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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

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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$^+$ 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 Tyr_{29} in CDR1α, an *N*-nucleotide–encoded Tyr_{94} in CDR3α, and a conserved His_{81} 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 His_{81} 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

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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 expressing glutamate in position 69 of the DP β chain (14–16). Glu_{69} has been implicated in the binding of Be (14) as well as of Co (11).

We have chosen to study nickel as a model of metal-induced T cell activation because Ni represents one of the most common contact allergens in humans (4). This study concentrates on one particular $CD4^+$, Ni-reactive human T cell clone, clone SE9. This clone belongs to a group of TCRBV17-expressing T cells that we and others found overrepresented among Ni-specific $CD4^+$ T cells of patients suffering from particularly severe Ni contact hypersensitivity (17, 18). However, unlike $VB17^+$ clones from 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 $NiSO_4 \times 6H_2O$, 1 μg/ml phytohemagglutinin (PHA-P; Murex), 20 ng/ml staphylococcal enterotoxin B (SEB;* Serva), and rat spleen Con A supernatant (10%) served as a source of IL-2 to maintain CTLL cells. Growth medium for T cell hybridomas (RPMI-FCS) was RPMI 1640 supplemented with 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol (Roth), 10% heat-inactivated FCS, and 10 mM Hepes (GIBCO BRL). Culture conditions for human T cell clones have been previously described (19, 20). Monoclonal antibodies against HLA-DR (L243; American Type Culture Collection [ATCC]), HLA-DP (B7.21; ATCC), or HLA-DQ (SVPL3; ATCC) were used as 1:10 diluted culture supernatants. Stainings for flow cytometry were performed with FITC-conjugated anti-huVβ17 (E17.5F3.15.13; Beckman Coulter).

Cell Lines and T Cell Clones. The Ni-specific human T cell clone SE9 and the murine hybridoma T913 carrying the SE9 TCR have been previously described (19). The T cell donor SE expressed HLA-DRB1*0101, DRB1*1501, and DR51. The TCR^- huCD4+ murine T cell hybridoma 54ζ17 (21) was provided by O. Acuto (Institut Pasteur, Paris, France). HLA-DR homozygous B cell lines JESTHOM (International Histocompatibility Workshop [IHW] no. 9004, DRB1*0101), HOM-2 (IHW no. 9005, DRB1*0101), WT47 (IHW no. 9063, DRB1*1302, DR52), and SWEIG (IHW no. 9037, DRB1*1101, DR52) were from the IHW. Mouse fibroblasts transfected with human HLA-DRB1*0101, DRB1*0401, DRB1*1501, or DR53, designated as L-DR1 (L544.H8), L-DR4 (L243.6), L-DR15 (L466.1), and L-DR53 (L257.6), were obtained from F. Sinigaglia (Roche Ricerce, Milano, Italy). The mouse fibroblast cell line Dap-3 (L cells) and wild-type or mutated HLA-DR1 liter cell transfectants (22, 23) as well as the DR1 cotransfectants of 293 cells with wild-type or mutated invariant chain (Ii; 24) have been previously described.

Expression Vectors for Wild-type and Mutated TCR. The constructs for the CDR2α mutated or wild-type TCR α genes were cloned into the pV2–15α vector (25) and transfected into 54ζ17 cells by electroporation as previously described (19). Mutation of the CDR2α amino acids Asp_{53} and Asp_{54} to Ala was obtained by overlapping PCR as previously described (19) using the following complementary primer pair (mutated nucleotides underlined, only sense orientation shown): GCCACGAAGGCTGCTGCCAAGGGAAG. For mutations in CDR1 and 3, cDNA's coding for TCR α and β chains were amplified by RT-PCR from RNA of hybridoma T913 using primers EcoHVA22 liter (sense) AAACCGgaattcCGGAGGAATGAACTATTCTCCAGG (EcoRI site in lower case) and CAendBam (antisense) TTCTCGCggatccGCGCAGACCTCAACTGGAC (BamHI site in lower case). Upon cloning into pCR®-Blunt (Invitrogen), inserts were sequenced using the Big Dye sequencing kit (Applied Biosystems). Sequences were read on a 310 Genetic Analyzer (Applied Biosystems). 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). Correspondingly, the wild-type T913 β chain was amplified by RT-PCR from T913 RNA using the primers EcoHVB17 liter (sense) AAACCGgaattcCGGAGGAATGAGCAACCAGGTGCT and CB2endBam (antisense) TTCTCGCggatccGCGCATGTCTCAGGAATTTTTTTT, and cloned into pLXSP (puromycin resistance; reference 27). We used the BOSC packaging cell line and followed published procedures (26) to express the various TCR αβ combinations in 54ζ17 hybridoma cells. TCR^+ cell lines were cloned by limiting dilution and assayed for IL-2 responsiveness to SEB or $NiSO_4$. Sequencing primers were huLVβ17 sense (ATGAGCAACCAGGTGCTCTGC), huVβ17 sense (TTTCAGAAAGGAGATATAGCT), huVα22 sense (CCTCCTGAAAGCCACGAAGGCTGA), Cα antisense (TGTCCTGAGACCGAGGATCT), murine Cβ antisense (TGATGGCTCAAACAAGGAGAC), as well as commercial M13f and M13r primers (Invitrogen). Mutation primers (only sense primers shown, mutated nucleotides underlined) were for CDR1: MutVA22CDR1G-P s: GTACACAGCCACACCATACCCTTCC; MutVA22CDR1T-A s: GTACACAGCCGCAGGATACCCTTC; MutVA22CDR1Y-A s: GCCACAGGAGCCCCTTCCCTTTTC; MutVA22CDR1Y-H s: CACAGCCACAGGACACCCTTCC. For CDR3: MutVA22CDR3+AvG s: CTTC-

Abbreviations used in this paper: Ii, invariant chain; SEB, staphylococcal enterotoxin B.

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TGTGCTCTGGCTGGGTATACCGGCAC; MutVA22CDR3G-P s: CTTCTGTGCTCTGCCGTATACCGG; MutVA22CDR3T-A s: GCTCTGGGGTATGCCGGCACTGCC; MutVA22CDR3Y-A s: GTGCTCTGGGGGCTACCGGCACTG; MutVA22CDR3Y-F s: TGTGCTCTGGGGTTTACCGGCA; MutVA22CDR3Y-H s: TCTGTGCTCTGGGGCATACCGG.

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

Results

Functional Characterization of Clone SE9 and Its TCR Expressed 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$^+$/VB17$^+$ 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-γ (unpublished data). Expression of the variable parts of the SE9 TCR, fused to murine constant α and β segments, together with human CD4 in the mouse hybridoma 54ζ17 (21) resulted in the transfectant T913, which possessed an identical pattern of specificity as clone SE9 (19). Thus, the specificity of SE9 is defined exclusively by its TCR.

Ni reactivity was determined by proliferation ([^{3}H]thymidine incorporation) for clone SE9 and by IL-2 secretion (proliferation of IL-2–dependent CTLL cells) for hybridoma 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 donors as well murine L cells transfected with a variety of individual HLA-DR alleles served as APC, albeit with variable 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 α chain could be combined with unrelated β 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$^+$ α chain, the activation is not due to a superantigen-like coupling of HLA-DR by Ni to genomically determined amino acids of AV22. A hybrid TCR containing the T913 β chain together with the AV22$^+$ α 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 responded to SEB stimulation, but only the SE9 TCR was activated by $NiSO_4$ (Fig. 2, C and D). Hence, the Ni con-

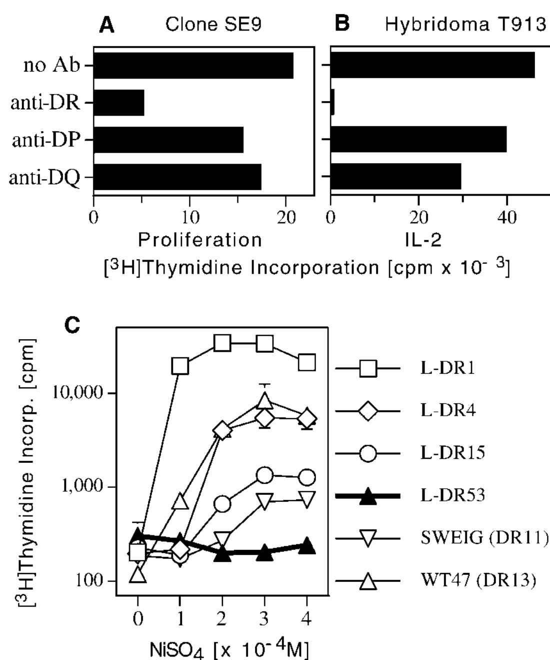

Figure 1. Promiscuous HLA-DR restriction of hybridoma T913. (A) Ni-specific proliferation of clone SE9 in the presence or absence of antibodies against HLA-DR, HLA-DP, or HLA-DQ. (B) Effects of the same antibodies on Ni-induced IL-2 production by hybridoma T913. (C) Concentration-dependent Ni responses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

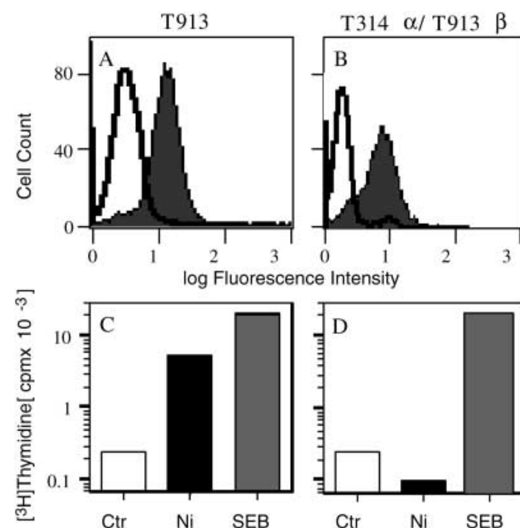

Figure 2. No superantigen-like usage of AV22 by nickel. Hybridoma T913 (A and C) is compared with transfectant T314A/T913B (B and D) combining the TCR β chain of T913 with the α chain of the Ni-reactive human T cell clone 3.14 (reference 17). The T314 α chain contains AV22 like T913, but differs in joining and J sequences. (A and B) FACS® staining with FITC-conjugated anti-huVβ17 (shaded) compared with isotype control (open) cells. (C and D) IL-2 production of T913 (C) or T314α/T913β (D) on HOM-2 alone (open bars) with 2×10^{-4} M $NiSO_4$ (solid bars), or with 20 ng/ml SEB (hatched bars).

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tacts 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 $NiSO_4$ 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 cells cotransfected 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 $HA_{307-319}$ of the influenza hemagglutinin or by its mutated variant in which Lys_{316} had been replaced by His (HA(K>H); see legend to Fig. 4 for sequences). In the Ii-$HA_{307-319}$ 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 $NiSO_4$ (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 $HA_{307-319}$. 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.

His_{81} 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

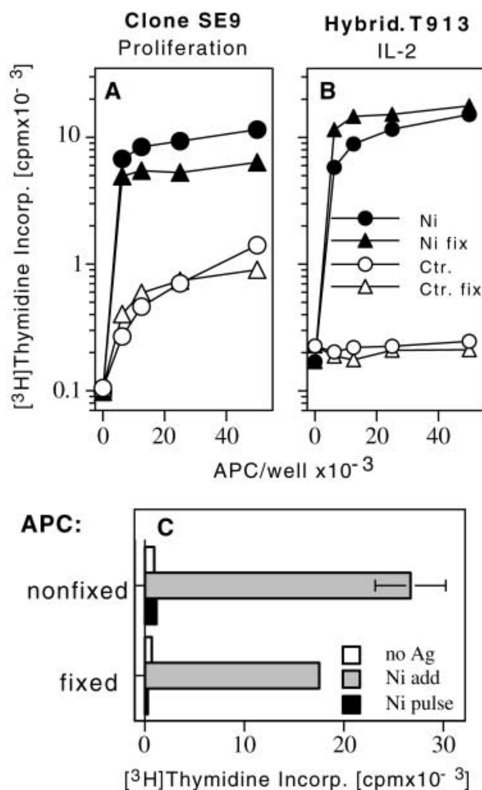

Figure 3. Effects of fixation and antigen pulsing on Ni presentation by APC. 2×10^4/well JESTHOM B cells were fixed or not with 0.05% glutaraldehyde for 45 s at room temperature in RPMI without FCS and used for presentation of Ni to clone SE9 or hybridoma T913. (A) Proliferation of SE9. (B) IL-2 production by T913. ○△, in absence, ●▲, in presence of 10^{-4} M $NiSO_4$ (clone SE9) or 2.5×10^{-4} M $NiSO_4$ (hybridoma T913). ○● for unfixed and △▲ for fixed APCs. (C) APCs, fixed or unfixed, were either untreated (open bars) or pulsed (solid bars) with 10^{-3} M $NiSO_4$ for 1 h, washed, and then coincubated with SE9 cells. Control cultures (gray bars) contained 10^{-4} M $NiSO_4$ in the medium.

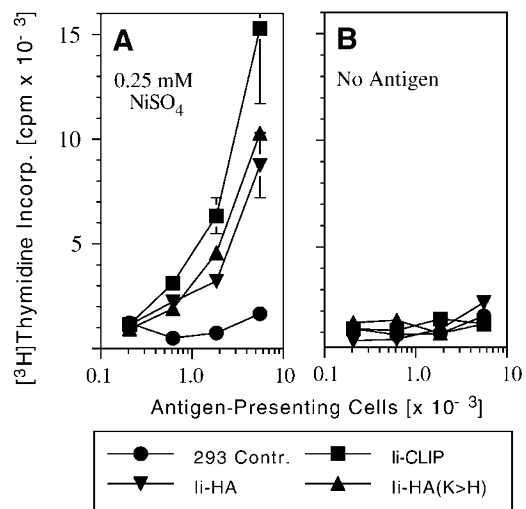

Figure 4. Peptide-independent presentation of Ni to hybridoma T913. 5×10^4/well T913 cells were incubated with 2.5×10^{-4} M $NiSO_4$ (A) or without Ni (B) in the presence of graded numbers of different 293 transfectants as APCs, and IL-2 production was determined as described in Materials and Methods. 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-$HA_{309-317}$) or by a HA mutant in which K_{316} was replaced by H (Ii-HA(K>H)). The core peptides presented by the respective DR1-expressing cells are: CLIP, MRMATPLLM; $HA_{309-317}$, YVKQNTLKL; HA(K>H), YVKQNTLHL.

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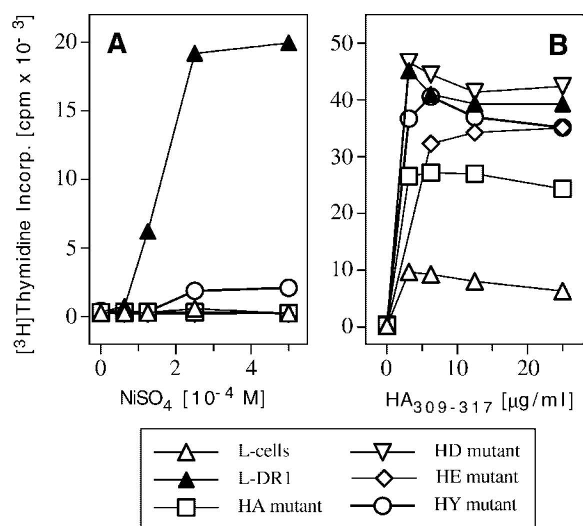

Figure 5. Effects of His_{81} mutation in DRB1*0101 on Ni and peptide presentation. (A) Ni-specific IL-2 production of T913 in the presence of L cells (DAP-3) transfected with DR1 wild-type (▲) or different His_{81} mutants of the DR1 β chain (as indicated in the figure). The small but significant response to the H-Y mutant (○) was repeated in several independent experiments. (B) Proliferative response of the DR1-restricted, hemagglutinin-specific T cell clone HACoH8 to $HA_{309–317}$ peptide on the different DAP transfectants. Untransfected DAP cells served as control APCs in both experiments (A and B).

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 His_{81} is missing. Using L cells transfected either with wild-type DRB1*0101 or with DR1 harboring point mutations replacing His_{81} by Ala, Asp, Glu, or Tyr, we found (Fig. 5 A) that mutations of His_{81} 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 $HA_{309–317}$, 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 Lys_{51} 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 Thr_{95}, and Ala, Phe, or His mutations of Tyr_{94} (Table I). As shown in Fig. 7 A, CDR3 elongation had absolutely no effect on Ni reactivity and the exchange of Thr_{95} for Ala only partially impaired the reaction. However, mutations of Tyr_{94} to Ala (Fig. 7 A), Phe, or His (Fig. 7 B), completely abolished antigen reactivity of the recombined TCR.

For CDR1, Fig. 7 C reveals that exchange of Thr_{27} for Ala did not affect Ni recognition at all and even the Gly_{28} to Pro mutation, probably inducing a notable sterical alteration of the CDR1 loop, resulted in only partial reduction of reactivity. However, mutation of Tyr_{29} 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*

	CDR1	CDR2	CDR3
a.a. position[a]	25	48	92
AV22 wild-type[b]	TA**TGY**PS	KAT**K**A**DD**K	L G**YT**GTASKLT
AV22 mutated[c]	- - A - - - -	- - - S - - - -	- A - - - - - - - - - -
	- - - P - - -	- - - - - A A -	- - - A - - - - - - -
	- - - - A - -		- - A - - - - - - - -
	- - - - H - -		- - H - - - - - - - -

[a]Position of first amino acid in each of the CDR sequences. CDR1, CDR2, and CDR3 are as previously defined (reference 47).
[b]Amino acid sequences in single letter code. Targets for point mutations in bold.
[c]Amino acid exchanges in 11 individual TCR α mutants. Dots indicate identity with wild-type sequence.

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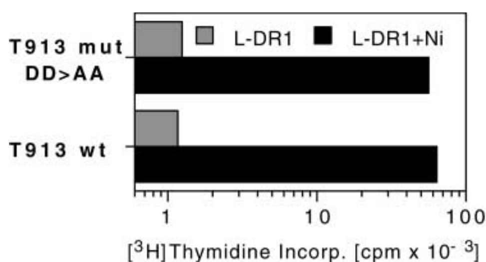

Figure 6. Ni responsiveness of a CDR2α-mutated T913 receptor. Comparison of wild-type (T913 wt) and mutated TCR transfectants stimulated with L-DR1 cells in the absence (dotted bars) or presence (solid bars) of 2×10^{-4} M $NiSO_4$. Secreted IL-2 was determined as described in Materials and Methods. D_{53} and D_{54} in CDR2α were replaced by alanines in the mutant. Refer to Table I for sequences.

ment of Tyr_{29} by His left the TCR reactivity surprisingly unaltered (Fig. 7 D). Hence, antigen contact is clearly mediated by Tyr_{29} 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).

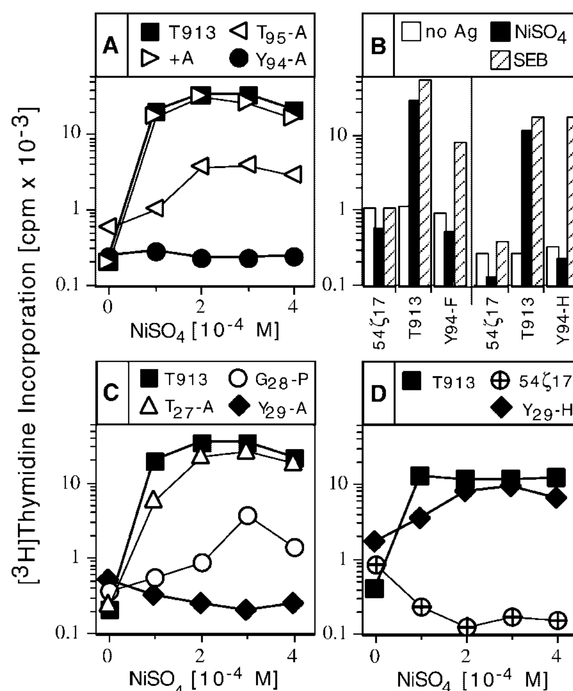

Figure 7. Essential tyrosines in CDR1 and CDR3 of the T913 α chain. Ni-induced IL-2 responses of T913 as compared with transfectants mutated in CDR3 (A and B) or CDR1 (C and D) of their TCR α chains. L-DR1 cells were used as APC. (A) CDR3 mutated by Ala insertion between positions 92 and 93 (+A) or Ala replacements of T_{95} (T_{95}-A) or Y_{94} (Y_{94}-A). (B) Exchange of Y_{94} in CDR 3 for Phe (Y_{94}-F) or His (Y_{94}-H), fixed concentration of 2×10^{-4} M $NiSO_4$. (C) CDR1 mutated by Ala replacements of T_{27} (T_{27}-A) or Y_{29} (Y_{29}-A), or by Pro exchange for G_{28} (G_{28}-P). (D) Exchange of Y_{29} in CDR1 for His (Y_{29}-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.

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 His_{81} in the β chain of HLA-DR52 in forming a Ni/MHC/peptide epitope for clone ANi2.3. However, although His_{81} 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). As in the 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 His_{81} 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 His_{81} 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 His_{81} may explain the promiscuous DR restriction of clone SE9 (Figs. 4 and 5). His_{81} has been recognized as a site for Zn coordination

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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. His_{81} is potentially one of the central attachment sites for allergenic Ni epitopes. Epitopes 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 His_{81} 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 prepulsing with Ni of the T cells, i.e. SE9 or T913, did not mediate reactivity (unpublished data). Thus, neither APCs nor the SE9 TCR alone express enough coordination sites to bind Ni tightly enough to prevent its dissociation in the absence of surplus Ni^{2+} in the medium. 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 His_{81}) 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 germline-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 germline-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 $TCR/HA_{307-319}$–HLA-DR1 complex (39) reveals close contacts between residue Pro_{29} in the TCR CDR1α loop and the His_{81} in the HLA-DR1 β chain helix. The fact that our SE9 TCR reacts to Ni on the very same $HA_{307-319}$–HLA-DR1 complex suggests a similar orientation, including close proximity between the TCR α chain residue Tyr_{29} and His_{81} 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, Tyr_{29} 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 Tyr_{94} by Phe destroyed reactivity, indicating that not the hydrophobic properties of the aromatic ring, but rather the p-hydroxy group of Tyr determines Ni specificity. Finally, the only mutation of His_{81} 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), Lys_{51} 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 Asp_{53} and Asp_{54} 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 Vβ 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).

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