A New Type of Metal Recognition by Human T Cells: Contact Residues for Peptide-independent Bridging of T Cell Receptor and Major Histocompatibility Complex by Nickel

Katharina Gamerdinger,1,2 Corinne Moulon,1 David R. Karp,3 Jeroen van Bergen,4 Frits Koning,1 Doris Wild,1 Ulrike Pflugfelder,1 and Hans Ulrich Weltzien1

1Max-Planck-Institut für Immunobiologie, D-79108 Freiburg, Germany
2Fakultät für Biologie, Universität Freiburg, D-79104 Freiburg, Germany
3Rheumatic Diseases Division, University of Texas Southwestern Medical Center, Dallas, TX 75390
4Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, 2300 RC Leiden, Netherlands

Abstract

In spite of high frequencies of metal allergies, the structural basis for major histocompatibility complex (MHC)-restricted metal recognition is among the unanswered questions in the field of T cell activation. For the human T cell clone SE9, we have identified potential Ni contact sites in the T cell receptor (TCR) and the restricting human histocompatibility leukocyte antigen (HLA)-DR structure. The specificity of this HLA-DR–promiscuous VA22/VB17/H11001 TCR is primarily harbored in its α chain. Ni reactivity is neither dependent on protein processing in antigen-presenting cells nor affected by the nature of HLA-DR–associated peptides. However, SE9 activation by Ni crucially depends on Tyr29 in CDR1α, an N-nucleotide–encoded Tyr94 in CDR3α, and a conserved His81 in the HLA-DR β chain. These data indicate that labile, nonactivating complexes between the SE9 TCR and most HLA-DR/peptide conjugates might supply sterically optimized coordination sites for Ni ions, three of which were identified in this study. In such complexes Ni may effectively bridge the TCR α chain to His81 of most DR molecules. Thus, in analogy to superantigens, Ni may directly link TCR and MHC in a peptide-independent manner. However, unlike superantigens, Ni requires idiotypic, i.e., CDR3α-determined TCR amino acids. This new type of TCR–MHC linkage might explain the high frequency of Ni-reactive T cells in the human population.

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

Introduction

Studies dealing with the characterization of antigenic epitopes for T cells have primarily focused on protein antigens. However, T cells may also be triggered by a vast variety of low molecular haptens that frequently result in allergic hyperreactivities (1–3). A subclass of these allergens consists of metals that might be encountered repeatedly via cutaneous, respiratory, oral, or intramuscular routes. These contacts may result in immune-mediated pathologies such as contact hypersensitivity to Au, Be, Co, and in particular to Ni (1, 4), pulmonary granulomatous disease, also called chronic beryllium disease (5), cobalt-induced hard metal lung disease (6), or nephropathy as a result of treatment with gold salts (7). Contact allergy or granulomas may also be caused by aluminium hydroxide, a commonly used adjuvant in vaccines. Thus, 620 cases of itching granulomas and contact allergy to aluminium were recently reported for children undergoing diphtheria-tetanus-pertussis vaccination (8). Although the capacity of these metals to induce MHC-restricted T cell activation is well established (9), the nature of the allergenic epitopes recognized by metal-specific T cells remains unknown in most cases.

Several models have been proposed to explain the activation of metal-reactive T cells: (a) analogous to typical haptens such as TNP or penicillin (1), metal-specific TCR
may react to determinants formed by a complex of metal ions with MHC-embedded self-peptides (9, 10), (b) TCR may recognize metal-modified amino acid residues of the MHC molecule itself or metal-provoked conformational changes in the MHC (11), and (c) metals may affect the processing of self-antigens, resulting in T cells reactive to cryptic self-peptides (12, 13).

None of these models is mutually exclusive and each metal may use several pathways to activate T cells. The first and third of these models have been suggested for Ni- (10, 13) as well as for Au-reactive T cells (9, 12). The second model is favored in the case of Co (hard metal lung disease) and Be (chronic beryllium disease) where the development of disease has been correlated to HLA-DPB1 alleles expressed in the case of clone SE9. This clone belongs to a group of other patients, the TCR of clone SE9 exhibited a dominance of its TCR α chain in determining antigen specificity. Its α chain retained HLA-DR–restricted Ni reactivity even upon pairing with unrelated β chains (19), indicating that in this case the usage of BV17 is not related to Ni specificity. We took advantage of this apparently selective localization of Ni contact sites in the TCR α chain to investigate the relevance of the different activation models listed above in the case of clone SE9.

Materials and Methods

Antigens, Reagents, and Media. If not specified otherwise, reagents were used at the following concentrations: 10^{-4} M NiSO_{4} × 6H_{2}O, 1 μg/ml phytohemagglutinin (PHA-P; Murex), 20 ng/ml staphyloccocal enterotoxin B (SEB; Serva), and rat spleen Con A supernatant (10%) served as a source of IL-2 using the QuickChange Site-Directed Mutagenesis Kit (Stratagene) and appropriate mutation primers (see below), point mutations were introduced into the α chain's CDR1 and 3 sequences. All α chain constructs, including the wild-type T913 α chain, were then excised with EcoRI and BamHI and recloned into the pLXSN retroviral vector (neomycin resistance) as described by Backstrom et al. (26) and Naeher et al. (27). Corre-
The Journal of Experimental Medicine
1347
Gamerdinger et al.

TGTGCTCTGGCTGGTATACCGGAC; MutVA22CDR3G-P
s: CTTCTGTGCTCTGGCAG; MutVA22CDR3T-A
s: GTGTGCTCTGGCTGGTC; MutVA22CDR3Y-A
s: TGTGCTCTGGCTGGTACCGGCAC; MutVA22CDR3G-P
s: CTTCTGTGCTCTGCC
s: GCTCTGGGGTATG
s: CA; MutVA22CDR3Y-A
s: GTGCTCTGGGGTATG
s: CA; MutVA22CDR3Y-F
s: TGTGCTCTGGGGTATG
s: CA; MutVA22CDR3Y-H
s: TCTGTGCTCTGGGGTATG

IL-2 Secretion and Proliferation Assays. Supernatants of 20 h cocultures of $5 \times 10^5$/H11003
10^4 TCR transfectants and $5 \times 10^5$/X-irradiated
APCs with or without antigen were assayed for IL-2 by prolifera-
tion ($[^3H]$thymidine incorporation) of an IL-2–dependent T cell
line as previously reported (28). Antigen-specific proliferation of
clon SE9 was determined by incorporation of $[^3H]$thymidine as
previously described (19).

Results

Functional Characterization of Clone SE9 and Its TCR Ex-
pressed in the Mouse Hybridoma T913. The Ni-reactive
human T cell clone SE9 was isolated from the peripheral
blood of donor SE. It expresses a VA22/H11001/VB17/H11001
TCR, the
CD4 coreceptor, and the skin-homing molecule CLA (19
and unpublished data). Upon stimulation with NiSO$_4$, SE9
secretes large amounts of IL-4 and IL-5, less IL-10 and IL-
2, and little to no IFN-$\gamma$ (unpublished data). Expression of
the variable parts of the SE9 TCR, fused to murine con-
stant and
segments, together with human CD4 in the
mouse hybridoma 54/17 (21) resulted in the transfectant
T913, which possessed an identical pattern of specificity as
clon SE9 (19). Thus, the specificity of SE9 is defined ex-
clusively by its TCR.

Ni reactivity was determined by proliferation ($[^3H]$thymi-
dine incorporation) for clone SE9 and by IL-2 secretion
(proliferation of IL-2–dependent CTLL cells) for hy-
bridoma T913. Both stimuli were inhibited by mAbs to
HLA-DR, but not to HLA-DP nor HLA-DQ (Fig. 1, A and
B). However, in both cases, HLA-DR restriction was
highly promiscuous in that B cell lines from different do-
nors as well murine L cells transfected with a variety of in-
dividual HLA-DR alleles served as APC, albeit with vari-
able efficacy (Fig. 1 C; reference 19). These variations do
not relate to differences in MHC expression (unpublished
data), but probably reflect differences in the overall fit of
the SE9 TCR to the different HLA-DR alleles. In contrast,
HLA-DR53 was completely ineffective in mediating Ni
activation (Fig. 1 C).

TCR transfections had further shown that the T913 $\alpha$
chain could be combined with unrelated $\beta$ chains from
other human or even murine TCR without loss of its DR-
promiscuous Ni specificity (19). Although this implied that
the specificity of the T913 TCR is largely determined by
its AV22$/\beta$ chain, the activation is not due to a superanti-
gen-like coupling of HLA-DR by Ni to genomically de-
termined amino acids of AV22. A hybrid TCR containing
the T913 $\beta$ chain together with the AV22$\alpha$ chain of a
different Ni-reactive clone (clone 3.14; reference 17) was
expressed in transfectants comparably to the original TCR
in hybridoma T913 (Fig. 2, A and B). Both hybridomas re-
sponded to SEB stimulation, but only the SE9 TCR was
activated by NiSO$_4$ (Fig. 2, C and D). Hence, the Ni con-

Figure 1. Promiscuous HLA-DR restriction of hybridoma T913. (A)
Ni-specific proliferation of clone SE9 in the presence or absence of anti-
bodies against HLA-DR, HLA-DP, or HLA-DQ. (B) Effects of the same
antibodies on Ni-induced IL-2 production by hybridoma T913. (C)
Concentration-dependent Ni responses of T913 with HLA-DR–trans-
fected murine L cells or DR homozygous human B cells as APCs.

Figure 2. No superantigen-like usage of AV22 by nickel. Hybridoma
T913 (A and C) is compared with transfectant T314A/T913B (B and D)
combining the TCR $\beta$ chain of T913 with the $\alpha$ chain of the Ni-reactive
human T cell clone 3.14 (reference 17). The T314 $\alpha$ chain contains
AV22-like T913, but differs in joining and J sequences. (A and B) FACS
staining with FITC-conjugated anti-huV$\beta$17 (shaded) compared with
isotype control (open) cells. (C and D) IL-2 production of T913 (C) or
T314A/T913B (D) on HOM-2 alone (open bars) with $2 \times 10^{-4}$ M
NiSO$_4$ (solid bars), or with 20 ng/ml SEB (hatched bars).
Peptide-independent TCR-Ni-MHC Contacts apparently involve unique sequences of the T913 α chain, most likely within the V/J joining CDR3 loop.

**Ni Recognition in the Absence of Antigen Processing.** Clone SE9 as well as the transfectant T913 both reacted to Ni in the presence of glutaraldehyde-fixed as well as unfixed APC (Fig. 3, A and B). Thus, antigen processing was not required and cryptic self-peptides could be excluded as Ni-induced determinants for clone SE9. However, SE9 cells did not proliferate in response to APC (fixed or unfixed), which had been preincubated with NiSO₄ for 1 h and subsequently washed (pulsed APC; Fig. 3 C). These findings, indicating the necessity of permanent availability of Ni ions in the medium, argue against the existence of preformed Ni-MHC determinants for the SE9 TCR.

**Ni Recognition Is Independent of the Nature of MHC-associated Peptides.** The promiscuous HLA-DR restriction infers that the nature of the DR-associated Ni epitopes might be independent of the sequences of DR-associated peptides. To directly address this question, we stimulated T913 cells in the presence of a series of human 293 cell transfectants with HLA-DRB₁*0101 as well as with different human Ii constructs (24). These constructs encoded either wild-type Ii, containing the original CLIP sequence, or an Ii variant in which CLIP was replaced either by the DRB₁*0101-restricted peptide HA₃₀⁷–₃₁⁹ of the influenza hemagglutinin or by its mutated variant in which Lys₃₁₆ had been replaced by His (HA(K>H); see legend to Fig. 4 for sequences). In the Ii-HA₃₀⁷–₃₁⁹ transfectants, the vast majority of DR1 molecules have been shown to present different length variants of the artificially introduced HA peptide sequence in their peptide binding grooves (24).

As shown in Fig. 4 A, all three 293 transfectants were absolutely comparable in presenting Ni to T913, and the extent of this activation was similar to Ni responses induced by DR1-expressing human B cells (Fig. 3 B). Ni specificity of the reaction is demonstrated by the lack of responses in the absence of NiSO₄ (Fig. 4 B). In experiments not shown here we demonstrated that transfectants expressing either one of the two HA peptide variants, but not CLIP, both effectively stimulate DR1-restricted human T cell clones induced by HA₃₀⁷–₃₁⁹. In this context it is worth noting that unlike the results of Romagnoli et al. (10), these reactions were not inhibited by the addition of Ni, even when the His-containing mutant peptide was presented.

**His₈₁ in the DR1 β Chain, A Possible Contact Site for Nickel.** As shown above, Ni reactivity of the SE9/T913 TCR is restricted by human HLA-DR molecules encoded by a variety of different DR alleles (Fig. 1 B) and is not affected by the nature of DR-associated peptides (Fig. 4). Therefore, we assumed that a Ni coordination complex

---

**Figure 3.** Effects of fixation and antigen pulsing on Ni presentation by APC. 2 × 10⁵/well JESTHOM B cells were fixed or not with 0.05% glutaraldehyde for 45 s at room temperature in RPMI without FCS and used for presentation of Ni to clone SE9 or hybridoma T913. (A) Proliferation of SE9. (B) IL-2 production by T913. □Δ, in absence; ○●, in presence of 10⁻⁴ M NiSO₄ (clone SE9) or 2.5 × 10⁻⁴ M NiSO₄ (hybridoma T913). ○● for unfixed and □Δ for fixed APCs. (C) APCs, fixed or unfixed, were either untreated (open bars) or pulsed (solid bars) with 10⁻⁴ M NiSO₄ for 1 h, washed, and then coincubated with SE9 cells. Control cultures (gray bars) contained 10⁻⁴ M NiSO₄ in the medium.

**Figure 4.** Peptide-independent presentation of Ni to hybridoma T913. 5 × 10⁵/well T913 cells were incubated with 2.5 × 10⁻⁴ M NiSO₄ (A) or without Ni (B) in the presence of graded numbers of different 293 transfectants as APCs, and IL-2 production was determined as described in Materials and Methods. APCs were either untransfected (293 Contr) or transfected with HLA-DR1 plus human Ii construct (Ii-CLIP). In two cell lines, the CLIP sequence of Ii was replaced either by the hemagglutinin peptide 309–317 (Ii-HA₃₀⁹–₃₁⁷) or by a HA mutant in which K₃₁₆ was replaced by H (Ii-HA(K>H)). The core peptides presented by the respective DR1-expressing cells are: CLIP, MRMATPLLM; HA₃₀⁹–₃₁⁷, YVKQNTLKL; HA(K>H), YVKQNTLHL.
might bridge conserved amino acid side chains of the HLA-DR β chain with hypervariable amino acids of the TCR α chain. The lack of Ni presentation by HLA-DR53 (Fig. 1 C) pointed to histidine in position 81 of the DR β chain because DR53 is the only one of all DR alleles tested (Fig. 1 C) in which the highly conserved His81 is missing. Using L cells transfected either with wild-type DRB1*0101 or with DR1 harboring point mutations replacing His81 by Ala, Asp, Glu, or Tyr, we found (Fig. 5 A) that mutations of His81 to Ala, Asp, or Glu completely eliminated the presentation of Ni whereas mutation to Tyr strongly reduced, but did not eliminate reactivity. This result is not due to major structural distortions in the mutated DR1 molecules because the DR1-restricted human T cell clone HACoH8, specific for the hemagglutinin peptide HA309–317, responded to its antigen in the presence of APCs expressing either wild-type DR1 or DR1 molecules carrying the various mutations in position 81 (Fig. 5 B).

**Antigen Contacts within the TCR Hypervariable Sequences.** As demonstrated above, the Ni reactivity of the SE9/T913 receptor, although largely defined by its α chain (19), does not resemble a superantigen-like activation via the genomically defined VA22 sequence (Fig. 2). Therefore, to determine antigen contact sites within the T913 α chain we introduced a series of defined point mutations into all of its hypervariable regions, including N-nucleotide determined amino acids of the CDR3 loop. Table I depicts the amino acid sequences of the three hypervariable regions in single letter code and indicates the point mutations introduced by PCR technology. Unexpectedly, and unlike results of TCR mutations in other systems (29), most of these mutations do not, or only partially, reduce TCR reactivity to Ni (Figs. 6 and 7). Replacement of Lys94 by Ser in CDR2 had already been shown not to affect Ni recognition (19). Here we show that Ala mutations of the two aspartic acids in positions 53 and 54 (Fig. 6) also did not impair TCR specificity, making antigen contacts via the CDR2 loop rather unlikely.

The effects of CDR3 mutations are summarized in Fig. 7, A and B. These mutations included an elongation of CDR3α by insertion of Ala between positions 92 and 93, an Ala mutation of Thr95, and Ala, Phe, or His mutations of Tyr94 (Table I). As shown in Fig. 7 A, CDR3 elongation had absolutely no effect on Ni reactivity and the exchange of Thr95 for Ala only partially impaired the reaction. However, mutations of Tyr94 to Ala (Fig. 7 A), Phe, or His (Fig. 7 B), completely abolished antigen reactivity of the recombinant TCR.

For CDR1, Fig. 7 C reveals that exchange of Thr28 to Ala did not affect Ni recognition at all and even the Gly28 to Pro mutation, probably inducing a notable sterical alteration of the CDR1 loop, resulted in only partial reduction of reactivity. However, mutation of Tyr29 to Ala completely destroyed the reactivity for Ni (Fig. 7 C). A Tyr to Phe mutation was not tested in this position, but replace-

**Table I. Point Mutations in the TCR α Chain of Hybridoma T913**

<table>
<thead>
<tr>
<th>CDR1</th>
<th>CDR2</th>
<th>CDR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>48</td>
<td>92</td>
</tr>
<tr>
<td>AV22 wild-type</td>
<td>TATGYP</td>
<td>KATKADDK</td>
</tr>
<tr>
<td>AV22 mutated</td>
<td>- - A - - - -</td>
<td>- - S - - - -</td>
</tr>
<tr>
<td></td>
<td>- - P - - - -</td>
<td>- - - A A -</td>
</tr>
<tr>
<td></td>
<td>- - - A - - -</td>
<td>- - A - - - -</td>
</tr>
<tr>
<td></td>
<td>- - - - H - -</td>
<td>- - - - H -</td>
</tr>
</tbody>
</table>

*aPosition of first amino acid in each of the CDR sequences. CDR1, CDR2, and CDR3 are as previously defined (reference 47).

*bAmino acid sequences in single letter code. Targets for point mutations in bold.

*cAmino acid exchanges in 11 individual TCR α mutants. Dots indicate identity with wild-type sequence.
ment of Tyr29 by His left the TCR reactivity surprisingly unaltered (Fig. 7 D). Hence, antigen contact is clearly mediated by Tyr29 and its functional replacement by His points toward a direct involvement of Ni in this contact.

In this context it is important to note that all mutated TCR α chains effectively paired with the T913 β chain and the resulting TCR were expressed well on the surface of transfected hybridomas (unpublished data). Regardless of their reactivity or nonreactivity to Ni, all TCR transfectants were strongly activated by the VB17-reactive superantigen SEB (unpublished data).

**Discussion**

HLA-restricted αβ T cells with specificity for Ni ions have been isolated from peripheral blood as well as from skin lesions of Ni-allergic patients (20, 30–32) and are regarded as essential elements in nickel-contact dermatitis (33). The finding that Ni blocked MHC-restricted T cell recognition of a His-containing peptide has lead to the assumption that Ni-specific T cells recognize complexes of Ni with HLA-associated peptides (10). This rather indirect evidence was supported by our observation that some T cell clones reacted to Ni in the context of their respective HLA restriction element on one type of APC, but not on others (28). More recently, Lu et al. (34) demonstrated the requirement of particular (though not yet identified) peptides for presentation of Ni to one of our previously described AV1/BV17+ T cell clones (ANi2.3). It appears from these data that Ni-induced epitopes for T cells may, indeed, be formed by a complex of Ni ions with selective MHC-associated peptides in a hapten-like fashion. The same study also revealed a significant contribution of His81 in the β chain of HLA-DR52 in forming a Ni/MHC/peptide epitope for clone ANi2.3. However, although His81 is conserved in most HLA-DR β chains, clone ANi2.3 is selectively restricted to only one DR allele (34, 35). This might be related to the restrictive requirements concerning the sequences of DR-associated peptides (34) as well as to the previously described requirement for an Arg-Asp motif in the CDR3β loop of the ANi2.3 TCR (35). 

In this case with peptide-specific TCR, the receptor of clone ANi2.3 will recognize its specific epitope on only a small fraction of MHC molecules expressed on APCs, a number even more reduced by the very low Ni concentrations available in vivo.

However, such hapten-like interaction with MHC–peptide complexes is clearly not the only way for Ni to activate T cells. Here we describe clone SE9 (or hybridoma T913) as an example of a quite different type of TCR–Ni–MHC interaction. This receptor reacts to Ni independently of the nature of MHC–associated peptides (Fig. 4) or of the presenting HLA-DR allele (Fig. 1). Essential antigen contact sites had previously been shown to localize to the α chain of the SE9 TCR (19) and antigen presentation by processing inactive APCs pointed to Ni ions as part of the antigenic epitope (Fig. 3). We have identified the conserved His81 residue in HLA-DR β chains as a major contact site for the SE9 TCR. The fact that the same residue is essential for Ni presentation to clone ANi2.3 (34) strongly points to His81 in HLA-DR β chains as a major coordination site for Ni. The lack of peptide participation in Ni presentation and the involvement of the conserved His81 may explain the promiscuous DR restriction of clone SE9 (Figs. 4 and 5). His81 has been recognized as a site for Zn coordination.

**Figure 6.** Ni responsiveness of a CDR2α-mutated T913 receptor. Comparison of wild-type (T913 wt) and mutated TCR transfectants stimulated with L-DR1 cells in the absence (dotted bars) or presence (solid bars) of $2 \times 10^{-5} \text{M NiSO}_4$. Secreted IL-2 was determined as described in Materials and Methods. D13 and D15 in CDR2α were replaced by alanines in the mutant. Refer to Table I for sequences.

**Figure 7.** Essential tyrosines in CDR1 and CDR3 of the T913 α chain. Ni-induced IL-2 responses of T913 as compared with transfectants mutated in CDR3 (A and B) or CDR1 (C and D) of their TCR α chains. L-DR1 cells were used as APC. (A) CDR3 mutated by Ala insertion between positions 92 and 93 (+A) or Ala replacements of T95 (T95-A) or Y94 (Y94-A). (B) Exchange of Y94 in CDR3 for Phe (Y94-F) or His (Y94-H), fixed concentration of $2 \times 10^{-5} \text{M NiSO}_4$. (C) CDR1 mutated by Ala replacements of T27 (T27-A) or Y29 (Y29-A), or by Pro exchange for G28 (G28-P). (D) Exchange of Y29 in CDR1 for His (Y29-H). All transfectants expressed comparable amounts of TCR and regardless of their responses to Ni reacted strongly to stimulation with SEB (not depicted). For TCR sequences refer to Table I.
in the context of Zn-dependent superantigens (23, 36–38). However, the finding that it participates in direct presentation of metal ions such as Ni to DR allele-specific as well as to DR-promiscuous Ni-reactive T cells is of particular interest in the context of metal allergies. His81 is potentially one of the central attachment sites for allergenic Ni epitopes. Epitope involving additional Ni coordination sites on DR-associated peptides would be more likely restricted to defined DR alleles, like the one recognized by clone ANi2.3, whereas peptide-independent clones such as SE9 would tend to be DR promiscuous.

The two clones further differ in that Ni-epitopes specific for clone SE9 (Fig. 3), but not those for clone ANi2.3 (35), are lost by washing of Ni-treated APCs. Again, this might be explained by the different role of peptides in epitope formation. Although Lu et al. (34) presented evidence for stable Ni–MHC coordination complexes involving His81 as well as peptide-determined amino acids, we found no peptide contribution to SE9-specific epitopes and, hence, probably fewer coordination sites on the DR surface. In fact, we not only were unable to prepulse APC with Ni for clone SE9 (Fig. 3), but also prewashing with Ni of the T cells, i.e. SE9 or T913, did not mediate reactivity (unpublished data). Thus, neither APC nor the SE9 TCR alone express enough coordination sites to bind Ni tightly enough to prevent its dissociation in the absence of surplus Ni2+. Therefore, we assume that only short-lived and normally unproductive complexes of the SE9/T913 TCR with HLA-DR provide a sterically perfect arrangement of amino acids (including His81) to accommodate Ni in a stable coordination complex. In that sense we envisage Ni like a bolt that may connect two parts only during a state of accurate positioning, requiring its permanent presence in the surrounding medium.

Concerning the role of the TCR α chain in this complex, we excluded a superantigen-like interaction via germ-line-determined sequences of the AV22 element (Fig. 2). Ala mutations within the three α chain hypervariable regions identified two absolutely essential Tyr residues: one in position 29 of the germ-line-encoded CDR1 common to all AV22 sequences, and the other in the N-nucleotide–determined position 94 of the CDR3, which is unique to SE9 (Fig. 7 and Table I). Interestingly, the crystal structure of a TCR/HAS0–319-HLA-DR1 complex (39) reveals close contacts between residue Pro29 in the TCR CDR1α loop and the His81 in the HLA-DR1 β chain helix. The fact that our SE9 TCR reacts to Ni on the very same HAS0–319-HLA-DR1 complex suggests a similar orientation, including close proximity between the TCR α chain residue Tyr29 and His81 in HLA-DR1.

The potential role of tyrosine residues as coordination sites for Ni in our TCR–MHC complex is corroborated by several additional observations. On the one hand, Tyr29 could be replaced by His, probably the best chelator of Ni, without any loss of specificity (Fig. 7 D). On the other hand, the exchange of Tyr64 by Phe destroyed reactivity, indicating that not the hydophobic properties of the aromatic ring, but rather the p-hydroxy group of Tyr determines Ni specificity. Finally, the only mutation of His81 in the DR β chain that left the Ni-presenting properties at least partially intact was a change to Tyr (Fig. 5).

Even though Tyr is not generally regarded as a ligand for Ni, it has been reported as a coordination partner for Cu in bacterial RNase (40). Moreover, in the Ni complex of bacterial urease two of six Ni coordination sites are occupied by oxygens of water molecules (41, 42). The more acidic OH group of Tyr would thus certainly appear as a possible electrophilic partner in a complex. However, a definitive decision as to whether the identified amino acids in TCR and MHC indeed serve as coordination sites for Ni or rather are involved in protein–protein interactions can only be made on the basis of a crystal structure of the complete complex.

No specificity could be assigned to the CDR2 loop of the T913 α chain (Fig. 6). Even though position 51 of several TCR α chains has been reported to be essential for MHC contacts (43, 44), Lys51 in the SE9 α chain has been shown not to be required for Ni reactivity (19). Similarly, Ni specificity was not impaired by mutation of Asp53 and Asp54 in CDR2 (Fig. 6), although aspartic acid has been described to be involved in metal-mediated superantigen binding (38) as well as in the binding site for Ni in human serum albumin (45, 46).

All together, our findings reveal the TCR of clone SE9 as an example of a so far unpredicted possibility for antigen-specific and MHC-restricted T cell activation. Metal ions, and possibly other low molecular weight compounds as well, may form and stabilize intramolecular bridges between TCR and MHC, independent of the primary structure of MHC-associated peptides. Most superantigens achieve a similar connection by bridging nonpolymorphic MHC regions to VB family-specific sequences of the TCR. In the SE9 TCR, in contrast, Ni ions require an N-region–determined Tyr in the α chain CDR3 loop, i.e. within the idiotypic TCR sequences. The flexibility of such interactions is stressed by our finding that introduction as well as removal of the structural constraints of proline residues within the CDR1 or CDR3 loops was well tolerated, and that even elongation of CDR3 by one amino acid did not affect Ni reactivity (Fig. 7).

We detected these mechanisms due to the exceptional features of clone SE9, particularly the dominance of its TCR α chain in determining specificity. It is well conceivable that other clones might use both α and β chains of their TCR to form similar peptide-independent complexes, however, their identification would be extremely more difficult. Relating to Ni allergy, the impact of such types of TCR is apparent. APCs present significantly more Ni epitopes to peptide-independent than peptide-dependent T cells, a difference further increased for clones like SE9 recognizing Ni on different HLA-DR alleles. Even if such receptors represent a minority within the Ni-reactive repertoire, their effectiveness and amplifying properties in skin responses to Ni might be significant. The question whether the proposed model also applies to the allergic cross-reactivities to other metals remains unanswered be-
cause clone SE9 did not cross-react to Cu, Pd, Co, nor Cr (unpublished data).

The authors wish to thank Dr. E. Palmer for supply of retroviral vectors and technical advice, Dr. Jörg Vollmer for fruitful discussions, and Dr. Ian Haidl for critically revising the manuscript.

This work was supported by the German Ministry of Education and Research within the Clinical Research Group “Pathomechanisms in Allergic Inflammation” (BMBF FKZ 01GC0102).

Submitted: 23 January 2003
Revised: 24 March 2003
Accepted: 7 April 2003

References


