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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 Ni2+ 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) Ni2+ 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 Benezza, 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 NiSO4 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 Ni2+ 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 trinitrotrophenyl 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...
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: NiSO₄·6H₂O, 10⁻³ M; CoCl₂·6H₂O, 10⁻³ M; PdCl₂, 10⁻⁵ M (all obtained from Sigma, Deisenhofen, Germany); phytohemagglutinin (PHA-P) (Murex, Dartford, U.K.), 1 µg per ml; bacterial supernatant SE2 (staphylococcal enterotoxin C2) (Serva, Heidelberg, Germany), 0.1 µg per ml. Rat Con A 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 TCR Dβ (H75–597) (Kubo et al., 1989). Mouse anti-human MoAb used included: FITC-conjugated and non-conjugated TCRBV13S6 (U74–39), FITC-conjugated CD80 (MAB104), FITC-conjugated ICAM-2 (B–T1), FITC-conjugated CD58 (AICD58), PE-conjugated CD40 (MoAb89), and PE-conjugated ICAM-3 (HP2/19) (all from Immunootech, Marseille, 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 IgG and goat anti-human IgG (Dianova, Hamburg, Germany). Streptavidin–RED670TM (Becton/BRL, Eggenstein, Germany) and FITC-conjugated mouse IgG1 (MOPC-21) (Sigma) were also used. For flow cytometric analysis, 2 × 10⁶ 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 lymphoblasts, transfected 54PPN8 was stained with CD4 MoAb and co-receptor loss variants were sorted with a FACSort separator (Becton-Dickinson).

Cells and growth media

The Ni-specific human CD4⁺ positive T cell clone PNN82 was isolated from the Ni-allergic donor PNN (Piotto et al., 1995) and was cultured as described previously (Moulin et al., 1995). The murine T cell hybridoma 54C17 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 58–6β (Letourneur and Malissen, 1989), transfected with vectors for the human CD4 molecule (confering resistance to mycophenolic acid) and the murine CD8α chain (coexpression). 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 × 10⁻³ M 2-mercaptoethanol (Roth, Karlsruhe, Germany), and 10% heat-inactivated fetal calf serum (RPMI-FCS). After 20 h at 37°C, 100 µl of the supernatant was measured in a beta-counter (INOTECH, Aichach, Germany) after another 18 h.

Proliferation assay

T cell clones (2 × 10⁶ cells) were co-cultured in duplicate or triplicate with 2 × 10⁴ irradiated (6000 rad) B cells (line BG, if not indicated otherwise) in 200 µl RPMI-FCS with 5 × 10⁴ irradiated B cells (line BG, if not indicated otherwise) with or without the different metal salts. After 48 h at 37°C, cultures were incubated with 0.5 µCi [³H]thymidine (Amersham Buchler, Braunschweig, Germany) and incorporation of radioactivity was measured in a beta-counter. 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 60 min at 37°C with 10⁻⁶ M [³H]thymidine (Amersham), washed 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) as MoAb as a 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 Vβ18, 5′-GGGTCACATTGAGTACATGTTGGAAGAATCCTTTG-3′; J6-β-Span-BamHI and J6-β-Span-Sall-82, 5′-CAGGATCCCTGTTCTACAGATGGAGTGTGTG-3′.

RESULTS

Functional expression of a human Ni-specific TCR in a mouse T cell hybridoma

The human CD4⁺ T cell clone

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 60 min at 37°C with 10⁻⁶ M [³H]thymidine (Amersham), washed 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) as MoAb as a 1:10 culture SN. IL-2 secretion was determined as above.

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The human CD4⁺ T cell clone
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. 1a). The surface expression of VB13 was confirmed by FACS analysis (Fig. 2b).

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. 1b) 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 human CD4 (Blank et al., 1993). IL-2 secretion by the transfectant followed the same dose dependence of NiSO₄ as the proliferative response of the original clone. In contrast, the TCR-recipient cell line 54ζ17 was not stimulated by NiSO₄ in

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 NiSO₄ (Fig. 3a). IL-2 secretion by the transfectant followed the same dose dependence of NiSO₄ as the proliferative response of the original clone. In contrast, the TCR-recipient cell line 54ζ17 was not stimulated by NiSO₄ in
Materials and Methods

Standard deviations did not exceed 10% (ζ (for 54PPN8 and 54 cpmp NiSO4-pulsed APC were washed prior to stimulation of the TCR or Cu was observed, and activation by Ni was abolished when clone PPN82, no activation of the transfectant with Zn, Pd, Co, or absence of fixed APC (B cell line JESTHOM). IL-2 secretion was measured as in (a) and results are given in cpm 6 SD. For details see Materials and Methods.

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 3b). 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 of the presence of both APC and NiSO4. As for the original clone PPN82, no activation of the transfected with Zn, Pd, Co, or Cu was observed, and activation by Ni was abolished when NiSO4-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.

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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 4a) 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 (Moulin 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 4c). 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 in the HLA-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.

**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 (Gläichenhaus 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 5a–c) and one CD4+ transfectant clone (Fig 5d). 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-TCR13S6 MoAb (Fig 5e); however, the magnitude of the response to NiSO4 was clearly reduced for the CD4− cell variants (Fig 5f).

**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 to identifying the antigenic epitopes involved in Ni-specific T cell activation have so far been unsuccessful (Sinagiglia, 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 (Meinel 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; Bukowska 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...
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. Ni2+ 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 4a versus 4c). 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 Ni2+ 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 Ni2+ 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 coreceptors 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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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– (O) clones and 54PPN8 (D) was measured following stimulation with anti–TCRBV13S6 MoAb. (f) IL-2 response to graded concentrations of NiSO4 in the presence of irradiated B cells BG. Production of IL-2 in (e) and (f) was determined by [3H]thymidine incorporation into IL-2 dependent CTLL cells. Data are expressed as cpm with SD not exceeding 10%.
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NICKEL-REACTIVE DQ-RESTRICTED TCR TRANSFECTANT