Check for updates

Received: 4 November 2019 | Revised: 24 March 2020 | Accepted: 31 March 2020

DOI: 10.1111/all.14322

ORIGINAL ARTICLE

Basic and Translational Allergy Immunology

TCRs with segment TRAV9-2 or a CDR3 histidine are overrepresented among nickel-specific CD4+ T cells

Marina Aparicio-Soto[1] | Franziska Riedel[1] | Melanie Leddermann[1] | Petra Bacher[2,3] | Alexander Scheffold[2] | Heiner Kuhl[4] | Bernd Timmermann[4] | Dmitriy M. Chudakov[5,6,7] | Sonja Molin[8,9] | Margitta Worm[10] | Guido Heine[10,11] | Hermann-Josef Thierse[1] | Andreas Luch[1] | Katherina Siewert[1]

[1]Department of Chemical and Product Safety, German Federal Institute for Risk Assessment, Berlin, Germany

[2]Institute of Immunology, Christian-Albrechts Universität zu Kiel and Universitätsklinik Schleswig-Holstein, Kiel, Germany

[3]Institute of Clinical Molecular Biology, Christian-Albrechts Universität zu Kiel, Kiel, Germany

[4]Sequencing Core Facility, Max-Planck-Institute of Molecular Genetics, Berlin, Germany

[5]Genomics of Adaptive Immunity Department, Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Science, Moscow, Russia

[6]Center for Precision Genome Editing and Genetic Technologies for Biomedicine, Pirogov Russian National Research Medical University, Moscow, Russia

[7]Center of Molecular Medicine, CEITEC, Masaryk University, Brno, Czech Republic

[8]Division of Dermatology, Queen's University, Kingston, ON, Canada

[9]Department of Dermatology and Allergy, Ludwig Maximilian University, Munich, Germany

[10]Division of Allergy and Immunology, Department of Dermatology, Venerology, and Allergy, Charité – Universitätsmedizin Berlin, Berlin, Germany

[11]Department of Dermatology and Allergy, University Hospital Schleswig-Holstein, Kiel, Germany

Correspondence
Katherina Siewert, Department of Chemical and Product Safety, German Federal Institute for Risk Assessment (BfR), Max-Dohrn-Str. 8-10, 10589 Berlin, Germany.
Email: Katherina.Siewert@bfr.bund.de

Funding information
Bundesinstitut für Risikobewertung, Grant/Award Number: SFP 1322-595, SFP 1322-663, SFP 1322-718 and SFP 1322-719; Ministry of Science and Higher Education of the Russian Federation, Grant/Award Number: 075-15-2019-1789

Abstract

Background: Nickel is the most frequent cause of T cell–mediated allergic contact dermatitis worldwide. In vitro, CD4+ T cells from all donors respond to nickel but the involved αβ T cell receptor (TCR) repertoire has not been comprehensively analyzed.

Methods: We introduce CD154 (CD40L) upregulation as a fast, unbiased, and quantitative method to detect nickel-specific CD4+ T cells ex vivo in blood of clinically characterized allergic and non allergic donors. Naïve (CCR7+ CD45RA+) and memory (not naïve) CD154+ CD4+ T cells were analyzed by flow cytometry after 5 hours of stimulation with 200 µmol/L $NiSO_4$., TCR α- and β-chains of sorted nickel-specific and control cells were studied by high-throughput sequencing.

Results: Stimulation of PBMCs with $NiSO_4$ induced CD154 expression on ~0.1% (mean) of naïve and memory CD4+ T cells. In allergic donors with recent positive patch test, memory frequencies further increased ~13-fold and were associated with markers of in vivo activation. CD154 expression was TCR-mediated since single clones could be

Abbreviations: APC, antigen presenting cell; CDR, complementarity determining region; CLA, cutaneous lymphocyte-associated antigen; CMV, cytomegalovirus; MHC, major histocompatibility complex; Ni, nickel; PBMCs, peripheral blood mononuclear cells; PMA-I, PMA-ionomycin; PT, patch test; SEB, staphylococcus enterotoxin B; TCR, T cell receptor; TRAV9-2, TCR α-chain V segment.

Marina Aparicio-Soto and Franziska Riedel equally contributed to this work.

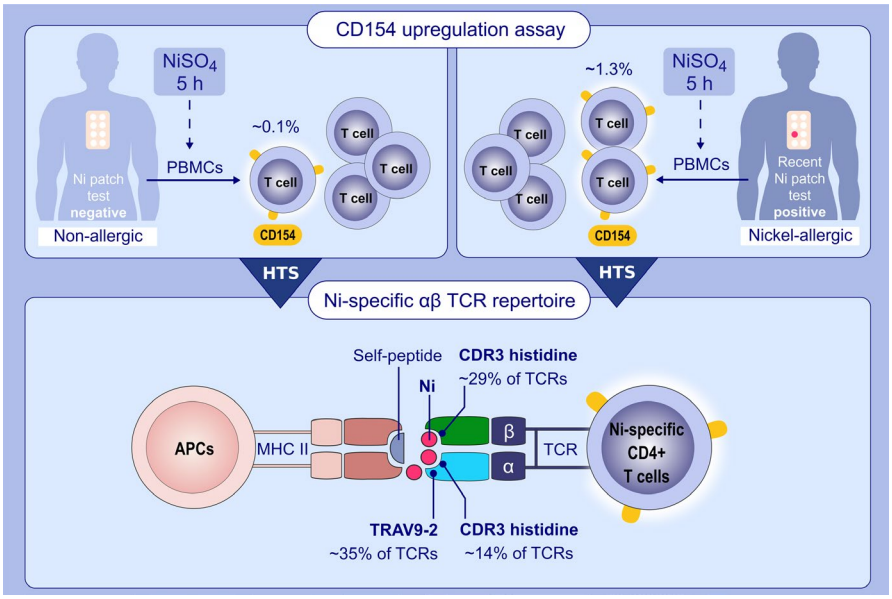
This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
© 2020 The Authors. *Allergy* published by John Wiley & Sons Ltd.

specifically restimulated. Among nickel-specific CD4+ T cells of allergic and non allergic donors, TCRs expressing the α-chain segment TRAV9-2 or a histidine in their α- or β-chain complementarity determining region 3 (CDR3) were highly overrepresented.

Conclusions: Induced CD154 expression represents a reliable method to study nickel-specific CD4+ T cells. TCRs with particular features respond in all donors, while strongly increased blood frequencies indicate nickel allergy for some donors. Our approach may be extended to other contact allergens for the further development of diagnostic and predictive in vitro tests.

KEYWORDS

CD154 upregulation assay, CDR3 histidine, human allergic contact dermatitis, nickel-specific CD4+ T cells, TCR α-chain segment TRAV9-2



GRAPHICAL ABSTRACT

We find increased frequencies of Nickel-specific memory CD4+ T cells in PBMCs of some nickel allergic individuals, applying a new approach based on CD154 upregulation. High-throughput sequencing of αβ T cell receptors reveals an overrepresentation of α-chain segment TRAV9-2 and CDR3 histidines.

Abbreviations: CDR, complementarity determining region; HTS, high throughput sequencing; MHC, major histocompatibility complex; Ni, nickel; $NiSO_4$, nickel sulfate; PBMCs, peripheral blood mononuclear cells; TCR, T cell receptor; TRAV9-2, TCR α-chain V-segment.

1 | INTRODUCTION

Nickel (Ni) contact allergy affects approximately 11% of the general population.[1] It is a T cell–mediated type 4 hypersensitivity reaction directly linked to Ni exposure[2,3] with little genetic influences.[4]

In allergic individuals, skin contact with Ni triggers allergic contact dermatitis mediated by allergen-specific CD4+ and CD8+ T cells.[5-8] Patch testing reproduces this reaction as current diagnostic standard because no reliable in vitro test exists.

Ni-specific T cells have been analyzed in vitro by proliferation or cytokine secretion assays (eg, lymphocyte transformation test, ELISpot assay). These studies found similar blood frequencies for many allergic and non allergic donors, especially in the CD4+ T cell compartment,[9-12] a limited correlation with patch test results,[11-13] and mixed T_H1/T_H2 cytokine secretion.[14-16] A

possible reason for the frequent in vitro activation of CD4+ T cells by Ni could be bystander activation due to unspecific mitogenic effects of Ni.[11,17] However, Ni-reactive clones were often generated[9,14] arguing for T cell receptor (TCR)-mediated activation.

Several mechanisms for the interaction of Ni with the TCR-peptide-major histocompatibility complex (MHC) II surface area have been proposed while the exact epitopes remain unknown.[19] Ni can bind to histidines on MHC II-presented peptides as has been shown for a single peptide.[20] Also, peptide mimotopes have been identified that activate a Ni-specific TCR and likely replace an unknown Ni-loaded peptide.[21] Alternatively, an interaction with TCR β-chain segment TRBV19 (Vβ17 in Arden nomenclature) has been discussed[17,22,23] but mutation analysis of three TRBV19 clones rejected an involvement in Ni binding.[24] Still, the idea that conserved TCR segments are associated with the recognition of Ni seems intriguing since it could explain the activation of a larger fraction of TCRs.

So far, a comprehensive analysis of the Ni-reactive TCR repertoire has been missing due to technological limitations. Only β-chains were analyzed because many V segment antibodies are available and the limited number of J segments facilitates multiplex PCR approaches.[22,25,26]

To analyze the Ni-reactive αβ TCR repertoire more comprehensively, we here adopted induced CD154 (CD40L) expression[27-29] and combined it with high-throughput sequencing of both TCR α- and β-chains[30]. Our approach revealed that Ni mainly activates TCRs with certain characteristics providing an explanation for the high numbers of Ni-specific CD154+ CD4+ T cells in non allergic individuals.

2 | METHODS

2.1 | Blood samples

Blood samples (~50 mL) were obtained from individuals with defined status regarding Ni sensitization and allergic contact dermatitis with written informed consent according to the current version of the declaration of Helsinki at the Department of Dermatology and Allergology (Charité, Berlin) and at the German Federal Institute for Risk Assessment (BfR, Berlin; ethic votes EA4/071/13, EA2/228/17). Details on donors and methods are given in Table S1 and Appendix S1 "Supplemental Methods."

2.2 | Antigen stimulation assays

Peripheral blood mononuclear cells (PBMCs) were stimulated with 200 μmol/L $NiSO_4$ in the presence of CD40 blocking antibody (1 μg/mL, HB14, Miltenyi Biotec).[27,28,29] After 5 hours, cells were stained for CD154 and other markers and analyzed by flow cytometry. CD154+ CD4+ T cells were sorted for TCR sequencing or expanded as clones for restimulation assays. Details on antigen stimulation assays and further methods are provided in Appendix S1 "Supplemental Methods."

2.3 | TCR sequencing

RNA- and UMI-based high-throughput sequencing of TCR α- and β-chains was performed on the Illumina MiSeq platform basically as described.[30] Appendix S1 "Supplemental Methods" lists full methodological details, Table S2 lists sorted cells and TCR sequence numbers. For primers and PCR conditions, see Table S3 and for sequences of single clones, see Table S4. Raw reads from αβ TCR sequencing are available on the European Nucleotide Archive (study accession no. PRJEB37836, https://www.ebi.ac.uk/ena/data/view/PRJEB37836).

3 | RESULTS

3.1 | Detection of Ni-specific CD4+ T cells by induced CD154 expression

To assess whether Ni-specific CD4+ T cells are detectable by CD154 (CD40L) upregulation similar to protein-specific cells,[27,28,30] blood was collected from non allergic donors (n = 12, Table S1) and from allergic donors with recent positive Ni patch test (n = 11). As a third group, donors with former positive Ni patch test were included (n = 8, ≥3 weeks between patch test and blood draw).

PBMC cultures were left without antigen or stimulated with 200 μmol/L $NiSO_4$. After 5 hours, we analyzed CD154 expression on naïve (CCR7+ CD45RA+, gating strategy Figure S1A) and memory (non-naïve) CD4+ T cells by flow cytometry. Without antigen stimulation, CD154 expression was low (Figure 1A shows two representative donors). After stimulation with 200 μmol/L $NiSO_4$, increased CD154 expression was detectable at various frequencies for 53 out of 62 naïve and memory CD4+ T-cell populations from all 31 donors on a 96-well plate (Figure 1B, Table S1).

We then compared Ni-induced CD154 expression among the different groups. Similar increased frequencies were observed for non allergic donors and for donors with former positive Ni patch test (~0.1%, Figure 1B). For donors with recent positive Ni patch test, memory frequencies increased significantly to 1.3%. Receiver operating characteristics curve analysis yielded a cutoff value of 0.53% that discriminates donors with recent positive patch test from nonallergic donors with 100% specificity and 90% sensitivity (Figure 1C). Therefore, many Ni-allergic individuals with recent positive patch test but not those with former positive patch test could be discriminated from non allergic controls.

Stimulation of PBMC cultures with the superantigen staphylococcus enterotoxin B (SEB) confirmed that T cells from the different groups did not differ in their general capacity to express CD154 (Figure S1B). Only CD4+ T cells expressing the SEB-interacting TCR

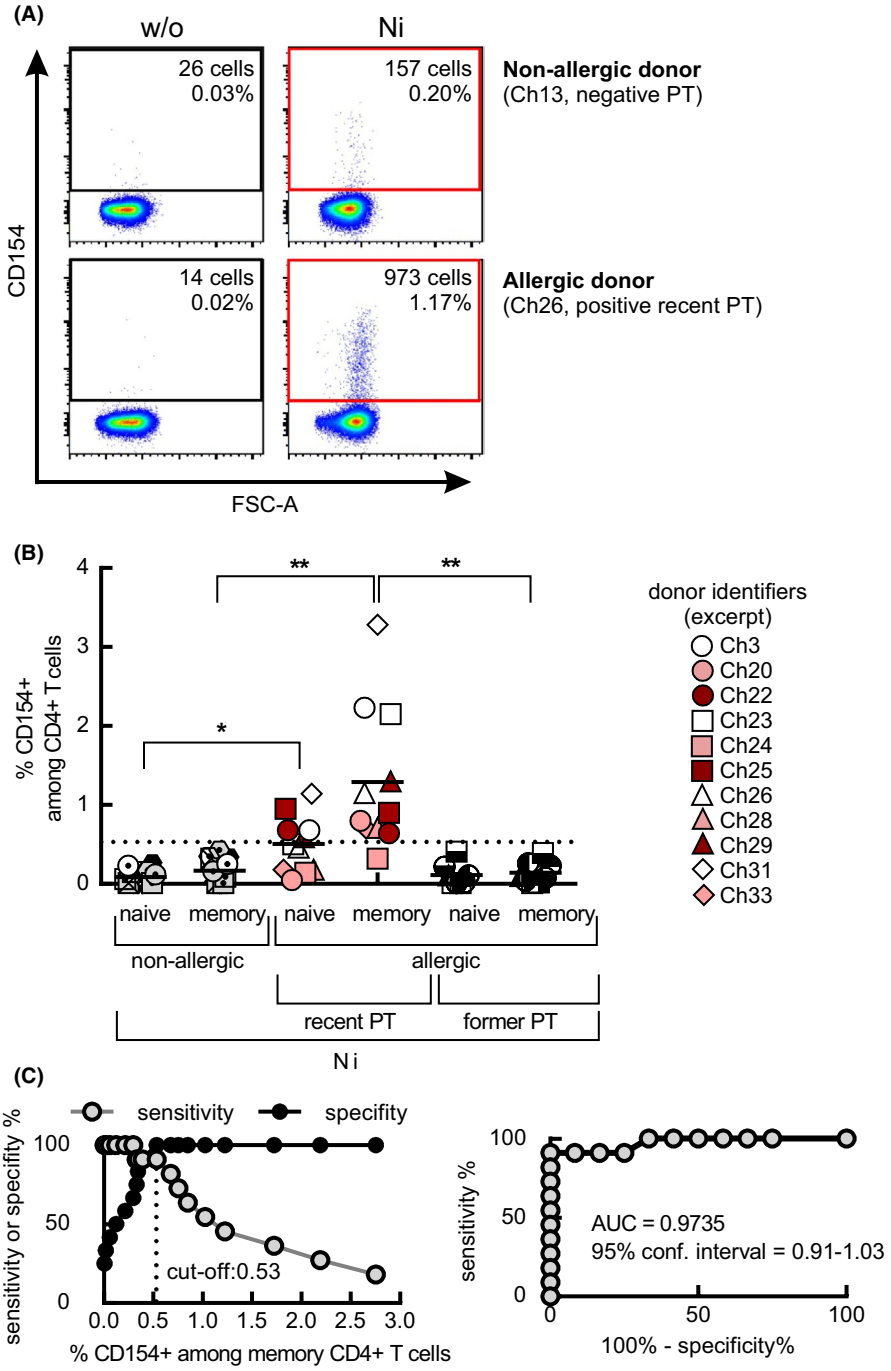


FIGURE 1 Detecting Ni-specific CD4+ T cells by CD154 upregulation assay. (A) Dot plots showing frequencies and cell numbers of CD154+ memory CD4+ T cells without antigen stimulation (w/o, left panels) and after 5 h of stimulation with 200 µM $NiSO_4$ (Ni, right panels) on a 96-well plate. Data are from one representative non allergic (Ch13) and from one Ni-allergic donor with recent positive patch test (PT; Ch26, gating scheme Figure S1A). (B) Frequencies of Ni-specific naïve and memory CD154+ CD4+ T cells in blood from all donors. Values from samples without antigen stimulation were subtracted. Donors were separated into three groups (non allergic, n = 12; allergic with recent positive PT, n = 11; and allergic with former positive PT, n = 8). For all donor identifiers and frequency values, see Table S1. Lines indicate the means. The dashed line indicates a cutoff value obtained by receiver operator characteristic (ROC) curve. One-way nonparametric ANOVA analysis (Kruskal-Wallis) with Dunn's test for multiple comparisons was used to assess differences between groups (*P < .05, **P < .01). (C) ROC curve analysis for frequencies of Ni-specific memory CD4+ T cells from allergic donors with recent positive PT and from non allergic donors discriminates between both groups: area under the curve (AUC) = 0.9735 (95% CI: 0.91-1.03), cutoff: 0.53% for 90% sensitivity and 100% specificity

β-chain TRVB19 (Vβ17) but not the noninteracting β-chain TRBV6-5 (Vβ13) stained CD154+ (Figure S1C). This confirms TCR-mediated and not bystander-mediated CD154 expression even if large fractions of T cells become activated, analogue to previous findings.[27] We did not find interferences with unrelated immune responses. For instance, donor Ch32 (non allergic) had many cytomegalovirus (CMV) pp65-specific but hardly Ni-specific cells (Figure S1D). Vice versa, donor Ch23 (allergic but CMV IgG negative) had increased frequencies of Ni-specific but not CMV pp65-specific CD4+ T cells.

In additional experiments, we further characterized Ni-induced CD154 expression. Upregulation peaked 2-4 hours after SEB and Ni stimulation and then stayed constant for more than 12 hours (Figure S1E). CD154 expression was dependent on PBMC density, that is, the presence of antigen presenting cells (APCs), arguing for TCR-mediated activation, whereas PMA-ionomycin (PMA-I) stimulation was APC-independent (Figure S1F). $NiSO_4$ titration showed increasing frequencies up to ~1 mmol/L (Figure S1G) while toxic effects, for example, decreasing monocyte numbers, started at ~400 µmol/L. We then chose 200 µmol/L $NiSO_4$ as standard concentration.

Regarding Ni-specific CD154+ CD8+ T cells, we observed a trend toward higher frequencies for allergic donors with outliers

among non allergic donors (Figure S1H). Since only ~20% of memory CD8+ T cells express CD154[32] and because Ni-specific CD8+ T cells seem less frequent in general,[9] they cannot be reliably detected on a 96-well plate format.

3.2 | Effector immune responses in donors with recent positive Ni patch test

To further estimate the in vivo relevance of Ni-specific CD4+ T cells, we analyzed co-expression of activation markers for donors with recent positive patch test. This was possible due to the high numbers of responding cells in this group (mean 1049 cells/well, 96-well plate).

Cutaneous lymphocyte antigen (CLA) has been associated with skin homing and Ni allergy.[33,34] Among Ni-activated CD154+ memory CD4+ T cells, 26% (mean) expressed CLA compared to 22% of total T cells (Figure 2A, Table S1). Increased CLA expression was not observed for non-skin–associated CMV pp65- or SEB-specific cells (12% and 14%, respectively). Since Ni is also contained in some foods, we determined the expression of the gut-homing chemokine receptor CCR9 to dissect food-associated from skin-associated Ni-specific T cells.[35] CCR9 was expressed by few total and CD154+ memory CD4+ T cells (<5%, Figure S2A), arguing against gut involvement in most cases.

Ni-specific cells of some donors expressed Ki-67, a marker for an active cell cycle,[36] to a higher percentage compared to the total cell pool and SEB-stimulated cells (Figure 2B; means 5%, 2%, 2%). Ki-67

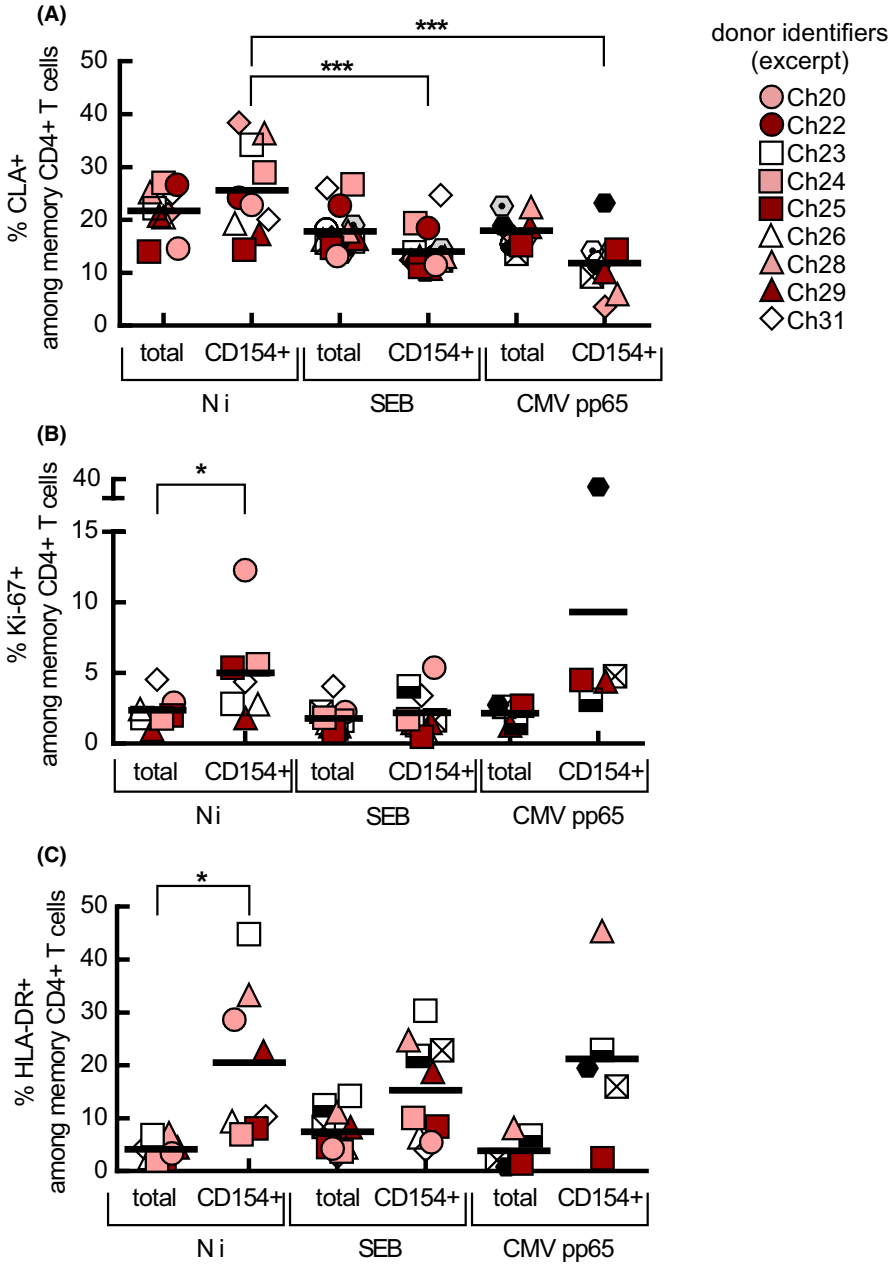


FIGURE 2 In vivo activation of Ni-specific T cells in allergic donors. Expression of (A) CLA, (B) Ki-67, and (C) HLA-DR by total or antigen-specific CD154+ CD4+ memory T cells in donors with recent positive patch test after 5 h of in vitro stimulation . Only populations with ≥20 cells are shown. Lines indicate the means. For all donor identifiers and frequency values, see Table S1. One-way ANOVA analysis (Kruskal-Wallis) with Dunn's test for multiple comparisons was used to assess differences between populations stimulated with different antigens. Wilcoxon signed-rank test was used to observe paired differences between total and CD154+ CD4+ T cells after stimulation with the same antigen (*P < .05, ***P < .001)

expression was also increased among CMV pp65-specific cells in some donors with positive CMV IgG titer (9%), likely due to ongoing chronic immune responses.

HLA-DR, a late activation marker,[37] was also more frequently expressed by Ni-specific T cells (Figure 2C). Nur77 and CD69, markers for TCR-mediated activation,[38,39] were induced on nearly all CD154+ CD4+ T cells after ~5 hours of stimulation (Figure S2B,C) arguing against bystander activation.

Co-expression of CLA, Ki-67, and HLA-DR differed among donors. For some donors, increased expression by Ni-specific cells

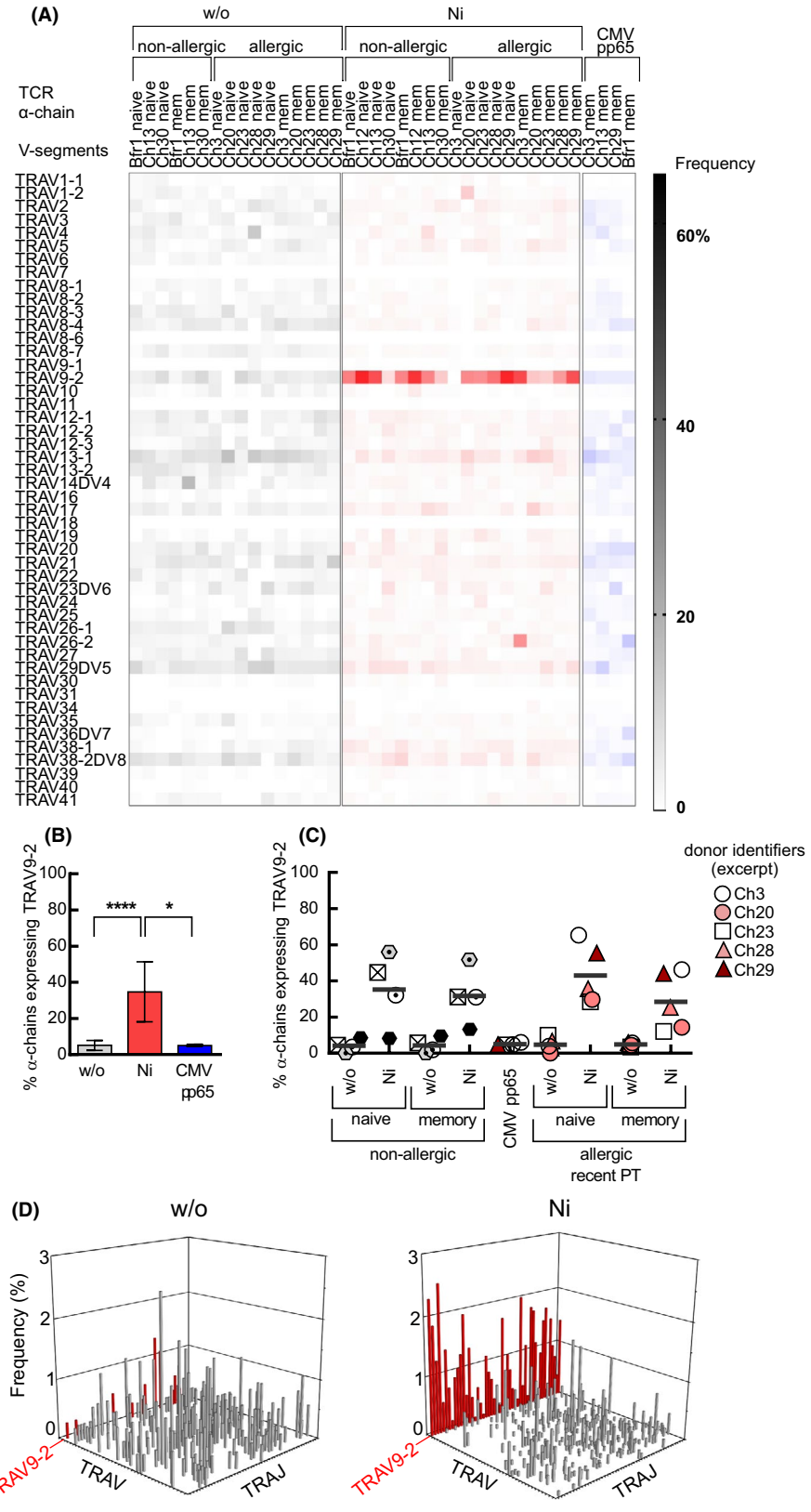


FIGURE 3 TCR α-chain segment TRAV9-2 is overrepresented among Ni-specific TCRs. (A) Heat plot depicting TCR α-chain V segment use among randomly sorted (without antigen stimulation, w/o, grey), Ni-specific (red), and CMV pp65-specific (blue) CD4+ T cells (frequencies). Cells were sorted from CD154 upregulation assays (~1000-3000 cells per sample, Table S2), and their TCRs were analyzed by high-throughput sequencing. (B) Mean frequencies of TRAV9-2+ TCRs among Ni- and CMV pp65-specific or randomly sorted (w/o) CD4+ T cells (means ± SD). (C) Frequencies of TRAV9-2+ TCRs among the indicated T-cell populations from individual donors (same data as in (A)). For all donor identifiers, see Table S1. One-way ANOVA analysis (Kruskal-Wallis) with Dunn's test for multiple comparisons was used to assess differences between groups (B and C, *P < .05; ****P < .0001). (D) Representative 3D bar plots depicting TCR α-chain V- and J segment use for randomly sampled CD4+ T cells (left, w/o—without antigen stimulation) and for Ni-specific CD154+ CD4+memory T cells (right; frequencies, donor Ch29)

was observed (eg, Ch20) arguing for ongoing Ni-related immune responses. On the contrary, donor Ch29 had a strongly positive patch test (+++) and a high frequency of Ni-specific cells (1.3%) but showed no signs of recent immune activation, that is, no increased percentages of CLA+ or Ki-67+ T cells among Ni-specific cells. This donor also lacked recent dermatitis (personal report) and may therefore have a long-term increased memory response.

Ni-specific CD154+ CD4+ T cells expressed different cytokines (Figure S3), in line with previous reports.[14-16] Many expressed INFγ (T_H1), with similar or lower percentages compared to SEB-activated CD154+ cells. For some donors, IL-17A or IL-4 was expressed by a relatively high percentage of Ni-activated cells compared to SEB-activated cells indicating outgrowth of T_H17 or T_H2 cells during Ni allergy. For non allergic donors and allergic donors with former positive patch test, cytokine-producing cells were below detection limits (≤20 cells) but most cytokines tended to be elevated in Ni-stimulated cell culture supernatants. We observed no correlation for the type of Ni-specific cytokine response with the atopic status of the donor. As expected, CMV pp65-specific CD4+ T cells mainly expressed IFNγ.

3.3 | Ni-specific CD4+ T cells are linked to TCRs expressing α-chain segment TRAV9-2

Given the relatively high frequencies of Ni-specific CD4+ T cells in nonallergic donors, we wondered whether certain αβ TCR repertoire features are involved and whether repertoires differ between allergic and nonallergic individuals. To address this, we sorted Ni-specific CD154+ naïve and memory CD4+ T cells from 5 allergic donors and from 4 nonallergic donors (Table S2). Typically, 1000-3000 cells were collected from ~10 million stimulated PBMCs from a 12-well plate. As controls, random naïve and memory CD4+ T cells from samples without antigen stimulation, CMV pp65-specific T cells, and Jurkat T cells were sorted. In total, we analyzed 102 TCR α- and β-chain libraries, yielding 42 713 functional sequence reads (counts) of 20 397 distinct clonal lineages (TCR diversity; Table S2). On average, we obtained 0.2 ± 0.2 (mean ± SD) TCR cDNA counts per sorted cell for TCR α- and β-chains, similar to literature data.[40] Slightly higher cDNA counts were observed for activated CD154+ CD4+ T cells (0.3 ± 0.2) compared to randomly sorted cells (0.1 ± 0.1 counts/cell) for both naïve and memory CD4+ T cells. This is probably due to a slightly higher TCR mRNA amounts in the former.

In samples from Jurkat T cells, we only found the expected αβ TCRs (except for one erroneous α-chain with one sequence count among 1380 correct sequence counts) indicating that TCR sequences were correctly identified and that little spillover occurred between samples.

TCR β-chain segment TRBV19, which has been hypothesized to be involved in Ni allergy,[17,22-24] was equally expressed by randomly sorted CD4+ T cells (~5% of sequence counts) and by Ni-specific CD154+ CD4+ T cells (~3% of sequence counts, Figure S4).

Instead, we found a Ni-related overrepresentation of TCR α-chain segment TRAV9-2 (Figure 3A,B). TRAV9-2 is one out of 47 functional α-chain gene segments with ~5% background expression among randomly sorted CD4+ T cells. Among Ni-specific naïve and memory cells, TRAV9-2 was expressed by 43% ± 15% (mean ± SD) and 28% ± 14% of T cells, respectively, in allergic individuals and by 35% ± 18% and 32% ± 14% of T cells in nonallergic individuals (Figure 3C). Ni-specific TRAV9-2+ TCRs combined with different J segments (Figure 3D).

Because ~5% of randomly sampled T cells express TRAV9-2 and just ~0.1% of T cells respond to Ni in nonallergic donors, not all TRAV9-2+ T cells react to Ni. As a rough estimate, less than ~1 out of 100 TRAV9-2+ cells recognizes Ni.

A prior mutation study with a single TRAV9-2+ Ni-reactive T cell clone showed Ni recognition via $tyrosine_{36}$ in the CDR1 of TRAV9-2.[41,42] Although TRAV40 has a similar CDR1 compared to TRAV9-2 (TRAV40: STG**Y**PT, TRAV9-2: ATG**Y**PS), the TRAV40 segment was rarely expressed among randomly sorted T cells and not commonly enriched among Ni-reactive CD4+ T cells. Summarizing, apart from TRAV9-2, no other V- or J segments were commonly enriched and/or had relevant sequence counts among Ni-reactive CD4+ T cells (Figure S4).

3.4 | Increased abundance of a histidine residue among Ni-specific CDR3

Given that only ~35% of Ni-specific TCRs express TRAV9-2, we further analyzed the CDR3 which is mainly responsible for peptide antigen recognition. We observed an increased use of the Ni-binding amino acid histidine among Ni-specific CDR3 (Figure 4A). Among randomly sorted, Ni-specific, and CMV pp65-specific α-chain CDR3, 3%, 14%, and 5% contained a histidine, respectively. Among β-chain CDR3, 17%, 29%, and 21% contained a histidine. No other amino acid was similarly increased (Figure 4B).

We mainly found one histidine per CDR3. Two or more histidines were present in 4% and 13% of Ni-specific TCR α- and β-chains, with similar results for random, Ni, or CMV pp65-specific TCRs (Figure 4C). Only few Ni-specific TRAV9-2+ TCR contained a CDR3 histidine arguing for largely independent mechanisms (Figure 4D).

As for TRAV9-2 overrepresentation, we observed a trend for an increased occurrence of a CDR3 histidine among Ni-specific naïve and memory CD4+ T cells of allergic and nonallergic donors (Figure 4E). The proportion of Ni-specific TCRs expressing a CDR3 histidine seemed less frequent among memory CD4+ T cells of allergic donors. This effect was not significant but could indicate less efficient in vivo selection.

Every donor had different Ni-specific TCRs. Not one overlapping clone was observed on CDR3 amino acid or nucleotide sequence level for β-chains (Figure S5A). To assess differences in TCR repertoire diversity, we calculated the Shannon index, which considers TCR diversity and the abundance of each clone in a sample. As expected, repertoire diversity was higher for naïve compared to memory T cells (Figure S5B). Ni-specific and CMV pp65-specific

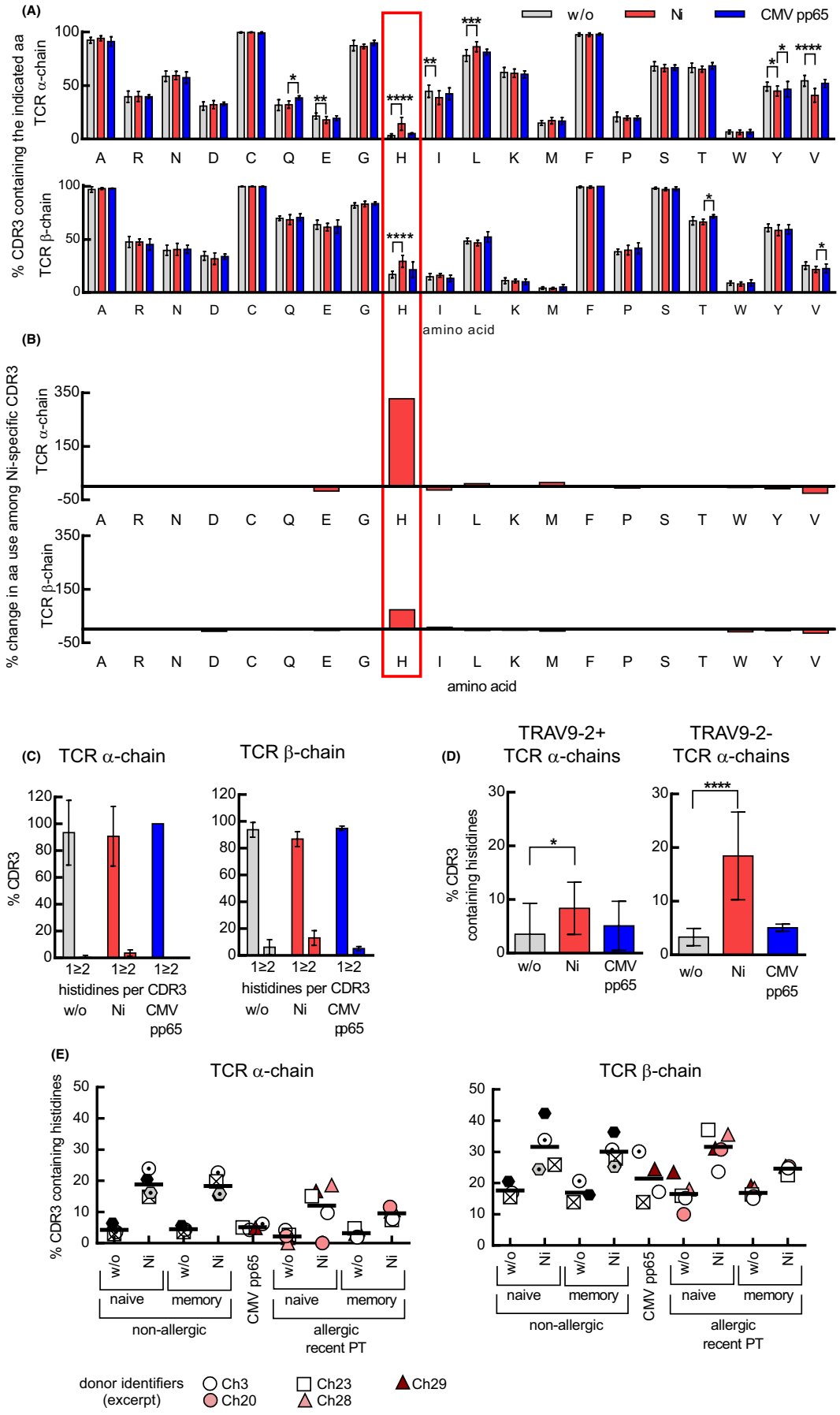

(A)
w/o
Ni
CMV pp65
% CDR3 containing the indicated aa
TCR α-chain
TCR β-chain
A R N D C Q E G H I L K M F P S T W Y V
amino acid
(B)
% change in aa use among Ni-specific CDR3
TCR α-chain
TCR β-chain
amino acid
(C)
TCR α-chain
TCR β-chain
% CDR3
1≥2 1≥2 1≥2
histidines per CDR3
w/o Ni CMV pp65
(D)
TRAV9-2+ TCR α-chains
TRAV9-2- TCR α-chains
% CDR3 containing histidines
w/o Ni CMV pp65
(E)
TCR α-chain
TCR β-chain
% CDR3 containing histidines
w/o Ni w/o Ni CMV pp65 w/o Ni w/o Ni
naive memory naive memory
non-allergic
allergic recent PT
donor identifiers (excerpt)
Ch3 Ch20 Ch23 Ch28 Ch29

FIGURE 4 Ni-specific TCR α- and β-chains frequently contain a histidine in their CDR3. (A) Percentage of α- and β-chain CDR3 that contain the indicated amino acids among randomly sorted (without antigen stimulation, w/o, grey), Ni-specific (red), and CMV pp65-specific (blue) CD4+ T cells (diversity). Cells were sorted from CD154 upregulation assays (~1000-3000 cells per sample, Table S2), and their TCRs were analyzed by high-throughput sequencing. The first CDR3 cysteine (C) and last phenylalanine (F) were included in the analysis. Data were pooled according to antigen specificity (w/o n = 16, Ni n = 18, CMV pp65 n = 4; means ± SD, diversity values). (B) Percent change in amino acid use for α- and β-chain CDR3 of Ni-specific TCRs compared to randomly sampled TCRs. The same data as in (A) were used. (C) Analysis of the number of histidines in α- or β-chain CDR3 that are specific for the indicated antigens (means ± SD). (D) Percentage of CDR3 containing a histidine among TRAV9-2+ and TRAV9-2- TCRs for the indicated antigens (means ± SD). (E) Percentage of CDR3 with histidine for the indicated CD4+ T cell populations from individual donors. For all donor identifiers, see Table S1. One-way ANOVA analysis (Kruskal-Wallis) with Dunn's test for multiple comparisons was used to assess differences between groups (*P < .05, **P < .01, ***P < .001, ****P < .0001)

memory T cells had a lower diversity compared to randomly sorted cells indicating similar selection of antigen-specific TCRs. In general, there was no difference in repertoire diversity between allergic and nonallergic donors, that is, in terms of clonal expansions. Frequency and TCR diversity percentages were similar for the different repertoire features (Figure 5SC). However, donor Ch3 (allergic) had two expanded Ni-reactive clones occupying 26% (TRAV26-2) and 17% (TRAV9-2) of α-chain sequences (Figure 4A) causing a low Shannon index (Figure S5B). Since we observed β-chains with similar frequencies (33% TRBV12-4 and 13% TRBV27), matching TCR αβ chains may be assigned from bulk sequencing data in this case.[40]

3.5 | Ni-specific CD154+ CD4+ T cell clones can be specifically restimulated

Single Ni- or CMV pp65-specific CD154+ memory CD4+ T cells were sorted, expanded, and restimulated with the original and control antigens (Figure 5A). Restimulation frequencies were similar for Ni- and CMV pp65-specific CD4+ T cells with 34/47 (72%) of Ni-specific clones and 16/21 (76%) of CMV pp65-specific clones reacting to their respective original antigen (Figure 5B). This shows that sorted cells were indeed antigen-specific and that background expression of CD154 hardly interferes. We did not observe cross-reactive clones; for example, 0/17 CMV pp65-specific clones were Ni-reactive and *vice versa* (0/20 clones). Among Ni-specific clones, 27% (6/22) expressed the TRAV9-2 gene segment and 41% (9/22) a CDR3 histidine (8 clones β-chain, 1 clone α-chain), reproducing data from bulk sequencing (Table S4). Some clones from nonallergic and allergic donors reacted to 2 μmol/L $NiSO_4$ (3/16) and some did not require additional APCs (2/6 TRAV9-2+, 3/9 TRAV9-2-). Ni- and CMV pp65-induced CD154 expression was blocked to a similar extent by the addition of anti-MHC blocking antibodies (clones tu39 and ac122) in both bulk PMBC cultures and clone restimulation assays (Figure 5D) indicating TCR-mediated CD154 expression.

4 | DISCUSSION

The present study characterizes the frequencies and αβ TCR repertoires of Ni-specific CD154+ CD4+ T cells in blood of allergic and nonallergic donors.

The CD154 upregulation assay has several advantages.[27,28,30] It is fast, quantitative, and unbiased because it does not depend on proliferative or cytokine-producing capacity and it includes even naïve cells. Prior epitope knowledge is not required, and natural background expression of CD154 is low. Ni-induced CD154 expression was mainly TCR- but not bystander-mediated as demonstrated by the specific restimulation of clones and inhibited activation after the addition of MHC blocking antibodies, in agreement with previous work.[9,25]

We observed relatively high frequencies of Ni-specific CD4+ T cells for nonallergic individuals, similar to proliferation-based studies (ie, ~0.1% with 200 μmol/L $NiSO_4$ here vs 0.02% with ~40 μmol/L $NiSO_4$[9]). We used a high nontoxic concentration of $NiSO_4$ to also capture low-affinity clones. The importance of low-affinity clones becomes increasingly recognized even in chronic T cell responses,[43] in contrast to antibodie responses that undergo affinity maturation. The identified Ni-specific cells seem relevant in vivo, given elevated frequencies and expression of in vivo activation markers like CLA, Ki-67, and HLA-DR in some allergic donors. Lower Ni concentrations do not appear to improve the distinction of allergic and nonallergic individuals.[11]

Analyzing thousands of Ni-specific αβ TCRs, we identified two commonly shared characteristics: increased expression of the α-chain segment TRAV9-2 (~35% of TCRs) or a CDR3 histidine (~14% of α-chains, 29% of β-chains). Apart from these features, TCRs differed for each donor as expected since individual repertoires usually comprise ≥ 100 million, mainly unique, clonotypes.[44]

The use of the TRAV9-2 segment has been previously described for only one Ni-specific clone termed "SE9".[41,42] Mutation studies of "SE9" showed that it recognizes Ni via $tyrosine_{36}$ in the CDR1 of TRAV9-2 and via $histidine_{81}$ in the β-chain of human MHC II proteins, which is in close proximity and forms a major contact site in the canonical docking mode of TCRs.[45] Given that MHC II $histidine_{81}$ is expressed by most HLA-DRB1 alleles and no polymorphisms are known for the CDR1 of TRAV9-2 (IMGT database, February 24, 2020), TRAV9-2-mediated Ni recognition could occur in most donors and is compatible with a lack of HLA association in Ni allergy.[4,46] Contrary, Beryllium disease, another metal allergy, has a HLA allele-associated mechanism where Be^{2+} has no direct TCR contact.[47] Additionally, drugs may cause type IV hypersensitivity by noncovalent binding to certain HLA alleles via "pharmacological interactions".[48,49] This is, to our knowledge, the first report of an antigen-specific immune response linked to one conserved segment of the adaptive immune receptor repertoire.

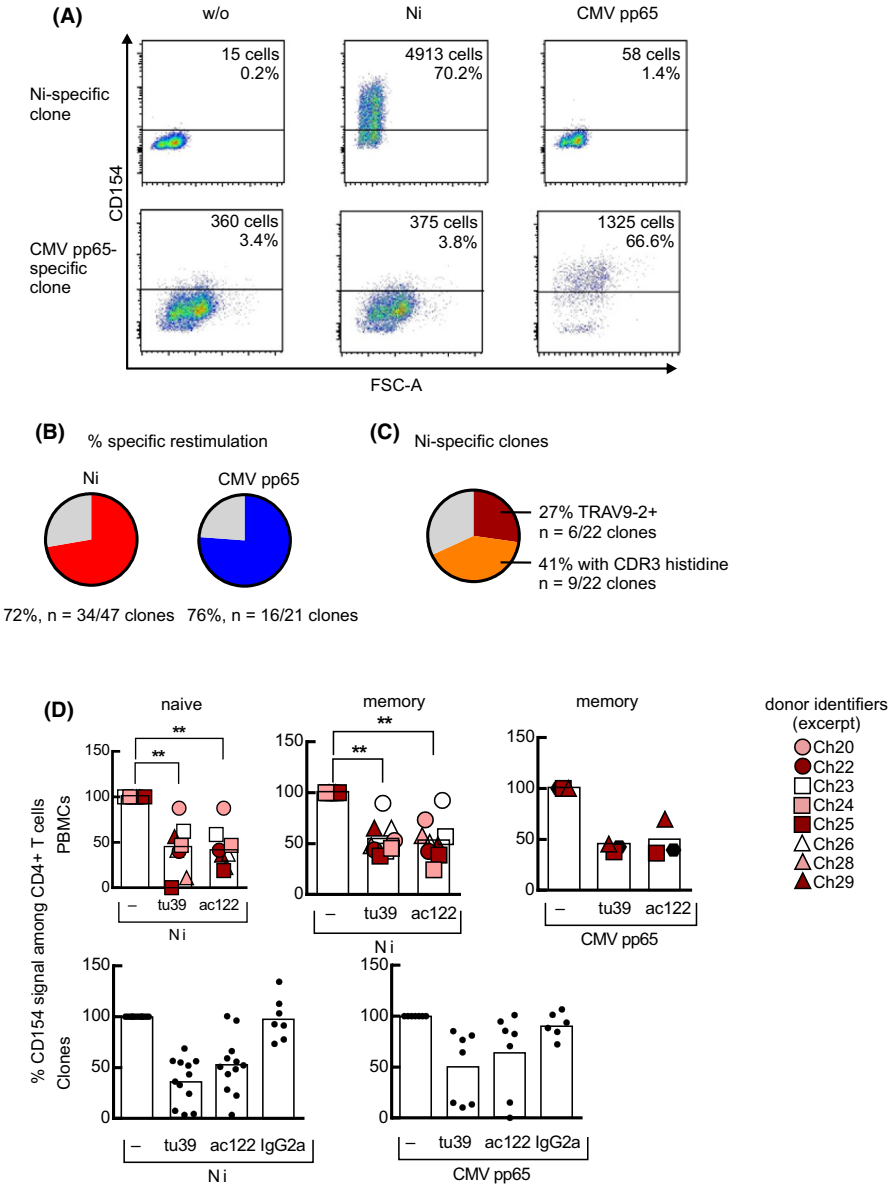


FIGURE 5 Ni-induced CD154 expression is TCR-mediated. (A) Representative dot plots depicting CD154 expression after 5 h of restimulation of a Ni- and a CMV pp65-specific T cell clone with the different antigens. Clones were derived from single CD154+ CD4+ memory T cells that were sorted from a CD154 upregulation assay. Cells were expanded in vitro for 2-3 wk and restimulated with autologous APCs and the indicated antigens. Gated on live, CD3+, single, CD4+ T cells. W/o—without antigen stimulation. (B) Restimulation efficiencies for Ni- (red) and CMV pp65-specific clones (blue, clones from n = 9 donors). (C) Frequency of Ni-specific clones expressing TRAV9-2 or a CDR3 histidine (n = 22 clones from 3 donors, TCR sequences are listed in Table S4). (D) Inhibited CD154 expression after the addition of MHC blocking antibodies to PMBCs bulk cultures (upper panels; n = 3-9 donors; for donor identifiers see Table S1) and single antigen-specific CD4+ T cell clones in restimulation assays (lower panel; n = 7-12 clones). MHC blocking antibodies (clones tu39, ac122) were added to the culture medium 30 min prior to stimulation with Ni or CMV pp65. After 5 h, expression of CD154 was monitored on CD4+ T cells. Graphs illustrate the percentage in signal reduction due to MHC block. Cultures without MHC blocking antibodies (upper panels) or with IgG2a isotype control antibody (lower panels) served as controls. White bars represent the means. One-way nonparametric ANOVA analysis (Kruskal-Wallis) with Dunn's test for multiple comparisons was used to assess differences between groups (**$P < .01$)

Only a fraction of TRAV9-2+ T cells reacted to Ni, indicating that it is not a classical superantigen-like interaction. The presented self-peptide may interfere with Ni binding or may not provide enough interactions to overcome a signaling threshold. Clone "SE9" lost its reactivity when tyrosine$_{108}$ in the α-chain CDR3 was mutated to histidine, suggesting a role in peptide but not Ni binding.[41] Accordingly, some Ni-reactive clones are dependent on peptides presented by certain APCs, as reported here and elsewhere.[50,51]

As second feature, TCRs with a CDR3 histidine were commonly enriched among Ni-specific TCRs (mainly TRAV9-2

negative), suggesting Ni binding via a CDR3 histidine is one mechanism of Ni-mediated T cell activation. This has not been hypothesized before. Future mutation or crystallization studies could solve structural details of the recognition mechanism. Together, TRAV9-2–mediated and CDR3 histidine-mediated Ni recognition cover a large proportion of Ni-specific TCRs (~78%) in both nonallergic and allergic donors with increased frequencies indicating that the identified cells are recruited in vivo. This renders repertoire features or clonal expansions unsuitable for the detection of Ni allergy.

Usually, specific memory CD4+ T cells comprise less than ~0.05% of all cells in the absence of effector responses, as shown for tetanus toxoid, measles virus,[52] vaccinia virus (IFNγ-read-out),[53] or food allergens.[30] Ni activates already ~0.1% of cells in nonallergic individuals. Therefore, only strongly increased blood frequencies can be linked to Ni allergy. For these cases, CD154 upregulation could represent an alternative in vitro test if patch tests cannot be applied or are less reliable, for example, for implant-related allergies. We do not expect strong interferences with unrelated immune responses since only ~0.1% of TCRs are cross-reactive but this requires further confirmation.

Given the relatively high frequencies of Ni-specific CD4+ T cells in blood of nonallergic donors, we hypothesize that the local density of Ni-specific CD4+ and CD8+ T cells in the skin is decisive for the Ni-allergic state and patch test results.[5,6] However, proof for the existence of global skin-resident nickel-specific CD4+ or CD8+ T-cell memory is missing.

For allergic donors without increased blood frequencies of Ni-specific CD4+ T cells, in vitro tests remain challenging. Possibly, rarer subpopulations within the Ni-specific pool are linked to Ni allergy.[15,16] Outgrowth of cytokine-producing clones may be detected by the CD154 upregulation assay if compared to unspecifically stimulated cells or to cells from non allergic donors (but not by simple cytokine analysis of cell culture supernatants). This requires large input cell numbers and magnetic enrichment of Ni-reactive T cells[28] and was incompatible with TCR sequencing here.

Summarizing, this study shows for the first time that TCRs with α-chain segment TRAV9-2 and a histidine in their α- or β-chain CDR3 are linked to Ni-specific CD4+ T cell activation. The CD154 upregulation assay may help to identify active allergy and may be extended to other contact allergens for the future development of diagnostic and predictive tests.[54-56]

ACKNOWLEDGMENTS

We thank PD Dr Klaus Abraham and Prof Cornelia Weikert for collecting blood samples at the BfR, PD Dr Burkhard Malorny and Maria Borowiak for their excellent assistance in Illumina MiSeq sequencing, and Daniel Siewert and Dr Julia Katharina Schlichting for help in analyses involving R.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

MAS, FR, ML, and KS performed experiments and analyzed data. GH, MW and SM provided samples and patient data. BT and HK performed library quantification and assisted in sequencing reactions and sequencing data analysis. DMC assisted in TCR sequencing experiments and TCR data analysis. KS, PB, AS, HJT, GH, and AL conceived the project, and KS directed the project. KS, MAS, and FR prepared the figures and wrote the manuscript. AL, BT, MW, GH, DMC, HJT, and AS corrected the manuscript. All authors approved the submitted version.

ORCID

Marina Aparicio-Soto https://orcid.org/0000-0002-5483-1366
Franziska Riedel https://orcid.org/0000-0002-2395-5454
Margitta Worm https://orcid.org/0000-0002-3449-1245
Guido Heine https://orcid.org/0000-0003-1467-9862
Katherina Siewert https://orcid.org/0000-0003-0380-1594

REFERENCES

1. Alinaghi F, Bennike NH, Egeberg A, Thyssen JP, Johansen JD. Prevalence of contact allergy in the general population: a systematic review and meta-analysis. *Contact Dermatitis*. 2019;80(2):77-85.
2. Kasper-Sonnenberg M, Sugiri D, Wurzler S, et al. Prevalence of nickel sensitization and urinary nickel content of children are increased by nickel in ambient air. *Environ Res*. 2011;111(2):266-273.
3. Schuttelaar MLA, Ofenloch RF, Bruze M, et al. Prevalence of contact allergy to metals in the European general population with a focus on nickel and piercings: the EDEN Fragrance Study. *Contact Dermatitis*. 2018;79(1):1-9.
4. Bryld LE, Hindsberger C, Kyvik KO, Agner T, Menne T. Genetic factors in nickel allergy evaluated in a population-based female twin sample. *J Invest Dermatol*. 2004;123(6):1025-1029.
5. Gaide O, Emerson RO, Jiang X, et al. Common clonal origin of central and resident memory T cells following skin immunization. *Nat Med*. 2015;21(6):647-653.
6. Schmidt JD, Ahlstrom MG, Johansen JD, et al. Rapid allergen-induced interleukin-17 and interferon-gamma secretion by skin-resident memory CD8(+) T cells. *Contact Dermatitis*. 2017;76(4):218-227.
7. Vocanson M, Hennino A, Chavagnac C, et al. Contribution of CD4(+)and CD8(+) T-cells in contact hypersensitivity and allergic contact dermatitis. *Expert Rev Clin Immunol*. 2005;1(1):75-86.
8. Esser PR, Martin SF. Pathomechanisms of contact sensitization. *Curr Allergy Asthma Rep*. 2017;17(12):83.
9. Cavani A, Mei D, Guerra E, et al. Patients with allergic contact dermatitis to nickel and nonallergic individuals display different nickel-specific T cell responses. Evidence for the presence of effector CD8+ and regulatory CD4+ T cells. *J Invest Dermatol*. 1998;111(4):621-628.
10. Lindemann M, Bohmer J, Zabel M, Grosse-Wilde H. ELISpot: a new tool for the detection of nickel sensitization. *Clin Exp Allergy*. 2003;33(7):992-998.
11. Stander S, Oppel E, Thomas P, Summer B. Evaluation of lymphocyte transformation tests as compared with patch tests in nickel allergy diagnosis. *Contact Dermatitis*. 2017;76(4):228-234.
12. Blomberg-Van Der Flier MV, Burg CKHVD, Pos O, et al. In vitro studies in nickel allergy: diagnostic value of a dual parameter analysis. *J Invest Dermatol*. 1987;88(4):362-368.
13. Al-Tawil NG, Berggren G, Emtestam L, Fransson J, Jernselius R, Marcusson JA. Correlation between quantitative in vivo and in vitro responses in nickel-allergic patients. *Acta Derm Venereol*. 1985;65(5):385-389.

14. Kapsenberg ML, Wierenga EA, Stiekema FE, Tiggelman AM, Bos JD. Th1 lymphokine production profiles of nickel-specific CD4+T-lymphocyte clones from nickel contact allergic and non-allergic individuals. *J Invest Dermatol*. 1992;98(1):59-63.
15. Minang JT, Troye-Blomberg M, Lundeberg L, Ahlborg N. Nickel elicits concomitant and correlated in vitro production of Th1-, Th2-type and regulatory cytokines in subjects with contact allergy to nickel. *Scand J Immunol*. 2005;62(3):289-296.
16. Spiewak R, Moed H, von Blomberg BM, et al. Allergic contact dermatitis to nickel: modified in vitro test protocols for better detection of allergen-specific response. *Contact Dermatitis*. 2007;56(2):63-69.
17. Cederbrant K, Anderson C, Andersson T, Marcusson-Stahl M, Hultman P. Cytokine production, lymphocyte proliferation and T-cell receptor Vbeta expression in primary peripheral blood mononuclear cell cultures from nickel-allergic individuals. *Int Arch Allergy Immunol*. 2003;132(4):373-379.
18. Thierse HJ, Gamerdinger K, Junkes C, Guerreiro N, Weltzien HU. T cell receptor (TCR) interaction with haptens: metal ions as non-classical haptens. *Toxicology*. 2005;209(2):101-107.
19. Thierse HJ, Moulon C, Allespach Y, et al. Metal-protein complex-mediated transport and delivery of Ni2+ to TCR/MHC contact sites in nickel-specific human T cell activation. *J Immunol*. 2004;172(3):1926-1934.
20. Romagnoli P, Labhardt AM, Sinigaglia F. Selective interaction of Ni with an MHC-bound peptide. *EMBO J*. 1991;10(6):1303-1306.
21. Yin L, Crawford F, Marrack P, Kappler JW, Dai S. T-cell receptor (TCR) interaction with peptides that mimic nickel offers insight into nickel contact allergy. *Proc Natl Acad Sci U S A*. 2012;109(45):18517-18522.
22. Budinger L, Neuser N, Totzke U, Merk HF, Hertl M. Preferential usage of TCR-Vbeta17 by peripheral and cutaneous T cells in nickel-induced contact dermatitis. *J Immunol*. 2001;167(10):6038-6044.
23. Vollmer J, Fritz M, Dormoy A, Weltzien HU, Moulon C. Dominance of the BV17 element in nickel-specific human T cell receptors relates to severity of contact sensitivity. *Eur J Immunol*. 1997;27(8):1865-1874.
24. Vollmer J, Weltzien HU, Moulon C. TCR reactivity in human nickel allergy indicates contacts with complementarity-determining region 3 but excludes superantigen-like recognition. *J Immunol*. 1999;163(5):2723-2731.
25. Werfel T, Hentschel M, Kapp A, Renz H. Dichotomy of blood- and skin-derived IL-4-producing allergen-specific T cells and restricted V beta repertoire in nickel-mediated contact dermatitis. *J Immunol*. 1997;158(5):2500-2505.
26. Bechara R, Pollastro S, Azoury ME, et al. Identification and characterization of circulating naive CD4+ and CD8+ T cells recognizing nickel. *Front Immunol*. 2019;10:1331.
27. Frentsch M, Arbach O, Kirchhoff D, et al. Direct access to CD4+ T cells specific for defined antigens according to CD154 expression. *Nat Med*. 2005;11(10):1118-1124.
28. Bacher P, Schink C, Teutschbein J, et al. Antigen-reactive T cell enrichment for direct, high-resolution analysis of the human naive and memory Th cell repertoire. *J Immunol*. 2013;190(8):3967-3976.
29. Jakob A, Mussotter F, Ohnesorge S, et al. Immunoproteomic identification and characterization of Ni(2+)-regulated proteins implicates Ni(2+) in the induction of monocyte cell death. *Cell Death Dis*. 2017;8(3):e2684.
30. Archila LD, Chow IT, McGinty JW, et al. Ana o 1 and Ana o 2 cashew allergens share cross-reactive CD4(+) T cell epitopes with other tree nuts. *Clin Exp Allergy*. 2016;46(6):871-883.
31. Britanova OV, Putintseva EV, Shugay M, et al. Age-related decrease in TCR repertoire diversity measured with deep and normalized sequence profiling. *J Immunol*. 2014;192(6):2689-2698.
32. Frentsch M, Stark R, Matzmohr N, et al. CD40L expression permits CD8+ T cells to execute immunologic helper functions. *Blood*. 2013;122(3):405-412.
33. Santamaria Babi LF, Picker LJ, Perez Soler MT, et al. Circulating allergen-reactive T cells from patients with atopic dermatitis and allergic contact dermatitis express the skin-selective homing receptor, the cutaneous lymphocyte-associated antigen. *J Exp Med*. 1995;181(5):1935-1940.
34. Moed H, Boorsma DM, Stoof TJ, et al. Nickel-responding T cells are CD4+ CLA+ CD45RO+ and express chemokine receptors CXCR3, CCR4 and CCR10. *Br J Dermatol*. 2004;151(1):32-41.
35. Zabel BA, Agace WW, Campbell JJ, et al. Human G protein-coupled receptor GPR-9-6/CC chemokine receptor 9 is selectively expressed on intestinal homing T lymphocytes, mucosal lymphocytes, and thymocytes and is required for thymus-expressed chemokine-mediated chemotaxis. *J Exp Med*. 1999;190(9):1241-1256.
36. Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab U, Stein H. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. *J Immunol*. 1984;133(4):1710-1715.
37. Evans RL, Faldetta TJ, Humphreys RE, Pratt DM, Yunis EJ, Schlossman SF. Peripheral human T cells sensitized in mixed leukocyte culture synthesize and express Ia-like antigens. *J Exp Med*. 1978;148(5):1440-1445.
38. Ashouri JF, Weiss A. Endogenous Nur77 is a specific indicator of antigen receptor signaling in human T and B cells. *J Immunol*. 2017;198(2):657-668.
39. Testi R, Phillips JH, Lanier LL. T cell activation via Leu-23 (CD69). *J Immunol*. 1989;143(4):1123-1128.
40. Egorov ES, Merzlyak EM, Shelenkov AA, et al. Quantitative profiling of immune repertoires for minor lymphocyte counts using unique molecular identifiers. *J Immunol*. 2015;194(12):6155-6163.
41. Gamerdinger K, Moulon C, Karp DR, et al. 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. *J Exp Med*. 2003;197(10):1345-1353.
42. Vollmer J, Weltzien HU, Gamerdinger K, Lang S, Choleva Y, Moulon C. Antigen contacts by Ni-reactive TCR: typical alphass chain cooperation versus alpha chain-dominated specificity. *Int Immunol*. 2000;12(12):1723-1731.
43. Andargachew R, Martinez RJ, Kolawole EM, Evavold BD. CD4 T cell affinity diversity is equally maintained during acute and chronic infection. *J Immunol*. 2018;201(1):19-30.
44. Robins HS, Campregher PV, Srivastava SK, et al. Comprehensive assessment of T-cell receptor beta-chain diversity in alphabeta T cells. *Blood*. 2009;114(19):4099-4107.
45. Rudolph MG, Stanfield RL, Wilson IA. How TCRs bind MHCs, peptides, and coreceptors. *Annu Rev Immunol*. 2006;24:419-466.
46. Friedmann PS, Sanchez-Elsner T, Schnuch A. Genetic factors in susceptibility to contact sensitivity. *Contact Dermatitis*. 2015;72(5):263-274.
47. Clayton GM, Wang Y, Crawford F, et al. Structural basis of chronic beryllium disease: linking allergic hypersensitivity and autoimmunity. *Cell*. 2014;158(1):132-142.
48. Pichler WJ, Beeler A, Keller M, et al. Pharmacological interaction of drugs with immune receptors: the p-i concept. *Allergol Int*. 2006;55(1):17-25.
49. Illing PT, Vivian JP, Purcell AW, Rossjohn J, McCluskey J. Human leukocyte antigen-associated drug hypersensitivity. *Curr Opin Immunol*. 2013;25(1):81-89.
50. Kapsenberg ML, Res P, Bos JD, Schootemijer A, Teunissen MB, Van Schooten W. Nickel-specific T lymphocyte clones derived from allergic nickel-contact dermatitis lesions in man: heterogeneity based on requirement of dendritic antigen-presenting cell subsets. *Eur J Immunol*. 1987;17(6):861-865.

51. Lu L, Vollmer J, Moulon C, Weltzien HU, Marrack P, Kappler J. Components of the ligand for a Ni++ reactive human T cell clone. *J Exp Med*. 2003;197(5):567-574.
52. Okhrimenko A, Grun JR, Westendorf K, et al. Human memory T cells from the bone marrow are resting and maintain long-lasting systemic memory. *Proc Natl Acad Sci U S A*. 2014;111(25):9229-9234.
53. Hammarlund E, Lewis MW, Hansen SG, et al. Duration of antiviral immunity after smallpox vaccination. *Nat Med*. 2003;9(9):1131-1137.
54. Gibbs S, Martin SF, Corsini E, Thierse H-J. Identification of contact allergens by in vitro cell culture-based methods. In: John SM, Johansen JD, Rustemeyer T, Elsner P, Maibach HI, eds. *Kanerva's Occupational Dermatology*. Cham: Springer International Publishing; 2018:1-20.
55. van Vliet E, Kuhnl J, Goebel C, et al. State-of-the-art and new options to assess T cell activation by skin sensitizers: Cosmetics Europe Workshop. *Altex*. 2018;35(2):179-192.
56. Thierse H-J, Luch A. Consumer protection and risk assessment: sensitising substances in consumer products. *Allergo J Int*. 2019;28(6):167-182.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Aparicio-Soto M, Riedel F, Leddermann M, et al. TCRs with segment TRAV9-2 or a CDR3 histidine are overrepresented among nickel-specific CD4+ T cells. *Allergy*. 2020;75:2574–2586. https://doi.org/10.1111/all.14322