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Brokering the peace: the origin of intestinal T cells

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In designating the thymic origin of the cells, the T in T cell seems simple enough, and the impressive unfolding of how the differentiation and selection of conventional CD4 and CD8 T cells are supported by the uniquely capable thymic stroma seems prima facie to leave little left to uncover. But, as the initial uncovering of T-cell receptor (TCR) γ-chain genes forewarned, there are myriad "unconventional T cell" subtypes whose development is not easily explained by current understanding. Such cells, either TCRαβ$^+$ or TCRγδ$^+$, rarely express either CD4 (a coreceptor for major histocompatibility complex (MHC) II) or CD8αβ (a coreceptor for MHC I).[2] Instead, they are CD4, CD8 double-negative (DN) or express a homomeric CD8αα molecule. However, rather than being mere fringe players, worthy only of "page 2, column 3,"[3] these unconventional T cells compose a substantial fraction of perhaps the most abundant and most active T cells in the body—the intraepithelial lymphocytes (IELs)—that populate several body surfaces, including the gut. There, they seemingly contribute to the physiologic homeostasis that embraces epithelial integrity, the measured immune response to commensals, and the adaptive tolerance toward self-antigens. When this homeostasis is disrupted, IELs may also contribute to inflammatory and wound-healing responses. Given this, a strong interest in their origin is appropriate.

EXTRATHYMIC IEL DEVELOPMENT

About 50% of intestinal IELs ("type a" cells[4]) seemingly compose conventional, MHC-restricted CD8αβ TCRαβ$^+$ effector-memory cells, which home to the gut during infection. These cells develop in the thymus from CD4$^-$CD8$^-$ (DN) progenitors which, upon productive TCRβ expression, enter a CD4$^+$CD8$^+$ ("double-positive" (DP)) pool (the most abundant thymocyte subset), where cells are positively selected by "light-touch" peptide–MHC interactions in the thymic cortex, and then pass into the complex medullary stroma where strongly self-reactive DP cells are purged by MHC–peptide engagement (**Figure 1**). In contrast to this, the remaining DN and CD8αα$^+$ "type b" cells are plainly detectable in athymic mice, albeit less abundantly than in euthymic mice (**Table 1**). As these cells seemingly lack peptide–MHC restriction, there is *a priori* no need for them to endure the complex developmental progression of DP cells. Thus, unconventional T-cell development would seem to exploit very little of what the thymus has to offer, possibly requiring only a simple stroma to support it.

Such a primitive stroma was arguably identified along the intestinal wall by Ishikawa and co-workers with their landmark discovery of cryptopatches (CPs)[5]—small organized structures containing immature CD25$^+$ IL7R$^+$ c-kit$^+$ hematopoietic cells, which when isolated from athymic donors, reconstituted the T-cell compartment of irradiated severe combined immuno deficient mice.[6] Furthermore, by proposing an alternative mechanism of differentiation, CPs offered an explanation for the unusual "agonist-selection" of unconventional T cells. Thus, T progenitors expressing a transgenic autoreactive TCR precociously early in development do not all die upon autoantigen engagement, as would be predicted for conventional development, but rather may develop as unconventional αβ T cells.[7] Agonist selection is considered a signature of unconventional T-cell biology, evidenced in the gut by type b cells with "forbidden" TCRαβ combinations that are purged from the conventional thymic repertoire by stromal presentation of autologous "super-antigens."[8] Supporting the capacity of gut structures to nurture T-cell development, a hierarchy of immature T-lineage cells was identified in the intestine.[9] Moreover, intestinal γδ-cell development (including γ-gene rearrangement), which depends on IL7, can be rescued in IL7-null mice by gut epithelium-specific IL-7 expression.[10]

Nonetheless, one needs to accommodate the fact that CD8αα$^+$ IEL numbers are reduced by as much as 90% in athymic mice (**Table 1** and Figure 6a in ref. 1). As unconventional T cells are characteristically oligoclonal, they may have a high potential for homeostatic expansion in immuno-depleted mice, in which case the frequency of their precursors in athymic mice may be very low indeed.

THYMIC IEL DEVELOPMENT REVISITED

Formally, the thymus might promote gut T-cell development *in trans*, via a "thymic hormone." However, our increasing recognition that the thymus will support unconventional development offers a

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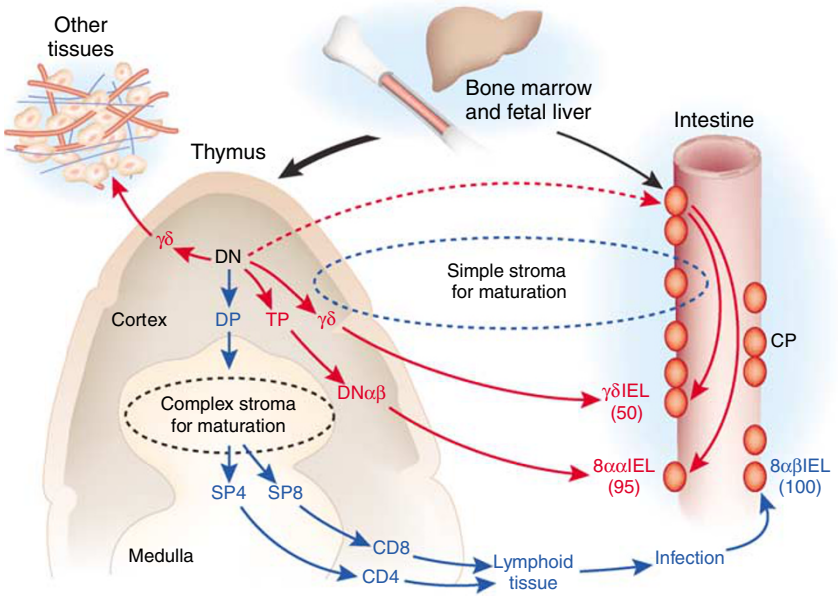

Figure 1 A proposed scheme for combining the thymic and extrathymic development of intestinal T cells. Red font denotes unconventional T-cell maturation; blue font represents conventional T-cell maturation; numbers in brackets represent this author's estimate of possible percentages of each cell type that are thymically derived in euthymic mice.

Table 1 Occurrence of gut T-cell subtypes in euthymic and athymic mice

	Type b (unconventional)		Type a
	TCRγδ$^+$	TCRαβ$^+$	
Intestinal T-cell subset		**CD8αα$^+$**	**CD8αβ$^+$**
Euthymic mice	+++++	+++++	+++++
Athymic mice	++++	++	–

TCR, T-cell receptor.

more straightforward explanation. Most obviously, many γδ T-cell subsets are thymically derived (**Figure 1**). They do not pass through the DP stage, maturing as DN T cells, seemingly on the basis of strong signaling fluxes through the TCR and other receptors. Indeed, TCRγδ$^+$ thymocytes atypically mature as DPs if their TCR–CD3 complex signals weakly.[11]

γδ cells share with other unconventional T cells a gene expression profile distinct from that of DP cells and their conventional CD4 and CD8T cell progeny.[12] This profile collectively depicts an "activated-yet-resting," highly differentiated phenotype, akin to that attained by "type a" IELs following infection and homing to the gut. This "pseudomemory" state is associated with the capacity of unconventional T cells to respond much more rapidly in the periphery than naive conventional T cells.[2,4,12] Thus, it was hypothesized that other unconventional T cells may also differentiate from DN thymocytes via differential signaling and, quite recently, this was supported by reports of novel unconventional T-cell differentiation pathways associated with mutations in thymocyte signaling pathways.[13] Nonetheless, CD8αα$^+$TCRαβ$^+$ thymocytes are near impossible to detect in the thymus, and their thymic origin remained unproven.

In this context, Eberl and Littman[14] described a fate-mapping experiment in which a *gfp* marker was activated by a *cre*-mediated excision event, driven by the promoter for RoRγt, a transcription factor expressed by fetal lymphoid tissue-inducer cells that promote lymphoid organogenesis. It is worth noting that RoRγt was reportedly expressed by all CP cells, and by DP thymocytes where it promotes survival. The finding that *gfp* marked most TCRαβ$^+$ CD8αα IELs implied that most progenitors pass through either a CP or DP intermediate. Interestingly, TCRγδ$^+$ IELs were not marked. Given that γδ cells are known not to pass through the DP stage, a reasonable explanation lies in all IELs developing in the thymus, with CD8αα αβ T cells being derived from the DP stage. This clearly explained why DP survival and CD8αα IELs were substantially depleted in RoRγt$^{-/-}$ mice, whereas TCRγδ$^+$ IELs were not. Although CPs were also reportedly absent in RoRγt$^{-/-}$ mice, DP survival and CD8αα IELs were rescued by a Bcl-xL transgene, whereas CPs were not.[14] This elegant study delivered a body blow to the extrathymic development of CD8αα IELs, which was further compounded when Cheroutre and co-workers described rare thymic DPs that express CD8αα (so-called "triple-positive" (TP) cells), and that, in contrast to normal DP cells, survive and adopt the unconventional phenotype when exposed to agonist ligands in organ culture.[15] Moreover, the intrathymic transfer of TP to sublethally irradiated recipients reconstituted the TCRαβ$^+$CD8αα$^+$ IEL compartment. The slight surprise in these experiments was that the CD4$^+$CD8αβ$^+$CD8αα$^+$ intermediates seem to shut off all coreceptors, maturing in the thymus via DN TCRαβ$^+$ cells, which may explain previously reported agonist-selected DN TCRαβ$^+$ thymocytes.[16] CD8αα is then re-expressed in the gut. Possibly, the type b progenitors pass through an early DP stage merely because CD4 and CD8 are automatically induced by the preTCR that signals productive TCRβ gene expression in late DN thymocytes, but there is no evidence that they functionally utilize CD4 or CD8αβ.

What then of RoRγt-expressing CPs? Rather than a site of T-cell development, Littman and co-workers proposed them as dynamic structures in which RoRγt-dependent adult counterparts of fetal lymphoid tissue-inducer-like cells respond to gut inflammation by providing sites for effector maturation (e.g., RoRγt-dependent Th-17 cells[17]) rather than *de novo* differentiation. However, the current study from Ishikawa's group challenges this as the sole role of CPs.[1] By fine dissection of CPs from the mice

generated by Littman's group on both euthymic and athymic backgrounds, the study establishes that RoRγt$^+$ lymphoid tissue-inducer-like cells compose <50% of CPs, with other RoRγt$^{(-)}$ or RoRγt^{lo} cells within and around CPs comprising immature cells with hallmarks of the T lineage—e.g., CD3ε and preTCR RNA, and TCRβ and γ-chain gene rearrangements. Consistent with this, the study shows that CPs are not absent from RoRγt$^{-/-}$ mice, merely sevenfold depleted, albeit that most seem small and poorly defined. Accepting that RoRγt profoundly affects CP development, Ishikawa considers them as aggregates where RoRγt-dependent lymphoid tissue-inducer-like cells nurture T progenitors that may transiently express RoRγt, but that are RoRγt-independent. Indeed, in contrast to the fate-mapping paper, the latest study confirms other reports that CD8αα IEL depletion in RoRγt$^{(-)}$ mice is marginal on euthymic or athymic backgrounds. Thus, Naito *et al.*[1] claim that their study reasserts the local origin of the body's largest T-cell subset.

A RESOLUTION

The abundance of TCRγδ$^+$ IELs in athymic mice (Figure 6a, ref. 1) coupled to the capacity of gut-specific IL7 to rescue γδ development in IL7$^{(-)}$ mice asserts that the gut stroma can support T-cell differentiation. The simplicity of gut relative to thymic stroma may render it incapable of supporting conventional T-cell differentiation, but it may still nurture unconventional differentiation of bone marrow- or fetal liver-derived progenitors that arrive there (**Figure 1**). That some such precursors enter the gut, as opposed to exclusively entering the thymus, is not improbable. Moreover, Lambolez and Rocha[18] reported that immature DN thymocytes may, during the perinatal period, exit to the gut and complete maturation there. Although CPs are not likely to be obligatory for extrathymic development, their formation shortly after birth renders them obvious candidates for attracting progenitors and concentrating their differentiation. Nonetheless, the data that CD8αα TCRαβ$^+$ IEL progenitors more generally pass through a TP intermediate argue that their differentiation requires further maturation steps than γδ differentiation, which may be more challenging (although not impossible) for the gut to support, and hence the cells' overwhelming thymic derivation in euthymic mice. At the same time, one wonders whether TPs derive from distinct, precommitted DN progenitors more prone to enter the gut than conventional thymocyte progenitors permitting local, albeit inefficient differentiation in aythmic mice. Such precommitted cells may respond to agonist engagement via a completely different signaling machinery than a conventional progenitor, thus explaining agonist selection.

If most CD8αα TCRαβ$^+$ IELs mature in CPs only in athymic conditions, how relevant are CDPs? Perhaps very relevant. The potent, differentiated phenotype of IELs and their capacity for rapid responsiveness would predict them to be victims of activation-induced cell death. But if so, how are these repertoires maintained in animals that undergo thymic involution, where the microbial challenges at body surfaces continue to change throughout life? The most extreme case of this is in the murine skin, where the repertoire of TCRγδ$^+$ IELs is generated exclusively in the fetal thymus, yet lasts the lifetime of the mouse.[19] Given this, one has to consider the possibility of local repositories of partially differentiated cells, protected from activation-induced cell death, and capable of replenishing the mature compartment. Is it conceivable that CPs compose such repositories? Again, their confinement to the antigen-exposed postnatal period may be a clue.

In short, the latest paper and many that precede it collectively permit a revised perspective on unconventional T cells. Key questions remain. Aside from the "missing repositories," why do agonist-selected cells mature as potentially murderous depots of cytolytic mediators? Clearly, identifying the physiologic agonists is a high priority that might be usefully pursued in the aftermath of the T-cell development controversies.

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DISCLOSURE

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REFERENCES

1. Naito, T. *et al.* RoRγt is dispensable for the development of intestinal mucosal T cells. *Mucosal. Immunol.* (2008) this issue.
2. Pennington, D. *et al.* The integrated responses of conventional and unconventional T cells. *Adv. Immunol.* **87,** 27–59 (2005).
3. Cook, W. *Goodbye Again: the definitive Peter Cook and Dudley Moore*, (Arrow, London, 2005).
4. Hayday, A., Theodoridis, E., Ramsburg, E. & Shires, J. Intraepithelial lymphocytes: exploring the Third Way in immunology. *Nat. Immunol.* **2,** 997–1003 (2001).
5. Kamamori, Y. *et al.* Identification of novel lymphoid tissues in murine intestinal mucosa where clusters of c-kit$^+$ IL-7R$^+$ Thy1$^+$ lympho-hemopoietic progenitors develop. *J. Exp. Med.* **184,** 1449–1459 (1996).
6. Saito, H. *et al.* Generation of intestinal T cells from progenitors residing in gut cryptoopatches. *Science* **280,** 275–278 (1998).
7. Bruno, L., Fehling, J. & von Boehmer, H. The alpha beta T cell receptor can replace the gamma delta receptor in the development of gamma delta lineage cells. *Immunity* **5,** 342–352 (1996).
8. Rocha, B., Vassalli, P. & Guy-Grand, D. The V-beta repertoire of mouse gut homodimeric alpha CD8$^+$ intraepithelial T cell receptor alpha beta$^+$ lymphocytes reveals a major extrathymic pathway of T cell differentiation. *J. Exp. Med.* **173,** 483–486 (1991).
9. Lambolez, F. *et al.* Characterization of T cell differentiation in the murine gut. *J. Exp. Med.* **195,** 437–449 (2002).
10. Laky, K. *et al.* Enterocyte expression of interleukin 7 induces development of gd T cells and Peyer's patches. *J. Exp. Med.* **191,** 1569–1580 (2000).
11. Hayes, S.M., Li, L. & Love, P.E. TCR signal strength influences alphabeta/gammadelta lineage fate. *Immunity* **22,** 583–593 (2005).
12. Pennington, D. *et al.* The inter-relatedness and interdependence of mouse T cell receptor γδ$^+$ and TCRαβ$^+$ cells. *Nat. Immunol.* **4,** 991–998 (2003).
13. Veillette, A., Dong, Z. & Latour, S. Consequence of the SLAM–SAP signaling pathway in innate-like and conventional lymphocytes. *Immunity* **27,** 698–710 (2007).
14. Eberl, G. & Littman, D. Thymic origin of intestinal alpha beta T cells revealed by fate-mapping of RoR-gamma-t$^+$ cells. *Science* **305,** 248–251 (2004).
15. Gangadharan, D. *et al.* Identification of pre- and postselection TCRαβ$^+$ intraepithelial lymphocyte precursors in the thymus. *Immunity* **25,** 631–641 (2006).
16. Hanke, T., Mitnacht, R., Boyd, R. & Hunig, T. Induction of interleukin 2 receptor beta chain by self-recognition in the thymus. *J. Exp. Med.* **180,** 1629–1636 (1994).
17. Invanov, I.I. *et al.* The orphan nuclear receptor RORgamma-t directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* **126,** 1121–1133 (2006).
18. Lambolez, F. *et al.* The thymus exports long-lived fully committed T cell precursors that can colonize primary lymphoid organs. *Nat. Immunol.* **7,** 76–82 (2006).
19. Havran, W. & Allison, J.P. Origin of Thy-1+ dendritic epidermal cells of adult mice from fetal thymic precursors. *Nature* **344,** 68–70 (1990).