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Functional Expression and Analysis of a Human HLA-DQ Restricted, Nickel-Reactive T Cell Receptor in Mouse Hybridoma Cells

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Nickel-induced contact dermatitis represents a T cell mediated delayed type hyperreactivity. The elucidation of the molecular basis of T cell activation by Ni^{2+} ions may serve as a model for the understanding of other metal allergies. We describe here the expression of hybrid T cell antigen receptor (TCR) α- and β-genes, containing rearranged human Ni-reactive variable and mouse constant regions, together with human CD4 in a mouse T cell hybridoma. The resulting hybridoma specifically responds to IL-2 secretion to Ni, but not to other metal ions in the presence of HLA-matched antigen-presenting cells. Loss of CD4 decreases, but does not completely abrogate this reactivity. The restricting HLA-DQ element is identified as consisting of DQA1*0101 and DQB1*0501; however, only some of the B cell lines homozygous for these molecules effectively present Ni to the hybridoma. We interpret these data to show that (i) Ni-reactivity is definitely mediated by αβ TCR variable regions; (ii) as for peptide-specific TCR, the CD4 co-receptor enhances Ni-reactivity, but is not absolutely essential; (iii) Ni^{2+} ions like nominal peptide antigens require HLA (here class II) molecules of the APC for presentation; (iv) the restricting molecule may require a special conformation or the association with a particular type of peptide or an as yet unidentified other surface structure on the antigen-presenting cell for effective Ni-presentation. ***Key words: TCR/nickel-specificity. J Invest Dermatol 113:175–181, 1999***

The αβ type of the T cell antigen receptor (TCR) is typically activated by major histocompatibility complex (MHC)-associated peptides on the surface of antigen-presenting cells (APC) (Jorgensen *et al*, 1992; Germain, 1994). The recent literature, however, also reveals examples for αβ as well as γδ TCR that interact with non-peptide molecules such as carbohydrates, pyrophosphates, or lipoglycans (Corinti *et al*, 1997; Harding *et al*, 1993; Haurum *et al*, 1994; Morita *et al*, 1995; Sieling *et al*, 1995; Tanaka *et al*, 1995). Haptens in the form of drugs or chemicals in cosmetics or the environment represent major sources of allergens or contact sensitizers (Dupuis and Benezra, 1982). Such contact sensitivities to haptens are initiated and determined in their specificity by hapten-specific T cells (Kapsenberg *et al*, 1992). The most common form of contact dermatitis in the caucasian population is induced by metal ions from nickel-containing alloys (e.g., in jewellery and clips) and is known as nickel allergy (Basketter *et al*, 1993; Schaller *et al*, 1994). T cells from the peripheral blood or skin lesions of Ni-allergic patients have been shown to proliferate in response to $NiSO_4$ and to require HLA-matched APC (Sinigaglia *et al*, 1985; Kapsenberg *et al*, 1987; Emtestam *et al*, 1989; Moulon *et al*, 1995). It has therefore been argued that Ni^{2+} ions, which form square-planar coordination complexes with amino acids of certain proteins or peptides (Martin, 1988), may function like covalently reactive haptens to form antigenic epitopes for T cells (Romagnoli *et al*, 1991; Basketter *et al*, 1995).

Indeed, mouse model-haptens, such as di- or trinitrophenyl reagents, urushiol, p-azobenzene-arsonate, and others, have been shown to form covalent conjugates with MHC-associated peptides as epitopes for T cells (Nalefski and Rao, 1993; Romero *et al*, 1993; Weltzien *et al*, 1996; Gelber *et al*, 1997). Also in the case of human penicillin allergy, HLA-binding penicilloyl peptides have been identified as T cell antigenic determinants (Padovan *et al*, 1997). In contrast, the structural identity of the antigenic epitopes that activate Ni-reactive T cells remains obscure. So far, it is unclear whether the metal ions are actually part of the antigenic determinants or only indirectly involved in their production (Sinigaglia, 1994; Lepoittevin and Leblond, 1997). It also remains to be investigated how strictly the phenomenon of MHC restriction applies to metal reactive T cells and whether the TCR is the only structure to mediate Ni-reactivity.

In murine systems, hapten-reactive T cell hybridomas have greatly contributed to the elucidation of relevant T cell epitopes as well as of their molecular interactions with the respective TCR (Nalefski *et al*, 1990; von Bonin *et al*, 1993). Because human T cells do not form stable T cell hybridomas, this study deals with the functional co-expression of TCR α- and β-chain genes isolated

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Abbreviation: CDR, complementarity determining region.

from human, Ni-reactive T cell clones together with human CD4 in a receptor-deficient mouse T cell hybridoma line.

MATERIALS AND METHODS

T cell stimulants and growth factors The following reagents were applied at the indicated concentrations, if not specified otherwise: $NiSO_4 \times 6H_2O$, 10^{-4} M; $CoCl_2 \times 6H_2O$, 5×10^{-5} M; $ZnSO_4 \times 7H_2O$, 7.5×10^{-6} M; $CuSO_4 \times 5H_2O$, 10^{-5} M; $PdCl_2$, 5×10^{-4} M (all obtained from Sigma, Deisenhofen, Germany); phytohemagglutin (PHA-P) (Murex, Dartford, U.K.), 1μg per ml; bacterial superantigen SEC2 (staphylococcal enterotoxin C2) (Serva, Heidelberg, Germany), 0.1μg per ml. Rat ConA supernatant, a 24 h supernatant of ConA-stimulated rat spleen cells, served as a source of IL-2 to maintain CTLL cells (Grabstein *et al*, 1986).

Antibodies and flow cytometry The following hamster monoclonal antibodies (MoAb) were used for flow cytometry: anti-murine CD3ε (145–2C11) (Leo *et al*, 1987) and anti-murine TCRαβ (H57–597) (Kubo *et al*, 1989). Mouse anti-human MoAb used included: FITC-conjugated and non-conjugated TCRBV13S6 (JU74.39), FITC-conjugated CD4 (13B8.2), HLA-DQ (SLVP3), FITC-conjugated ICAM-1 (84H10), FITC-conjugated CD80 (MAB104), FITC-conjugated ICAM-2 (B-T1), FITC-conjugated CD58 (AICD58), FITC-conjugated CD11a (25.3.1), PE-conjugated CD40 (MoAb89), and PE-conjugated ICAM-3 (HP2/19) (all from Immunotech, Marseilles, France), FITC-conjugated HLA-DR (B8.12.2) (Becton-Dickinson, San Jose, NM), and Biotin-conjugated CD86 (IT2.2) (Pharmingen, San Diego, NM). Secondary antibodies used were: FITC-conjugated goat anti-mouse Ig and goat anti-hamster Ig (Dianova, Hamburg, Germany). Streptavidin-RED670TM (Gibco/BRL, Eggenstein, Germany) and FITC-conjugated mouse IgG1 (MOPC-21) (Sigma) were also used. For flow cytometric analysis, 2×10^5 cells were stained at 4°C in 96 well round bottom plates either directly with a fluorescence-labelled MoAb or with an unlabelled MoAb, followed by staining with the appropriate secondary MoAb. Fluorescence was determined in a FACScan instrument (Becton-Dickinson). For sorting of hybridomas, transfectant 54PPN8 was stained with CD4 MoAb and co-receptor loss variants were sorted with a FACS sorter (Becton-Dickinson).

Cells and growth media The Ni-specific human CD4-positive T cell clone PPN82 was isolated from the Ni-allergic donor PPN (Pistoor *et al*, 1995) and was cultured as described previously (Moulon *et al*, 1995). The murine T cell hybridoma 54ζ17 was a gift of O. Acuto (Institut Pasteur, Paris, France), and has been described previously (Blank *et al*, 1993). This hybridoma is a variant of the TCR-negative T cell hybridoma $58\alpha^-\beta^-$ (Letourneur and Malissen, 1989), transfected with vectors for the human CD4 molecule (conferring resistance to mycophenolic acid) and the murine CD3ζ chain. All T cell hybridomas were maintained in RPMI 1640 supplemented with 2 mM L-glutamine, 100 μg kanamycin per ml (all from Gibco/BRL), 5×10^{-5} M 2-mercaptoethanol (Roth, Karlsruhe, Germany), and 10% heat-inactivated fetal calf serum (RPMI-FCS). The following EBV-transformed B cell lines were used as APC and known HLA-DR and -DQ haplotypes are given in parentheses: BG (DRB1*0101, DRB1*14); JESTHOM (DRB1*0101, DQA1*0101, DQB1*0501); EHM (DRB1*0101, DQA1*0101, DQB1*0501); LWAGS (DRB1*0102, DQA1*0101, DQB1*0501). In one set of experiments, additional nickel-specific T cells [the human T cell clone 3 and the T cell transfectant T913 (TCR of clone 9)] were used as responder cells. Both were derived from the donor SE.

Proliferation assay T cell clones (2×10^4 cells) were co-cultured in duplicate or triplicate with 2×10^4 irradiated (6000 rad) B cells (line BG, if not indicated otherwise) in 200 μl of complete RPMI 1640 with or without the different metal salts. After 48 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.

IL-2 secretion assay Hybridomas or transfectants (5×10^4 cells) were co-cultured for 20 h in triplication in 200 μl RPMI-FCS with 5×10^4 irradiated B cells (line BG, if not indicated otherwise) with or without the different metal salts. For stimulation with immobilized purified CD3ε MoAb or TCRBV13S6 MoAb, round-bottom microwells were incubated with different concentrations of either of the two mentioned MoAb in phosphate-buffered saline without fetal calf serum for 1 h at 37°C, washed, and T cell hybridomas were added at 5×10^4 cells per well in 200 μl RPMI-FCS. After 20 h at 37°C, 100 μl of the supernatant were used for a CTLL assay as described previously (Grabstein *et al*, 1986). In some experiments, APC were fixed as described by Shimonkevitz *et al* (1983). Briefly, B cells were suspended in 1 ml phosphate-buffered saline containing 0.05% glutaraldehyde (Gibco/BRL) for 45 s at room temperature. The reaction was stopped by adding 1 ml of 0.2 M L-lysine (Gibco/BRL) for an additional 45 s. Cells were then washed and in some experiments pulsed for 90 min at 37°C with 10^{-4} M $NiSO_4$ and washed before being used as APC as described above. For T cell restriction specificity, T cells were cultured with B cells, metal-salt, and either anti-DR (L243, ATCC), anti-DP (B7.21, ATCC), or anti-DQ (SVPL3, ATCC) MoAb as 1:10 culture SN. IL-2 secretion was determined as above.

PCR methodology and TCR expression vectors Oligonucleotide primers employed in this study were as follows: Leader Vα18, ACTCC-AGTGGCTCAGAAAATGGAGAAGAATCCTTTG; Jα-Splice-BamHI, GATC**GGATCC**acttacCTGGATTCACGGTTAAGG; mutα18/s, TTC-AGGCTGGGAACCTTTGAT; mutα18/as, ATCAAAGGTTCCCAGC-CTGAA; Pr-Vα4 CAATGTTTTTCACAGCTGTGCATCA; Pr-Vα18, CATTTTCTGAGCCACTGGAGTGTGTG; Leader Vβ13, ACCTGC-CTTGGTCCCAAGATGAGCATCAGCCTCCTG; Jβ-Splice-SalI-82, GATC**GTCGAC**tcttacCTGTCACAGTGAGCCTGGTCCCG; Pr-Vβ8-EcoRI, GATC**GAATTC**TTTGTGTAAGGAAGGGTGTGGT; Pr-Vβ13, CATCTTGGGACCAAGGCAGGTTCTTG; P5′DQA1, ATGG-TGTAAACTTGTACCAGT; P3′DQA1, TTGGTAGCAGCGGTAGA-GTTG; P5′RevM13DQB1, CAGGAAACAGCTATGACCCATGTG-CTACTTCACCAACGG; P3′DQB95011b, CAGGATCCCGCGGTA-CGCCA. Restriction sites for BamHI, SalI, and EcoRI are in bold print. The primer pair mutα18/s and mutα18/as was used to eliminate an endogenous BamHI site in the TCRAV18S2 element prior to the final cloning steps without altering the amino acid sequence (mutated nucleotides are underlined). Splice sites (indicated by lower-case letters) were introduced into the two primers Jα-Splice-BamHI and Jβ-Splice-SalI-82 to allow for correct splicing of the genomically arranged exons of the TCRA and TCRB constant regions. Standard PCR procedures included 36 cycles of 1.2 min at 95°C, 2 min at 55°C, and 2 min at 72°C in 100 μl volumes using 200 μM dNTP (Pharmacia, Freiburg, Germany), 10 μM of each primer, 1×Pfu buffer (Stratagene, Heidelberg, Germany), and 2.5U Pfu polymerase (Stratagene). Total RNA was extracted from 5×10^6 cells of clone PPN82, transcribed into cDNA, and screened by PCR for rearranged VA- and VB-elements as previously described to determine the TCRJ-elements and to ensure functionality of the rearrangements (Vollmer *et al*, 1997). The primer pairs P5′DQA1P3′DQA1 and P5′RevM13DQB1/P3′DQB95011b were used for specific amplification of the second exon of the DQA1 gene and of the DQB1*05 allele after DNA extraction (NatuTec, Frankfurt, Germany). PCR procedures for DQ amplification included 1 cycle of 2 min at 98°C, 5 cycles of 1 min at 95°C, 2 min at 60°C, and 1 min at 72°C followed by 32 cycles of 1 min at 95°C, 30 s at 55°C, and 1 min at 72°C. PCR was performed in 13 μl volumes using 20 μM dNTP, 1 μM of each primer, 2 mM $MgCl_2$ (Eurogentec, Seraing, Belgium), 1×Taq buffer (Eurogentec), and 0.5U Taq polymerase (Eurogentec). PCR reaction products were purified and sequenced. Sequencing was performed using the Big Dye terminator cycle sequencing ready action kit (Applied Biosystems, Warrington, U.K.) on the ABI automatic sequencer (Applied Biosystems). To replace the variable mouse TCR regions in the expression vectors pV2-15α and pV2-15β (Casorati *et al*, 1993), the mouse TCR sequences were excised by either SacI and BamHI (pV2-15α) or EcoRI and SalI for pV2-15β (all enzymes from Gibco/BRL) (**Fig 1*a***). Human variable regions, including the partially removed mouse promoter sequences, were constructed in a series of PCR reactions with overlapping intermediates (**Table I** and **Fig 1*b***). In the case of the α-chain, it included the mutational destruction of an internal BamHI restriction site by the primer pair mutα18. PCR product 3 was cloned into pTZ19 (Amersham) and sequenced (Vollmer *et al*, 1997) to ensure mutation of the BamHI restriction site. The final PCR products 5 and 8 were cloned into pTZ19, sequenced, excised with SaI/BamHI or EcoRI/SalI, respectively, and recloned into the digested vectors pV2–15α or pV2–15β. The resulting mouse/human hybrid expression vectors were designated pV2-82α18 and pV2-82β13 (**Fig 1*b***). These vectors were linearized for transfection with ClaI (α-vector) or EcoRI (β-vector).

Transfection of the mouse T cell hybridoma 54ζ17 Eight × 10^6 54ζ17 recipient cells were transfected with 1 μg pV2-82α18 and 4 μg pV2–82β13 in a Gene Pulse Cuvette (BIORAD, Hercules) by electroporation. After 48 h, the cells were selected in 1.5 mg G418 per ml (Gibco/BRL). Resistant cultures were analysed by FACS for surface expression of TCR/CD3.

RESULTS

Functional expression of a human Ni-specific TCR in a mouse T cell hybridoma The human $CD4^+$ T cell clone

a)

TCRV		CDR3		TCRJ
VA18S2	CA	FWSGGSNYKLT	FG	JA53
VB13S6A2T	CAS	SYAGYNSPLH	FG	JB1S6

b)

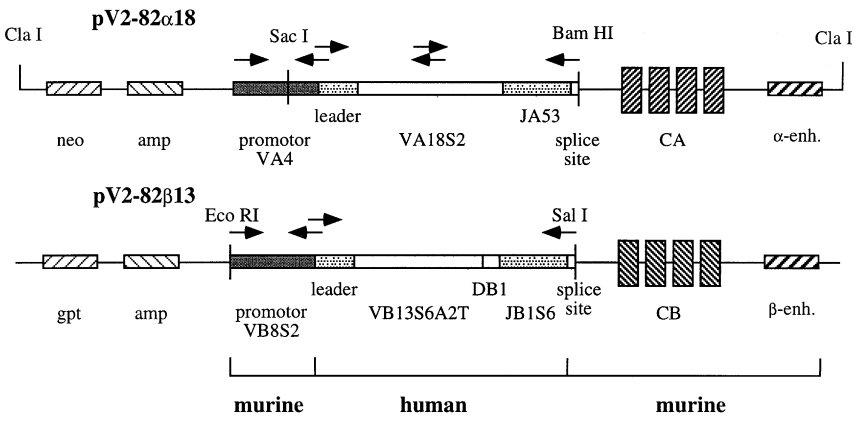

Figure 1. Amino acid sequences of TCR CDR3 regions of clone PPN82 and schematic representation of TCR expression vectors pV2–82α18 and pV2–82β13. (*a*) PCR amplified TCRA and TCRB chains of clone PPN82 were sequenced and amino acid sequences of the in-frame CDR3 regions are indicated using the single-letter code. The assignment of the CDR3 loops is according to Arden *et al* (1995). Amino acids encoded by N or D/N nucleotides contributing to each CDR3 region are underlined. The TCRAV18 element has not yet been described and sequence data of this new allele, termed TCRAV18S2, is available from EMBL under accession number Y16433, sequence data of the TCRB chain is available under accession number Y16434. (*b*) Constructs are based on the vectors pV2-15α and pV2-15β of Casorati *et al* (1993). The chimeric expression vectors consist of human variable regions and murine constant, promoter and enhancer regions of the T cell receptor as indicated. Vectors are shown in the linearized form and distances are not to scale. *Arrows* indicate the location of PCR primers used for construction (see *Materials and Methods* and **Table I**).

Table I. PCR protocols for construction of TCRA- and TCRB-expression vectors[a]

		Primers		
	PCR No.	sense	antisense	Template
TCRA chain	1	Leader Vα18	mutα18/as	PPN82, cDNA
	2	mutα18/s	Jα-Splice-BamHI	PPN82, cDNA
	3	Leader Vα18	Jα-Splice-BamHI	PCR 1 + 2
	4	Pr-Vα4	Pr-Vα18	pV2-15α
	5	Pr-Vα4	Jα-Splice-BamHI	PCR 3 + 4
TCRB chain	6	Leader Vβ13	Jβ-Splice-Sal I-82	PPN82, cDNA
	7	Pr-Vβ8-EcoRI	Pr-Vβ13	pV2–15β
	8	Pr-Vβ8-EcoRI	Jβ-Splice-Sal I-82	PCR 6 + 7

[a]For primer sequences see *Materials and Methods*. Approximate locations of primer sequences in the constructs are indicated by *arrows* in **Fig 1(*b*)**.

PPN82 (isolated from the peripheral blood of the Ni-allergic donor PPN) was screened by PCR for TCRA and TCRB use. One in-frame TCRB-chain (VB13S6A2T, DB1, JB1S6, CB1) and two TCRA-chains were identified, one of the latter (VAD14S1, JA44) was rearranged out of frame. The functional TCRA sequence contained a new member of the VA18 family (VA18S2) rearranged to JA53 (**Fig 1*a***). The surface expression of VB13 was confirmed by FACS analysis (**Fig 2*b***).

The rearranged V(D)J-regions of the functional TCRA- and TCRB-chains were amplified by PCR and cloned into previously described expression vectors (Casorati *et al*, 1993; von Bonin *et al*, 1996) as described in the *Materials and Methods* and **Table I**. The resulting vectors pV2-82α18 and pV2-82β13 (**Fig 1*b***) contained human variable sequences together with mouse constant and regulatory TCR sequences. They were transfected into the TCR-negative murine T cell hybridoma 54ζ17, which also expressed the human CD4 co-receptor (Blank *et al*, 1993), but no endogenous murine CD4 molecule (Glaichenhaus *et al*, 1991). This recipient cell line also lacks expression of endogenous TCRA and TCRB chains as shown in **Fig 2(*a*)**. A panel of transfectant lines was analysed for surface expression of TCR and co-receptor using MoAb against VB13S6 and human CD4. T cell hybridoma 54PPN8 was chosen as a representative hybridoma for further experiments as it homogeneously expressed the chimeric TCR as well as the human CD4 co-receptor. FACS data are shown in **Fig 2(*a*)** in comparison with the TCR donor clone PPN82 (**Fig 2*b***).

Functionality of the expressed TCR was assessed by stimulation with MoAb specific for either mouse CD3ε or human VB13S6 or with staphylococcal superantigen SEC2 in the presence of the irradiated B cell line BG. As shown in **Fig 2(*c*)**, the transfectant 54PPN8, but not the untransfected hybridoma 54ζ17, responded to each of the three stimuli.

Nickel-specificity and HLA-DQ restriction of the TCR transfectant The transfectant 54PPN8 as well as the TCR donor clone PPN82 were co-cultivated with the irradiated B cell line BG and graded concentrations of $NiSO_4$ (**Fig 3*a***). IL-2 secretion by the transfectant followed the same dose dependence of $NiSO_4$ as the proliferative response of the original clone. In contrast, the TCR-recipient cell line 54ζ17 was not stimulated by $NiSO_4$ in

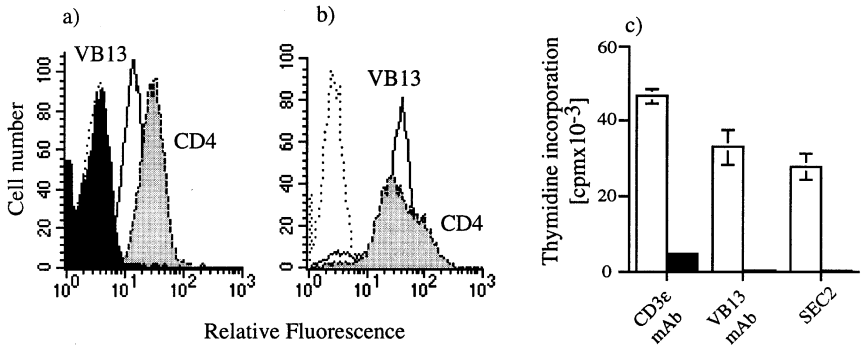

Figure 2. Phenotype and functional integrity of the transfectant 54PPN8 expressing a human/mouse chimeric TCR. (*a*,*b*) FACS histograms are shown for staining of transfectant 54PPN8, untransfected recipient cell line 54ζ17 (*a*), and of T cell clone PPN82 (*b*) with anti-TCRBV13S6 (open histogram) or anti-CD4 MoAb (shaded histograms). Staining of 54ζ17 with anti-TCRBV13S6 is shown by the left filled histogram in (*a*). Isotypic controls are indicated by the left dotted histograms. (*c*) Secretion of IL-2 upon culturing on immobilized anti-CD3ε MoAb (0.5 μg per well) and anti-TCRBV13S6 MoAb (0.5 μg per well) or in the presence of 0.1 μg SEC2 per ml and irradiated B cell line BG are shown for the TCR transfectant 54PPN8 (□) and the cell line 54ζ17 (■). IL-2 was determined in triplicate by [^{3}H]thymidine incorporation of CTLL cells and results are expressed as cpm±SD. For details see *Materials and Methods*.

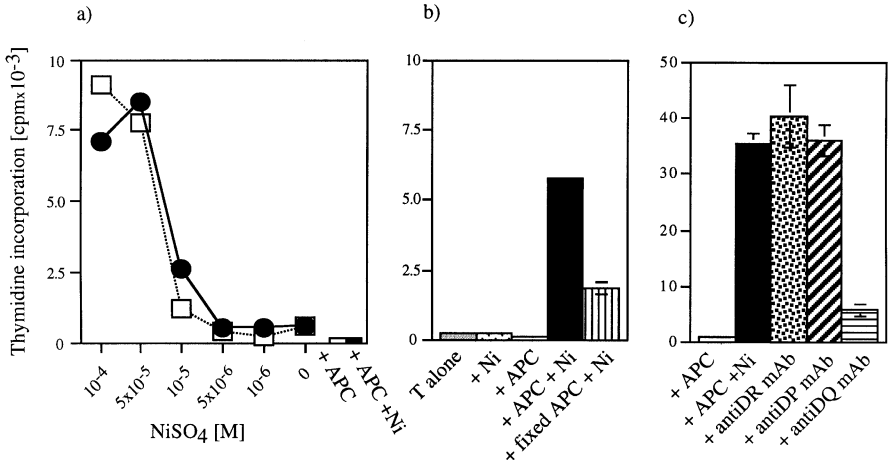

Figure 3. Nickel-specific reactivity of the TCR transfectant and the role of APC in Ni-presentation. (*a*) Transfectant 54PPN8 (□) and clone PPN82 (●) were incubated with graded concentrations of $NiSO_4$ in the presence of irradiated B cells BG. The right bars in (*a*) show data obtained after cultivation of the untransfected recipient cell line 54ζ17 with or without 10^{-4}M $NiSO_4$ in the presence of the irradiated B cell line BG. IL-2 secretion (for 54PPN8 and 54ζ17) and proliferation (for PPN82) were determined as described in *Materials and Methods*. Results in cpm are means of triplicates. Standard deviations did not exceed 10%. (*b*) Hybridoma cells 54PPN8 were cultivated for 20 h at 37°C with or without 10^{-4}M $NiSO_4$ in the presence or absence of fixed APC (B cell line JESTHOM). IL-2 secretion was measured as in (*a*) and results are given in cpm ± SD. For experimental details see *Materials and Methods*. (*c*) IL-2 release of 54PPN8 in response to 10^{-4}M $NiSO_4$ on JESTHOM APC was determined in the absence or presence of culture supernatants of hybridomas L243 (anti-DR), B7.21 (anti-DP), or SVPL3 (anti-DQ) at 1:10 final dilutions. IL-2 secretion was determined as in (*a*) and results are given in cpm ± SD.

the presence of the irradiated B cell line BG (**Fig 3***a*). In **Fig 3**(***b***) as well as in **Fig 3**(***c***), the B cell line JESTHOM was used as APC because the donor PPN was HLA-typed as DQA1*0101, DQB1*0501, and DQB1*0201/0202, and this B cell line is homozygous for the HLA-genes DQA1*0101 and DQB1*0501. **Figure 3**(***b***) reveals that the Ni-specific response requires the simultaneous presence of both APC and $NiSO_4$. As for the original clone PPN82, no activation of the transfectant with Zn, Pd, Co, or Cu was observed, and activation by Ni was abolished when $NiSO_4$-pulsed APC were washed prior to stimulation of the TCR transfectant (data not shown). **Figure 3**(***b***) also illustrates that glutaraldehyde fixation reduced but never completely abolished the antigen-presenting capacities of the APC. Fixation of B cells JESTHOM was done under conditions previously shown to completely abolish the stimulatory capacity of APC for some Ni-reactive T cell clones (Moulon *et al*, 1995). Thus, PPN82 and the resulting transfectant 54PPN8 belong to the class of Ni-reactive T cells we described earlier (Moulon *et al*, 1995), which do not require antigen processing for Ni-recognition.

Concerning the specificity of HLA-restriction, it first appeared that only HLA-DR1 expressing APC mediated the reaction (not shown); however, as shown in **Fig 3**(***c***), the Ni-reactivity was effectively inhibited by MoAb to HLA-DQ, but not to HLA-DR or HLA-DP. Furthermore, 54PPN8 was stimulated in the presence of APC homozygous for DQA1*0101 and DQB1*0501 (**Fig 3***b*). The anti-DQ antibody was also effective in blocking the Ni-reactivity of clone PPN82 (not shown). Consequently, B cells negative for DQB1*0501 failed to stimulate 54PPN8 (not shown).

Influence of different HLA-DQ5 positive B cell lines on Ni-recognition We further compared the Ni-presenting potential of the APC line BG with several reference EBV-transformed B cell lines, i.e., EHM, LWAGS, and JESTHOM, all of which are homozygous for the HLA class II genes DQA1*0101 and DQB1*0501. All of these APC supported SEC2-mediated activation of 54PPN8 as demonstrated in **Fig 4**(***a***). The HLA-DQ molecules expressed on the cell lines JESTHOM and BG were perfectly able to present Ni to the transfectant and the reaction was inhibited by anti-DQ MoAb; however, the other two B cell lines (EHM and LWAGS) were extremely poor stimulators in spite

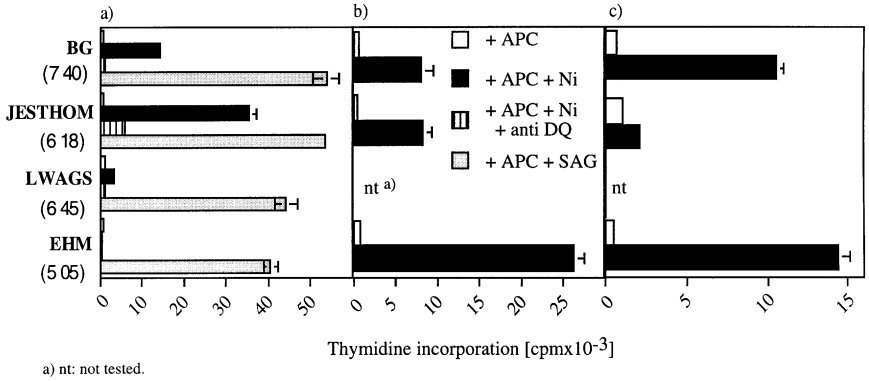

Figure 4. Differential Ni-presentation by several HLA-DQ matched B cell lines. (*a*) IL-2 response of hybridoma 54PPN8 to $NiSO_4$ (10^{-4} M) was determined in the presence of the irradiated B cell lines EHM, LWAGS, JESTHOM, or BG. Controls include IL-2 responses of 54PPN8 with APC in the absence of $NiSO_4$ (+ APC). Inhibition of Ni-specific IL-2 secretion by anti-DQ MoAb was performed a in **Fig 3**. Superantigen (SAG) SEC2 was used at a concentration of 0.1 μg per ml. Data are given as means of triplicates in cpm ± SD. Numbers in parentheses indicate the mean fluorescence of the different cell lines after FACS staining with anti-DQ MoAb. For experimental details see *Materials and Methods*. (*b*) Stimulation of the Ni-specific TCR transfectant T913 (Moulon and Vollmer, unpublished) by the different B cell lines in the presence of 10^{-4} M $NiSO_4$ (*c*) Clone 3, obtained from donor SE (Moulon, unpublished), was incubated with or without 10^{-4}M $NiSO_4$ in the presence of the B cell lines BG, JESTHOM, or EHM. Proliferation was determined as described in *Materials and Methods* and results are given in cpm ± SD.

of expressing the same HLA-DQA/B combination. This result was not due to variations in HLA-DQ expression as the mean fluorescence intensity of anti-HLA-DQ staining (numbers in parentheses in **Fig 4***a*) indicated a very similar surface density of HLA-DQ on all four APC. We also did not find any significant differences in the expression of the adhesion molecules or costimulatory molecules ICAM-1, ICAM-2, ICAM-3, LFA-1, LFA-3, B7-1, B7-2, and CD40 (not shown), which are, among others, important for interaction of T cells with APC. Functional integrity of all four APC lines was indicated by their ability to mediate SEC2 activation of 54PPN8. In addition, because three of the four APC (except for LWAGS) also expressed the DRB1*0101 allele, they were tested as Ni-presenters for the TCR transfectant T913, which reacts to Ni presented on DRB1*0101-expressing APC (Moulon and Vollmer, unpublished data). As shown in **Fig 4**(***b***), all three DRB1*0101-expressing APC including EHM induced a Ni-specific release of IL-2 from this transfectant. We also tested the stimulatory capacity of the APC lines BG, JESTHOM, and EHM on a Ni-specific, DQ-restricted human T cell clone (clone 3, **Fig 4*c***). In contrast to the hybridoma 54PPN8, clone 3 was strongly activated in the presence of Ni by EHM, but only marginally by JESTHOM. Thus, our data reveal opposing stimulatory capacities of the DQ5-positive B cell lines JESTHOM and EHM for different T cells, including a TCR transfected hybridoma as well as a genuine T cell clone.

To investigate whether variations in the individual HLA-DQ5 sequences were responsible for the observed differences in Ni-presentation, we sequenced exon 2, coding for the polymorphic regions of HLA-DQA1*0101 and DQB1*0501, of the APC lines EHM and JESTHOM. Both sequences revealed complete identity to the previously published HLA-DQA1*0101 and DQB1*0501 sequences (available under accession numbers L34082 and X03068, respectively) (data not shown).

Influence of the CD4 co-receptor on Ni-recognition It is well established that CD4 and CD8 co-receptor molecules play an important part in TCR-mediated T cell activation (Glaichenhaus *et al*, 1991; Garcia *et al*, 1996; König *et al*, 1996); however, it is also known that the influence of the co-receptors may vary significantly (Janeway, 1992) and that in some cases co-receptor is even not required for antigen recognition (Shi *et al*, 1998). We therefore have isolated several CD4-negative variants of 54PPN8 by FACS-sorting and cloning. **Figure 5** compares FACS profiles of three $CD4^-$ transfectant clones (**Figs 5*a–c***) and one $CD4^+$ transfectant clone (**Fig 5*d***). These clones all showed TCR expression similar to the original TCR transfectant 54PPN8 as analysed in **Fig 2**(***a***), whereas for the CD4-negative clones, no co-receptor expression was detectable. All five cell types revealed indistinguishable IL-2 responses to stimulation with anti-TCRB13S6 MoAb (**Fig 5*e***); however, the magnitude of the response to $NiSO_4$ was clearly reduced for the CD4 loss variants (**Fig 5*f***).

DISCUSSION

Contact hypersensitivity to nickel represents the most common of numerous metal-induced human allergic disorders and may serve as a model to elucidate the cellular and molecular basis for these types of allergies. It is well established that Ni-reactive T cells represent the specificity-determining elements in Ni-allergy (Kapsenberg *et al*, 1992); however, studies aiming at identifying the antigenic epitopes involved in Ni-specific T cell activation have so far been unsuccessful (Sinigaglia, 1994; Griem *et al*, 1998).

In mouse systems, the production of class I and class II MHC-restricted T cell hybridomas has spurred the identification of peptide as well as of hapten epitopes (Nalefski *et al*, 1990; von Bonin *et al*, 1993). Human T cells, in contrast, cannot be fused to produce stable hybridomas, and the only way to immortalize them is either by virus transformation (Meinl *et al*, 1995) or by transfection of human TCR genes into Jurkat cells or receptor-less variants of mouse T cell hybridomas (Ohashi *et al*, 1985; Bukowski *et al*, 1995; Fleischer *et al*, 1996). We have adopted the latter technology and combined it with a method originally developed to express mouse TCR (Casorati *et al*, 1993; von Bonin *et al*, 1996). This approach also circumvents the problem of self-presentation of antigen in the absence of APC because mouse hybridomas, unlike activated human T cells, do not express human HLA class I or class II molecules.

In this study, therefore, we constructed hybrid human/mouse expression vectors for the TCR α- and β-chain of a human Ni-specific T cell clone. Similar to other published human/mouse TCR hybrids (Blank *et al*, 1993), these constructs contained the complete constant regions of the mouse TCR joined to the rearranged human variable regions. The induction of IL-2 secretion in the resulting TCR transfectant 54PPN8 by antibody-, by superantigen-, and especially by antigen-stimulation proves that these chimeric molecules are capable of signal transmission from the plasma membrane to the nucleus in response to antigen. The identical antigen-specificity and -sensitivity of the transfectant and the T cell clone (**Fig 3**) identifies the TCR of a Ni-reactive T cell clone as the only structure required to define its Ni-specificity. Moreover, the quantitative dependence of Ni-specific responses on the presence of CD4 co-receptors strengthens the point that the MHC-restricted interaction of TCR with Ni-determinants more

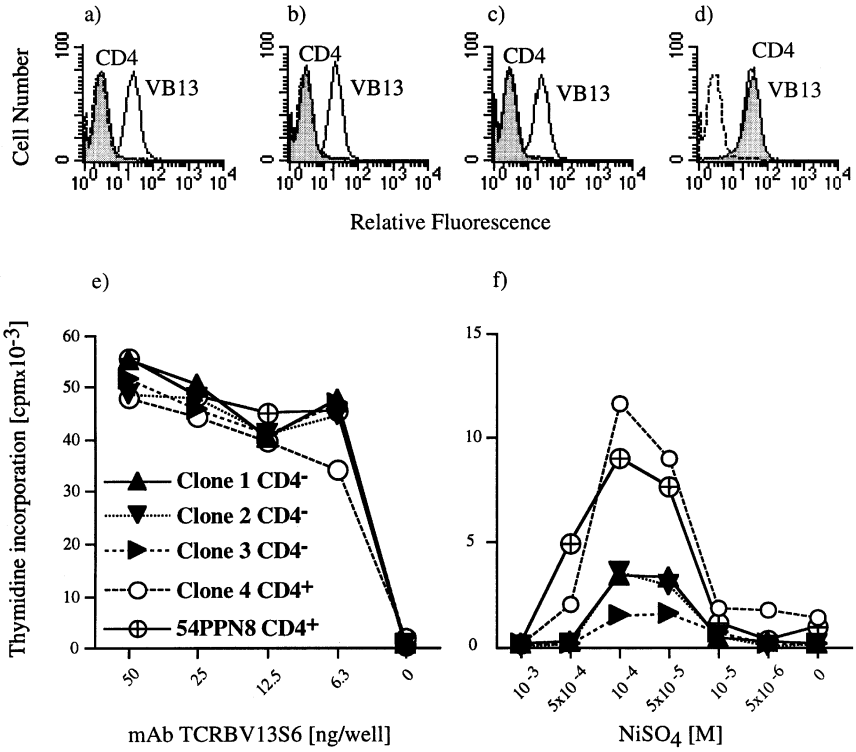

Figure 5. Contribution of the CD4 molecule to nickel-reactivity of the hybridoma 54PPN8. (*a–d*) FACS sorted and cloned $CD4^-$ (*a–c*) and $CD4^+$ (*d*) variants of 54PPN8 were stained for expression of human VB13 and CD4 by flow cytometry. FACS staining of three $CD4^-$ and one $CD4^+$ clones using anti-CD4 (shaded histograms) and anti-TCRBV13S6 (open histograms) MoAb. Isotype controls are indicated by the dotted left lines. For experimental details see *Materials and Methods*. (*e*) IL-2 production of $CD4^-$ (closed symbols) and $CD4^+$ (○) clones and 54PPN8 (⊕) was measured following stimulation with anti-TCRBV13S6 MoAb. (*f*) IL-2 response to graded concentrations of $NiSO_4$ in the presence of irradiated B cells BG. Production of IL-2 in (*e*) and (*f*) was determined by [^{3}H]thymidine incorporation into IL-2 dependent CTLL cells. Data are expressed as cpm with SD not exceeding 10%.

closely resembles interactions of TCR with nominal peptide antigens (Hampl *et al*, 1997) than CD4-independent interactions with certain peptides (Hastings *et al*, 1996; Shi *et al*, 1998) or with superantigens and mitogens. The failure to completely abolish Ni-reactivity by glutaraldehyde fixation of the APC places clone PPN82, as well as the transfectant 54PPN8, among those Ni-reactive T cells (Moulon *et al*, 1995), which apparently do not require entry of Ni into the cell to form relevant determinants. Ni^{2+} ions, therefore, may be envisaged as forming structural parts of the antigenic determinants for such clones. It is still unclear, however, whether the epitope depends on the nature of the peptides embedded within the binding groove of the restricting HLA molecule or whether the metal ions form peptide-independent complexes between TCR and HLA (Sinigaglia, 1994).

In this respect, the data of **Fig 4** are of particular relevance. There we demonstrated that the restricting DQ5 element consisting of DQA1*0101 and DQB1*0501, when expressed on different human B cell lines homozygous for these HLA-DQ genes, exhibited dramatic variations in its capability to present Ni to 54PPN8. Some of the B cell lines (EHM and LWAGS) were poor stimulators for this transfectant, although all APC were comparable in superantigen-mediated activation of 54PPN8. These differences were not due to a general inability of some of the APC to present antigen, as the B cell Line EHM effectively presented Ni to another Ni-reactive TCR transfectant in a DRB1*0101-restricted manner. Moreover, the variable Ni-presenting capacity of identical HLA-DQ5 molecules on different B cell lines was not restricted to mouse T cell hybridomas but was also observed for a human T cell clone. The nickel-specific T cell clone 3 and the hybridoma 54PPN8 (both DQ5-restricted) showed opposing responses to the two B cell lines JESTHOM and EHM in antigen-mediated TCR activation (**Fig 4*a*** *versus* **4*c***). This latter finding in addition to rather small differences in the surface expression of HLA-DQ and a variety of adhesion and costimulatory molecules, suggests qualitative differences between sequence-identical DQ5-molecules on different cells. A similar phenomenon has been reported by Emtestam and Olerup (1996) for a DR4-restricted, Ni-reactive, human T cell clone. Our data support their hypothesis that variations of the endogenous peptide pool in the MHC binding groove might be responsible for the observed effects. It remains to be seen whether this indicates a hapten-like interaction of Ni^{2+} ions with specific MHC-bound peptides (Romagnoli *et al*, 1991) or a particular, peptide-induced MHC-conformation. Factors independent of the nature of the HLA-associated peptides may also be involved, however. It should also be considered that the phenomenon described here may represent one of several different mechanisms for the activation of T cells by Ni^{2+} ions. In fact, the complexity of T cell activation by Ni salts may reflect the situation that individual TCR substantially differ in their molecular mode of interaction with Ni-treated APC.

The approach we applied in this study resulted in immortalization of human Ni-specific TCR. This simplified culture conditions and allowed the investigation of the role of TCR and TCR co-receptors in Ni-recognition. In addition, this method provides us with a new tool, which will now greatly facilitate studies aiming at the elucidation of Ni-induced T cell epitopes and their molecular interactions with TCR by mutational analysis.

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