Published in final edited form as:
Nat Immunol. ; 13(8): 705–706. doi:10.1038/ni.2347.

Lower TCR repertoire diversity in TRAJ18-deficient mice

Romain Bedel[1], **Jennifer Matsuda**[1], **Manfred Brigl**[2], **Janice White**[3], **John Kappler**[1,3,5], **Philippa Marrack**[1,3,4,6], and **Laurent Gapin**[1]

[1]Integrated Department of Immunology, National Jewish Health and University of Colorado Denver, Denver, CO 80206, USA

[2]Department of Pathology, Brigham and Women's Hospital, Boston, MA 02115, USA

[3]Howard Hughes Medical Institute and [3] Department of Medicine, University of Colorado Denver, Aurora, CO 80220, USA

[4]Department of Pharmacology, University of Colorado Denver, Aurora, CO 80220, USA

[5]Program in Biomolecular Structure, University of Colorado Denver, Aurora, CO 80220, USA

[6]Department of Biochemistry and Molecular Genetics, University of Colorado Denver, Aurora, CO 80220, USA

To the Editor

Natural Killer T (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]. As such, NKT cells serve as an important link between the innate and adaptive immune systems and are promising targets for immune therapy. Type I NKT cells, also named iNKT cells, are the most prevalent NKT cells in mice and have similar properties in humans. iNKT cells have evolved to recognize lipid-based antigens that are presented by the non-classical Major Histocompatibility Complex (MHC)-like molecule, CD1d. Numerous studies in humans and mice have reported a strong association between iNKT cell defects and an increased susceptibility to autoimmune diseases and cancers. In addition, iNKT cells are known to play important roles during infection with bacterial, viral, protozoan, and fungal pathogens[2].

Because iNKT cells are highly conserved between mice and humans[3], mouse models of iNKT cell deficiencies represent useful tools to immunologists. To this end, two similar but not equivalent, models of iNKT cell deficiencies exist. One makes use of mice in which the CD1d-encoding genes have been deleted[4], thereby preventing the development of any CD1d-reactive T cells, including iNKT cells. Another model directly targets the *TRAJ18* gene segment[5], which in combination with *TRAV11* gene is absolutely required to form the iNKT T-cell receptor (TCR) with the appropriate antigenic specificity[6].

RAG-1 and *RAG-2* recombinases drive successive TCR β- and α-chain gene rearrangements during thymocyte development. Primary rearrangements of TCRα VJ gene segments are initiated in CD4+CD8+ double positive (DP) thymocytes and, if successful, lead to the audition of TCR-expressing thymocytes for a productive interaction between TCR and self major histocompatibility complex (MHC) molecules. If positive selection does not occur, secondary Vα-Jα rearrangement proceeds to replace ineffective primary

Competing Financial Interests:
The authors declare no competing financial interests.

rearrangements[7]. TCRα gene recombination is thought to begin at the 5′ end of the Jα cluster and to progress to the 3′ Jαs during thymocyte maturation, though recent results showed that choice of the Jαs to which Vαs rearrange 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 against virtually any antigen[9].

We isolated DP CD69− thymocytes from the thymuses of wildtype (C57BL/6), CD1d1d2−/−[4] and Jα18−/−[5] mice and amplified the TCR rearrangements for three TCR Vα gene families (Vα14 (TRAV11), Vα3 (TRAV9) and Vα8 (TRAV12) using specific forward primers for each Vα family and a specific reverse primer for the Cα gene (TRAC). PCR products were subjected to high throughput sequencing using the Roche 454 platform and the extent of Jα usage for each Vα gene family was analyzed (Fig. 1). The B6 Jα locus contains 60 TRAJ genes of which 22 are classified as pseudogenes (http://www.imgt.org). Transcripts containing sequences for these pseudogenes were indeed absent, except for five TRAJ (TRAJ47, TRAJ44, TRAJ26, TRAJ7 and TRAJ4), in agreement with recent results[8]. Focusing on productive in-frame rearrangements in wildtype animals, we found sequences coding for all Vα-Jα combinations although the frequency of Jα usage was different for each V gene family. This Jα usage gene frequency was reproducible in separate samples and was not affected by the absence of CD1d molecules. In contrast, transcripts using TRAJs upstream TRAJ18 were nearly absent in TRAJ18−/− mice, and we estimate that about 60% of TCRα repertoire diversity is actually lacking in these mice (supplementary Fig. 1). These results are not the consequence of genetic drift or other environmental factors as they were entirely reproducible using TRAJ18-deficient mice from another facility that have been maintained independently from our colony for at least 10 years. Analyses of out-of-frame sequences for which potential protein products cannot be subjected to any selection event, demonstrated a similar pattern of Jα usage frequencies to the in-frame sequences for each of the three strains examined (supplementary Fig. 1), arguing that the observed effects are the consequences of a genetic event. Introduction of a neomycin resistance gene driven by the phosphoglycerate kinase promoter (pgk-neo^r) can inadvertently have dramatic effects on both transcription and rearrangements by introducing a constitutively open chromatin configuration and competing for transcription factors[10]. We propose that this unexpected mechanism is at play in TRAJ18−/− mice, where pgk-neo^r, transcribed in the opposite orientation to the Jαs, replaces the TRAJ18 gene[5]. We previously observed a partial suppression of 5′ rearrangements relative to 3′ rearrangements in TRAJ18−/− mice[11]. However, it is unclear whether the pgk-neo^r cassette really affects rearrangement in these mice, as the results might have been biased by the analysis of total thymocytes[11], Nevertheless, the pgk-neo^r cassette clearly causes a nearly complete shutdown of transcription/splicing to create mature TCRα transcripts.

Consequently, any change in immunological behavior found associated with these mice and for which a role was ascribed to iNKT cells, needs to be reassessed. This does not apply only to studies that contrast Jα18$^{-/-}$ and CD1d$^{-/-}$ mice to determine a role for type I or type II NKT cells respectively, but also to many disease model studies, developmental studies etc., which have used or make use of these mice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Matsuda JL, Mallevaey T, Scott-Browne J, Gapin L. CD1d-restricted iNKT cells, the 'Swiss-Army knife' of the immune system. Curr Opin Immunol. 2008; 20:358–368. [PubMed: 18501573]
2. Cohen NR, Garg S, Brenner MB. Antigen presentation by CD1: Lipids, T cells, and NKT cells in microbial Immunity. Advances in Immunology. 2009; 102:1–94. [PubMed: 19477319]
3. Brossay L, et al. CD1d-mediated recognition of an α-galactosylceramide by natural killer T cells is highly conserved through mammalian evolution. J Exp Med. 1998; 188:1521–1528. [PubMed: 9782129]
4. Chen YH, Chiu NM, Mandal M, Wang N, Wang CR. Impaired $NK1^+$ T cell development and early IL-4 production in CD1− deficient mice. Immunity. 1997; 6:459–467. [PubMed: 9133425]
5. Cui J, et al. Requirement for Vα14 NKT cells in IL-12-mediated rejection of tumors. Science. 1997; 278:1623–1626. [PubMed: 9374462]
6. Godfrey DI, Rossjohn J, McCluskey J. The fidelity, occasional promiscuity, and versatility of T cell receptor recognition. Immunity. 2008; 28:304–314. [PubMed: 18342005]
7. Yannoutsos N, et al. The role of recombination activating gene (RAG) reinduction in thymocyte development in vivo. J Exp Med. 2001; 194:471–480. [PubMed: 11514603]
8. Genolet R, Stevenson BJ, Farinelli L, Osteras M, Luescher IF. Highly diverse TCRα chain repertoire of pre-immune CD8(+) T cells reveals new insights in gene recombination. Embo J. 2012; 31:1666–1678. [PubMed: 22373576]
9. Jenkins MK, Chu HH, McLachlan JB, Moon JJ. On the composition of the preimmune repertoire of T cells specific for Peptide-major histocompatibility complex ligands. Annu Rev Immunol. 2010; 28:275–294. [PubMed: 20307209]
10. Riegert P, Gilfillan S. A conserved sequence block in the murine and human TCR J alpha region: assessment of regulatory function in vivo. J Immunol. 1999; 162:3471–3480. [PubMed: 10092803]
11. Hager EJ, Hawwari A, Matsuda JL, Krangel MS, Gapin L. Multiple constraints at the level of TCR alpha rearrangement impact Vα14i NKT cell development. J Immunol. 2007; 197:2228–2234. [PubMed: 17675483]

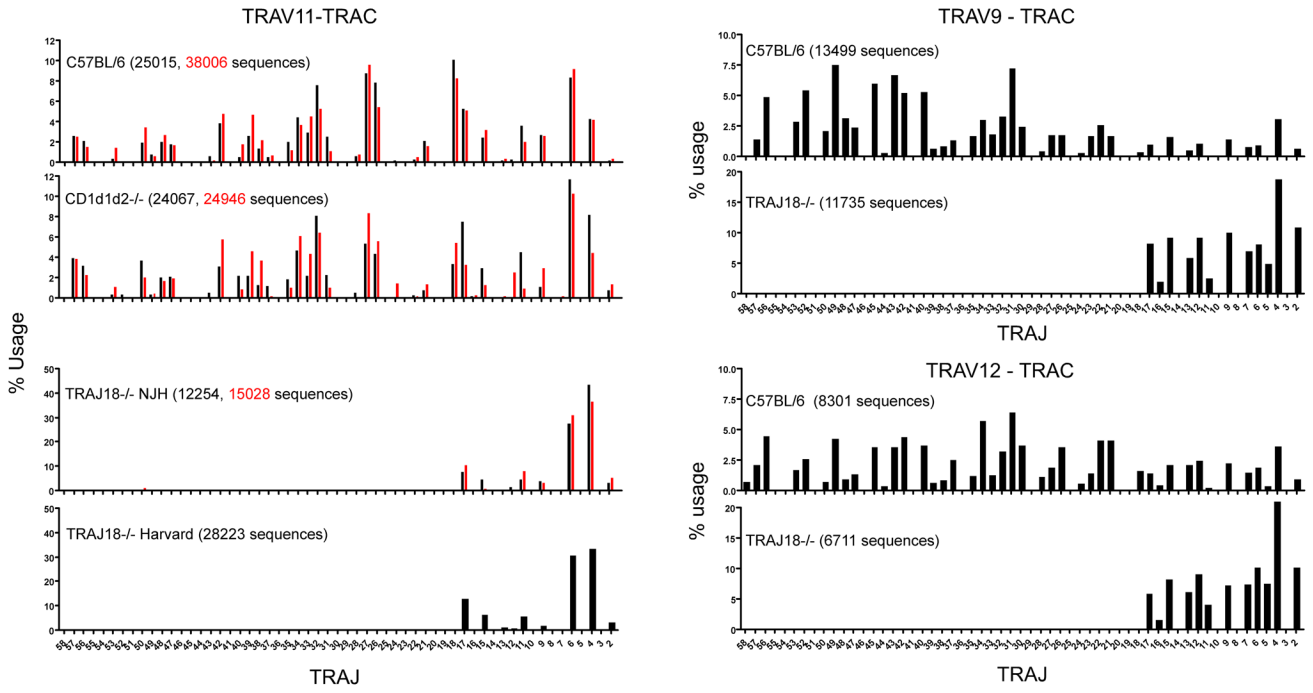

Fig 1.

Impaired TCRα diversity in TRAJ18−/− mice. The frequency of TRAJ gene usage for productive, in-frame, rearrangements involving the TRAV11, TRAV9 and TRAV12 family gene segments in sorted CD69− double positive (CD4+CD8+) thymocytes from C57BL/6, CD1d1d2−/− and TRAJ18−/− mice is shown. Rearrangements for each V gene family were amplified by PCR with specific V primers and a specific reverse C primer. PCR products were subjected to high throughput sequencing using the 454 platform. Sequences were analyzed using an in-house software and identity of each TRAV and TRAJ genes was assigned based on sequence alignment to published sequences (www.imgt.org). The number of analyzed sequences for each sample is indicated in parenthesis. The black and red bars indicate replicate experiments from independent samples. TRAJ18−/− that have been maintained in the animal facility at National Jewish Health (NJH), were compared to the same mice that had been maintained at the Harvard Medical school mouse facility. All mice were on the C57BL/6 background and were housed in specific pathogen-free conditions. All animal studies were approved by NJH Animal Care and Use Committee. TRAJ genes are ordered from 5′ to 3′, similar to their position in the TRAJ locus.