Lower TCR repertoire diversity in *Traj18*-deficient mice

To the Editor:

Natural killer T cells (NKT cells) constitute a distinct subset of T lymphocytes that can modulate immune responses through the rapid release of cytokines and direct interactions with other cells of the immune system[1]. Thus, NKT cells serve as an important link between the innate and adaptive immune systems and are promising targets for immunotherapy. Type I NKT cells (*i*NKT cells) are the most prevalent NKT cells in mice and have similar properties in mice and humans. The *i*NKT cells have evolved to recognize lipid-based antigens presented by the nonclassical major histocompatibility complex (MHC)-like molecule CD1d. Many studies of humans and mice have reported a strong association between defects in *i*NKT cells and greater susceptibility to autoimmune disease and cancer. In addition, *i*NKT cells are known to have important roles during infection with bacterial, viral, protozoan and fungal pathogens[2].

Because *i*NKT cells are highly conserved in mice and humans[3], mouse models of deficiency in *i*NKT cells represent useful tools for immunologists. Two similar but not equivalent models of deficiency in *i*NKT cells exist. One makes use of mice deficient in CD1d (*Cd1d1*$^{-/-}$*Cd1d2*$^{-/-}$ mice)[4], which prevents the development of any CD1d-reactive T cells, including *i*NKT cells. Another model directly targets *Traj18* (which encodes the T cell antigen receptor (TCR) α-chain joining region 18 (J_α18))[5], which in combination with *Trav11* (which encodes the TCR α-chain variable region 14 (V_α14)) is absolutely required for formation of an *i*NKT TCR with the appropriate antigenic specificity[6].

The RAG-1 and RAG-2 recombinases drive successive rearrangement of genes encoding TCR β- and α-chains during thymocyte development.

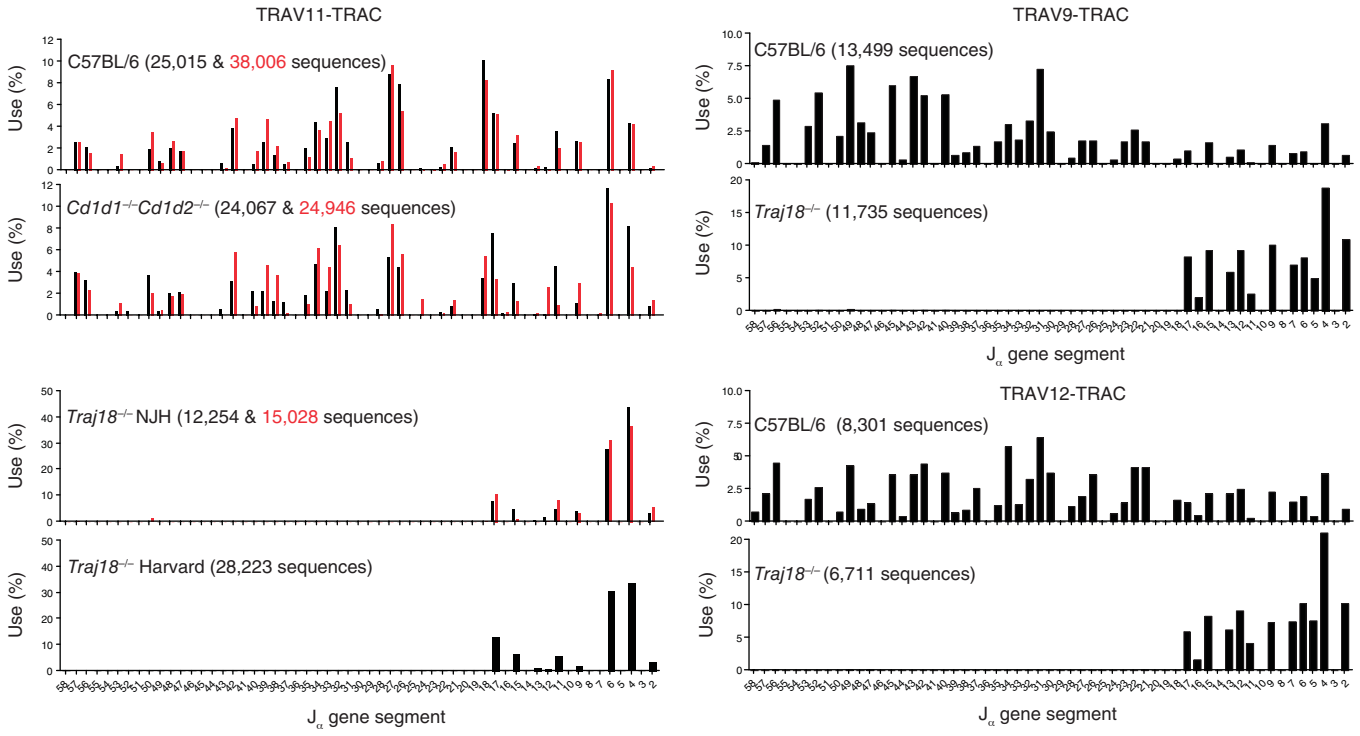

Figure 1 Impaired TCRα diversity in *Traj18*$^{-/-}$ mice. PCR analysis of the frequency of use of genes encoding J_α for productive, in-frame, rearrangements involving gene segments of the *Trav11*, *Trav9* or *Trav12* family in sorted CD69$^-$ double-positive (CD4$^+$CD8$^+$) thymocytes from C57BL/6, *Cd1d1*$^{-/-}$*Cd1d2*$^{-/-}$ and *Traj18*$^{-/-}$ mice. Order of gene segments along horizontal axes (left to right) is similar to their 5′ to 3′ organization in the mouse genome. Numbers in parenthesis indicate sequences analyzed for each sample; black and red indicate replicate analysis of independent samples. Rearrangements for each V-gene family were amplified by PCR with V-specific primers and a C-specific reverse primer (above plots), followed by high-throughput sequencing with the Roche 454 platform and sequence analysis with in-house software, and gene identity was assigned on the basis of sequence alignment with published sequences (International ImMunoGeneTics Information System). *Cd1d1*$^{-/-}$*Cd1d2*$^{-/-}$ mice and *Traj18*$^{-/-}$ mice were maintained in the animal facility at National Jewish Health (NJH), and *Traj18*$^{-/-}$ mice were maintained at the mouse facility of Harvard Medical School. All mice were on the C57BL/6 background and were housed in specific pathogen–free conditions. All animal studies were approved by the Animal Care and Use Committee of NJG. Data are representative of one experiment (TRAV9-TRAC and TRAV12-TRAC) or two experiments (TRAV11-TRAC) with different samples used for TRAV11-TRAC than for TRAV9-TRAC and TRAV12-TRAC.

Primary rearrangement of genes encoding TCR V_α and J_α regions is initiated in $CD4^+CD8^+$ (double-positive) thymocytes and, if successful, leads to the 'audition' of TCR-expressing thymocytes for productive interaction between TCRs and self MHC molecules. If positive selection does not occur, secondary rearrangement of genes encoding V_α-J_α proceeds to replace ineffective primary rearrangements[7]. Recombination of TCRα genes is thought to begin at the 5′ end of the J_α cluster and to progress to the 3′ J_α regions during thymocyte maturation, although published results indicate that use of the J_α regions with which V_α regions recombine probably results from a more complicated procedure[8]. The diversity of the TCR repertoire generated by this combinatorial process largely determines the ability of the immune system to mount a proper immune response to almost any antigen[9].

We isolated $CD4^+CD8^+$ (double-positive) $CD69^-$ thymocytes from the thymuses of wild-type (C57BL/6) mice, CD1d-deficient (*Cd1d1*$^{-/-}$ *Cd1d2*$^{-/-}$) mice[4] and mice deficient in J_α18 (*Traj18*$^{-/-}$ mice)[5] and amplified the TCR rearrangements for genes encoding three TCR V_α regions through the use of specific forward primers for each V_α family (TRAV11 for V_α14, TRAV9 for V_α3 and TRAV12 for V_α8) and a specific reverse primer for the gene encoding the α-chain constant region (C_α; TRAC). We sequenced the PCR products and analyzed the extent of J_α use for each V_α gene family (**Fig. 1**). The C57BL/6 J_α locus contains 60 genes, of which 22 are classified as pseudogenes (according to the International ImMunoGeneTics Information System). Transcripts containing sequences for these pseudogenes were indeed absent, except for five (*Traj47*, *Traj44*, *Traj26*, *Traj7* and *Traj4*), in agreement with published results[8]. Focusing on productive in-frame rearrangements in wild-type mice, we found sequences encoding all V_α-J_α combinations, although the frequency of J_α use was different for each V-gene family. This frequency of J_α use was reproducible in separate samples and was not affected by the absence of CD1d. In contrast, transcripts of genes encoding J_α regions upstream of *Traj18* were almost completely absent from *Traj18*$^{-/-}$ mice, and we estimated that about 60% of the diversity of the TCRα repertoire was actually lacking in these mice (**Supplementary Fig. 1**). These results were not a consequence of genetic drift or other environmental factors, as they were entirely reproducible in *Traj18*-deficient mice from another facility that have been maintained independently from our colony for at least 10 years. Analysis of out-of-frame sequences for which potential protein products cannot be subjected to any selection event demonstrated a pattern of frequency of J_α use similar to that of the in-frame sequences for each of the strains examined (**Supplementary Fig. 1** and data not shown), which indicated that the effects observed were the consequences of a genetic event. The introduction of a gene encoding neomycin resistance driven by the promoter of the gene encoding phosphoglycerate kinase (a *PGK-neo*^{r} cassette) can have inadvertent substantial effects on both transcription and rearrangements through the introduction of a constitutively open-chromatin configuration and competition for transcription factors[10]. We propose that this unexpected mechanism acts in *Traj18*$^{-/-}$ mice, in which the *PGK-neo*^{r} cassette, transcribed in an orientation opposite to that of the J_α regions, replaces *Traj18* (ref. 5). Partial suppression of 5′ rearrangements relative to 3′ rearrangements in *Traj18*$^{-/-}$ mice has been noted[11]. However, it is unclear whether the *PGK-neo*^{r} cassette actually affects rearrangements in these mice, as those results might have been biased by the analysis of total thymocytes[11]. Nevertheless, the *PGK-neo*^{r} cassette clearly caused nearly complete shutdown of transcription and splicing to create mature transcripts for TCRα.

Consequently, any change in immunological activity associated with these mice and for which a role has been ascribed to *i*NKT cells needs to be reassessed. This applies not only to studies that contrast *Traj18*$^{-/-}$ and *Cd1d1*$^{-/-}$*Cd1d2*$^{-/-}$ mice to investigate a role for type I NKT cells or type II NKT cells, respectively, but also to many disease-model studies, developmental studies and so on that have used or make use of these mice.

Note: Supplementary information is available in the online version of the paper.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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