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Post-thymic maturation: young T cells assert their individuality

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Abstract

T cell maturation was once thought to occur entirely within the thymus. Now, evidence is mounting that the youngest peripheral T cells in both mice and humans comprise a distinct population from their more mature, yet still naive, counterparts. These cells, termed recent thymic emigrants (RTEs), undergo a process of post-thymic maturation that can be monitored at the levels of cell phenotype and immune function. Understanding this final maturation step in the process of generating useful and safe T cells is of clinical relevance, given that RTEs are over-represented in neonates and in adults recovering from lymphopenia. Post-thymic maturation may function to ensure T cell fitness and self tolerance.

Recent thymic emigrants (RTEs) are T cells that have recently completed thymic development and egress, and they comprise a crucial but understudied population connecting thymic output to the recirculating T cell pool. RTEs are a clinically relevant population because they constitute a considerable proportion of the T cell pool in neonates, infants and young adults^{1–3}. Moreover, they maintain T cell receptor (TCR) repertoire diversity in young and middle-aged adults⁴ and foster recovery from unintentional or therapeutic lymphoablation⁵. Thus, understanding RTE biology is key to predicting the responses to immune assault of neonates, ageing individuals and those recovering from lymphoablation. In addition, RTEs may have a role in several disease states, including ulcerative colitis⁶, chronic myeloid leukaemia⁷ and autoimmune thyroid disease⁸. A roadblock to studying the RTE compartment has been the lack of an unambiguous cell surface marker to distinguish RTEs from the remainder of the peripheral T cell pool (TABLE 1). However, newly developed tools are providing ways to specifically and reliably label RTEs^{3,9}, thereby allowing immunologists to analyse for the first time the phenotype and function of RTEs from unmanipulated donors.

In this Review, we highlight differences between adult mouse RTEs and their more mature, although still naive, T cell counterparts. Owing to space constraints, we do not cover in depth the biology of neonatal mouse RTEs or human RTEs, but these are the subjects of several recent and thorough reviews^{10–12}.

RTE population dynamics

Maintenance of the peripheral T cell pool is achieved through both homeostatic turnover of peripheral T cells and thymic output, which provides new recruits to the pool of recirculating T cells. These new recruits are responsible for maintaining a diverse TCR repertoire, allowing useful T cell responses against newly encountered pathogens and antigens⁴. Indeed, female mice that have been thymectomized gradually lose reactivity to

the male-specific HY antigen¹³. Moreover, during chronic viral infection, thymic output is necessary for efficient viral clearance¹⁴ and helps to preserve antiviral CD8⁺ T cell populations¹⁵. These data provide strong evidence that newly generated T cells play a crucial role in sustaining effective immunity.

Although the absolute number of RTEs in mice peaks at approximately 6 weeks of age, the proportion of the peripheral T cell population comprised of RTEs drops from 100% at 0–3 weeks of age to ~20% in the young adult mouse¹. The number of T cells leaving the thymus decreases as the thymus involutes, but RTEs are readily detectable even in aged mice¹. Adoptive transfer experiments show that, once in the periphery, RTEs accumulate in the spleen less well than mature naive T cells do¹⁶. This imbalance is ameliorated by overexpression of either the interleukin-7 receptor α -chain (IL-7R α) or the anti-apoptotic molecule B cell lymphoma 2 (BCL-2), suggesting that RTEs exhibit diminished survival compared with mature naive T cells. The transcriptional regulator early growth response protein 1 (EGR1) may also have a role in RTE survival, because EGR1-deficient mice show poor accumulation of RTEs¹⁷.

Do RTEs occupy a distinct cellular niche?

Studies of RTEs from hyperthymic (thymus-grafted) mice suggest that, on a population level, RTEs are preferentially incorporated into the peripheral T cell pool at the expense of their mature naive T cell counterparts¹⁸. More recently, however, adoptive transfer experiments have demonstrated that RTEs and mature naive T cells occupy largely overlapping niches¹⁶. Depleting the RTE compartment by thymectomy only marginally improves the accumulation of RTEs relative to co-transferred mature naive T cells. Thus, in the healthy adult, RTEs and mature naive T cells compete for a limiting niche, and RTEs, whether polyclonal or TCR transgenic, are the disadvantaged competitors¹⁶.

RTE homeostasis is context dependent

RTEs can enter a periphery that is either empty, as in neonates or in adults recovering from lymphoablation, or full, as in healthy lymphoreplete adults. Depending on the lymphocyte occupancy of the immediate environment, RTEs can be expected to receive distinct homeostatic signals. Adoptive transfer of RTEs and mature naive T cells into lymphoreplete or lymphopenic mice demonstrates that RTEs preferentially accumulate only in a lymphopenic environment¹⁶. Perhaps their higher expression level of CD24 (REF. 9) — a molecule required for optimal T cell homeostatic proliferation in a lymphopenic setting¹⁹ — allows RTEs to settle into an empty compartment more readily than their mature counterparts. Although the ability of RTEs to undergo homeostasis is influenced by their environment, whether CD4⁺ and CD8⁺ RTEs can sense the occupancy of their separate compartments remains to be tested.

RTEs are a distinct T cell subset

A long-standing question in T cell development is whether intrathymic maturation generates fully functional thymic emigrants²⁰. Early studies focused on similarities between the RTE compartment and mature peripheral T cells, with the intention of elevating the thymus from a mere factory for hormone production to its unique status as the site for T cell maturation^{21,22}. More recent studies, however, have shown that RTEs display a cell surface phenotype distinct from that of mature naive T cells (FIG. 1), with higher expression levels of CD24 and TCR-CD3 and lower expression levels of QA2, CD45RB, IL-7R α and CD28 (REFS 9,23,24). That functional differences underlie this distinct phenotype has been proven experimentally.

CD8⁺ RTEs are functionally distinct

CD8⁺ RTEs are unique in their high-level expression of α E integrin and CC-chemokine receptor 9 (CCR9) compared with mature naive T cells, and this enables their more efficient migration on a CC-chemokine ligand 25 (CCL25) gradient and consequent access to the gut epithelium^{25,26}. However, these markers do not clearly distinguish RTEs from bulk peripheral CD8⁺ T cells. *In vitro* studies demonstrate that the CD8⁺ RTE population contains a lower frequency of cytolytic precursors than the mature T cell compartment, suggesting that these young T cells may possess functional defects⁹. Indeed, CD8⁺ RTEs produce significantly less tumour necrosis factor (TNF) in response to CD3- and CD28-specific stimulation *in vitro*²⁷. During bacterial or viral infections *in vivo*, activated CD8⁺ T cells derived from RTEs have lower levels of cytokine production than those derived from mature naive T cells and generate fewer IL-7R α^{hi} KLRG1^{low} memory precursor CD8⁺ T cells^{28,29}. These cytokine production defects are found even 60 days after infection, well after the 3 week RTE maturation period has expired²⁸. Thus, a T cell's maturation status at the time of antigen encounter may determine cell fate decisions.

CD4⁺ RTEs are functionally distinct

In vitro studies have provided valuable insights into the functional differences between CD4⁺ RTEs and mature T cells. Under non-polarizing conditions (no exogenous cytokines), activated CD4⁺ RTEs exhibit lower proliferation rates, decreased production of IL-2, IL-4 and interferon- γ (IFN γ), and reduced expression of CD25 (the high-affinity IL-2 receptor α -chain) compared with mature CD4⁺ T cells^{9,30,31}. Defective IL-2 production is exacerbated in CD4⁺ RTEs from aged mice³². These functional differences also extend to the ability of RTEs to differentiate into various T helper (T_H) cell lineages. Compared with their mature T cell counterparts, CD4⁺ RTEs are defective in their commitment to the T_H1 and T_H17 cell lineages, in that they show reduced proliferation and cytokine production^{3,31}. Analyses of cytokine production as a function of cell division indicate that lower proliferation rates are not solely responsible for the reduced cytokine production³¹. It should be emphasized that CD4⁺ RTE function is not globally defective, and is influenced by the cytokine and cellular environment. Proliferative defects are more pronounced under T_H1 cell-polarizing than T_H17 cell-polarizing conditions, and T_H1 cell-associated cytokine production is restored when RTEs are cultured in the presence of mature naive T cells³¹. Most strikingly, despite dramatic proliferative and cytokine defects under non-polarizing conditions, CD4⁺ RTEs surpass their mature T cell counterparts under T_H2 cell-polarizing conditions, as they have enhanced T_H2 cell-associated cytokine production and effector function both *in vitro* and *in vivo*^{31,33,34}.

What drives these functional differences?

That RTEs are a functionally distinct subset of the peripheral T cell pool has been firmly established only recently. Thus, there is a dearth of experimental evidence on the pathways or molecules that orchestrate the distinct functions of RTEs and mature T cells. It is unlikely, however, that any single factor is responsible. Analyses of transcription factor and cytokine receptor expression by cells taken directly *ex vivo* suggest that CD4⁺ RTEs are not inherently biased towards the T_H2 cell lineage but, rather, are biased away from the T_H1 cell lineage³¹. Indeed, unlike neonatal CD4⁺ T cells, RTEs from young adults do not produce T_H2 cell-associated cytokines directly *ex vivo*^{33,34}. Reduced steady-state expression by RTEs of T_H1 cell-associated molecules, such as T-bet and IL-12R, probably drives the gradual acquisition of a bias towards a T_H2 cell phenotype³¹.

Poor IL-2 secretion by activated CD4⁺ and CD8⁺ RTEs and differential secretion of IL-4 by CD4⁺ RTEs under non-polarizing and T_H2 cell-polarizing conditions suggest that these cytokine loci may be subject to distinct epigenetic regulation in RTEs and mature naive T

cells^{9,28,31}. Indeed, preliminary DNA methylation studies from our laboratory on CD4⁺ RTEs and mature naive T cells show that the *Il2* and *Il4* promoter regions are hypermethylated in unstimulated adult RTEs (D.W.H. and P.J.F., unpublished observations).

It is tempting to speculate that antigen recognition and subsequent signalling through the TCR have a role in the distinct function of RTEs. In response to TCR stimulation, a number of factors — including signal strength and the presence of co-stimulatory molecules and cytokines — influence the balance between a full response and the induction of anergy or non-responsiveness^{35–37}. Although RTEs express higher levels of CD3–TCR than mature T cells, they are characterized by lower levels of cell surface CD28 (REF. 9), suggesting that reduced co-stimulation in certain environments may play a part in RTE functional defects. Studies comparing the efficacy of signalling through the TCR and co-stimulatory pathways in RTEs and mature T cells are ongoing. In the presence of anergizing stimuli, CD4⁺ T cells initiate a gene expression programme that dampens proliferation and IL-2 secretion³⁸. Anergy-associated genes include those encoding transcription factors — such as EGR2, EGR3 and Ikaros — and E3 ubiquitin ligases, such as gene related to anergy in lymphocytes (GRAIL) and CBLB. Of future interest would be a comparison of the expression of such key regulatory proteins in RTEs and mature T cells.

Are there consequences to these functional differences?

Studies in which RTEs and mature naive T cells were mixed at varying ratios *in vitro* suggested that RTEs behave differently when they constitute the entire T cell compartment from when they comprise a small fraction of the population³¹. These data indicate the need to consider RTE function in both lymphopenic and lymphoreplete animals. The main evolutionary advantage for dampened cytokine production combined with efficient homeostatic proliferation in an empty periphery would be displayed in neonates, who are lymphopenic and whose TCR repertoires are cross-reactive and have an average avidity lower than that of adult repertoires^{39,40}.

Driving CD8⁺ RTEs towards terminal effector differentiation at the expense of generating good memory T cells may benefit the host. In lymphopenic neonates and lymphoablated adults, mobilizing all available T cells, the majority of which are RTEs, may be essential to mount an effective primary immune response, without which a memory immune response would be useless. In the presence of a highly inflammatory secondary challenge, however, the response of CD8⁺ RTE-derived cells is similar to that of cells derived from mature naive CD8⁺ T cells, suggesting that the RTE-derived memory compartment can rise to the challenge²⁸.

Similarly, dampened cytokine production by CD4⁺ RTEs, especially in response to antigen recognition under non-polarizing conditions, may ensure that RTEs do not respond inappropriately to self antigens expressed only in the periphery and not in the thymus^{41,42}. Reducing production of highly pro-inflammatory T_H1 and T_H17 cell-associated cytokines and developing a gradual T_H2 cell bias may be beneficial to the host during RTE maturation, particularly when RTEs constitute a large proportion of the T cell population. This is especially crucial in neonates, whose RTEs enter a lymphopenic environment that can promote autoimmune responses⁴³. However, the T_H2 cell response is not completely innocuous, and recent data demonstrate that antigen-specific CD4⁺ RTEs drive enhanced eosinophilia compared with mature T cells in a mouse model of induced allergic airway inflammation³¹.

What triggers RTE maturation?

The acquisition by RTEs of a mature phenotype and function is not solely a product of selective survival or proliferation⁴⁴ but a result of maturation at the individual cell level. What, then, triggers this maturation?

Entry into secondary lymphoid organs is required

One question that arises is whether RTE maturation is on ‘autopilot’, or whether specific signals drive this process. Intrathymic sequestration of RTEs blocks their full maturation. In addition to the need for thymic egress, RTE phenotypic and functional maturation requires access to secondary lymphoid organs, such as the lymph nodes or the spleen⁴⁴. The only structural requirement elucidated so far is the presence of a full dendritic cell compartment.

The usual suspects are not involved

T cell homeostasis is driven by IL-7 and TCR engagement with self peptide–MHC complexes, two factors that are concentrated in secondary lymphoid organs⁴⁵. Work from our laboratory has ruled out self peptide–MHC complexes as candidates for driving RTE maturation⁴⁶. Studies are ongoing to investigate the role of IL-7 in promoting RTE maturation, but preliminary data from our laboratory suggest that RTE maturation is IL-7 independent (E. G. Houston Jr and P.J.F., unpublished observations). Recent work shows that the transcriptional repressor NKAP may regulate post-thymic maturation, because T cells lacking NKAP do not undergo complete maturation⁴⁷. As it is currently unclear how NKAP exerts its influence on RTEs, these data deepen the mystery behind the identity of the trigger for RTE maturation.

Why do RTEs need further maturation?

Understanding why post-thymic T cell maturation may be necessary requires a careful comparison between the phenotypes and functions of mature single-positive thymocytes and RTEs. Mature CD4⁺ thymocytes stimulated under non-polarizing conditions produce more IL-4 than similarly treated mature CD4⁺ peripheral T cells^{48,49}, whereas CD4⁺ RTEs make less³¹. Given that CD4⁺ thymocytes are hypomethylated at the *Il4* promoter compared with peripheral T cells⁴⁹, studies on the methylation status of the *Il4* locus in RTEs are clearly needed. The IL-4 expression levels and the unique gene and protein expression pattern in RTEs compared with single-positive thymocytes and mature naive T cells^{3,9,23,24,50} suggest that RTEs are not simply the cellular midpoint between progenitor thymocytes and mature peripheral T cells. What is gained by requiring that T cells transit through this distinct developmental stage?

Does maturation help to define homeostatic fitness?

If the maturation process is required to test the ability of RTEs to respond to homeostatic signals, then these signals probably do not include IL-7 or MHC molecules^{17,46}. Alternatively, maturation may help to ensure metabolic fitness in RTEs, given that homeostasis, migration and activation all entail metabolic demands. In support of this notion, RTEs express lower levels of CD28 (REF. 9), and CD28 signals are required for the regulation of glucose uptake by T cells⁵¹. Maturation may also test the ability of a young cell to transmigrate through endothelial cells, a notion bolstered by the differences in integrin expression between RTEs and mature naive T cells²⁶. Perhaps movement from the cloistered environment of the thymus out into the peripheral lymphoid system requires time and suspension in a somewhat quiescent state.

Does post-thymic maturation influence self tolerance?

Although central tolerance culls a considerable proportion of self-reactive T cells⁵², the inevitable escape of autoreactive T cells must be adequately dealt with. Recent evidence shows that tissue-restricted antigens are expressed in secondary lymphoid organs and mediate peripheral tolerance by driving the deletion of autoreactive T cells^{41,42}. That RTE maturation requires access to secondary lymphoid organs with an intact dendritic cell compartment offers circumstantial evidence for the influence of post-thymic maturation on self tolerance⁴⁴. More concrete evidence comes from studies indicating that RTEs do not elicit lethal acute graft-versus-host disease, even when the number injected is eight times the dose of splenic T cells required to mediate acute lethality²⁴. These data suggest that RTEs become tolerized on antigen encounter, but their function may also be kept in check by inhibitory molecule expression⁵⁰. The preference of RTEs to home to the gut²⁶ — the tissue with the greatest extrathymic antigenic load⁵³ — may promote exposure to gut-associated antigens at a tolerance-prone maturational stage. With chronic exposure to environmental antigens from the gut, there is an apparent loss of TCR specificities in the responding RTE pool⁵⁴, and this suggests a tolerance mechanism. Collectively, these data support a role for post-thymic maturation in maintaining peripheral T cell tolerance, although further experiments to interrogate this role for RTE maturation are clearly needed.

Conclusions

RTEs are a key T cell population that help to maintain TCR repertoire diversity throughout life and comprise the majority of the T cell population in neonates and in adults recovering from lymphopenia. Recent tools for identifying and analysing RTE phenotype and function have revealed that RTEs represent a distinct T cell subset undergoing a recently discovered phase of post-thymic maturation. Many questions about RTE biology remain, and future studies must elucidate additional factors that drive RTE maturation, as well as those that trigger the distinct phenotype and function of RTEs. The continued expansion in our understanding of post-thymic maturation is likely to help us to implement improvements in neonatal vaccine immunity and to accelerate the recovery of the peripheral T cell compartment following lymphoablation.

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Glossary

Recent thymic emigrants	T cells that have completed thymic development and have recently entered the lymphoid periphery. These young T cells undergo a maturation process that includes changes in function and cell surface phenotype.
Lymphoreplete	A lymphoid periphery that is relatively full of lymphocytes.
Lymphopenic	A lymphoid periphery that is depleted of lymphocytes.
Homeostasis	The controlled turnover of cell populations in which the balance between cell proliferation and cell death maintains constancy in the size of the lymphocyte pool.

Memory precursor CD8⁺ T cells	A subset of effector CD8 ⁺ T cells, defined as IL-7R α^{hi} KLRG1 ^{low} that have an enhanced potential to become long-lived memory CD8 ⁺ T cells.
DNA methylation	A repressive epigenetic modification in which methyl groups are present on cytosine bases that are followed by guanine bases (CpGs). CpG-rich areas are typically found in gene promoter and other regulatory regions.
Anergy	A state of immune unresponsiveness. Anergic B and T cells do not respond fully to their cognate antigens.
Secondary lymphoid organs	Organs, including the spleen and lymph nodes, that support lymphocyte homeostasis, maturation and activation-induced differentiation.
Graft-versus-host disease	(GVHD). A disease that results from the immunological attack on target recipient organs or tissues (such as the skin or gut) by donor allogeneic T cells that are transferred along with the allograft (such as bone marrow, liver or gut allografts). GVHD occurs in graft recipients that are unable to eliminate the host-reactive donor T cells owing to immunosuppression, immunological immaturity or tolerance.

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Figure 1. RTEs exhibit a distinct cell surface phenotype

Over a period of approximately 3 weeks, recent thymic emigrants (RTEs) undergo phenotypic maturation in the lymphoid periphery. This is characterized by the downregulation of surface CD24 and T cell receptor (TCR)–CD3 expression and the upregulation of surface expression of CD45RB, QA2, interleukin-7 receptor α -chain (IL-7R α) and CD28. Symbol sizes indicate the relative cell surface expression level on each cell type.

Table 1

Methods for studying RTE biology

Model system	Method	Advantages	Disadvantages	Refs
Intrathymic FITC injection	Labels developing thymocytes; FITC ⁺ cells in the periphery are RTEs	Allows the phenotypic and functional analysis of the youngest RTEs by flow cytometry	- Causes surgical stress - Short time frame for FITC detection - Labels mature T cells recirculating to the thymus	55,56
BrdU incorporation	BrdU is taken up by dividing thymocytes; RTEs are BrdU ^{low}	A non-surgical procedure that allows the analysis of RTE phenotype	- BrdU label is also taken up by dividing mature peripheral T cells - Functional studies are precluded	57
TRECs	Quantification by PCR of excised DNA fragments generated during TCR rearrangement; RTEs are enriched for TRECs	Allows the determination of thymic output and the relative proportion of RTEs in the human T cell population	- Precludes phenotypic and functional analysis - Does not indicate time since thymic egress - Cannot be used for analysis of single cells - TREC⁺ cells may or may not be RTEs	58,59
Thymic lobe grafts	Congenetic thymic lobes are grafted under the kidney capsule	Allows the phenotypic and functional analysis of RTEs by flow cytometry for up to 3 weeks	- Creates an artificially full peripheral T cell compartment - Causes surgical stress	18,60
Fetal thymus organ culture	Fetal thymic lobes are placed in culture; RTEs are harvested 1–12 days later	Allows the phenotypic and functional analysis of RTEs by flow cytometry	An <i>in vitro</i> system may not accurately reflect the <i>in vivo</i> environment	24
RAG2–GFP transgenic mice or RAG1–GFP knock-in mice	GFP signal remains detectable after RAG expression has ceased; GFP ⁺ peripheral T cells are RTEs	Allows the phenotypic and functional analysis of RTEs from unmanipulated mice, and GFP signal intensity correlates with time since thymic egress	- Division dilutes the GFP signal - GFP signal is lower in CD8⁺ RTEs than in CD4⁺ RTEs	9,47,61
Cell surface phenotype	Mouse RTEs are QA2 ^{low} CD24 ^{hi}	Allows for phenotypic and functional analysis of RTEs by flow cytometry	Excludes ~85% of RTEs	9
	Human RTEs are CD31 ⁺ PTK7 ⁺		Markers are only expressed by CD4⁺ RTEs	3,62

BrdU, 5-bromodeoxyuridine; FITC, fluorescein isothiocyanate; GFP, green fluorescent protein; PTK7, protein tyrosine kinase 7; RAG, recombination-activating gene; RTE, recent thymic emigrant; TCR, T cell receptor; TREC, TCR, rearrangement excision circle.