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A new mouse strain for the analysis of invariant NKT cell function

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To the editor

Invariant natural killer T cells (*i*NKT cells) are a T lymphocyte population with restricted T cell antigen receptor (TCR) diversity. They make up a distinct subset of T lymphocytes that is activated by glycolipid antigens presented by CD1d, a non-classical major histocompatibility complex class I-like molecule. The invariant TCR of *i*NKT cells consists of a specific α -chain rearrangement, of α -chain variable region 14 and α -chain joining region 18 ($V_{\alpha}14$ - $J_{\alpha}18$), encoded by *Tcra* gene segments *Trav11*-*Traj18* in mice, and $V_{\alpha}24$ - $J_{\alpha}18$ (*TRAV10*-*TRAJ18*) in humans, and a limited β -chain repertoire¹.

*i*NKT cells are associated with early immune responses and have been reported to exert a crucial influence on a wide variety of immune and inflammatory responses². Mice deficient in *i*NKT cells provide a key tool for analysis of the role of these cells in various contexts. There are two widely used mouse models of *i*NKT cell deficiency, but neither provides a completely specific defect. Mice lacking the *Cd1d1* and *Cd1d2* loci cannot positively select *i*NKT cells, but they also do not have type II NKT cells³, which are CD1d-reactive T lymphocytes with a more diverse α -chain repertoire⁴. Mice lacking the *Traj18* gene segment cannot form the TCR α chain that is essential for *i*NKT cell development⁵. However, a published study has reported that rearrangements of all the J_{α} regions upstream of *Traj18* were suppressed in the available strain of mice with deletion of the *Traj18* gene segment⁶. In this strain, the gene encoding the neomycin-resistance selection marker, driven by the promoter of the phosphoglycerate kinase gene (*Pgk-neo^r*), is not removed. Therefore, the authors proposed that the transcription of *Pgk-neo^r*, which is in an orientation opposite to that of the J_{α} regions, led to the suppression of rearrangement of the majority of J_{α} regions and thereby caused substantial distortion of the TCR α repertoire.

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Note: Any Supplementary Information and Source Data files are available in the [online version of the paper](#)

AUTHOR CONTRIBUTIONS

S.C., M.Z., A.B., J.D., Z.F., L.S., D.S. and M.K. designed and analyzed the experiments, which were performed by S.C., M.Z., A.d.M.P., J.D., L.S. and D.S.; and S.C., A.B., D.S. and M.K. wrote the paper.

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Here we describe a new mouse strain with deletion of *Trajl8* created on the C57BL/6 background in which *neo^r* was deleted along with the *Trajl8* gene segment. These mice lacked *i*NKT cells, defined as TCR β^+ lymphocytes in spleen and other tissues that reacted with CD1d tetramers loaded with α -galactosyl ceramide (Fig. 1a and Supplementary Fig. 1), and they did not produce interferon- γ (IFN- γ) or interleukin 4 (IL-4) at 2 h or 24 h after injection of α -galactosyl ceramide (Supplementary Fig. 2). Although the minor population of tetramer-positive cells with a V α 10-J α 50 rearrangement would not be affected by deletion of *Trajl8*, we did not find definitive evidence of these cells, which are rare in C57BL/6 mice, by tetramer staining or by PCR (data not shown). To analyze the TCR α repertoire, we isolated CD4⁺CD8⁺ (double-positive) CD69⁻ thymocytes either from C57BL/6 wild-type mice or from *Trajl8*-deficient mice and performed PCR amplification of genes encoding three TCR α chains, V α 14 (*Trav11*), V α 3 (*Trav9*) and V α 8 (*Trav12*), by using a specific forward primer for each V α -encoding sequence and a reverse primer for sequence encoding the TCR α constant region. We purified and sequenced PCR products. Our results indicated that except for *Trajl8*, the frequency of J α use for each of these rearranged V α regions was similar for wild-type mice and *Trajl8*-deficient mice (Fig. 1b and Supplementary Fig. 3a,b). Therefore, the data indicated that the overall TCR repertoire was not perturbed in these mice.

This new mouse strain should allow investigators to assess the function of *i*NKT cells specifically, without the complicating factor of the reduction in the diversity of TCR J α regions. Furthermore, it is unlikely that the type II NKT cells with more diverse TCRs were affected, although we did not analyze this. As a proof of concept, we analyzed airway inflammation and airway hyper-reactivity, which have been shown in mouse models of asthma to require the presence of *i*NKT cells⁷. We used ovalbumin (OVA)-induced and cockroach antigen-induced models of pulmonary inflammation. For the OVA model, we sensitized mice by intraperitoneal administration of OVA weekly for 2 weeks. At 2 weeks after the second OVA sensitization, we challenged mice intranasally on 3 consecutive days with OVA. We harvested bronchoalveolar lavage fluid 72 h after the final OVA challenge. Our results showed that no airway eosinophilia was induced in *Trajl8*-deficient mice (Fig. 1c), and they also had considerably fewer neutrophils and total leukocytes than their wild-type counterparts had (data not shown). We assessed airway resistance in response to methacholine challenge 48 h after the final OVA challenge and found that *Trajl8*-deficient mice showed significantly less pulmonary resistance than did their wild-type counterparts (Supplementary Fig. 4). For the cockroach antigen model, we challenged mice intranasally with the antigen for 2 weeks on days 1, 3, 6, 8, 10, 13 and 15 and assessed airway resistance in response to methacholine 24 h after the final challenge. In this model as well, *Trajl8*-deficient mice showed significantly less pulmonary resistance than did their wild-type counterparts (Supplementary Fig. 5).

In conclusion, we have generated and characterized a new and potentially useful strain of mice that lack *i*NKT cells while maintaining an essentially complete TCR α repertoire. We showed that these mice had diminished airway inflammation and resistance, consistent with published studies showing the importance of *i*NKT cells⁷, but we have also provided definitive evidence that *i*NKT cells were not only sufficient but also necessary in these

contexts. Given other experimental systems in which analysis of mice with deletion of *Trajl18* has indicated *i*NKT cells are important, we consider it likely that in many cases, the crucial role of *i*NKT cells will be confirmed by analysis of the mice of our strain. This will have to be confirmed, however, on a case-by-case basis.

METHODS

Methods and any associated references are available in the [online version of the paper](#).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Tra18-deficient mice lack iNKT cells but have a normal J α -region repertoire and show significantly fewer eosinophils in an airway hyper-reactivity model. **(a)** Flow cytometry of total splenocytes isolated from a wild-type mouse (WT) and a *Tra18*-deficient mouse (*Tra18*^{-/-}). Numbers adjacent to outlined areas indicate percent live CD19⁻CD8 α ⁻ TCR β ⁺ lymphocytes reactive with the CD1d- α -galactosyl ceramide (α -GalCer) tetramer. **(b)** PCR analysis assessing the frequency of J α use for *Trav9* in CD4⁺CD8⁺ (double-positive) CD69⁻ thymocytes from wild-type mice (458,170 sequences) and *Tra18*-deficient mice (176,431 sequences). **(c)** Airway hyper-reactivity in mice sensitized with OVA for 2 weeks and challenged with OVA for 3 consecutive days, assessed by quantification of eosinophils in bronchoalveolar lavage fluid (BALF). Each symbol represents an individual mouse ($n = 10$ per genotype); small horizontal lines indicate the mean. * $P < 0.0001$ (Mann-Whitney test). Data are from one experiment (**a,c**) or are representative of one experiment with one of two mice per genotype analyzed (**b**).