Received: 11 February 2020 | Revised: 12 May 2020 | Accepted: 26 May 2020

DOI: 10.1111/ajt.16122

ORIGINAL ARTICLE

AJT

Mucosal associated invariant T cells are differentially impaired in tolerant and immunosuppressed liver transplant recipients

Arne Sattler[1*] | Lion G. Thiel[1*] | Annkathrin H. Ruhm[1] | Yasmin Bergmann[1] | Theresa Dornieden[1] | Mira Choi[2] | Fabian Halleck[2] | Frank Friedersdorff[3] | Dennis Eurich[4] | Katja Kotsch[1]

[1]Department for General, Visceral and Vascular Surgery, Charité–Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, Berlin, Germany

[2]Department for Nephrology and Internal Intensive Care, Charité–Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, Berlin, Germany

[3]Department for Urology, Charité–Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, Berlin, Germany

[4]Department for Surgery, Charité–Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, Berlin, Germany

Correspondence
Arne Sattler
Email: arne.sattler@charite.de

Funding information
Bundesministerin für Wirtschaft und Energie, Zentrales Innovationsprogramm Mittelstand, Grant/Award Number: ZF4245603AJ7; Sonnenfeldstiftung, Grant/Award Number: Personal student award to A.H.R.

Mucosal associated invariant T (MAIT-) cells represent a semi-invariant T cell population responsive to microbial vitamin B metabolite and innate cytokine stimulation, executing border tissue protection and particularly contributing to human liver immunity. The impact of immunosuppressants on MAIT cell biology alone and in context with solid organ transplantation has not been thoroughly examined. Here, we demonstrate that in vitro cytokine activation of peripheral MAIT cells from healthy individuals was impaired by glucocorticoids, whereas antigen-specific stimulation was additionally sensitive to calcineurin inhibitors. In liver transplant (LTx) recipients, significant depletion of peripheral MAIT cells was observed that was largely independent of the type and dosage of immunosuppression, equally applied to tolerant patients, and was reproducible in kidney transplant recipients. However, MAIT cells from tolerant LTx patients exhibited a markedly diminished ex vivo activation signature, associated with individual regain of functional competence toward antigenic and cytokine stimulation. Still, MAIT cells from tolerant and treated liver recipients exhibited high levels of PD1, accompanied by functional impairment particularly toward bacterial stimulation that also affected polyfunctionality. Our data suggest interlinked effects of primary liver pathology and immunosuppressive treatment on overall MAIT cell fitness after transplantation and propose their monitoring in context with tolerance induction protocols.

KEYWORDS

basic (laboratory) research/science, flow cytometry, immunobiology, immunosuppressant, immunosuppression/immune modulation, liver transplantation/hepatology, lymphocyte biology: activation, T cell biology, tolerance, translational research/science

Abbreviations: CyA or C, cyclosporine A; GranB, granzyme B; HC, healthy control(s); IS, immunosuppression; ISD, immunosuppressive drug; KTx, kidney transplant; LTx, liver transplant; MAIT cell, mucosal associated invariant T cell; MPA or M, mycophenolic acid; Pred or P, prednisolone; Tac or T, tacrolimus.

*These authors contributed equally.

1 | INTRODUCTION

Mucosal-associated invariant T (MAIT) cells constitute an innate-like T cell subset in mammals[1] that is gaining increasing attention because of its unconventional antigen specificity[2] and an emerging role in host protection[3] and tissue repair.[4] MAIT cells are uniquely identified based on coexpression of a semi-invariant T cell receptor (TCR Vα7.2-Jα33 in humans[5]) along with the C-type lectin CD161 or interleukin 18 receptor alpha;[6] they are restricted by the HLA-Ib major histocompatibility complex (MHC)-related protein I (MR1) presenting vitamin B metabolites produced by a multitude of microbes and fungi that employ the riboflavin synthesis pathway.[2] Accumulating data, including our own,[7,8] indicate that alternative activation modes via inflammatory cytokines exist, positioning MAIT cells in between innate and adaptive immunity. Apart from their abundance in peripheral blood[1] and at mucosal surfaces such as in the intestine[9] or lung,[10] MAIT cells account for up to 45% of intrahepatic T cells[6,11,12] where they likely contribute to an intestine-downstream firewall protecting bile ducts and sinusoids.[13] Several studies have reported a disturbed MAIT cell biology in liver diseases; as a common motif, MAIT cells are significantly depleted from the periphery in autoimmune hepatitis, primary biliary cholangitis, alcoholic liver disease, or chronic hepatitis C, a phenomenon being associated with changes in their functional repertoire.[14-17] So far, it has not been addressed whether MAIT cell homeostasis is restored in transplanted individuals and how diverse immunosuppressive regimens affect its reconstitution. Based on prominent expression of the multidrug resistance protein 1, MAIT cells are protected from xenotoxic stress, for example, induced by common cytostatic therapy.[6] With respect to immunosuppressive agents, however, there is only limited information on how these drugs affect MAIT cell effector functions in vitro and in vivo.

Given their emerging role in host protection, we studied peripheral MAIT cell responses toward bacterial and innate cytokine stimuli in the presence of common immunosuppressive medication in healthy individuals in vitro. Furthermore, we comprehensively extended our analysis to the examination of peripheral blood derived MAIT cell frequencies, activation state, and effector functions in immunosuppressed and tolerant liver transplant recipients ex vivo. Although not exactly reflecting intra-organ biology, our choice of peripheral MAIT cell analysis overcomes experimental limitations imposed by biopsy material, enables repeated sampling, and allows direct comparison with the aforementioned studies on liver diseases. Our data pave the way toward understanding how immunosuppressive therapy and/or the postorgan transplantation state per se affects MAIT cell biology—with possible implications for immunological competence of patients at risk of opportunistic infections.

2 | MATERIAL AND METHODS

2.1 | Study approval

The study protocol was approved by the ethics committee of the Charité-Universitätsmedizin Berlin (No. EA2/028/13, EA2/035/16 and EA4/127/17) and carried out in compliance with its guidelines. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

2.2 | Study participants

Details of individuals enrolled are summarized in Tables 1-3. Liver transplant (LTx) recipients initially diagnosed with hepatitis C virus (HCV) infection did not show viral reactivation at least within 3 years prior to the study. For some analyses, LTx patients treated with immunosuppression (IS) drugs were grouped according to drug levels. Group 1 included patients with serum levels of tacrolimus (Tac) < 3 ng/mL or cyclosporine A (CyA) < 50 ng/mL ± up to 500 mg mycophenolate mofetil (MMF) (1-0-1), Group 2 with Tac 3-5 ng/mL or CyA 50-100 ng/mL ± up to 500 mg MMF (1-0-1), Group 3 with Tac > 5 ng/mL or CyA > 100 ng/mL ± up to 500 mg MMF (1-0-1).

2.3 | Cell isolation

Peripheral blood was collected from liver and kidney transplanted individuals and from healthy controls; peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Paque™ density gradient centrifugation and freshly analyzed.

2.4 | Stimulation conditions

PMBC were cultured in RPMI1640 media containing 0.3 mg/mL glutamine, 100 U/mL penicillin, 0.1 mg/mL streptomycin, and 10% human AB serum (all Biochrom, Cambridge, UK). In all functional assays, cells were stimulated for 22 hours at 37°C. For cytokine stimulation, a combination of interleukin-12 (IL-12), IL-15 (5 ng/mL each, Miltenyi Biotec, Bergisch Gladbach, Germany), IL-18 (5 ng/mL, R&D, Minneapolis, MN), and TL1A (10 ng/mL, R&D) was used after determining optimal concentrations. Brefeldin A (BrefA, Sigma-Aldrich, St. Louis, MO) was present for the last 4 hours. Bacterial stimulation was performed with formalin-fixed *E. coli* (lab strain DH5α) at a pretitrated multiplicity of infection of 30 in the presence of anti-CD28 (1 μg/mL, 28.2, Biolegend, San Diego, CA) for costimulation. Specificity of bacterial stimulation was controlled by adding anti-MR1 (10 μg/mL, 26.5, Biolegend) or isotype control prior to bacterial stimulation. BrefA and Monensin (GolgiStop™, BD Biosciences, San Jose, CA) were added after 4 hours, enabling optimal CD107a and cytokine staining. In some experiments, PBMC were preincubated with immediate posttransplant consensus concentrations (based on Refs. 18 and 19-21 and according to local hospital guidelines) of tacrolimus (10 ng/mL, Astellas Pharma, Tokyo, Japan, Prograf®), cyclosporin A (120 ng/mL, Cayman Chemical, Ann Arbor, MI), mycophenolic acid (2.7 μg/mL, Sigma-Aldrich), or prednisolone (Prednisolut®, 0.57 μg/mL, Mibe, Sandersdorf-Brehna, Germany) for 2 hours and subsequently stimulated as described previously. Drug concentrations were titrated as indicated.

TABLE 1 Characteristics of LTx patients and healthy donors

Variable	All patients (n = 58)	Tolerant patients (n = 8)	Healthy donors (n = 19)
Age (y ± SD)[a]	61.78 (11.75)	63.8 (10.3)	61.26 (6.51)
Gender (females)[b]	21/58 (36.2%)	1/8 (12.5%)	10/19 (52.6%)
Time since transplantation (y ± SD)[a]	12.59 (7.2)	17.3 (5.5)	
Tolerant group (IS-free)[b]		8/58 (13.8%)	
Tolerant group: mean IS-free time[c]		36.8 (75.6)	
IS group 1[b,d]	20/58 (34.5%)		
IS group 2[b,d]	19/58 (32.8%)		
IS group 3[b,d]	11/58 (19%)		
IS medication[b]			
Tacrolimus + mycophenolate mofetil	19/58 (32.8%)		
Tacrolimus	16/58 (27.6%)		
None	8/58 (13.8%)		
Mycophenolate mofetil	6/58 (10.3%)		
Cyclosporin A + mycophenolate mofetil	5/58 (8.6%)		
Cyclosporin A	4/58 (6.9%)		
Primary disease[b]			
Nutritive-toxic cirrhosis	13/58 (22.4%)		
Hepatitis C	11/58 (19%)	5 (62.5%)	
Hepatocellular carcinoma	9/58 (15.5%)	1 (12.5%)	
Hepatitis B	9/58 (15.5%)	1 (12.5%)	
Primary biliary cholangitis	4/58 (6.9%)		
Cryptogenic cirrhosis	3/58 (5.2%)	1 (12.5%)	
Alpha-1 antitrypsin deficiency	2/58 (3.4%)		
Primary sclerosing cholangitis	2/58 (3.4%)		
Budd–Chiari syndrome	1/58 (1.7%)		
Caroli syndrome	1/58 (1.7%)		
Chemotherapy-induced cirrhosis	1/58 (1.7%)		
Secondary biliary cirrhosis	1/58 (1.7%)		
Wilson's disease	1/58 (1.7%)		

Abbreviations: IS, immunosuppression; LTx, liver transplant.

[a]Mean + SD.

[b]Count (%).

[c]Months + SD.

[d]For characterization of IS groups, see materials and methods section.

2.5 | Flow cytometric analysis

For surface stainings, antibodies against CD3 (SK7, Biolegend), CD4 (SK3, Becton Dickinson), CD8 (SK1, Ebioscience, San Diego, CA), CD8β (SIDI8BEE, eBioscience), CD25 (BC96, Biolegend), CD27 (M-T271, Biolegend), CD38 (HIT2, BD), CD45RA (HI100, Biolegend), CD56 (5.1H11, Biolegend), CD69 (FN50, Biolegend), CD103 (Ber-ACT8, Biolegend), CD161 (HP-3G10, Biolegend), TCRVα7.2 (3C10, Biolegend), IL-18Rα (H44, Biolegend), CCR7 (3D12, BD), KLRG1 (SA231A2, Biolegend), NKG2D (1D11, Biolegend), HLA-DR (L243, Biolegend), and PD1 (EH12.1, BD) were used. Unwanted cells were excluded via a "dump channel" containing $CD14^+$ (M5E2, Biolegend), $CD19^+$ (HIB19, Biolegend) and dead cells (fixable live/dead, Biolegend). The basic gating

TABLE 2 Characteristics of IS-free LTx patients

Patient number	01	02[a]	03[a]	04	05	06	07	08[a]
Age (y)	53	61	75	46	74	64	72	65
Gender (m/f)	m	m	m	f	m	m	m	m
Time since Tx (y)	19	9	14	26	12	22	22	14
IS-free time (mo)	19	6	9	223	9	21	3	4
Primary disease	HCV	Crypt.	HCV	HBV	HCV	HCV	HCC	HCV
Biopsy	Yes	Yes	Yes	Yes	No	Yes	No	No
Rejection	No	No	No	No	n.a.	No	n.a.	n.a.
Liver function[b]								
gGT (U/L)	211	300	52	27	20	17	14	28
AP (U/L)	158	135	56	86	75	62	48	73
Total bilirubin (mg/dL)	0.7	0.8	1.1	1.11	0.63	0.6	1.61	0.15
ALT/AST (U/L)	36/43	38/48	30/31	23/31	18/28	13/21	34/33	23/20
INR	1.03	0.92	1.14	1.35	0.99	1.23	1.12	n.d.

Abbreviations: ALT/AST, aspartate transaminase/alanine transaminase; AP, alkaline phosphatase; gGT, Gamma-glutamyltransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; INR, international normalized ratio; IS, immunosuppression; LTx, liver transplant; n.a., not applicable; n.d. not determined.

[a]Weaned during the study.

[b]At time of cellular analysis.

strategy to identify MAIT cells and other subsets within PBMC is shown in Figure S1A.

After stimulation, cells were fixed in FACS™ Lysing Solution (BD), permeabilized with FACS™ Perm II Solution (BD), and intracellularly stained with anti-tumor necrosis factor alpha (anti-TNFα) (MAb11, Biolegend), anti-interferon gamma (anti-IFNγ) (4SB3, Ebioscience), anti-granzymeB (GB11, BD), and anti-IL-17 (BL168, Biolegend). Where indicated, granzymeB or activated Caspase 3 (C92-605, BD) were intracellularly assessed ex vivo. For assessing expression of FoxP3 (150D, Biolegend), CTLA-4 (BNI3, Biolegend), and/or Ki67 (B56, BD), cells were fixed and permeabilized using the Foxp3 staining buffer set (Ebioscience). Cells were analyzed on a FACS™ Fortessa X20 (BD).

2.6 | Data analysis

FACS data were analyzed with FlowJo 10 (BD); polyfunctionality was assessed via Boolean gating. Statistical analysis and graph creation were conducted using GraphPad Prism 7.0 (GraphPad, La Jolla, CA). The Kolmogorov-Smirnov test was used to evaluate the distribution of each parameter. Depending on Gaussian distribution, analysis of variance (ANOVA) or Kruskal-Wallis test (with Dunn post hoc) was chosen for multiple comparisons; for 2-group comparisons with unpaired samples, *t* test with Welch's correction or Mann-Whitney test was used. For analysis of paired data (eg, titrations), paired *t* test or Wilcoxon matched-pairs signed rank test was performed depending on data distribution. In all tests, a value of $P < .05$ was considered significant with $^{*}P \leq .05$, $^{**}P \leq .01$, $^{***}P \leq .001$, and $^{****}P \leq .0001$.

3 | RESULTS

3.1 | Impairment of cytokine-induced MAIT cell functions by immunosuppressive drugs in vitro

To address the functional impact of immunosuppressive agents used in clinical routine after solid organ transplantation on human MAIT cells, PBMC from healthy donors were preincubated with tacrolimus, cyclosporine A, mycophenolic acid, or prednisolone. Thereafter, cells were stimulated with pretitrated (data not shown) concentrations of IL-12, IL-15, IL-18, and TL1A, a cytokine combination robustly inducing the effector molecules TNFα, IFNγ, and granzymeB in MAIT cells in a T cell receptor independent fashion.[7,8] Whereas prednisolone significantly inhibited TNFα and IFNγ secretion but did not reduce frequencies of MAIT cells producing the cytotoxic mediator granzymeB, none of the other drugs affected MAIT cell functionality (Figure 1A-D). Titration of immunosuppressive agents revealed that increasing concentrations by 10-fold further diminished $TNF\alpha^{+}$ MAIT cell frequencies significantly only in the presence of prednisolone (Figure S1B); higher concentrations of immunosuppressants did not show additional effects on frequencies of $IFN\gamma^{+}$ or $granzymeB^{+}$ cells (data not shown). Only prednisolone significantly affected multipotency of MAIT cells as mirrored by diminished portions of cells coexpressing all 3

TABLE 3 Characteristics of KTx patients

Variable	Patients (n = 23)
Age (y ± SD)[a]	58.57 (11.7)
Gender (females)[b]	8/23 (34.8%)
Time since transplantation (y ± SD)[a]	9.3 (5.0)
IS medication[b]	
Tacrolimus + mycophenolate mofetil + prednisolone	11/23 (47.8%)
Cyclosporin A + mycophenolate mofetil + prednisolone	8/23 (34.8%)
Tacrolimus + mycophenolate mofetil	2/23 (8.7%)
Tacrolimus + prednisolone	2/23 (8.7%)
Primary disease[b]	
Hypertensive nephropathy	5/23 (21.7%)
IgA nephropathy	3/23 (13%)
Glomerulonephritis	3/23 (13%)
Pyelonephritis	2/23 (8.7%)
Polycystic kidney disease	2/23 (8.7%)
Unknown	2/23 (8.7%)
Cystinosis	1/23 (4.3%)
Diabetic nephropathy	1/23 (4.3%)
Diabetic + hypertensive nephropathy	1/23 (4.3%)
Focal segmental glomerulosclerosis	1/23 (4.3%)
Obstructive nephropathy	1/23 (4.3%)
Systemic lupus erythematosus	1/23 (4.3%)

Abbreviations: IS, immunosuppression; KTx, kidney transplant.
[a]Mean + SD.
[b]Count (%).

effector molecules (Figure 1E,F), accompanied by an increase of cells expressing only one or no effector molecule (Figure 1F). Unless otherwise stated, live $CD3^+TCRV\alpha7.2^+CD161^+$ $CD8^+$ and $CD8^-CD4^-$ "DN" MAIT cells were identified within PBMC as depicted in the gating strategy in Figure S1A.

3.2 | Impairment of *E. coli* induced MAIT cell functions by immunosuppressive drugs in vitro

To decipher whether immunosuppressants influence MAIT cell functions induced by antigenic triggering, PBMC of healthy individuals were activated with formalin-fixed *E. coli*. IL-17 and the degranulation marker CD107a were included in the analysis being typically induced after TCR-dependent MAIT cell activation. Under these conditions, Tac, CyA, and prednisolone (Pred) significantly diminished frequencies of $TNF\alpha^+$ and $IL\text{-}17^+$ MAIT cells, whereas Tac and Pred also significantly reduced portions of $IFN\gamma^+$ cells; granzymeB expression was impaired only by Pred. Interestingly, the capacity of MAIT cells to release cytotoxic granula, being reflected by CD107a staining, was not affected by any of the drugs applied (Figure 2A-F). A dose dependency of immunosuppressive agents on frequencies of $TNF\alpha^+$ MAIT cells was evident for both Tac, CyA and Pred (Figure S1C). In context with bacterial stimulation, polyfunctionality of MAIT cells was significantly diminished following preincubation with Tac and CyA and moderately affected by Pred, as mirrored by reduced frequencies of cells expressing 4 effector molecules at a time (TNFα, IFNγ, granzymeB, and CD107a) (Figure 2G,H). As frequencies of $IL\text{-}17^+$ MAIT cells were very low, they were excluded from polyfunctional analyses. In line with earlier reports,[22] bacterial activation of MAIT cells proved to be largely, but not completely antigen-specific as mirrored by substantial, but not full abrogation of effector function upon MR1 blockade (Figure S1D).

3.3 | Depletion of peripheral MAIT cells in immunosuppressed and tolerant liver transplant recipients

To address how different immunosuppressive regimens affect MAIT cell biology in vivo, their frequencies were determined in treated liver transplant recipients in comparison to a group of tolerant patients as well as to age-matched healthy controls (Tables 1 and 2). Overall, frequencies of $TCRV\alpha7.2^+CD161^+$ MAIT cells within the $CD3^+$ T cell compartment were significantly reduced in LTx patients as compared to healthy controls (Figure 3B). This observation was largely independent of the type of IS regimen (Figure 3A,C) or the dosage of medication (Figure 3D) and equally accounted for tolerant patients (Figure 3A,C,D). These findings could be reproduced when only MAIT cells within the $CD8^+$ and DN compartment were quantified (Figure 3F-H). For a limited number of LTx patients, we assessed MAIT cells during immunosuppressive therapy and 4-9 months after cessation (Table 2). After successful weaning from medication (as reflected by stable graft function), we did not note consistent changes in MAIT cell frequencies both within the $CD3^+$ and the $CD8^+$/DN compartment (Figure 3E,I). Subset distribution analysis highlighted a significant rise in $CD4^+$ MAIT cells (Figure 3J) in treated, but not tolerant LTx patients at the expense of the $CD8^+$ population (Figure 3K), whereas the DN subset remained unaffected (Figure 3L). There was no significant correlation between MAIT cell frequencies and time since transplantation in all LTx patients (data not shown).

Contrary to MAIT cell subset distribution (Figure 3J,K), we observed an inverted ratio of conventional T cells with increased portions of $CD8^+$ at the expense of the $CD4^+$ population in all LTx patients regardless of being treated or not, as compared to healthy controls (Figure S2A,B). Whereas frequencies of $CD3^-CD56^+$ natural killer (NK) cells were similar in all groups (Figure S2C), we detected a significant drop of $CD3^+CD4^+CD25^+FoxP3^+$ natural regulatory T cells in patients treated with immunosuppressive drugs (ISDs) but not in tolerant patients, as compared to healthy controls (Figure S2D). Natural regulatory T cells (nTregs) of both patient groups were further characterized by significantly reduced portions of CTLA4, higher proliferation as mirrored by Ki67 expression, but no differences in frequencies of activated $HLA\text{-}DR^+$ cells (Figure S2E-G). nTregs from all LTx patients showed a substantial reduction of the naïve $CD45RA^+CCR7^+$ subset, counterbalanced by a rise in $CD45RA^-CCR7^-$ effector/memory nTregs (Figure S2H,I).

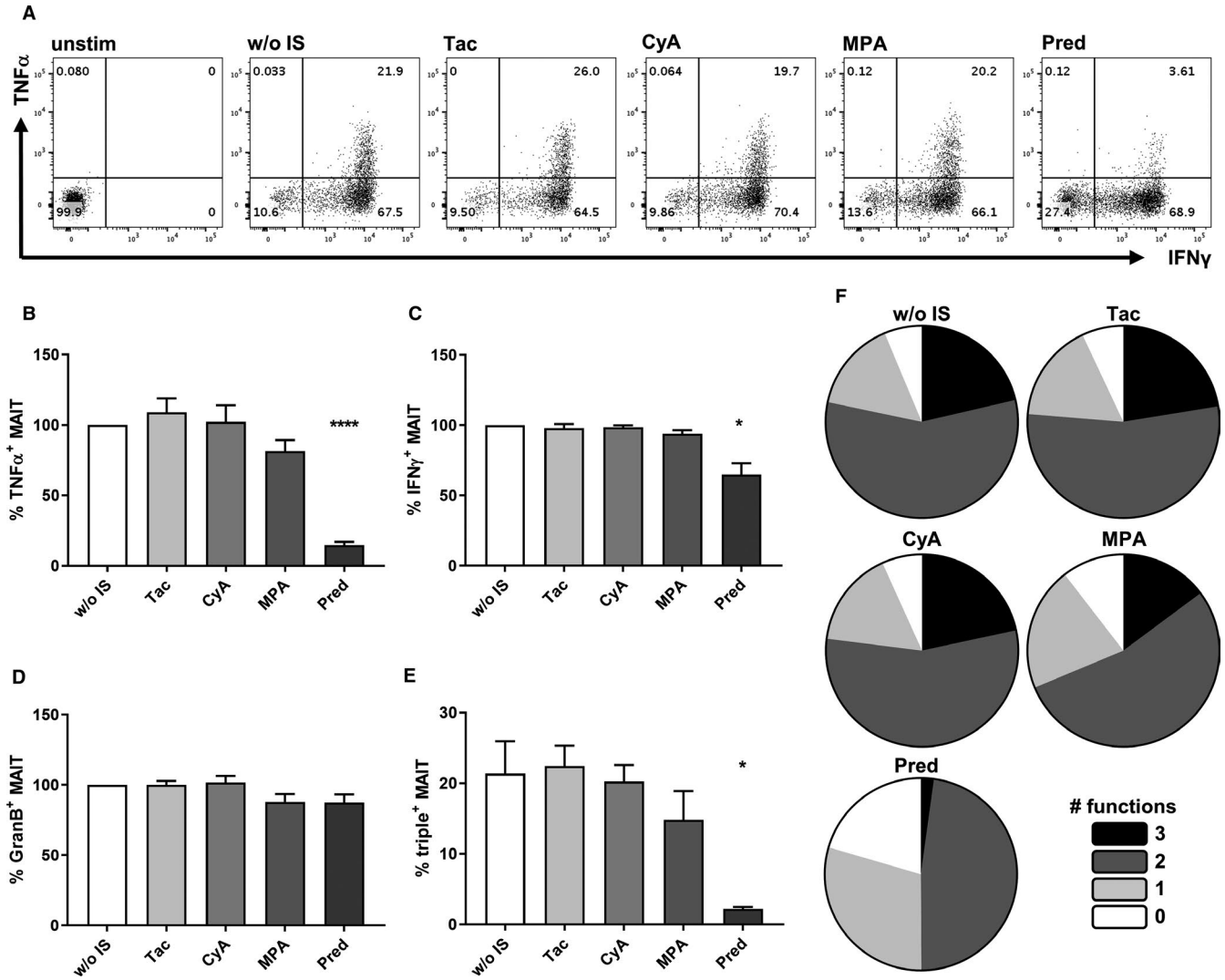


FIGURE 1 Impact of immunosuppressive drugs on cytokine-mediated MAIT cell activation in vitro. Peripheral blood mononuclear cells of healthy donors were preincubated with immunosuppressive drugs as indicated, followed by stimulation with IL-12, IL-15, IL-18, and TL1A. Frequencies of $CD8^+$ and $CD8^-CD4^-$ "DN" MAIT cells expressing TNFα (A,B), IFNγ (A,C), or granzymeB (D) were quantified by FACS. Polyfunctionality was assessed by Boolean gating in MAIT cells that express 3 (E,F), 2, 1, or no functions (TNFα, IFNγ, granzymeB) (F) at a time. Results are based on 5 individual donors obtained in 3 independent experiments. For polyfunctionality analysis (F), the respective means were used. In (B-D), values of stimulated cultures in the absence of ISDs were set to 100%, allowing normalization. All bar graphs show means ± SEM. In all statistical analyses with more than 2 groups, treatment groups were compared to the reference group (w/o IS). CyA, cyclosporine A; FACS, fluorescence-activated cell sorting; IFNγ, interferon gamma; IL, interleukin; IS, immunosuppression; ISD, immunosuppressive drug; MAIT, mucosal associated invariant T; MPA, mycophenolic acid; Pred, prednisolone; Tac, tacrolimus; TNFα, tumor necrosis factor alpha

3.4 | An ex vivo activation signature on MAIT cells distinguishes tolerant LTx patients from those receiving immunosuppressive therapy

To identify cellular signatures that are differentially regulated on MAIT cells isolated from immunosuppressed or tolerant LTx patients, a set of activation and differentiation related molecules was analyzed ex vivo by flow cytometry. First, frequencies of cells expressing the activation markers CD69 (Figure 4A), HLA-DR (Figure 4D), CD38 (Figure 4G), the inducible inhibitor PD1 (Figure 4M), the cytotoxic mediator granzymeB (Figure 4P), or coexpressing CD38 and HLA-DR (Figure 4J) were significantly upregulated in patients as compared to healthy controls. Stratification for treatment regimen, however, revealed that tolerant patients showed no significant differences in frequencies of $CD69^+$ (Figure 4B), HLA-DR^+ (Figure 4E), $CD38^+$ (Figure 4H), or granzymeB^+ (Figure 4Q) MAIT cells in comparison to healthy individuals, whereas these markers were significantly upregulated in most treated LTx recipients. Comparing this activation signature in patients during treatment and after cessation of medication, we noted a consistent reduction of MAIT cells being $CD38^+$ (Figure 4I), $CD38^+$HLA-DR^+ (Figure 4L), and granzymeB^+ (Figure 4R). Apart from diminished frequencies of IL-$18R^+$ MAIT cells in both tolerant and treated patients (Figure S3A) that did now show a distinct

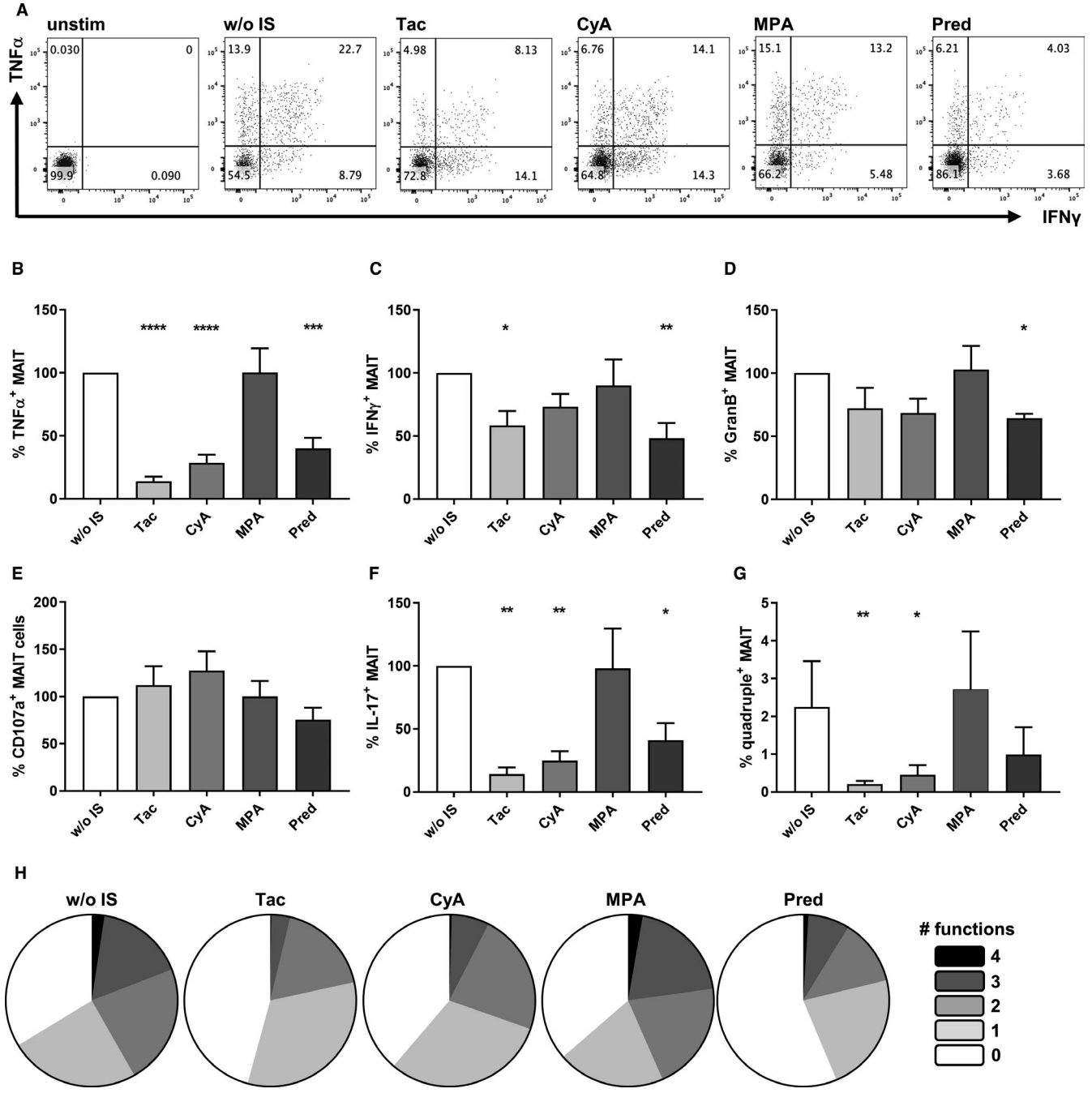


FIGURE 2 Impact of immunosuppressive drugs on bacterial MAIT cell activation in vitro. Peripheral blood mononuclear cells of healthy donors were preincubated with immunosuppressive drugs as indicated, followed by stimulation with fixed *E. coli*. Frequencies of MAIT cells expressing TNFα (A,B), IFNγ (A,C), granzymeB (D), CD107a (E), or IL-17 (F) were quantified by FACS. Polyfunctionality was assessed by Boolean gating in MAIT cells that express 4 (G,H), 3, 2, 1, or no functions (TNFα, IFNγ, granzymeB, CD107a) (H) at a time. Results are based on 6 individual donors obtained in 3 independent experiments. For polyfunctionality analysis (H), the respective means were used. In (B-F), values of stimulated cultures in the absence of ISDs were set to 100%, allowing normalization. All bar graphs show means ± SEM. In all statistical analyses with more than 2 groups, treatment groups were compared to the reference group (w/o IS). CyA, cyclosporine A; FACS, fluorescence-activated cell sorting; IFNγ, interferon gamma; IL, interleukin; IS, immunosuppression; ISD, immunosuppressive drug; MAIT, mucosal associated invariant T; MMF, mycophenolate mofetil; MPA, mycophenolic acid; Pred, prednisolone; TNFα, tumor necrosis factor alpha

expression in subgroups (data not shown), transplant recipients did not exhibit quantitative differences in MAIT cells expressing the pro-apoptotic marker activated caspase 3, or the differentiation related molecules CD56, CD27, or CD8β ex vivo; however, we found the NK-associated activating receptor NKG2D to be slightly upregulated in treated patients (Figure S3B-F).

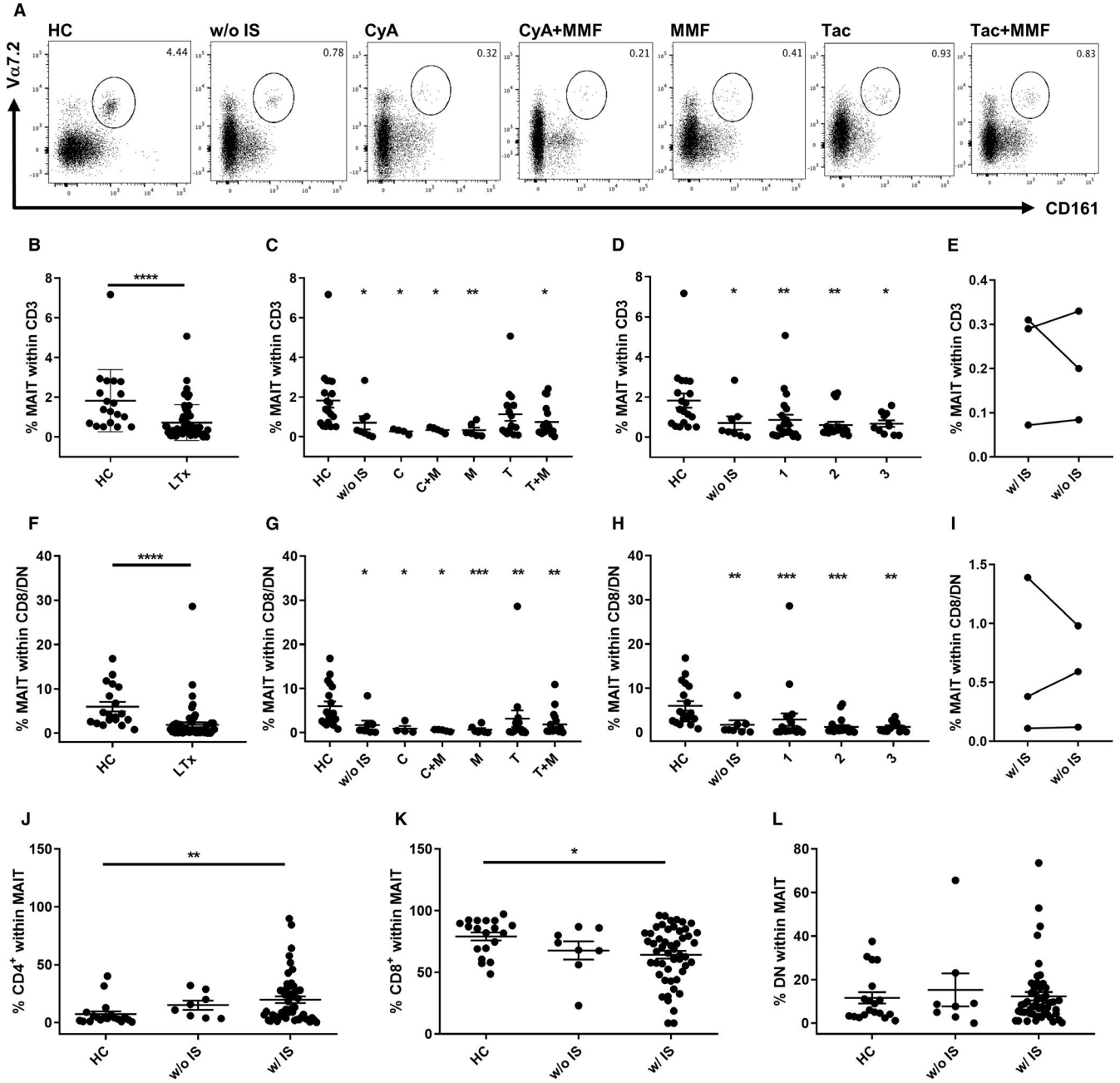
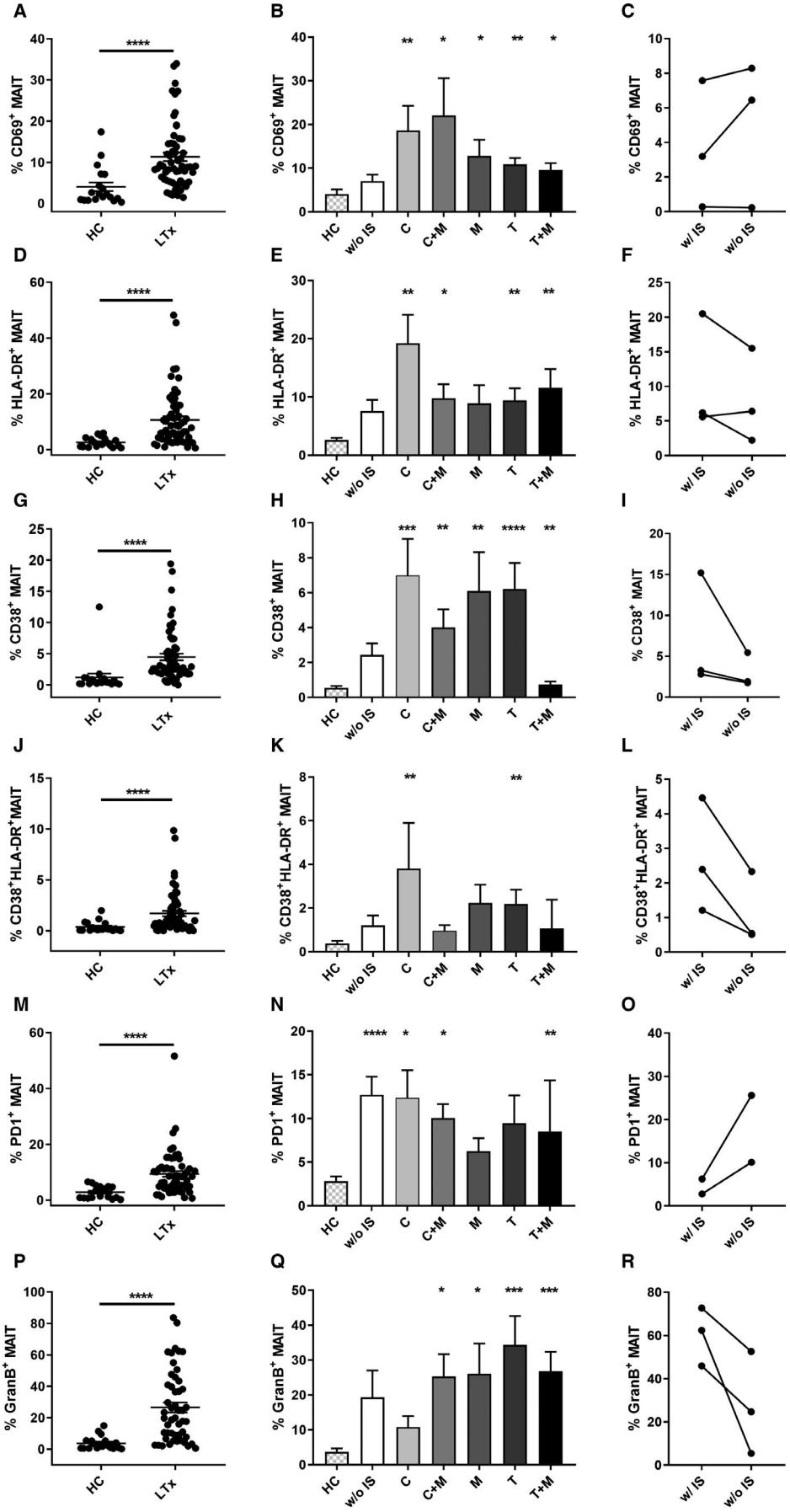


FIGURE 3 Quantitative impairment of MAIT cells in LTx patients. Frequencies of peripheral MAIT cells were determined in tolerant and treated liver transplant recipients and age-matched healthy controls (HC) by FACS within the total $CD3^+$ (B-D) or combined $CD8^+$/DN (A and F-H) compartment. Patients were further stratified according to their type (C + G) or dosage of treatment, or the absence thereof (see Table 1 for details) (D,H). The above mentioned analyses were further conducted in 3 liver transplant recipients during ISD treatment and 4-9 mo after complete drug weaning (E,I). $CD4^+$, $CD8^+$, and DN MAIT cell subsets were individually quantified in tolerant and treated patients and compared to healthy donors (J-L). All bar graphs show means ± SEM. In all statistical analyses with more than 2 groups, treatment groups were compared to the reference group (HC). C or CyA, cyclosporine A; FACS, fluorescence-activated cell sorting; HC, healthy controls; ISD, immunosuppressive drug; MAIT, mucosal associated invariant T; M or MPA, mycophenolic acid; Pred, prednisolone; T or Tac, tacrolimus

FIGURE 4 Tolerant and treated LTx patients are characterized by different activation patterns ex vivo. Frequencies of $CD8^+$/DN peripheral MAIT cells expressing CD69 (A,B), HLA-DR (D,E), CD38 (G,H), HLA-DR^+CD38 (J,K), PD1 (M,N), or granzymeB (P,Q) were quantified ex vivo by FACS in tolerant and treated liver transplant recipients in comparison with age-matched healthy controls. The marker set was further monitored in 3 liver transplant recipients during and after cessation of ISD treatment (C, F, I, L, O, and R). All bar graphs show means ± SEM. In all statistical analyses with more than 2 groups, treatment groups were compared to the reference group (HC). C, cyclosporine A; FACS, fluorescence-activated cell sorting; HC, healthy controls; IS, immunosuppression; ISD, immunosuppressive drug; LTx, liver transplant; M, mycophenolic acid; MAIT, mucosal associated invariant T; T, tacrolimus


A
% CD69+ MAIT
****
HC
LTx
B
% CD69+ MAIT
**
*
*
**
*
HC
w/o IS
C
C+M
M
T
T+M
C
% CD69+ MAIT
w/ IS
w/o IS
D
% HLA-DR+ MAIT
****
HC
LTx
E
% HLA-DR+ MAIT
**
*
**
**
HC
w/o IS
C
C+M
M
T
T+M
F
% HLA-DR+ MAIT
w/ IS
w/o IS
G
% CD38+ MAIT
****
HC
LTx
H
% CD38+ MAIT
***
**
**
****
**
HC
w/o IS
C
C+M
M
T
T+M
I
% CD38+ MAIT
w/ IS
w/o IS
J
% CD38+HLA-DR+MAIT
****
HC
LTx
K
% CD38+HLA-DR+ MAIT
**
**
HC
w/o IS
C
C+M
M
T
T+M
L
% CD38+HLA-DR+ MAIT
w/ IS
w/o IS
M
% PD1+ MAIT
****
HC
LTx
N
% PD1+ MAIT
****
*
*
**
HC
w/o IS
C
C+M
M
T
T+M
O
% PD1+ MAIT
w/ IS
w/o IS
P
% GranB+ MAIT
****
HC
LTx
Q
% GranB+ MAIT
*
*
***
***
HC
w/o IS
C
C+M
M
T
T+M
R
% GranB+ MAIT
w/ IS
w/o IS

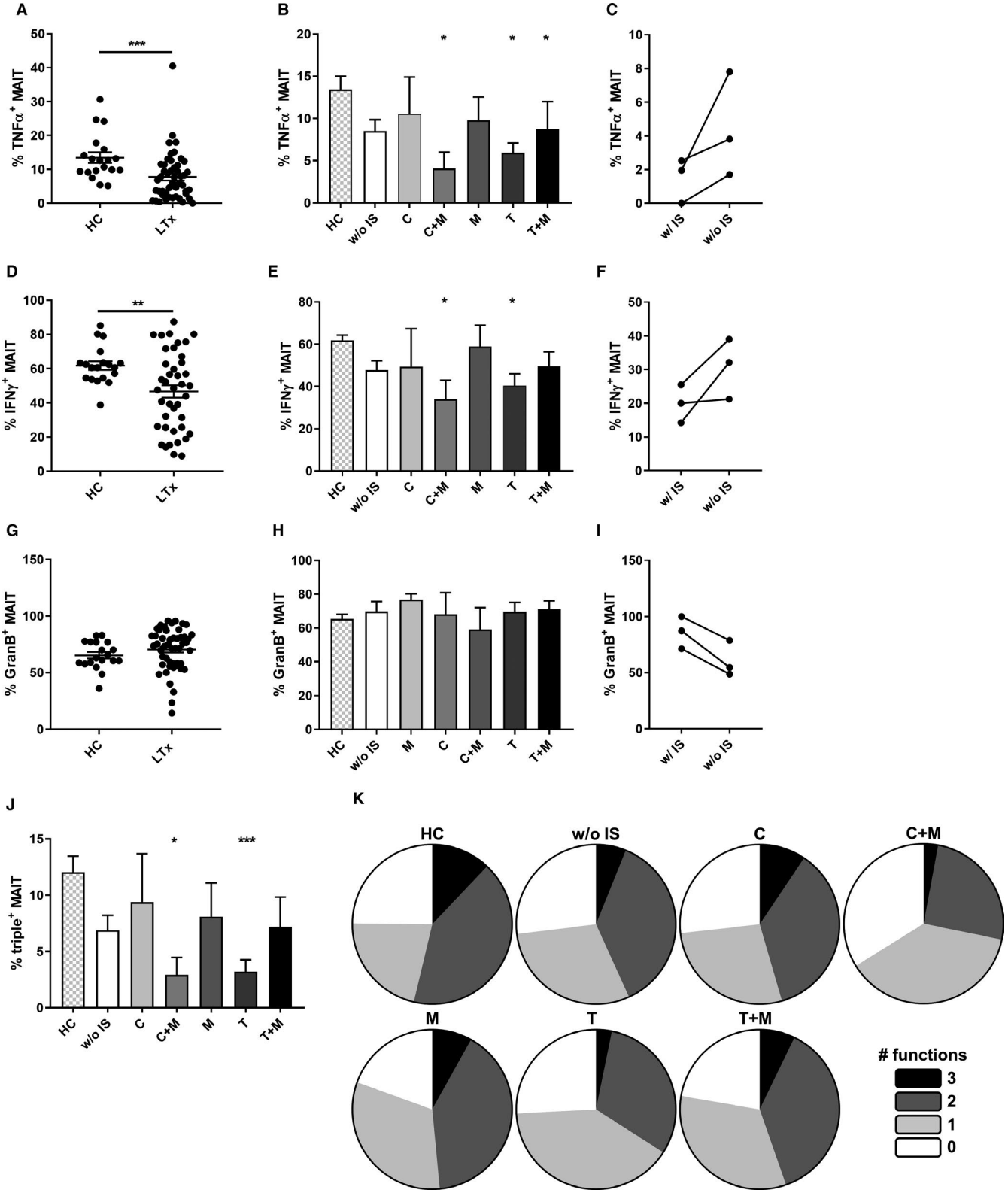


FIGURE 5 Differential impairment of MAIT cells from LTx patients in response to cytokine stimulation. Peripheral blood mononuclear cells from differently treated or tolerant LTx patients and healthy controls were stimulated with IL-12, IL-15, IL-18, and TL1A. Frequencies of MAIT cells expressing TNFα (A,B), IFNγ (D,E), or granzymeB (G,H) were quantified by FACS. MAIT cells from patients before and after ISD weaning were analyzed in an identical fashion (C, F, and I). Polyfunctionality was assessed by Boolean gating in MAIT cells that express 3 (J,K), 2, 1 or no functions (TNFα, IFNγ, granzymeB) (K) at a time. For polyfunctional analysis in (K), the respective means were used. All bar graphs show means ± SEM. In all statistical analyses with more than 2 groups, treatment groups were compared to the reference group (HC). C, cyclosporine A; FACS, fluorescence-activated cell sorting; HC, healthy controls; IFNγ, interferon gamma; IL, interleukin; IS, immunosuppression; LTx, liver transplant; M, mycophenolic acid; MAIT, mucosal associated invariant T; T, tacrolimus; TNFα, tumor necrosis factor alpha

3.5 | Impairment of cytokine-induced MAIT cell functions in LTx patients

To address whether MAIT cells isolated from LTx patients are functionally altered in response to inflammatory cytokines, cells were stimulated directly ex vivo with IL-12, IL-15, IL-18, and TL1A and analyzed for cytokine and cytotoxic molecule production. Collectively, transplanted individuals showed a significant impairment of TNFα and IFNγ production, whereas frequencies of cytokine-induced granzymeB expressing cells were not affected (Figure 5A,D,G). With respect to different treatment regimens, individuals receiving CyA+ MPA or Tac (±MPA with respect to TNFα production) exhibited significantly reduced frequencies of $TNF\alpha^+$ and $IFN\gamma^+$ cells (Figure 5B,E), accompanied by a drop in multipotency (Figure 5J,K). Importantly, whereas MAIT cells of patients weaned from immunosuppression regained their capacity to secrete higher levels of TNFα and IFNγ, they were characterized by a consistent drop in $granzymeB^+$ cells after cessation of medication (Figure 5C,F,I).

3.6 | Impairment of *E. coli* induced MAIT cell functions in LTx patients

In analogy to previous in vitro experiments (Figure 2), we compared the functional capacity of MAIT cells toward bacterial stimulation between LTx patients and healthy controls ex vivo. Overall, frequencies of *E. coli* reactive MAIT cells expressing TNFα (Figure 6A), IFNγ (Figure 6D), and CD107a (Figure 6J) were significantly reduced in patients as compared to controls, whereas those producing granzymeB showed a pronounced increase (Figure 6G). We observed a trend toward reduced frequencies of $TNF\alpha^+$, $IFN\gamma^+$, and $CD107a^+$ MAIT cells in patients regardless of the type of medication (Figure 6B,E,K) that reached significance for IFNγ not only in individuals treated with mycophenolic acid (MPA), Tac-, or Tac+ MPA but also in tolerant LTx patients (Figure 6E), whereas a drop in $CD107a^+$ cells was confined to the 3 aforementioned treatment groups only (Figure 6K). Assessment of polyfunctionality of bacteria-triggered MAIT cells revealed a significant reduction in cells coexpressing all 4 molecules in patients treated with Tac ± MPA as well as in tolerant patients (Figure 6M,N). Withdrawal from immunosuppression led to increased portions of cytokine and CD107a positive cells in all 3 individuals monitored (Figure 6C,F,L) but showed no consistency with respect to granzymeB expression (Figure 6I). Bacterial induction of IL-17 was neither significantly diminished in treated nor in tolerant LTx patients and not included into polyfunctional analyses because of low event counts (data not shown).

3.7 | Kidney transplanted individuals share quantitative and qualitative features of MAIT cells with patients after liver transplantation

To investigate whether prominent features of MAIT cells identified in LTx patients are shared with immunosuppressed individuals after kidney transplantation (KTx, Table 3), cells from a cohort with 23 patients were analyzed ex vivo by flow cytometry. Interestingly, KTx patients exhibited equally diminished frequencies of MAIT cells both within the $CD3^+$ (Figure 7A) and the $CD8^+$/DN (Figure 7B) subsets and we also noted a significant reduction of $CD8^+$ MAIT cells that was counterbalanced by a rise of the $CD4^+$ and DN subsets, although not reaching significance (Figure 7C-E). Whereas portions of $CD69^+$ and $CD38^+$ MAIT cells were similarly increased as observed for LTx recipients in comparison to healthy controls (Figure 7F,G), we could not detect significant changes in frequencies of HLA-DR- or PD1 expressing MAIT cells ex vivo (Figure 7H,I). Also in agreement with our data from liver transplant recipients, we observed an inverted CD4 to CD8 ratio in KTx patients (Figure 7J,K).

4 | DISCUSSION

In this report, we comprehensively examined how peripheral MAIT cell biology is affected by immunosuppressive medication in vitro and in individuals after liver and kidney transplantation ex vivo. Based on a comparison between tolerant or recently weaned LTx patients and treated individuals, we were able to attribute abnormalities in activation state, phenotype, and effector functions partially to the ISD treatment, whereas quantitative impairment is most likely related to the overall post liver transplantation state, because MAIT cell frequencies do not significantly recover after cessation of treatment.

Owing to their innate receptor repertoire, MAIT cells are not solely dependent on TCR mediated activation by bacterial ligands but also respond to cytokines, for example, released in the course of bacterial[23] and viral infections.[3] As recently published by us and others,[8,24] the TL1A containing cytokine combination applied herein robustly and predominantly induces production of the key antimicrobial mediators TNFα, IFNγ, and granzymeB in human MAIT cells. Under these conditions, only prednisolone significantly inhibited cytokine production and polyfunctionality in vitro, whereas cytotoxicity, based on granzyme expression, remained unaffected. In contrast, *E. coli* induced cytokine production and polyfunctionality were diminished both in the presence of calcineurin inhibitors and prednisolone, whereas only the latter significantly reduced frequencies of $granzymeB^+$ MAIT cells. Our data substantially extend the earlier finding that high expression of the multidrug resistance protein 1 does not rescue MAIT cell proliferation from inhibition by IS drugs,[25] because we now provide evidence for both impaired TCR-dependent and -independent effector functions. At the same time, our results mirror the respective signaling pathways required for effector molecule synthesis: whereas cytokine-mediated TNFα and IFNγ production involves phosphorylation of NFκB,[8,26] a component classically targeted by glucocorticoids,[27] *E. coli* induced activation involves TCR downstream calcineurin that is blocked by tacrolimus and cyclosporin A (reviewed in Ref. 28), as well as glucocorticoid targets such as AP-1 (reviewed in Ref. 29). The observation that individual MAIT cell effector functions are differentially regulated by ISD

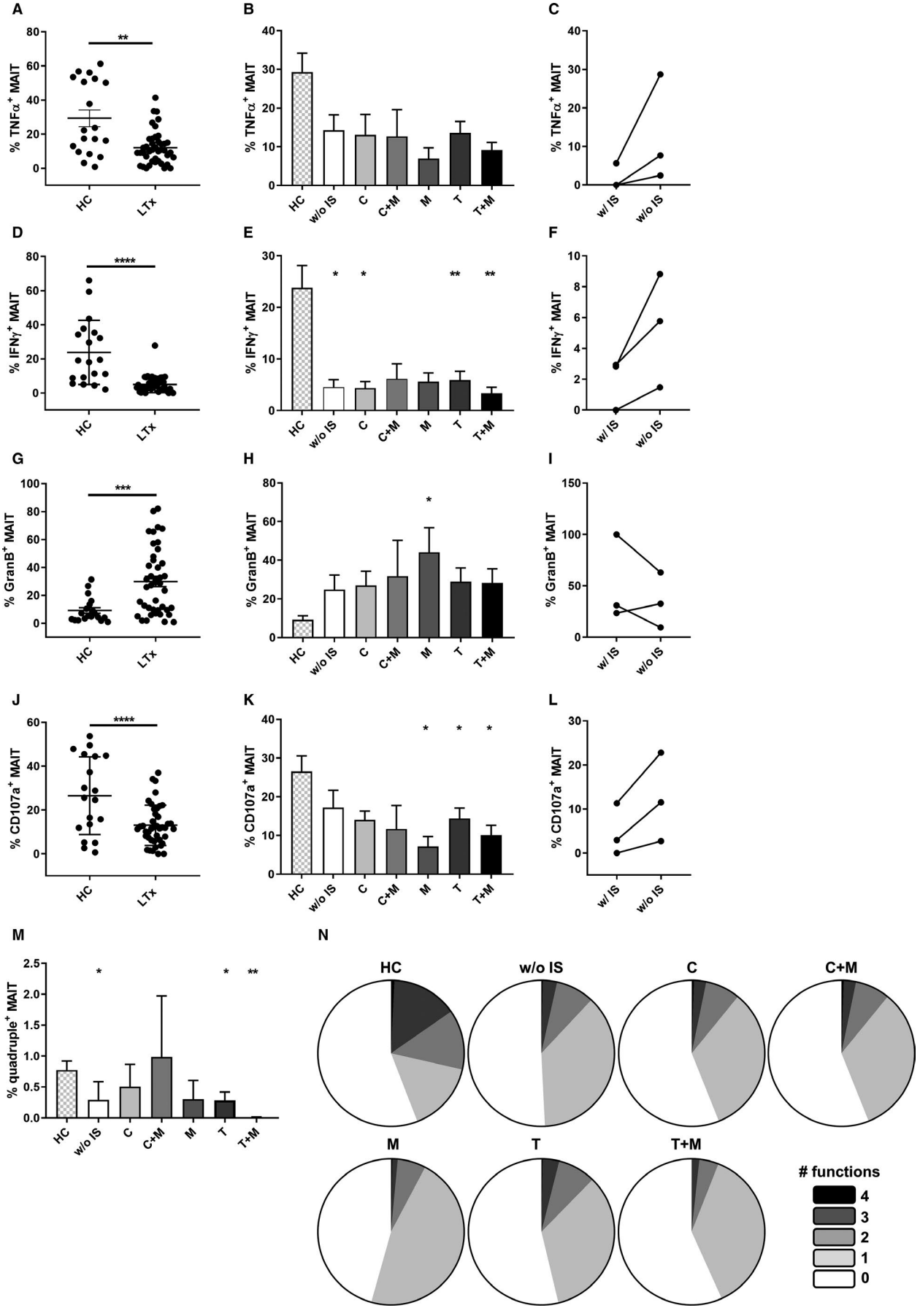

A
% TNFα+ MAIT
**
HC
LTx
B
% TNFα+ MAIT
HC
w/o IS
C
C+M
M
T
T+M
C
% TNFα+ MAIT
w/ IS
w/o IS
D
% IFNγ+ MAIT
****
HC
LTx
E
% IFNγ+ MAIT
* * ** **
HC
w/o IS
C
C+M
M
T
T+M
F
% IFNγ+ MAIT
w/ IS
w/o IS
G
% GranB+ MAIT
***
HC
LTx
H
% GranB+ MAIT
*
HC
w/o IS
C
C+M
M
T
T+M
I
% GranB+ MAIT
w/ IS
w/o IS
J
% CD107a+ MAIT
****
HC
LTx
K
% CD107a+ MAIT
* * *
HC
w/o IS
C
C+M
M
T
T+M
L
% CD107a+ MAIT
w/ IS
w/o IS
M
% quadruple+ MAIT
* * **
HC
w/o IS
C
C+M
M
T
T+M
N
HC
w/o IS
C
C+M
M
T
T+M
functions
4
3
2
1
0

FIGURE 6 Differential functional responses of MAIT cells from LTx patients toward bacterial stimulation. Peripheral blood mononuclear cells from differently treated or tolerant LTx patients and healthy controls were stimulated with formalin-fixed *E. coli*. Frequencies of MAIT cells expressing TNFα (A,B), IFNγ (D,E), granzymeB (G,H), or CD107a (J,K) were quantified by FACS. MAIT cells from patients before and after ISD treatment cessation were analyzed in an identical fashion (C, F, I, and L). Polyfunctionality was assessed by Boolean gating in MAIT cells that express 4 (M,N), 3, 2, 1, or no functions (TNFα, IFNγ, granzymeB, CD107a) (N) at a time. For the latter analysis, the respective means were used. All bar graphs show means ± SEM. In all statistical analyses with more than 2 groups, treatment groups were compared to the reference group (HC). C, cyclosporine A; FACS, fluorescence-activated cell sorting; HC, healthy controls; IFNγ, interferon gamma; IS, immunosuppression; LTx, liver transplant; M, mycophenolic acid; MAIT, mucosal associated invariant T; T, tacrolimus; TNFα, tumor necrosis factor alpha

treatment is supported by data focusing on conventional T cells: similarly to MAIT cells, TNFα production by virus-specific T cells showed a higher sensitivity toward inhibition by immunosuppressants than IFNγ secretion. Furthermore, the cytotoxic potential was only mildly affected by calcineurin inhibitors or prednisolone.[18] At least with respect to IFNγ and granzymeB production, our findings are mechanistically comprehensible as *E. coli* induced effector molecule synthesis in MAIT cells is not solely TCR-dependent, which we confirm in MR1 blocking experiments but could be augmented by cytokines like IL-12 being coreleased in context with bacterial triggering.[22,30] By this means, calcineurin-mediated activation (and thereby consequences of its inhibition by ISD) could be partially bypassed. To allow for translation, drug concentrations in our in vitro assays matched default initial plasma levels recommended for transplant recipients; in addition, titration experiments corroborated effects on MAIT cell functions only for those drugs that already showed potency at therapeutic levels.

As a hallmark, we demonstrate that LTx patients are characterized by a pronounced reduction of peripheral MAIT cells as compared to age-matched healthy controls. This feature comprised all organ recipients regardless of treatment regimen or dosage; surprisingly, it equally accounted for tolerant patients and was reproducible in KTx patients receiving standard IS medication. We cannot not fully exclude that transplantation itself modifies MAIT cell biology, which could, for example, be addressed in an autotransplant setting with nonhuman primates harboring similar MAIT cell frequencies as humans.[31] However, since depletion of MAIT cells is characteristic of severe liver[14-17] and kidney damage,[32] our observation supports the hypothesis that the primary disease likely predetermines the post-transplant MAIT cell pool and that its quantitative replenishment is largely independent from immunosuppression. Peripheral loss of MAIT cells is a common motif in liver diseases of autoimmune-,[14] infectious-[17] and lifestyle-related origin.[16] It further encompasses several nonhepatic pathologies, with HIV being most extensively

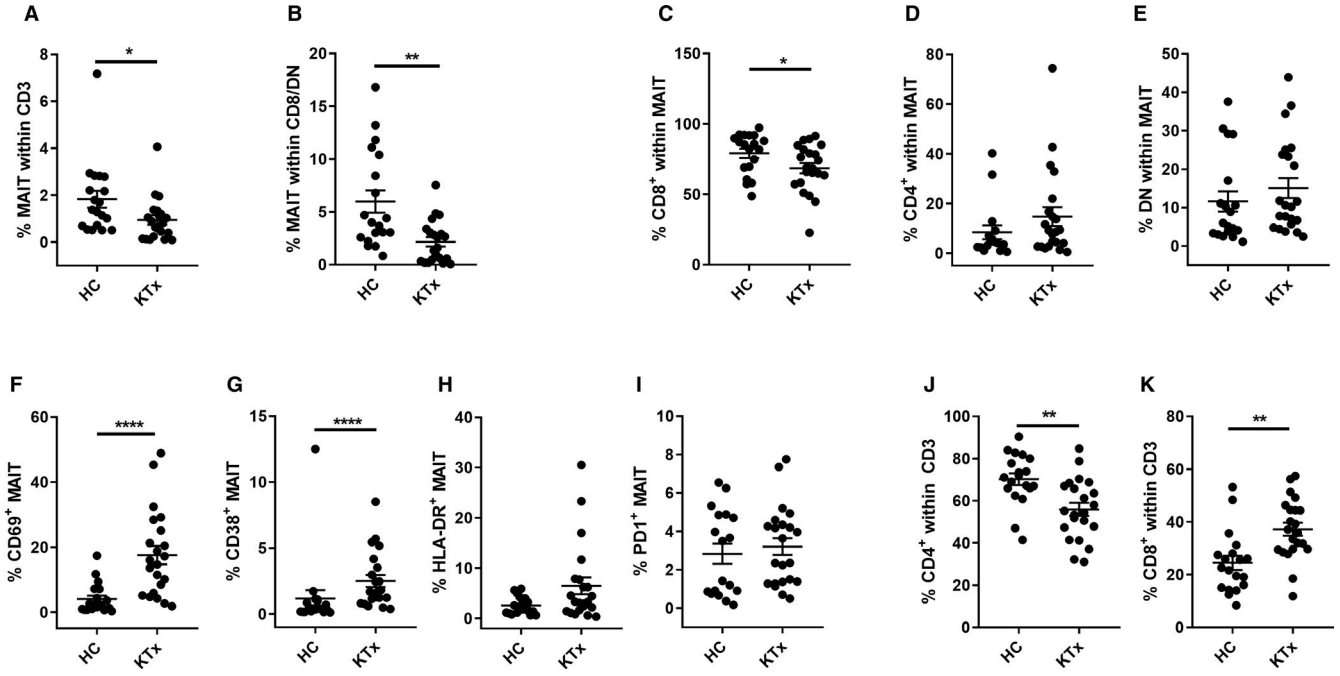


FIGURE 7 Quantitative and phenotypic alterations in MAIT- and conventional T cells from kidney transplanted patients. Frequencies of peripheral MAIT cells were determined in ISD treated kidney transplant recipients (Table 3) in comparison to healthy individuals (HC) by FACS within the total $CD3^+$ (A) or $CD8^+$/DN (B) compartment. Within total MAIT cells, portions of $CD8^+$, $CD4^+$, and DN subsets were further quantified (C-E). For determining the ex vivo activation and exhaustion state, expression of CD69 (F), CD38 (G), HLA-DR (H), and PD1 (I) was analyzed. Frequencies of conventional $CD4^+$ and $CD8^+$ T cells within the CD3 compartment are depicted in (J) and (K), respectively. All bar graphs show means ± SEM. FACS, fluorescence-activated cell sorting; HC, healthy controls; KTx, kidney transplant; MAIT, mucosal associated invariant T

studied.[33] The aforementioned conditions are also interlinked by the general inability to restore MAIT cell homeostasis despite successful treatment, a phenomenon possibly related to their comparatively poor proliferative potential, as reflected, for example, by low portions of peripheral Ki67$^+$ MAIT cells[34] and a high pro-apoptotic propensity.[35] At least with respect to the latter, however, we did not detect elevated frequencies of activated caspase 3 positive MAIT cells, thus questioning the relevance of this mechanism at least within the time frame (on average >10 years since transplantation) of our analyses.

Overall, it still remains obscure what factors promote self-renewal of this unconventional T cell population once thymic output declines and what niches are critical for their survival. Within the transplantation field, the only comprehensive data sets available derive from stem cell transplantation trials. Whereas in an autologous context, MAIT cells recover early,[36] their frequencies do not significantly increase within 2 years after allogeneic stem cell transplantation,[37] suggesting an impact of the inflammatory milieu on their reconstitution. As extra-thymic MAIT cell expansion is possibly depending on interactions with commensals,[38] it is tempting to speculate that in concert with (subclinical) alloinflammation, dysbiosis, established not only during early liver pathology, but also partially maintained after LTx,[39,40] contributes to curtailment of MAIT cell regeneration. Based on data from alcoholic hepatitis, it has already been hypothesized that gut dysbiosis might initially lead to chronic MAIT cell hyperactivation, followed by functional exhaustion, finally promoting their depletion.[16,41] MAIT cells traversing such developmental path are characterized by high expression of CD69, HLA-DR, CD38, PD1, and granzymeB, a signature we observe ex vivo in the majority of treated LTx patients and partially in kidney graft recipients. Interestingly, we demonstrate that this activation pattern is largely downregulated in tolerant individuals, with the exception of PD1, suggesting the possibility of partial normalization of allo- and/or dysbiosis-related triggers. These changes also include the general composition of the MAIT cell pool as the CD4$^+$ subset, being quantitatively expanded in treated patients at the expense of the CD8$^+$ population, was not significantly different from normal donors in treatment-free individuals. For a selected set of markers (CD38, CD38+HLA-DR, granzymeB), examined in patients before and after weaning from IS drugs, attenuation of activation, but not quantitative recovery, was achieved within months, accompanied by a consistent regain of effector cytokine production and cytotoxic capacity. In consequence, our data, together with studies on MAIT cells in severe liver diseases, indicate that transplantation does not entail an environment fostering quantitative regeneration of the MAIT cell pool. Clearly, future studies are needed in order to address whether our findings are restricted to the periphery or mirror local MAIT cell impairment within the liver. Among the few data sets available, a recent report with HCV-infected individuals demonstrated that peripheral MAIT cells might serve as surrogates reflecting the intraorgan situation: patients were characterized by both peripheral and intrahepatic depletion of MAIT cells that equally showed signs of ex vivo hyperactivation,[17] as we also report here.

With respect to patients successfully weaned from medication, it largely remains unclear what predisposes an organ recipient to develop tolerance against the graft, and it is still obscure what characterizes a tolerant immune system. In agreement with the few systematic reports on cellular immunity in operationally tolerant liver recipients,[42] we found normal frequencies of nTregs in tolerant, but not in ISD-treated patients. However, these differences did not extend to ex vivo proliferation, CTLA-4 expression, or differentiation state, thereby not allowing further speculation on their active involvement in the regulation of alloimmunity. Still, despite fingerprints of tolerance within the MAIT- and nTreg compartment, both treated and tolerant organ recipients share an overall attenuated response toward ex vivo stimulation: although not always reaching significance, both patient groups are particularly characterized by impaired cytokine production and cytotoxic capacity (as reflected by CD107a), but elevated portions of granzymeB$^+$ cells after bacterial triggering in comparison to controls. Because high PD-1 levels accompany this partially dysfunctional state also in patients after drug weaning, it needs to be addressed how and when functional exhaustion is imprinted and what consequences arise. Based on experimental limitations in humans, and given the complex individual contribution of alloimmunity, immunosuppression, dysbiosis, and opportunistic infections in our patient cohort, experimental models are needed to decipher in depth the impact of MAIT cells on overall health. However, livers of BALB/c or C57BL/6 mice contain roughly 30-50 times less MAIT cells than humans; furthermore, murine MAIT cells preferentially secrete IL-17 rather than IFNγ.[43] The few experimental studies available provide at least limited translational insights regarding how MAIT cells might contribute to both protective immunity and maintenance of eubiosis. With respect to the latter, MAIT cells are instrumental for regulating gut microbiota in experimental stem cell transplantation.[44] A similar link between quantitative MAIT cell reconstitution and diversity of gut microbiota after allogeneic stem cell transplantation was recently described for humans,[45] arguing in favor of such correlation analyses also after LTx. Infection models further indicated that MAIT cells contribute to decreased bacterial burden in urinary tract infections[46] and mediate protection against mycosis,[47] suggesting a role in these frequent opportunistic infections after kidney[48] or liver[49] transplantation, respectively.

ACKNOWLEDGMENTS

This project was supported by the Bundesministerium für Wirtschaft und Energie (Zentrales Innovationsprogramm Mittelstand, Project ZF4245603AJ7 to KK) and funded by a student award from the Sonnenfeld Stiftung (to AHR).

DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Arne Sattler https://orcid.org/0000-0003-2572-5641
Mira Choi https://orcid.org/0000-0002-0286-1491
Frank Friedersdorff https://orcid.org/0000-0002-3104-911X
Katja Kotsch https://orcid.org/0000-0002-3803-0964

REFERENCES

1. Treiner E, Duban L, Bahram S, et al. Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. *Nature*. 2003;422(6928):164-169.
2. Kjer-Nielsen L, Patel O, Corbett AJ, et al. MR1 presents microbial vitamin B metabolites to MAIT cells. *Nature*. 2012;491(7426):717-723.
3. van Wilgenburg B, Scherwitzl I, Hutchinson EC, et al. MAIT cells are activated during human viral infections. *Nat Commun*. 2016;7:11653.
4. Constantinides MG, Link VM, Tamoutounour S, et al. MAIT cells are imprinted by the microbiota in early life and promote tissue repair. *Science*. 2019;366(6464):eaax6624.
5. Tilloy F, Treiner E, Park SH, et al. An invariant T cell receptor alpha chain defines a novel TAP-independent major histocompatibility complex class Ib-restricted alpha/beta T cell subpopulation in mammals. *J Exp Med*. 1999;189(12):1907-1921.
6. Dusseaux M, Martin E, Serriari N, et al. Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17-secreting T cells. *Blood*. 2011;117(4):1250-1259.
7. Sattler A, Dang-Heine C, Reinke P, Babel N. IL-15 dependent induction of IL-18 secretion as a feedback mechanism controlling human MAIT-cell effector functions. *Eur J Immunol*. 2015;45(8):2286-2298.
8. Sattler A, Thiel LG, Ruhm AH, et al. The TL1A-DR3 axis selectively drives effector functions in human MAIT cells. *J Immunol*. 2019;203(11):2970-2978.
9. Fergusson JR, Hühn MH, Swadling L, et al. CD161(int)CD8+ T cells: a novel population of highly functional, memory CD8+ T cells enriched within the gut. *Mucosal Immunol*. 2016;9(2):401-413.
10. Wang H, D'Souza C, Lim XY, et al. MAIT cells protect against pulmonary Legionella longbeachae infection. *Nat Commun*. 2018;9(1):3350.
11. Tang X-Z, Jo J, Tan AT, et al. IL-7 licenses activation of human liver intrasinusoidal mucosal-associated invariant T cells. *J Immunol*. 2013;190(7):3142-3152.
12. Jeffery HC, van Wilgenburg B, Kurioka A, et al. Biliary epithelium and liver B cells exposed to bacteria activate intrahepatic MAIT cells through MR1. *J Hepatol*. 2016;64(5):1118-1127.
13. Kurioka A, Walker LJ, Klenerman P, Willberg CB. MAIT cells: new guardians of the liver. *Clin Transl Immunol*. 2016;5(8):e98.
14. Renand A, Habes S, Mosnier J-F, et al. Immune alterations in patients with type 1 autoimmune hepatitis persist upon standard immunosuppressive treatment. *Hepatol Commun*. 2018;2(8):968-981.
15. Seth E, Zimmer CL, Reuterwall-Hansson M, et al. Primary sclerosing cholangitis leads to dysfunction and loss of MAIT cells. *Eur J Immunol*. 2018;48(12):1997-2004.
16. Riva A, Patel V, Kurioka A, et al. Mucosa-associated invariant T cells link intestinal immunity with antibacterial immune defects in alcoholic liver disease. *Gut*. 2018;67(5):918-930.
17. Bolte FJ, O'Keefe AC, Webb LM, et al. Intra-hepatic depletion of mucosal-associated invariant T cells in hepatitis C virus-induced liver inflammation. *Gastroenterology*. 2017;153(5):1392-1403.e2.
18. Weist BJD, Wehler P, El Ahmad L, et al. A revised strategy for monitoring BKV-specific cellular immunity in kidney transplant patients. *Kidney Int*. 2015;88(6):1293-1303.
19. Post DJ, Douglas DD, Mulligan DC. Immunosuppression in liver transplantation. *Liver Transpl*. 2005;11(11):1307-1314.
20. Charlton M, Levitsky J, Aqel B, et al. International Liver Transplantation Society Consensus Statement on immunosuppression in liver transplant recipients. *Transplantation*. 2018;102(5):727-743.
21. Moini M, Schilsky ML, Tichy EM. Review on immunosuppression in liver transplantation. *World J Hepatol*. 2015;7(10):1355-1368.
22. Ussher JE, Bilton M, Attwod E, et al. CD161++ CD8+ T cells, including the MAIT cell subset, are specifically activated by IL-12+IL-18 in a TCR-independent manner. *Eur J Immunol*. 2014;44(1):195-203.
23. Chua WJ, Truscott SM, Eickhoff CS, Blazevic A, Hoft DF, Hansen TH. Polyclonal mucosa-associated invariant T cells have unique innate functions in bacterial infection. *Infect Immun*. 2012;80(9):3256-3267.
24. Leng T, Akther HD, Hackstein C-P, et al. TCR and inflammatory signals tune human MAIT cells to exert specific tissue repair and effector functions. *Cell Rep*. 2019;28(12):3077-3091.e5.
25. Fergusson JR, Ussher JE, Kurioka A, Klenerman P, Walker LJ. High MDR-1 expression by MAIT cells confers resistance to cytotoxic but not immunosuppressive MDR-1 substrates. *Clin Exp Immunol*. 2018;194(2):180-191.
26. Robinson D, Shibuya K, Mui A, et al. IGIF does not drive Th1 development but synergizes with IL-12 for interferon-gamma production and activates IRAK and NFkappaB. *Immunity*. 1997;7(4):571-581.
27. Auphan N, DiDonato JA, Rosette C, Helmberg A, Karin M. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. *Science*. 1995;270(5234):286-290.
28. Azzi JR, Sayegh MH, Mallat SG. Calcineurin inhibitors: 40 years later, can't live without. *J Immunol*. 2013;191(12):5785-5791.
29. Cain DW, Cidlowski JA. Immune regulation by glucocorticoids. *Nat Rev Immunol*. 2017;17(4):233-247.
30. Kurioka A, Ussher JE, Cosgrove C, et al. MAIT cells are licensed through granzyme exchange to kill bacterially sensitized targets. *Mucosal Immunol*. 2015;8(2):429-440.
31. Greene JM, Dash P, Roy S, et al. MR1-restricted mucosal-associated invariant T (MAIT) cells respond to mycobacterial vaccination and infection in nonhuman primates. *Mucosal Immunol*. 2017;10(3):802-813.
32. Juno JA, Waruk JLM, Wragg KM, et al. Mucosal-associated invariant T cells are depleted and exhibit altered chemokine receptor expression and elevated granulocyte macrophage-colony stimulating factor production during end-stage renal disease. *Front Immunol*. 2018;9:1076.
33. Leeansyah E, Ganesh A, Quigley MF, et al. Activation, exhaustion, and persistent decline of the antimicrobial MR1-restricted MAIT-cell population in chronic HIV-1 infection. *Blood*. 2013;121(7):1124-1135.
34. Leeansyah E, Loh L, Nixon DF, Sandberg JK. Acquisition of innate-like microbial reactivity in mucosal tissues during human fetal MAIT-cell development. *Nat Commun*. 2014;5:3143.
35. Gérart S, Sibéril S, Martin E, et al. Human iNKT and MAIT cells exhibit a PLZF-dependent proapoptotic propensity that is counterbalanced by XIAP. *Blood*. 2013;121(4):614-623.
36. Novak J, Dobrovolny J, Brozova J, Novakova L, Kozak T. Recovery of mucosal-associated invariant T cells after myeloablative chemotherapy and autologous peripheral blood stem cell transplantation. *Clin Exp Med*. 2016;16(4):529-537.
37. Solders M, Erkers T, Gorchs L, et al. Mucosal-associated invariant T cells display a poor reconstitution and altered phenotype after allogeneic hematopoietic stem cell transplantation. *Front Immunol*. 2017;8:1861.
38. Garner LC, Klenerman P, Provine NM. Insights into mucosal-associated invariant T cell biology from studies of invariant natural killer T cells. *Front Immunol*. 2018;9:1478.

39. Annavajhala MK, Gomez-Simmonds A, Macesic N, et al. Colonizing multidrug-resistant bacteria and the longitudinal evolution of the intestinal microbiome after liver transplantation. *Nat Commun*. 2019;10(1):4715.
40. Bajaj JS, Fagan A, Sikaroodi M, et al. Liver transplant modulates gut microbial dysbiosis and cognitive function in cirrhosis. *Liver Transpl*. 2017;23(7):907-914.
41. Li W, Lin EL, Liangpunsakul S, et al. Alcohol abstinence does not fully reverse abnormalities of mucosal-associated invariant T cells in the blood of patients with alcoholic hepatitis. *Clin Transl Gastroenterol*. 2019;10(6):e00052.
42. Lau AH, Vitalone MJ, Haas K, et al. Mass cytometry reveals a distinct immunoprofile of operational tolerance in pediatric liver transplantation. *Pediatr Transplant*. 2016;20(8):1072-1080.
43. Rahimpour A, Koay HF, Enders A, et al. Identification of phenotypically and functionally heterogeneous mouse mucosal-associated invariant T cells using MR1 tetramers. *J Exp Med*. 2015;212(7):1095-1108.
44. Varelias A, Bunting MD, Ormerod KL, et al. Recipient mucosal-associated invariant T cells control GVHD within the colon. *J Clin Invest*. 2018;128(5):1919-1936.
45. Konuma T, Kohara C, Watanabe E, et al. Reconstitution of circulating mucosal-associated invariant T cells after allogeneic hematopoietic cell transplantation: its association with the riboflavin synthetic pathway of gut microbiota in cord blood transplant recipients. *J Immunol*. 2020;204(6):1462-1473.
46. Cui Y, Franciszkiewicz K, Mburu YK, et al. Mucosal-associated invariant T cell-rich congenic mouse strain allows functional evaluation. *J Clin Invest*. 2015;125(11):4171-4185.
47. Dias J, Leeansyah E, Sandberg JK. Multiple layers of heterogeneity and subset diversity in human MAIT cell responses to distinct microorganisms and to innate cytokines. *Proc Natl Acad Sci USA*. 2017;114(27):E5434-E5443.
48. Vidal E, Torre-Cisneros J, Blanes M, et al. Bacterial urinary tract infection after solid organ transplantation in the RESITRA cohort. *Transpl Infect Dis*. 2012;14(6):595-603.
49. Hernandez Mdel P, Martin P, Simkins J. Infectious complications after liver transplantation. *Gastroenterol Hepatol*. 2015;11(11):741-753.

SUPPORTING INFORMATION

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

How to cite this article: Sattler A, Thiel LG, Ruhm AH, et al. Mucosal associated invariant T cells are differentially impaired in tolerant and immunosuppressed liver transplant recipients. *Am J Transplant*. 2021;21:87–102. https://doi.org/10.1111/ajt.16122