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Homeostatic Signals do not Drive Post-thymic T cell Maturation

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Abstract

Recent thymic emigrants, the youngest T cells in the lymphoid periphery, undergo a 3-week-long period of functional and phenotypic maturation before being incorporated into the pool of mature, naïve T cells. Previous studies indicate that this maturation requires T cell exit from the thymus and access to secondary lymphoid organs, but is MHC-independent. We now show that post-thymic T cell maturation is independent of homeostatic and costimulatory pathways, requiring neither signals delivered by IL-7 nor CD80/86. Furthermore, while CCR7/CCL19,21-regulated homing of recent thymic emigrants to the T cell zones within the secondary lymphoid organs is not required for post-thymic T cell maturation, an intact dendritic cell compartment modulates this process. It is thus clear that, unlike T cell development and homeostasis, post-thymic maturation is focused not on interrogating the T cell receptor or the cell's responsiveness to homeostatic or costimulatory signals, but on some as yet unrecognized property.

Keywords

recent thymic emigrants; IL-7; dendritic cells

1. Introduction

Recent thymic emigrants (RTEs), those peripheral T cells that have most recently completed thymic development and egress, are the subject of much current interest. These young T cells help replenish the diversity of the naïve peripheral T cell repertoire, and are of particular consequence both in adults recovering from lymphopenia, and in infants, whose lymphoid periphery is first being seeded with T cells (reviewed in [1]).

The study of RTE biology has been facilitated by the development of a tractable model system that allows unambiguous identification of RTEs from unmanipulated mice, enabling their ready isolation for functional and phenotypic analysis [2, 3]. Thus, in mice transgenic (Tg) for green fluorescent protein (GFP) under control of the RAG-2 promoter [4], GFP⁺ peripheral T cells are RTEs [2]. Furthermore, the intensity of the GFP signal can be used as a clock, being inversely proportional to the time the cells have spent in the lymphoid periphery, such that GFP^{hi} and GFP^{lo} RTEs have resided in the lymphoid periphery for ~1 and ~2 weeks, respectively [2, 5].

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Using RAG2p-GFP Tg mice, we and others have demonstrated that RTEs are both phenotypically and functionally distinct from their mature, yet still naïve (MN), peripheral T cell counterparts. Lower IL-7R α , CD28, Qa2, and CD45RB, and higher TCR, CD3, and CD24 cell surface expression characterize RTEs from mice of all ages [2, 3]. The functional distinctions between RTEs and MN T cells are equally stark. Stimulated RTEs exhibit dampened proliferation and cytokine production compared to MN T cells [2, 3, 6, 7], defective generation of memory precursor cells [8], and impaired skewing to effector lineages upon *in vitro* polarization [9]. In addition to RAG2p-GFP Tg mice, multiple other methods to tag and identify RTEs in both mice [10–13] and humans [14, 15] have been used to reach a similar conclusion: RTEs represent a T cell subset that is both functionally and phenotypically distinct from the bulk population of mature, naïve peripheral T cells (reviewed in [1]).

Using phenotypic markers as faithful indicators of RTE function, it has become clear that the transition from RTE to MN T cell is a result of cellular maturation, rather than selection and subsequent outgrowth of a small population of RTEs that already bear an MN-like surface phenotype. Thus, maturation occurs in the absence of selective survival or proliferation [16]. What triggers the maturational process that characterizes the first few weeks of post-thymic life for a T cell? Our previous work [16] has demonstrated that RTE maturation is an active process that requires both thymic egress and access to secondary lymphoid organs (SLOs). Given the need for tonic signaling through the T cell receptor (TCR) to regulate the survival and homeostasis of naïve peripheral T cells [17, 18] and our findings that RTEs and MN T cells interpret these homeostatic signals somewhat differently [19], we suspected that these signaling pathways might control post-thymic maturation. The central role played in thymocyte development by signals mediated through the TCR strengthened these suspicions. However, our previous work revealed that while RTE maturation is associated with subtle modulation of the TCR repertoire, the maturation process itself is unexpectedly MHC-independent [20]. Thus, signaling through the TCR initiated by recognition of self MHC/self peptide does not drive RTE maturation.

We now extend these studies to ask whether the obligatory entry of RTEs into SLOs facilitates delivery of either IL-7- or costimulation-dependent maturation signals, whether maturation requires RTE homing to specific T cell microenvironments, and whether the presence of an intact dendritic cell (DC) compartment is required to trigger RTE maturation. Our results offer the surprising conclusion that while IL-7, costimulation, and CCR7/CCL19,21-driven microenvironmental homing by T cells are all dispensable for post-thymic T cell maturation, DCs do modulate the transition of RTEs to the MN T cell compartment.

2. Materials and Methods

2.1. Mice

C57BL/6 (B6) mice were bred on site. RAG2p-GFP Tg mice [4] were originally a gift from M. Nussenzweig (The Rockefeller University) and were backcrossed in our lab at least 12 generations onto the B6 background. B6 mice Tg for human CD2 promoter-driven IL-7R [21], a gift from K. Elkon (University of Washington), were maintained as heterozygotes and crossed onto the RAG2p-GFP Tg background. CCL19/21^{-/-} mice [22] on the B6 background [23] were a gift from J. Cyster, (University of California, San Francisco), and were crossed onto the RAG2p-GFP Tg background. CD11c-diphtheria toxin receptor (DTR) Tg B6 mice [24] were a gift from M. Bevan (University of Washington), and CD11c-Cre [25] $\times$ inducible (i)DTR [26] Tg B6 mice were a gift from A. Rudensky (then at the University of Washington). Mice were used at 6–12 weeks of age, except for radiation chimeras, which were reconstituted at 6–8 weeks of age and analyzed 8 weeks later. RTE maturation follows a similar trend in mice throughout these age ranges [3]. All experiments

were performed in compliance with University of Washington Institutional Animal Care and Use Committee guidelines.

2.2. Mouse procedures

For blockade of IL-7R signaling, mice were given 200 μ g each of anti-IL-7 (M25; BioXCell) and anti-IL-7R α (A7R34; lab-purified) i.p. on d 0, 2, and 4. For blockade of CD28 signaling, mice were given 100 μ g each of anti-CD80 (16-10A1) and anti-CD86 (GL-1) i.p., both purchased from the University of California, San Francisco Monoclonal Antibody Core, on d 0, 2, and 4. For DC depletion, mice were given 60 μ g/kg body weight of diphtheria toxin (DT, from Sigma) in PBS i.p. on d 0, 1, 3, and 5. After titering the DT dose from 12.5–150 μ g/kg, we judged this dose to mediate effective DC ablation with acceptable weight loss.

To generate radiation chimeras, $\sim 5 \times 10^6$ T cell-depleted bone marrow cells from femurs and tibia were injected i.v. into lethally irradiated (1000 rads) RAG2p-GFP Tg recipient mice. Recipients were maintained on water containing neomycin sulfate (Mediatech, Inc.) and Polymyxin B (Invitrogen) from 1 d before to 14 d after irradiation. T cell depletion was achieved by incubating a single-cell suspension of bone marrow with lab-generated anti-CD4 (RC172.4R6), anti-CD8 (3.168.8), and anti-CD90.2 (13.4.6), followed by incubation with rabbit complement (Cedarlane).

2.3. Cell preparation, staining, enrichment and sorting

Single cell suspensions of brachial, axillary, inguinal, cervical, and mesenteric lymph nodes (LNs) and water-lysed splenocytes were prepared and counted. Where indicated, T cells were enriched using an EasySep mouse T cell enrichment kit (StemCell Technologies) according to the manufacturer's protocol. For flow cytometric analysis, FcR were blocked with anti-CD16/32 (2.4G2, BD Biosciences), and cells were stained as previously described [16] with antibodies conjugated to FITC, Phycoerythrin, Peridinin chlorophyll protein-Cyanine 5.5, Phycoerythrin-Cy7, Allophycocyanin, Allophycocyanin-eFluor 780, or biotin and against the following molecules: CD4 (RM4-5), CD8 (53-6.7), CD11c (N418), CD24 (M1/69), CD44 (Pgp-1), CD45RB (16A), CD62L (MEL-14), CD80 (16-10A1), CD86 (GL-1), Qa2 (1-1-2), and I-A^b (M5/114.15.2), all from eBioscience or BD Pharmingen. Biotinylated antibodies were detected with allophycocyanin- or Phycoerythrin-conjugated streptavidin (eBioscience). Events were collected on a FACSCanto (BD Biosciences) and data were analyzed on FlowJo software (TreeStar) after excluding doublets from live-gated samples. Fluorescence-Minus-One [27] samples were run when appropriate. While IL-7R, CD28, Qa2, TCR, CD3, CD24, and CD45RB levels all differ on RTEs and MN T cells, maturation can be most reliably assessed by tracking CD24, Qa2, and CD45RB expression. Data are shown for splenocytes; LN cells gave comparable results.

3. Results

3.1. RTE maturation is IL-7 independent

To determine whether IL-7 drives RTE maturation, given that it provides an important survival and homeostatic factor for naïve T cells [28], we co-administered anti-IL-7 plus anti-IL-7R α directly to RAG2p-GFP Tg mice for 6 d, and tracked the phenotypic maturation of CD4 and CD8 RTEs as a faithful reflection of their functional maturation [16]. The amount of administered antibody was sufficient to extinguish all peripheral IL-7R signaling, because on the day of analysis, not only were peripheral T cells maximally coated with anti-IL-7R α , but excess anti-IL-7R α was detected in the serum (Fig. 1A). In addition, the size of the CD4⁺CD8⁺ (double negative) 3 compartment, an IL-7-dependent stage of thymocyte development (reviewed in [28]), was severely reduced, from 1.6×10^6 in controls to 2×10^5 in

treated mice, demonstrating the efficacy of the IL-7R blockade. While the phenotypic maturation of the bulk population of GFP⁺ RTEs was not altered by IL-7R blockade, we concentrated our analysis of RTE maturation on the GFP^{hi} youngest ~10–25% of the RTE population [5] to identify those RTEs that had entered the periphery after the onset of antibody blockade. Consistent with the diminished IL-7R α expression that characterizes RTEs [2, 19], maturation occurred normally in both CD4 and CD8 GFP^{hi} RTEs in the absence of IL-7R signaling (Fig. 1B, 1C), demonstrating that IL-7 is dispensable for maturation. These GFP^{hi} RTEs had begun to undergo post-thymic maturation, as their phenotype was more mature than that of their immediate precursors in the thymus (Fig. 1B and C). Our short-term IL-7R blockade did not adversely affect T cell survival at the time of analysis, as the numbers of naïve T cells from PBS-treated and IL-7R-blocked mice were comparable. Furthermore, the GFP median fluorescence intensities (MFIs) of RTEs from both groups were comparable, suggesting that antibody inoculation did not alter RTE output at this timepoint. These results were corroborated by data we obtained after adoptive transfer of CD4 RTEs into IL-7 null mice (data not shown), but without introducing the undesirable possibility that, prior to transfer, RTEs received IL-7 signals sufficient to drive their maturation.

To further interrogate the relationship between IL-7R signaling and RTE maturation, we analyzed RAG2p-GFP Tg mice that carry an IL-7R α transgene [19, 21]. Despite the fact that RTEs from IL-7R α Tg mice overexpress IL-7R α , their phenotypic maturation closely mirrors that of wild-type RTEs (Fig. 2). Taken together, all of these data demonstrate that neither blocking nor enhancing IL-7 signaling impacts the phenotypic maturation of RTEs.

3.2. RTE maturation is not controlled by homing or costimulatory signals

We next hypothesized that the signals important for RTE maturation could be transmitted through cell surface receptors other than the IL-7R or the TCR. Naïve T cell homing to T cell zones in SLOs is dependent on CCR7 binding to its ligands CCL19 and CCL21. We therefore analyzed RTEs from CCR7 ligand-deficient (CCL19/21^{-/-}) RAG2p-GFP Tg mice. Phenotypic maturation occurred normally in RTEs present in the CCR7 ligand-deficient environment, and was indistinguishable from that of control RTEs (Fig. 3). These results demonstrate that the homing of naïve T cells to their proper microenvironment is unlikely to drive RTE maturation.

We next treated RAG2p-GFP Tg mice with antibodies against CD80 and CD86 for 6d to block basal CD28 signaling. CD80 and CD86 were maximally coated with blocking antibodies (Fig. 4A), and excess antibodies were detected in serum harvested at the time of RTE analysis (data not shown). No gross cellularity changes were detected. The maturation phenotype of CD28-blocked RTEs, both GFP^{hi} (Fig. 4B) and total GFP⁺ populations, was indistinguishable from that of control antibody-treated mice, suggesting that costimulatory signaling does not influence RTE maturation.

3.3. RTE maturation is influenced by DCs

In SLOs, naïve T cells interact with DCs and receive homeostatic, activation, and costimulatory signals. To test whether DCs also transmit the signals that drive the process of RTE maturation, we selectively depleted DCs in mice in which the promoter CD11c drives expression of DTR. Mice are not normally sensitive to DT, and as all murine DCs express CD11c, DT administration to CD11c-DTR [24] Tg mice or CD11c-Cre [25] $\times$ iDTR [26] Tg mice selectively and efficiently depletes this subset of cells when DTR expression is limited to the hematopoietic compartment [29].

To test the effect of DC depletion on RTE maturation, we crossed RAG2p-GFP Tg mice with each of the two aforementioned DC-DTR Tg lines to allow both RTE identification and DC depletion. Both lines gave similar results for all parameters measured. To avoid previously documented effects of DT treatment on non-hematopoietic cells [29], we used bone marrow from these mice to make radiation chimeras and treated the reconstituted chimeras with DT or left them untreated (Fig. 5A). Using a high dose of DT [30], we achieved ~90% depletion of the CD11c^{hi}I-A^b DC compartment by 6d (Fig. 5B), and the percent depletion was comparable whether spleens were processed with collagenase digestion (92% deletion) or without (97% deletion). Neither B cells nor T cells, including RTEs, were depleted by DT treatment (Fig. 5B and legend). When assessing the entirety of the RTE population (gated on all GFP⁺ CD4 or CD8 T cells), small but consistent defects were seen for both CD4 and CD8 RTE maturation in DC-depleted animals. However, given that 3 weeks is the limit of GFP detection in RTEs, many of the RTEs in the GFP⁺ gate had been in the periphery for longer than 6d. To restrict our analysis to only those RTEs that entered a DC-depleted periphery, we assessed maturation of GFP^{hi} RTEs, using a gate that eliminated the oldest ~80% of the total RTE population. A consistent defect in maturation was seen in GFP^{hi} CD8 RTEs from DC-depleted animals (relative to DT-treated DTR⁻RAG2p-GFP Tg mice) for the maturation markers Qa2 and CD45RB (Fig. 5C). Our analysis of GFP^{hi} CD4 RTEs in DC-depleted animals showed more subtle but still detectable maturation defects (Fig. 5D).

4. Discussion

We have previously shown that the maturation program RTEs undergo in the lymphoid periphery is an active process whose completion requires that RTEs both exit the thymus and enter SLOs. Accessing either the LN or the splenic compartment alone is sufficient to drive RTE maturation, while extensive node-to-node circulation appears unnecessary [16].

Based on this knowledge, we hypothesized that the maturation-inducing factor or factors provided to RTEs in SLOs would be previously described homeostatic factors known to regulate the size of the naïve T cell compartment, perhaps including the MHC/peptide ligand or IL-7. However, RTE maturation proved to be MHC-independent [20]. Although post-thymic maturation requires neither proliferation nor selective cell survival [16], we next turned our attention to IL-7, a cytokine encountered by naïve T cells in the SLOs, where it is produced mainly by fibroblastic reticular cells [31]. While overexpression of the IL-7R improves the long-term survival of RTEs in lymphoreplete hosts [19], we now show that RTE maturation is not influenced by IL-7, whether IL-7 signaling in RTEs is eliminated or enhanced. Thus, not only are the final stages of thymocyte development and thymic egress IL-7 independent [32], so too is the first phase of extrathymic T cell life.

Another likely candidate for driving post-thymic T cell maturation is the CD28-CD80/CD86 signaling axis. While CD28 signaling is required to trigger the full activation of naïve T cells, it has also been implicated in naïve T cell homeostasis [33, 34]. B cells, T cells, and DCs are all brought into close proximity in SLOs and constitutively express the CD28 ligands CD80 and CD86 [35]. However, blocking signals through the CD28-CD80/CD86 axis had no impact on RTE maturation.

We also evaluated the possibility that some of the chemokines that control naïve T cell homing and microenvironmental localization might also drive RTE maturation. The chemokines CCL19 and CCL21 bind CCR7 on naïve T cells in SLOs to trigger localization to the T cell zone, where these cells can “scan” colocalized DCs for foreign antigen [36]. We found that maturation still occurs normally in RTEs analyzed from mice congenitally lacking these CCR7 ligands, suggesting that chemokine-chemokine receptor signaling along

this axis may not be the event that promotes RTE maturation. However, our data do not rule out the possibility that other chemoattractants compensate for the loss of CCL19 and CCL21 to direct RTE microenvironmental localization and subsequent delivery of maturational signals.

We next hypothesized that DCs, which transmit many signals to naïve T cells in SLOs, may be an important component for driving RTE maturation. This idea is consistent with the role of DCs in promoting B cell maturation in the lymphoid periphery, as DCs are among the cell types expressing B cell activating factor and the Notch2 ligand Delta-like 1, molecules that promote transitional and marginal zone B cell maturation, respectively (reviewed in [37, 38]). Our data on the impact of DC depletion on RTE maturation suggest that DCs promote RTE maturation. An alternative interpretation, that DC ablation drives the premature emigration of RTEs from the thymus, is unlikely, given that the numbers of RTEs do not significantly differ in DT treated and control animals. Adoptive transfer experiments would be required to definitively test this possibility.

Our study may underestimate the role that DCs play in RTE maturation, given that in our hands, DC-depleted animals retained 4–12% of the CD11c^{hi} I-A^{b+} DC complement. The incomplete ablation of DCs may also explain why impaired maturation was less apparent for CD4 RTEs, as CD4 T cell function is less sensitive than that of CD8 T cells to DC depletion [39]. Depletion of the heterogeneous plasmacytoid DC and NK cell containing population of B220⁺CD11c^{int} cells [40] was variable, at 47–89% (for a mean of 73%). Importantly, the influence of DT treatment on RTE maturation was no more dramatic in mice in which B220⁺CD11c^{int} cells were 89% depleted than in those in which depletion of this population was limited to 47%. These data strengthen the notion that DCs are the relevant CD11c⁺ population with regard to RTE maturation, although we cannot definitively rule out the involvement of other CD11c⁺ cells, including NK cells and macrophages. In this regard, it would be interesting to determine whether injection of DCs into DC-ablated mice reverse the RTE maturation defect.

While the effect of DCs on RTE maturation may be direct, we cannot exclude the possibility that DC depletion indirectly affects this process, perhaps by altering SLO architecture. In fact, recent data show that DCs can regulate naïve T cell trafficking to LN by influencing high endothelial venules, the gatekeepers to LN entry [41]. This explanation alone cannot account for defective RTE maturation in DC-ablated mice, given that access to the spleen is not controlled by high endothelial venules, and yet is sufficient to induce RTE maturation [16]. However, our results do show that the reported DC redistribution incurred upon sphingosine-1-phosphate mimetic treatment [42] does not alter maturation, as RTEs restricted by such treatment to either the LNs or the spleen mature normally [16]. In addition, the weight loss often suffered by DT-treated DTR[−] mice was in our hands <15% and did not affect RTE maturation, as DT-treated DTR[−] RAG2p-GFP chimeras showed weight loss comparable to that of DC-depleted chimeras, but without a similar impairment of RTE maturation.

The factors that trigger RTE maturation appear to be localized to the SLO, and at least in part, present on or produced by DCs. However, while our combined studies eliminate CCL19,21-directed T cell microenvironmental homing and MHC, IL-7 and CD28 signals as triggers for RTE maturation, the identity of the definitive factor(s) that drive post-thymic T cell maturation remains elusive, although the transcriptional repressor NKAP appears to play a key role in regulating this process [43]. Thus, while successful intrathymic T cell development is centered around generating and testing TCRs for optimal self reactivity, it appears that post-thymic maturation is not focused on interrogating the TCR or the cell's

fitness for receiving IL-7- or costimulation-dependent survival or homeostatic signals, but on some as yet unrecognized property.

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Abbreviations

B6	C57BL/6
DC	dendritic cell
DT	diphtheria toxin
DTR	diphtheria toxin receptor
iDTR	inducible DTR
GFP	green fluorescent protein
LN	lymph node
MFI	median fluorescence intensity
MN	mature naïve peripheral T cell
RTE	recent thymic emigrant
SLO	secondary lymphoid organ
TCR	T cell receptor
Tg	transgenic

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Highlights

- The post-thymic maturation of recent thymic emigrants (RTEs) is IL-7 independent
- RTE maturation does not depend on signals delivered by CD80/86
- RTE microenvironmental homing within secondary lymphoid organs does not impact their maturation
- An intact dendritic cell compartment is required for full phenotypic maturation of RTEs

Figure 1. RTEs do not require IL-7R signaling to mature

(A) IL-7R on T cells is blocked following co-administration of anti-IL-7R α and anti-IL-7. RAG2p-GFP Tg mice were given antibodies on d 0, d 2, and d 4, and then on d 6, the level of anti-IL-7R α coating of TCR β ⁺ splenic T cells was determined. Splenocytes from antibody or PBS-treated mice were stained with a species-specific secondary antibody to determine the level of IL-7R α coating (left panel). Splenocytes from the same groups of animals were stained with additional anti-IL-7R α antibody to reveal available IL-7R α sites (middle panel). Sera from the same animals were diluted 1:3 and incubated with PBS-treated splenic T cells to determine the amount of available anti-IL-7R α (right panel). In all panels, the gray lines represent data from a Fluorescence-Minus-One control. (B, C) GFP^{hi} RTEs mature normally in the absence of IL-7R signaling. On d 6 following IL-7R blockade, CD24, Qa2, and CD45RB expression on GFP^{hi} RTEs from treated and untreated mice and on naïve GFP⁻ (MN) peripheral T cells from untreated mice was assessed. The gray dashed lines depict GFP⁺ CD4 and CD8 single positive thymocytes from untreated mice (pre-RTEs). Representative data are shown in (B), and data in (C) are averaged MFIs from 4 mice per group from 2 independent experiments, with error bars representing standard deviation. Differences were not statistically significant.

Figure 2. IL-7R overexpression does not influence RTE maturation

RTEs from age-matched IL-7R^{hi} Tg and wild type mice and naïve GFP⁻ (MN) peripheral T cells from wild type mice were analyzed for Qa2 and CD45RB expression. The bulk GFP⁺ gate was used to identify RTEs. Data are representative of at least 2 mice per group.

Figure 3. Naïve T cell homing ligand deficiency does not alter RTE maturation

RTEs from CCL19/21^{-/-} mice and RTEs and naïve GFP⁻ (MN) peripheral T cells from wild type littermate controls were analyzed for CD24, Qa2, and CD45RB expression. The bulk GFP⁺ gate was used to identify RTEs. Data are representative of at least 4 mice per group.

Figure 4. CD28 ligand blockade does not impair RTE maturation

(A) CD80 and CD86 are blocked following co-administration of anti-CD80 and anti-CD86. RAG2p-GFP Tg mice were co-administered CD80- and CD86-specific antibodies on d 0, d 2, and d 4, and then on d 6, blockade was assessed. Splenocytes from antibody- or isotype control-treated mice were stained with additional anti-CD80 and anti-CD86 to determine the level of available receptor binding sites. The dashed gray lines represent data from a Fluorescence-Minus-One control sample. (B) On d 6 following CD80 and CD86 blockade, CD24, Qa2, and CD45RB expression on GFP^{hi} RTEs were assessed and compared with that of RTEs and naïve GFP⁻ (MN) peripheral T cells from mice treated with isotype control antibodies. Data are representative of at least 2 mice per group.

Figure 5. RTE maturation is impaired by DC depletion

(A) Diagram of the experimental conditions. Bone marrow from RAG2p-GFP Tg × DC-DTR Tg (either CD11c-DTR Tg or CD11c-Cre × iDTR Tg) mice was used to reconstitute irradiated congenic B6 recipients. At least 8 weeks later, chimeras were treated with DT or PBS on d 0, 1, 3, and 5, and cells were analyzed on d 7. (B) Splenic DCs, but not RTEs, are selectively depleted by DT treatment. The % of CD11c^{hi}I-A^b DCs in spleens of DT- or PBS-treated animals (left panel) was determined for 6–10 mice per group, and the mean DT-mediated DC depletion was 92%. RTE representation among splenocytes (right panel) was calculated as % of RTEs in DT-treated animals normalized to that of controls for each experiment, and the mean RTE representation was determined for 6–10 mice per group from

3 independent experiments. Data are pooled from both the CD11c-DTR Tg and the CD11c-Cre $\times$ iDTR Tg systems, and no significant differences were found. Similarly, no significant differences in CD4 (2.2×10^6 in treated versus 2.0×10^6 in untreated) or CD8 (7.2×10^5 in treated versus 6.0×10^5 in untreated) RTE numbers were noted. Splenic B cell numbers (31.4×10^6 in treated versus 32.9×10^6 in untreated) and representation (41% in treated and untreated mice) were also not impacted by DT treatment. RTE maturation is impaired in CD8 (C) and CD4 (D) RTEs in a DC-depleted environment. Qa2 and CD45RB expression by GFP^{hi} RTEs was assessed using T-enriched splenocytes. Representative data (left panels), and the normalized MFIs (right panels) of Qa2 and CD45RB expression by GFP^{hi} CD8 (C) and CD4 (D) RTEs are shown from at least 8 animals per group in at least 3 independent experiments, in which the MFIs of experimental samples were normalized to those of controls (set at 1.0) for each experiment, and the mean calculated. *, $p < .05$; **, $p < .001$ as compared to control RTEs, using an unpaired Student's *t*-test.