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Deficiency of Mucosal-Associated Invariant T Cells in TCRJα18 Germline Knockout Mice

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ABSTRACT

Mucosal-associated invariant T (MAIT) cells and invariant NK T (iNKT) cells account for the major lymphocyte populations that express invariant TCRα-chains. MAIT cells mostly express the TCRVα19-Jα33 TCR in mice and the TCRVα7.2-Jα33 TCR in humans, whereas iNKT cells express the TCRVα14-Jα18 TCR in mice and the TCRVα24-Jα18 TCR in humans. Both MAIT and iNKT cells have the capacity to quickly produce a variety of cytokines in response to agonist stimuli and to regulate both innate and adaptive immunity. The germline TCRJα18 knockout (*Traj18*$^{-/-}$) mice have been used extensively for studying iNKT cells. Although it has been reported that the TCRα repertoire was narrowed and the level of *Trav19-ja33* transcript was decreased in this strain of mice, direct assessment of MAIT cells in these mice has not been reported. We demonstrate in this study that this strain of mice is also defective of MAIT T cells, cautioning data interpretation when using this strain of mice. *ImmunoHorizons*, 2019, 3: 203–207.

INTRODUCTION

Mucosal-associated invariant T (MAIT) and invariant NK T (iNKT) cells account for the major lymphocyte populations that express invariant TCRα-chains with a restricted TCR repertoire. MAIT cells mostly express the TCRVα19-Jα33 TCR in mice and the TCRVα7.2-Jα33 TCR in humans, whereas iNKT cells express the TCRVα14-Jα18 TCR in mice and the TCRVα24-Jα18 TCR in humans (1–5). Different from conventional TCRαβ$^+$ T cells, iNKT and MAIT cells do not respond to peptides presented by classic MHC molecules but instead recognize glycolipids and microbe-derived riboflavin (vitamin B2) metabolites presented by the MHC class I–related molecules CD1d and MR1, respectively (6, 7). Both iNKT and MAIT cells mature and differentiate into effector lineages in the thymus and are able to rapidly produce both proinflammatory and regulatory cytokines and exert other effector function (8–11). These cells play important roles in both innate and adaptive immunity against microbial infection and tumor but may also contribute to pathogenesis of diseases, such as allergy, asthma, and autoimmune diseases (2, 12–15). Many studies on iNKT cell pathophysiological functions have been performed using the *Trja18*$^{-/-}$ (TCRJα18$^{-/-}$) mice that lack iNKT cells because of an essential role of the signal from the TCRVα14-Jα18 TCR for iNKT cell development (16). In TCRJα18$^{-/-}$ mice, the *Traj18*

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J.X., Y.P., H.T., and P.W. designed and performed experiments, analyzed data, and were involved in manuscript preparation. Y.C. and J.G. were involved in data analyses and manuscript preparation. X.-P.Z. conceived the project, designed experiments, analyzed data, and wrote the manuscript.

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Abbreviations used in this article: BM, bone marrow; DP, double-positive; HSC, hematopoietic stem cell; iNKT, invariant NK T; LN, lymph node; MAIT, mucosal-associated invariant T; MR1Tet, MR-1 tetramer; WT, wild-type.

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segment is replaced with a neomycin resistance gene. Whereas narrowed TCRα repertoire and decreased *Trav19-ja33* transcript have been reported in TCRJα18$^{-/-}$ mice (17, 18), direct assessment of MAIT cells in this strain of mice has not been reported. We report in this article that TCRJα18$^{-/-}$ mice are virtually absent of MAIT cells in both thymus and peripheral organs, cautioning data interpretation when using this strain of mice.

MATERIALS AND METHODS

Mice

TCRJα18$^{-/-}$ mice in C57BL6/J background were kindly provided by Drs. Kim Nichols, Luc Van Kaer, and Masaru Taniguchi. Some of these mice were crossed with Th1.1^{+}CD45.1^{+} C57BL6/J mice. All animal experiments were performed according to protocols approved by the Institutional Animal Care and Use Committee of Duke University. Single-cell suspensions of the thymus, spleen, lymph nodes (LNs), and liver mononuclear cells were prepared as previously described (19). Single-cell suspensions of the lung were made after collagenase digestion, as reported previously (20). Cells were resuspended in IMDM containing 10% FBS (IMDM-10).

Abs and tetramers

Fluorochrome-conjugated anti-CD45.2 (clone 104), CD45.1 (A20), TCRγδ (clone GL3), TCRβ (clone H57-597), Gr1 (clone RB6-8C5), CD11b (clone M170), CD11c (clone N418), F4/80 (clone BM8), B220 (clone RA3-6B2), and 9/Erythroid Cells (clone TER-119) were purchased from BioLegend. PE- or allophycocyanin-conjugated 5-OP-RU loaded MR-1 tetramer (MR1Tet) (8, 21) was kindly provided by the National Institutes of Health tetramer facility. Dead cells were excluded using the Live/Dead Fixable Violet Dead Cell Stain (Invitrogen) or 7-AAD.

Enrichment of MAIT cells

MAIT cell enrichment was performed according to a published protocol (22) with modifications. Total thymocytes from individual mice in 200 μl of IMDM-10 were stained on ice with allophycocyanin- or PE-MR1Tet at 1:200 dilution for 30 min with brief shaking of the cells every 10 min. After having been washed twice with IMDM with 1% FBS, cells were resuspended in 200 μl of IMDM-10 with 10 μl of microbeads conjugated with an antiallophycocyanin Ab (Miltenyi Biotec). After incubation on ice for another 30 min with gentle shaking every 10 min, the cells were mixed with 5 ml of MACS buffer (PBS with 2 mM EDTA and 0.5% BSA), pelleted by centrifugation, resuspended in 500 μl of MACS buffer, and loaded on MACS LS columns (MACS no. 130-042-401) for positive selection, following the manufacturer's protocol. For MAIT cell analysis, single-cell suspensions with or without MR1Tet enrichment were stained with anti-TCRβ, CD24, CD44, and other Abs at room temperature for 30 min. For unenriched cells, PE- or allophycocyanin-conjugated 5-OP-RU–loaded MR1-Tet was also added to the staining. Lineage markers, including TCRγδ, CD11b, CD11c, F4/80, B220, Gr1, and Ter119, were included to exclude other cell lineages. MAIT cells were gated on live Lin^{-} TCRβ^{+}MR1-Tet^{+} cells.

Generation of chimeric mice

For data in Fig. 2A, CD45.2^{+} C57BL/6 mice were irradiated with a single dose of 800 rad x-ray and i.v. injected with 10–15 million cells of a mixture of bone marrow (BM) from CD45.1^{+}CD45.2^{+} wild-type (WT) mice and from CD45.1^{+} TCRJα18$^{-/-}$ mice at a 1:1 ratio. For data in Fig. 2B, CD45.1^{+} TCRJα18$^{-/-}$ mice were irradiated with a single dose of 600 rad x-ray and i.v. injected with 15 million BM cells from WT CD45.2^{+} C57BL/6 mice. Recipient mice were euthanized and analyzed 8 wk later.

Statistical analysis

The scarcity of MAIT cells causes variations between experiments. To overcome this issue, we performed individual experiments examining a pair of age- and sex-matched test and control mice housed in the same cage. Each pair of mice in individual experiments was marked by a connecting line between test and control mice. Scatter plots were pooled multiple experiments, and the numbers of pairs shown in the plots reflect the numbers of experiments performed. Comparisons were made by two-tailed Student *t* test using the Prism 5/GraphPad software. The p values <0.05 were considered significant.

RESULTS

Absence of MAIT cells in the thymus of TCRJα18$^{-/-}$ thymus

Using 5-OP-RU loaded MR1Tet to detect MAIT cells (21), it has been reported that MAIT cells are expanded in *Cd1d*$^{-/-}$ mice (22). We examined if MAIT cells would similarly expand in TCRJα18$^{-/-}$ mice. In contrast to *Cd1d*$^{-/-}$ mice, MR1Tet^{+}TCRβ^{+} MAIT cells were virtually absent in the thymus in TCRJα18$^{-/-}$ mice (Fig. 1A–C). Because MAIT cells are very rare in the thymus, we further enriched MAIT cells from total thymocytes using allophycocyanin- or PE-conjugated MR1Tet and anti-allophycocyanin or -PE Ab-conjugated magnetic beads. We were able to greatly enrich MAIT cells from WT thymocytes and confirm the absence of MAIT cells in the TCRJα18$^{-/-}$ thymus (Fig. 1D, 1E).

Intrinsic defect of MAIT cell generation in TCRJα18$^{-/-}$ thymus

Because TCRJα18$^{-/-}$ mice are germline knockout and MAIT cells are positively selected by MR1 expressed on CD4^{+}CD8^{+} double-positive (DP) thymocytes, we generated irradiated chimeric mice (CD45.2^{+}) with a mixture of BM cells from CD45.1^{+}CD45.2^{+} WT and CD45.1^{+} TCRJα18$^{-/-}$ mice at a 1:1 ratio to determine if cell-intrinsic mechanisms caused MAIT cell deficiency in TCRJα18$^{-/-}$ mice. Eight weeks after reconstitution, MAIT cells were solely derived from WT donor but not TCRJα18$^{-/-}$ donor. In contrast, CD4^{+}CD8^{+} DP thymocytes were equally generated from both origins, indicating equal reconstitution of hematopoietic stem cells (HSCs) from both origins (Fig. 2A). Additionally, in sublethally

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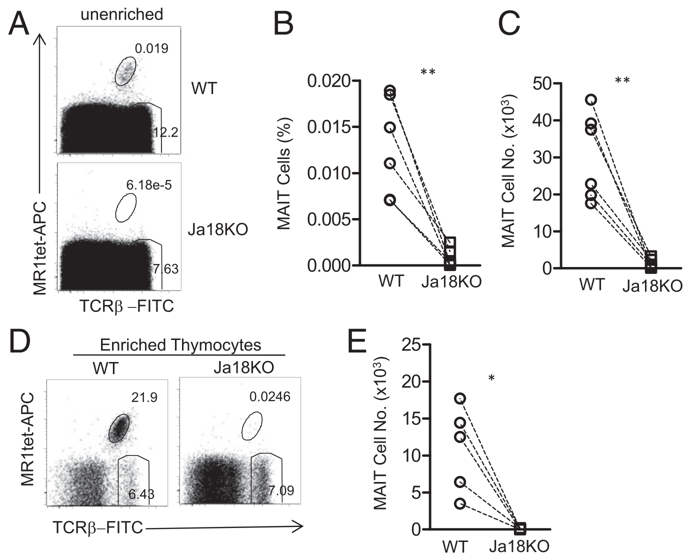

FIGURE 1. Defective MAIT cell generation in *TCRJα18*$^{-/-}$ mice.
(**A**–**C**) Total thymocytes from WT and TCRJα18$^{-/-}$ mice were stained with TCRβ and MR1Tet as well as lineage markers (CD11b, Gr1, F4/80, NK1.1, CD11c, Ter119, and B220) and LIVE/DEAD Viability/Cytotoxicity kit. (A) Representative dot plots of live-gated Lin^{-} thymocytes. (B and C) MAIT cell percentages (B) and numbers (C). (**D** and **E**) MAIT cells in thymocytes were enriched using PE- or allophycocyanin-MR1Tet and MACS beads, followed by staining and analysis similar to (A)–(C). Dotted line–connected samples represent sex- and age-matched WT and *Tcrja18*$^{-/-}$ mice examined in each experiment. Data shown are representative of or pooled from five experiments. *$p < 0.05$, **$p < 0.01$, determined by pairwise Student *t* test.

irradiated CD45.1^{+} TCRJα18$^{-/-}$ mice reconstituted with CD45.2^{+} WT BM cells, MAIT cells were generated only from donor WT BM HSCs, but TCRβ^{+} conventional T cells were generated from both donor and recipient HSCs (Fig. 2B), suggesting that there was no gross abnormality of thymic environment in TCRJα18$^{-/-}$ mice for MAIT cell development. Together, these data indicate that intrinsic mechanisms cause MAIT cell deficiency in TCRJα18$^{-/-}$ mice.

Virtual absence of MAIT cells in peripheral organs in TCRJα18$^{-/-}$ *mice*

MAIT cells are localized in both mucosal tissues and peripheral lymphoid and nonlymphoid organs. We further examined MAIT cells in the spleen, LNs, liver, and lung in TCRJα18$^{-/-}$ and WT control mice (Fig. 3). MAIT cells were virtually undetectable in these organs in TCRJα18$^{-/-}$ mice, with a decrease in total MAIT cell numbers ranging from 96.3 to 99.4% in these organs. Thus, defective MAIT cell generation caused virtual absence of these cells in the peripheral organs.

DISCUSSION

TCRJα18$^{-/-}$ mice have been widely used for studying iNKT cell functions because of their deficiency of iNKT cells. We have demonstrated in this study that this strain of TCRJα18$^{-/-}$ mice is also defective in MAIT cells. Virtually no MAIT cells are detected in both thymus and peripheral organs in these mice. Our data, together with the observations of a narrowed T cell repertoire and a severe decrease in *TCRVα19-Jα33* transcript in TCRJα18$^{-/-}$ mice (17, 18), support the notion that the presence of the *Neo*^{r} cassette in the *Traj18* region may reduce chromatin accessibility of *Traj* segments 5′ to the Neo^{r} distal to the *Tra* enhancer for *Trav-j* recombination or transcription. Given the extensive use of TCRJα18$^{-/-}$ mice for examining the pathophysiological functions of iNKT cells and important functions of MAIT cells, some of these data may need to be reexamined and reinterpreted. New strains of TCRJα18-deficient mice generated with either the Cre-LoxP technology (18, 23) or the transcription activator-like effector nuclease technology (24) should provide needed replacement of the TCRJα18$^{-/-}$ mice for interrogating iNKT cell function. These two strains of TCRJα18-deficient mice created by Cre-LoxP technology, the B6(Cg)-*Traj18*$^{tm1.1Kro}$/J mice (23), available in The Jackson Laboratory, and the other *Traj18*-deficient mice (18), display normal *Traj33* usage or *Trav1-ja33* expression within

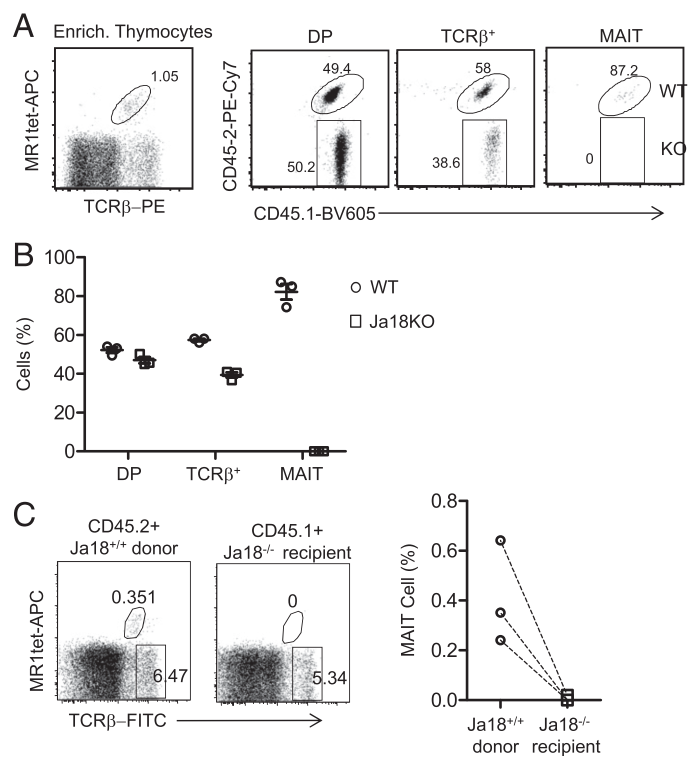

FIGURE 2. Assessment of MAIT cell development in irradiated chimeric mice.
(**A**) MAIT cells, CD4^{+}CD8^{+} DP, and TCRβ^{+} cells in the thymus of CD45.2^{+} recipient mice after irradiation and reconstitution with CD45.1^{+}CD45.2^{+} WT and CD45.1^{+} *TCRJa18*$^{-/-}$ BM cells at a 1:1 ratio. (**B**) Scatter plot shows percentages of indicated thymocyte populations from chimeric mice. (**C**) MAIT cells in sublethally irradiated CD45.1^{+} *TCRJa18*$^{-/-}$ mice 8 wk after reconstitution with CD45.2^{+} WT BM cells. Data shown are representative of or pooled from three experiments.

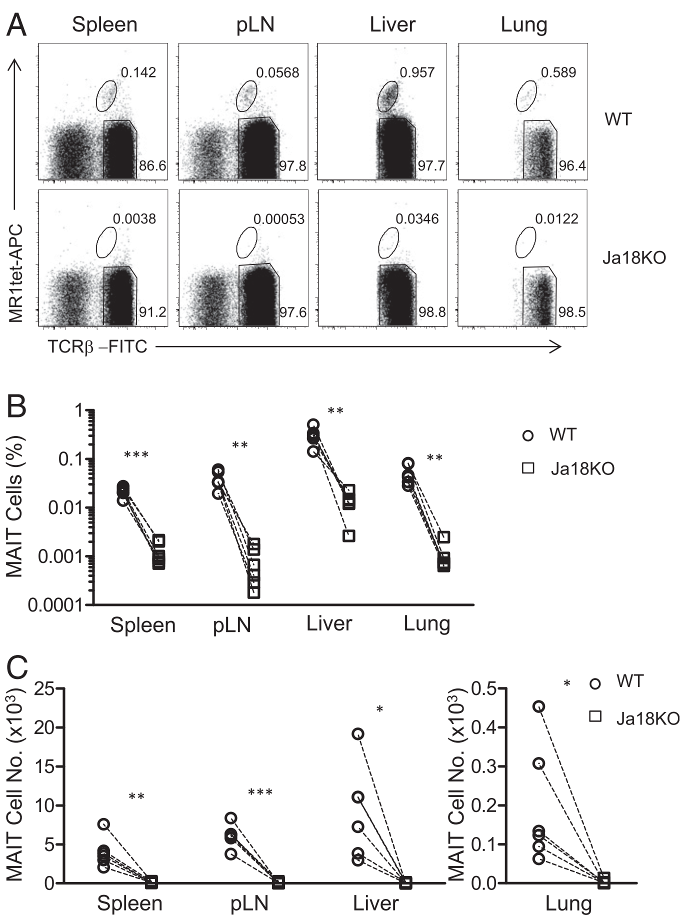

FIGURE 3. Deficiency of MAIT cells in the peripheral organs in *TCRJA18$^{-/-}$* mice.

(**A**) Representative dot plots showing TCRβ and MR1Tet staining in live-gated Lin^{-} cells from the spleen and peripheral LNs and Lin^{-}TCRβ^{+} cells from the lung and liver. (**B** and **C**) MAIT cell percentages (B) and numbers (C) in these organs. Dotted line–connected samples represent sex- and age-matched WT and *TCRJa18$^{-/-}$* mice examined in each experiment. Data shown are representative of or pooled from five to six experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, determined by pairwise Student t test.

T cells, indicating that MAIT cell generation in these mice is not impaired. The deficiency of both iNKT and MAIT cells in TCRJα18$^{-/-}$ mice and the potential competition of MAIT cells and iNKT cells for the same niche indicated by marked increases of MAIT cells in *Cd1d*$^{-/-}$ mice (22) suggest a possible use of the TCRJα18$^{-/-}$ mice for investigating MAIT cell function via reconstitution.

DISCLOSURES

The authors have no financial conflicts of interest.

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