Editorial

How does metabolism of an "immuno acid" (tryptophan) by commensal *Lactobacillus reuteri* educate resident intestinal intraepithelial lymphocytes?

Yuying Liu, J. Marc Rhoads

Division of Gastroenterology, Hepatology & Nutrition, Department of Pediatrics, the University of Texas Health Science Center at Houston McGovern Medical School, Houston, Texas, USA

Correspondence to: Yuying Liu, PhD, MEd. Division of Gastroenterology, Hepatology & Nutrition, Department of Pediatrics, the University of Texas Health Science Center at Houston McGovern Medical School, 6431 Fannin Street, MSB 3.140A, Houston, Texas 77030, USA.
Email: Yuying.Liu@uth.tmc.edu.

Comment on: Cervantes-Barragan L, Chai JN, Tianero MD, *et al.* Lactobacillus reuteri induces gut intraepithelial CD4(+)CD8αα(+) T cells. Science 2017;357:806-10.

Received: 03 April 2018; Accepted: 30 April 2018; Published: 09 May 2018.
doi: 10.21037/jlpm.2018.04.08
View this article at: http://dx.doi.org/10.21037/jlpm.2018.04.08

Intestinal intraepithelial lymphocytes (IELs) are a large and diverse population of lymphoid cells that reside between the intestinal epithelial cells (IECs) that form the intestinal mucosal barrier (1). Intestinal IELs can be classified into T cell receptor (TCR)$^-$ and TCR$^+$ subsets (1,2). TCR$^-$ IELs comprise subsets of cells resembling innate lymphoid cells (ILCs), intracellular-expressing CD3 cells (ICD3$^+$), and ICD8α$^+$ cells. TCR$^+$ IELs can be further divided into induced and natural IELs (2). The induced IELs are derived from antigen-experienced, conventional T cells that home to the intestinal epithelium, while natural IELs comprise cells that home immediately to the intestinal epithelium after their development. Induced IELs include TCRαβ$^+$CD4$^+$CD8αα$^+$ and TCRαβ$^+$CD8αβ$^+$CD8αα$^+$ T cells. In mice, about 25–50% of all TCRαβ$^+$CD4$^+$ IELs also express CD8αα (DP IELs), a finding shown by several research groups 20 years ago (3-5).

T cells expressing αβ T-cell receptors (TCRs) (hereafter simply "T cells"), comprising a majority of circulating T lymphocytes, can be classified into many subgroups. The first major classification comprises CD4$^+$ and CD8$^+$ T cells, which exert helper and cytotoxic functions, respectively, upon activation. Both lineages arise from a common pool of progenitors, namely double-positive thymocytes, although they subsequently diverge into each lineage depending on their ability to recognize different classes of the MHC: peptide complex by their TCRs (6). During lineage specification, the transcription factor Th-POK (also named c-Krox or Zfp67, encoded by Zbtb7b) plays an essential role for CD4$^+$ T cells whereas another transcription factor, Runx3, is crucial for CD8$^+$ T cells.

Both transcription factors are important for inducing lineage-specific genes as well as suppressing the other lineage by antagonizing the other's expression to ensure lineage stability (7,8). The second layer of classification is T-helper (Th) subsets, which subdivides effector CD4$^+$ T cells by their function and cytokine secretion. Several well-characterized Th subsets exist such as Th1, Th2, Th17, Treg, and Tfh, for which the so-called "master transcription factors" required for their differentiation have been identified (9). Although the central roles of these transcription factors are well established, their activity synergizes with other transcription factors to form a network and fine-tune their function.

In the past, the lineage-specifying gene expression program DP-IELs arise from conventional CD4$^+$ T cells through dynamic reorganization of the transcriptional network by the induction of Runx3 and subsequent down-regulation of Th-POK (a transcriptional factor that plays an essential role for CD4$^+$ T cells) (10-12). DP-IEL induction can be mimicked *in vitro* by activating naïve CD4$^+$ T cells in the presence of TGFβ, enhanced by additional factors such as retinoic acid (RA), IL-7, IFN-γ, and IL-27 (11,13,14).

DP IELs have been suggested to have a regulatory

function, based on several findings: (I) after $CD4^+$ T cells were cultured *ex vivo* under Th2-cell differentiation conditions and transferred into recombination activating gene (RAG)-deficient mice, transferred cells entered into the IEL compartment and gained CD8αα expression to become DP IELs. Secondary transfer of these DP IELs into RAG-deficient recipients protected the animals from colitis induced by co-transfer of pathogenic Th1 cells (15); (II) the frequency of DP IELs was decreased in patients suffering from chronic intestinal inflammation (16,17); and (III) DP IELs promoted tolerance to dietary antigens (18). In addition, DP IELs expressed large quantities of granzyme and had functional MHC-class II dependent cytotoxicity (10,19).

Gut microbiota shapes the immune functions in the intestinal mucosa, promoting the development and differentiation of immune cells, e.g., T cells (20,21). The lack of DP IELs in germ-free (GF) mice indicates that the intestinal microbiota is required for their development (10,18). Cervantes-Barragan and colleagues (22) have reported their findings that *Lactobacillus reuteri* (*L. reuteri*) regulates the differentiation of gut DP IELs. Based on their finding that the percentage of DP IEPs from C57BL/6 mice born in the SRF facility at Washington University School of Medicine (WUSM) was high while the percentage in mice born in the CSRB facility at WUSM was low, they performed oral gavage with ileal or fecal microbiota harvested from SRF mice to CSRB mice. They found that the frequency of DP IEL increased in CSRB mice 4 weeks after fecal transplantation. The frequency of DP IELs in mice from different vendors was also different, with high levels in Charles River (CR) mice and low levels in Jackson (Jax) mice. Similarly, fecal transplant from CR mice to Jax mice increased the DP IEL frequency of Jax mice, indicating that intestinal microbiome is an important factor that promotes the differentiation of DP IELs. The bacterial candidate suspected of inducing DP IELs was further narrowed down by selective antibiotic treatment, identifying neomycin-resistant Gram-positive bacterial taxa, which subsequently led to the identification of *L. reuteri*.

L. reuteri promotion of the differentiation of DP IELs was strain specific with *L. reuteri WU* and *L. reuteri 100-23* increasing the percentage of DP IELs after colonization of Jax mice, while *L. johnsonii* and *L. murinus* were ineffective. Mice housed in the absence of *L. reuteri* had significantly reduced numbers of DP IELs compared to mice harboring this bacterium. Interestingly, reconstitution of *L. reuteri* in mice lacking this organism was sufficient to induce the differentiation of DP IELs. This observation not only suggests that *L. reuteri* is responsible for the development of DP IELs but also raises a concern to all mouse investigators that the microbiota of mice from different commercial vendors may differ with respect to their commensal flora, resulting in variation in the prevalence of DP IELs.

Additional studies demonstrated that *L. reuteri* metabolizes tryptophan (Trp) into indole derivatives, including indole-3-lactic acid, a ligand of the aryl hydrocarbon receptor (AhR) which subsequently activates transcription factors regulating intestinal immunity and inflammation. Trp [or 2-Amino-3-(1H-indol-3-yl) propanoic acid] is an essential amino acid containing the indole ring, an alpha-carboxylic acid group, and an alpha amino group. It is best known for its metabolism to serotonin and melatonin. However, it is initially metabolized to kynurenine, kynurenic acid, xanthurenic acid and cinnabarinic acid in host cells. In the gut lumen microbiota convert Trp to indole, indole-3-aldehyde (IAld), indole-3-acetic acid (IAA), and indole-3-lactic acid (ILA). Trp metabolites are known to directly inhibit T cell proliferation; but they indirectly increase Treg-mediated immune suppression via AhR activation (23).

Previous studies have demonstrated that, under low Trp conditions, *Lactobacilli* switch from sugar metabolism to Trp metabolism, causing the degradation of Trp into indole derivatives such as IAld, which activates AhR to produce the anti-inflammatory cytokine IL-22. Under low Trp conditions, dietary Trp is metabolized into kynurenine (L-Kyn) by indoleamine 2,3-dioxygenase (IDO) expressed in the host IECs and tolerogenic dendritic cells (DCs), subsequently activating $Foxp3^+$ Tregs to produce anti-inflammatory IL-10, by which provides antifungal resistance and mucosal protection from damage (24). Other metabolites produced by *L. reuteri* such as reuterin also have anti-fungal activity (25).

DP IELs were significantly reduced in $AhR^{-/-}$ mice and mice lacking AhR in T cells, and $AhR^{-/-}$ T cells didn't differentiate into DP T cells *in vitro*, implying that AhR is required for DP IEL development. A mutant *L. reuteri* strain lacking a functional aromatic aminotransferase to convert amino acids into indolic AhR ligands did not induce DP IELs in Jax mice. This indicates that indolic AhR ligands are required to induce IELs. CR mice colonized with *L. reuteri* and other gut microbiota showed evidence of the differentiation of DP IELs when these animals were provided with a tryptophan-rich diet. The messages

inform that *L. reuteri*-generated indole derivatives activate the AhR receptor expressed in $CD4^+$ T cells, stimulating differentiation into DP IELs. In summary, Cervantes-Barragan and colleagues' studies indicate that a single species of *L. reuteri* and its Trp metabolites are enough for the development of DP IELs.

Currently, it remains unknown whether *L. reuteri* promotes the migration of certain subtypes of T cells such as $CD4^+$ T cells, naïve $CD4^+$ T cells, or even $Foxp3^+$ T cells to the intestinal epithelium to foster their development into DP IELs. We also do not know if there are other factors which promote the migration of T cells into the epithelium, and we do not know if *L. reuteri* is the only microbe to induce the differentiation of DP IELs. Cervantes-Barragan *et al.* did not examine whether these DP IELs express Foxp3, nor do they investigate potential immune regulatory mechanisms of the DP IELs. Recent experiments have shown that $CD4^+Foxp3^+$ Tregs in the lamina propria can migrate into the IEL compartment, where they acquire DP-$Foxp3^-$ phenotype. These DP-$Foxp3^-$ IELs possess regulatory properties that can compensate for the absence of $CD4^+Foxp3^+$ Tregs in the lamina propria to protect mice against intestinal inflammation (18).

It has been known that $CD4^+$ T cells that express Foxp3 and Tr1-like cells that produce IL-10 comprise the major regulatory leukocyte populations in the intestine. It has been reported that oral administration of *L. reuteri* promotes intestinal mucosal tolerance, associated with an increase in the percentage of $CD4^+Foxp3^+$ Tregs and a concomitant decrease in the percentage of proinflammatory $CD4^+CD44^+$ effector memory T cells (Teffs) during intestinal inflammation, for example in conditions such as experimental necrotizing enterocolitis (NEC) (26,27). The fascinating studies of Cervantes-Barragan *et al.* provide a rational basis for deeper investigation of probiotic *L. reuteri* and if coadministration of tryptophan-rich foods could aid in the treatment of chronic inflammatory disorders, such as inflammatory bowel disease (IBD).

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by Section Editor Dr. Shuli Zhao (The Center Lab, Nanjing First Hospital, Nanjing Medical University, Nanjing, China).

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jlpm.2018.04.08). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Cheroutre H, Lambolez F, Mucida D. The light and dark sides of intestinal intraepithelial lymphocytes. Nat Rev Immunol 2011;11:445-56.
2. Olivares-Villagómez D, Van Kaer L. Intestinal Intraepithelial Lymphocytes: Sentinels of the Mucosal Barrier. Trends Immunol 2018;39:264-75.
3. Aranda R, Sydora BC, McAllister PL, et al. Analysis of intestinal lymphocytes in mouse colitis mediated by transfer of CD4+, CD45RBhigh T cells to SCID recipients. J Immunol 1997;158:3464-73.
4. Morrissey PJ, Charrier K, Horovitz DA, et al. Analysis of the intra-epithelial lymphocyte compartment in SCID mice that received co-isogenic CD4+ T cells. Evidence that mature post-thymic CD4+ T cells can be induced to express CD8 alpha in vivo. J Immunol 1995;154:2678-86.
5. Mosley RL, Styre D, Klein JR. CD4+CD8+ murine intestinal intraepithelial lymphocytes. Int Immunol 1990;2:361-5.
6. Singer A, Adoro S, Park JH. Lineage fate and intense debate: myths, models and mechanisms of CD4- versus CD8-lineage choice. Nat Rev Immunol 2008;8:788-801.
7. Egawa T, Taniuchi I. Antagonistic interplay between ThPOK and Runx in lineage choice of thymocytes. Blood

Cells Mol Dis 2009;43:27-9.
8. Naito T, Taniuchi I. The network of transcription factors that underlie the CD4 versus CD8 lineage decision. Int Immunol 2010;22:791-6.
9. Fang D, Zhu J. Dynamic balance between master transcription factors determines the fates and functions of CD4 T cell and innate lymphoid cell subsets. J Exp Med 2017;214:1861-76.
10. Mucida D, Husain MM, Muroi S, et al. Transcriptional reprogramming of mature CD4(+) helper T cells generates distinct MHC class II-restricted cytotoxic T lymphocytes. Nat Immunol 2013;14:281-9.
11. Reis BS, Rogoz A, Costa-Pinto FA, et al. Mutual expression of the transcription factors Runx3 and ThPOK regulates intestinal CD4(+) T cell immunity. Nat Immunol 2013;14:271-80.
12. Kakugawa K, Kojo S, Tanaka H, et al. Essential Roles of SATB1 in Specifying T Lymphocyte Subsets. Cell Rep 2017;19:1176-88.
13. Konkel JE, Maruyama T, Carpenter AC, et al. Control of the development of CD8alphaalpha+ intestinal intraepithelial lymphocytes by TGF-beta. Nat Immunol 2011;12:312-9.
14. Van Kaer L, Rabacal WA, Scott Algood HM, et al. In vitro induction of regulatory CD4+CD8alpha+ T cells by TGF-beta, IL-7 and IFN-gamma. PLoS One 2013;8:e67821.
15. Das G, Augustine MM, Das J, et al. An important regulatory role for CD4+CD8 alpha alpha T cells in the intestinal epithelial layer in the prevention of inflammatory bowel disease. Proc Natl Acad Sci U S A 2003;100:5324-9.
16. Carton J, Byrne B, Madrigal-Estebas L, et al. CD4+CD8+ human small intestinal T cells are decreased in coeliac patients, with CD8 expression downregulated on intra-epithelial T cells in the active disease. Eur J Gastroenterol Hepatol 2004;16:961-8.
17. Senju M, Wu KC, Mahida YR, et al. Coexpression of CD4 and CD8 on peripheral blood T cells and lamina propria T cells in inflammatory bowel disease by two colour immunofluorescence and flow cytometric analysis. Gut 1991;32:918-22.
18. Sujino T, London M, Hoytema van Konijnenburg DP, et al. Tissue adaptation of regulatory and intraepithelial CD4(+) T cells controls gut inflammation. Science 2016;352:1581-6.
19. Sasahara T, Tamauchi H, Ikewaki N, et al. Unique properties of a cytotoxic CD4+CD8+ intraepithelial T-cell line established from the mouse intestinal epithelium. Microbiol Immunol 1994;38:191-9.
20. Lee YK, Mazmanian SK. Has the microbiota played a critical role in the evolution of the adaptive immune system? Science 2010;330:1768-73.
21. Mowat AM. To respond or not to respond - a personal perspective of intestinal tolerance. Nat Rev Immunol 2018. [Epub ahead of print].
22. Cervantes-Barragan L, Chai JN, Tianero MD, et al. Lactobacillus reuteri induces gut intraepithelial CD4(+) CD8αα(+) T cells. Science 2017;357:806-10.
23. Gao J, Xu K, Liu H, et al. Impact of the Gut Microbiota on Intestinal Immunity Mediated by Tryptophan Metabolism. Front Cell Infect Microbiol 2018;8:13.
24. Zelante T, Iannitti RG, Cunha C, et al. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. Immunity 2013;39:372-85.
25. Cleusix V, Lacroix C, Vollenweider S, et al. Inhibitory activity spectrum of reuterin produced by Lactobacillus reuteri against intestinal bacteria. BMC Microbiol 2007;7:101.
26. Liu Y, Fatheree NY, Dingle BM, et al. Lactobacillus reuteri DSM 17938 changes the frequency of Foxp3+ regulatory T cells in the intestine and mesenteric lymph node in experimental necrotizing enterocolitis. PLoS One 2013;8:e56547.
27. Liu Y, Tran DQ, Fatheree NY, et al. Lactobacillus reuteri DSM 17938 differentially modulates effector memory T cells and Foxp3+ regulatory T cells in a mouse model of necrotizing enterocolitis. Am J Physiol Gastrointest Liver Physiol 2014;307:G177-86.

doi: 10.21037/jlpm.2018.04.08
Cite this article as: Liu Y, Rhoads JM. How does metabolism of an "immuno acid" (tryptophan) by commensal *Lactobacillus reuteri* educate resident intestinal intraepithelial lymphocytes? J Lab Precis Med 2018;3:46.

