data not shown). The reactivity to mAb (Fig. 3B)- or SEB (Fig. 3D)-mediated triggering of the CDR3 mutants was also unaffected by the introduced mutations. However, in contrast to the CDR1 mutants, the reactivity of the two CDR3 mutants to Ni-induced epitopes in the presence of autologous APC of donor IF was completely abrogated (Fig. 3F). The same loss of activation was also true for the cross-reactivity to Cu (not shown). These data complete the observations made by shuffling of the TCR chains of clones 4.13 and ANi2.3. They allow us to conclude that the Ni-mediated activation of those two T cell clones is independent of the TCRJB region but directly involves the Arg95-Asp96 motif of their CDR3B sequences. We have also produced more conservative mutations in positions 95 and 96 by replacing Arg95 with Lys and Asp96 with Glu. Preliminary data (not shown) revealed that Asp-Glu exchange also abrogated Ni reactivity, whereas the Arg-Lys replacement had no effect; i.e., antigen contacts mediated by position 95 appear more flexible than those by position 96.

Discussion

Nickel ions are nonclassical Ags that specifically activate human αβ T lymphocytes in an HLA-restricted manner (18). Although these reactions form the basis for occasionally severe contact hypersensitivities in a large proportion of the caucasian population (45), the precise structure of the allergenic determinants involved and the mode of Ni-induced TCR activation remain poorly understood (22, 23). One way to address these questions is to study the major structural features of Ni-reactive TCR.

We have previously described an overrepresentation of VB171 TCR among CD4(1) Ni-induced T cell lines from the peripheral blood of patients with particularly severe contact hypersensitivity to Ni (24). Furthermore, others have reported the detection of elevated numbers of TCRBV171 Ni-reactive T cells in skin lesions (46). We took this to indicate an important role of so-far-unknown properties of this VB171 T helper population in mediating and defining the severity of Ni contact dermatitis. To study the influence of certain structural features of such TCR, we used our recently described system to express human-mouse hybrid TCR in the receptor-deficient mouse hybridoma 5417 together with human CD4 (28). Such hybridomas have two advantages over T cell clones: they clearly link recognition specificity to the transfected TCR and they lack the expression of MHC class II and, hence, the possibility of “self-presentation” of Ni.

A first experiment (Fig. 1E) revealed a complete CD4 independence of Ni recognition by at least one of the VB17+ TCR transfectants studied. In addition, the parent T cell clone (4.13) was inhibited only to a minor degree by mAb to the CD4 coreceptor (Fig. 1F), confirming that this Ni-specific TCR is independent of CD4 signaling. This may indicate a high TCR affinity for the DR13-Ni combination and/or that Ni2+ ions, in addition to conferring specificity, may be involved in an aggregation of TCR-MHC-Ni complexes. This CD4 independence resembles exceptional TCR interactions with peptide Ags (47) but also with
superantigens. It is not yet clear whether this effect is restricted to VB17 TCR, but earlier data concerning a DQ-restricted, VB13 TCR revealed some CD4 dependence of Ni reactivity (28). Different TCR might, therefore, recognize the Ni-induced antigenic determinant with distinct affinities. Thus, VB17 T cells might possess a higher affinity to the MHC-Ag complex in Ni recognition than most other TCRBV elements.

We also examined a potential TCRBV17-dependent superantigen-like T cell activation by Ni. It has indeed been previously reported in another hapten-mediated system of T cell activation that a transfer of Ag specificity (to p-azobenzenearsenonate) could be obtained alone by the TCR α-chain of the original TCR (48). From control experiments (not shown), we already knew that tetanus toxoid-specific CD4+ T cells (from the same donor, IF) did not cross-react with Ni. Moreover, pairing of the TCRBV17 chain of an HLA-DR13-restricted Ni-reactive T cell clone with the α-chain of a DR13-restricted T cells did not cross-react with Ni. Therefore, Ni does not activate the TCR in a superantigen-like fashion. Similar to nominal peptide Ags (26, 49, 50) and non-classical Ags (51), interactions with Ni for VB17 TCR also clearly depend on properties provided only by the specific combination of α- and β-chains.

Clones 4.13 and ANi1.3, the TCR of which were used in the above experiment, possessed different α-chains and also revealed large sequence differences in the CDR3 and J regions of their β-chains (Table II). In contrast, clone ANi2.3 expressed an α-chain differing from clone 4.13 by only one conservative amino acid exchange in its CDR3 loop. The β-chains of the two TCR differed in the J-proximal half of their CDR3 sequence as well as in the TCRBJ elements used, but exhibited the same TCRBV17 sequence and very similar amino acids in positions 93–98 of their CDR3 sequences (Table II). However, our data are in contrast to other studies identifying the J region to be responsible for a heterogeneous pattern of recognition (53, 54).
The different results obtained for the shuffling of the TCR α- and β-chains of clones 4.13, ANi1.3, and ANi2.3 might be explained by a difference in CDR3 length, leading to different TCR orientations above the antigenic determinant, as suggested for peptide Ags (13, 55). On the other hand, this puts into focus the V-proximal sequence of CDR3B, which is identical or very similar between the β-chains of clones 4.13 and ANi2.3. Those amino acids are located at or near the tip of the CDR3B loops in a variety of peptide- or hapten-specific TCR (10, 35, 56–58), indicating TCR contact sites with Ni determinants to be represented by the HV regions comparable with classical peptide Ags (5, 59). Crystallographic analysis of TCR even showed intimate contact of these amino acids in the CDR3B region to MHC-bound antigenic peptides (12, 37).

In this CDR3B region, we previously noticed a particular conservation of an Arg residues 26-Asp motif in Ni-reactive TCRBV17 chains of donor IF (24). Here we show that mutation of either of these two amino acids to Ala resulted in complete loss of Ni specificity (Fig. 3, D and F). This underlines the importance of these amino acids in HLA-DR-restricted Ni recognition and, again, stresses the similarity between the reactivity of Ni- and peptide-specific TCR. Indeed, for peptide-specific T cells, positions 95 and 96 in the β-chain CDR3 have repeatedly been shown to be involved in major contacts with MHC-associated antigenic peptides (6, 10, 56, 57, 60, 61). As Arg and Asp have been demonstrated to be involved in binding of Ni in peptides or in proteins such as arginase (42, 62), direct interactions with Ni ions are conceivable. Therefore, one could imagine the nucleophilic nitrogen groups of Arg and/or the negatively charged oxygen group of Asp as contributing one or two coordination bonds to a Ni complex. This would make Ni-mediated activation similar to hapten recognition by T cells of hapten-peptide-MHC complexes (15). Moreover, the fact that the same results were obtained for Cu ions clearly demonstrates that cross-reactive TCR adopt the same molecular interactions with Cu- as with Ni-induced antigenic determinants.

We have also compared the TCRBV17 sequence with other human TCRBV segments, particularly with regard to their CDR1 and CDR2 regions. Although His in position 27 is highly conserved in human CDR1B sequences (31, 38, 39), the amino acid motif His27–Asp28–Ala29 is unique for TCRBV17. This is interesting, as these same amino acids have been reported to participate in the complexing of Ni in several Ni-binding proteins such as human serum albumin or urease (40, 41, 63). The conservation of His27 in CDR1B led several authors to the conclusion that this amino acid might participate in conserved MHC-contacts or might be important for the overall structure of the TCR (38, 39, 64). Therefore, we assumed that the CDR1 of TCRBV17 might supply a second important site for Ni recognition and, thus, might explain the correlation of the TCRBV17 element with the severity of Ni hyperactivity. However, mutation of neither His27 nor Asp28 interfered in any way with the reactivity of the 4.13 TCR with Ni or with SEB (Fig. 3, C and E). It thus appears that the CDR1 loop of TCRBV17 in the receptor studied does not contribute significantly to the TCR-Ag, TCR-HLA, or TCR-SEB contacts. This is in contrast to several studies describing the involvement of especially the amino acids in positions 27 and 28 of CDR1B in hapten (35, 58), peptide (10, 35, 44, 65, 66), or MHC (38, 44, 45) contact.

In conclusion, we were able to define the amino acids Arg57 and Asp39 in the non-template-encoded region of the CDR3B loop as playing a crucial role in Ni recognition. We cannot exclude the possibility that amino acids other than the herein described Arg and Asp are involved in the recognition of Ni-induced antigenic determinants. Further mutational analysis of putative contact points in the TCR α as well as β-chains of Ni-specific T cell clones might clarify this point. In addition, we cannot yet explain which structural features of the human TCRBV17 element favor its preferred usage in Ni-reactive TCR of highly allergic individuals. However, a superantigen-like activation of VB17+ TCR mediated by Ni2+ ions could be excluded. The finding that TCR specificity and the sensitivity of its activation was untouched by removal of Cd4 implies a particularly high affinity of such receptor types for the Ni–MHC complex. This, in turn, may point to a dominant role of T cells bearing such receptors in the pathogenesis of contact dermatitis. In this respect, it should be recalled that overrepresentation of TCRBV17 among Ni-reactive TCR is restricted to Cd4+ T cells and was not observed for Cd8+ T cells of the same individual (24).

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