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The Role of IL-7 in Thymic and Extrathymic Development of TCRγδ Cells

K. Laky,* L. Lefranc¸ois,* U. von Freeden-Jeffry,2† R. Murray,2† and L. Puddington3*  

IL-7-deficient (IL-7−/−) mice have reduced numbers of B and TCRαβ cells, but lack mature TCRγδ cells. Although most T cell development occurs in the thymus, some intestinal intraepithelial lymphocytes (IEL), including TCRγδ cells, can develop extrathymically. Epithelial cells in both thymus and intestine synthesize IL-7, suggesting that TCRγδ cell development could occur in either site. To evaluate the role of thymic IL-7 in development of TCRγδ cells, newborn TCRβ-deficient (TCRβ−/−) thymi were grafted to IL-7−/− mice. Donor- and host-derived TCRγδ cells were recovered from thymus grafts, spleen, and IEL. However, when IL-7−/− thymi were grafted to TCRβ−/− mice, no development of graft-derived TCRγδ cells occurred, indicating that extrathymic IL-7 did not support TCRγδ IEL generation from newborn thymic precursors. In contrast, TCRγδ IEL development occurred efficiently in adult, thymectomized, irradiated C57BL/6J mice reconstituted with IL-7−/− bone marrow. This demonstrated that extrathymic development of TCRγδ IEL required extrathymic IL-7 production. Thus, intrathymic IL-7 was required for development of thymic TCRγδ cells, while peripheral IL-7 was sufficient for development of extrathymic TCRγδ IEL. The Journal of Immunology, 1998, 161: 707–713.

Mice deficient for IL-7 (IL-7−/−) or IL-7R (IL-7Rα-chain or γc-chain) are lymphopenic (1–5), yet completely lack mature TCRγδ cells. TCRγδ cells are absent from thymus, spleen, skin (dendritic epidermal T cells (DETCs)),4 and intestinal epithelium in IL-7−/− mice (2–4, 6). In contrast, the IL-7−/− or IL-7Rγc−/− fetal thymus (day 16–18) contains immature TCRγδlow/HAshigh thymocyte precursors to DETCs in normal frequency, but no mature TCRγδhigh/HAlow cells (5, 6). Moreover, although DETCs are readily detectable in the skin of newborn normal mice, no TCRγδ cells were detectable in the epidermis of newborn IL-7−/− mice (J. M. Lewis and R. E. Tigelaar, personal communication). The incomplete block in TCRαβ development in IL-7−/− and IL-7Rγc−/− mice indicates that some TCRαβ cell development is IL-7 independent, whereas the generation of mature TCRγδ cells absolutely requires IL-7. A role for IL-7 in promoting rearrangements of murine TCR genes has been demonstrated. Culture of day 14 fetal liver cells or adult bone marrow (BM) T cell precursors with IL-7 yields in-frame, junctionally diverse Vγ2 and Vγ4 transcripts or full-length VγL2.1 and Vγ2 transcripts, respectively (7–9). In both instances, TCRγ mRNA is not found in cells cultured without IL-7. These in vitro data are consistent with gene-deleted IL-7−/− or IL-7−/− mice in which TCRγ-chain gene rearrangements are severely reduced or absent, implying that TCRγ rearrangement is indeed a stage of TCRγδ cell development dependent upon signaling through the IL-7R (10–12). However, the presence of Vγδlow DETC precursors in IL-7−/− or IL-7Rγc−/− fetal thymi (5), and the incomplete restoration of TCRγδ cell numbers in IL-7Rγc−/− mice expressing a TCRγδ transgene (6), suggest that either survival of immature TCRγδ cells, or terminal differentiation steps within the TCRγδ lineage require IL-7. Since IL-7 is synthesized by cells at multiple anatomic locations, including BM reticular cells (13), cortical epithelial cells in thymus (14), and intestinal epithelial cells (15), we sought to determine at which anatomic location(s) IL-7 was required for development of TCRγδ cells, including intraepithelial lymphocytes (IEL) in the intestinal mucosa.

IEL are a distinct population of T lymphocytes that reside above the basement membrane, between epithelial cells lining the intestines. TCRγδ cells make up approximately 60 or 30% of small or large intestinal IELs, respectively, with the remainder being TCRαβ cells (16, 17). Using neonatally thymectomized (ntx) and thymus-grafted mice, we and others showed that neonatal thymus contains TCRγδ IEL precursors, and that either the thymus itself or thymus-derived factors are required for normal TCRγδ IEL development (18–20). Several lines of evidence suggest that other IEL develop extrathymically, including the presence of TCRγδ IEL in congenitally athymic nude mice (21, 22), and TCRαβ and TCRγδ IEL in thymectomized, irradiated, fetal liver-, or adult BM-reconstituted mice (21, 23–25). Moreover, similarities exist between immature thymocytes and potential IEL precursors, and between thymic and intestinal epithelium, which suggest that intestinal, extrathymic T cell development occurs. For example, stem cells in BM, immature thymocytes, and small and large intestine crypt patch cells express both e-Kit and IL-7Rα (26–28). Thymic and intestinal epithelial cells synthesize both IL-7 and stem cell factor (SCF) (15–17). Interactions between these growth factors and their receptors are important in early thymocyte development (27, 29–31). In addition, we have shown that SCF/e-Kit interactions are critical for maintenance of normal IEL populations (16,

4 Abbreviations used in this paper: DETC, dendritic epidermal T cell; ATx, adult thymectomized; BM, bone marrow; IEL, intestinal intraepithelial lymphocytes; nTx, neonatal thymectomy; PE, phycoerythrin; SCF, stem cell factor; TN, triple negative.

*Department of Medicine, University of Connecticut Health Center, Farmington, CT 06030; and DNA Research Institute of Cellular and Molecular Biology, Palo Alto, CA 94304

† Address correspondence and reprint requests to Dr. Lynn Puddington, Department of Medicine, MC-1310, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030-1310. E-mail address: lpudding@panda.uchc.edu

‡ Current address: EOS Biotechnology, Inc., 225A Gateway Boulevard, South San Francisco, CA 94080.

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17). Finally, thymocytes express RAG1 and RAG2 mRNA and protein, and subpopulations of IEL express RAG1 mRNA (32). Although this is characteristic of lymphocytes undergoing TCR gene rearrangements in the thymus (33, 34), the occurrence of extrathympic rearrangements has yet to be directly demonstrated.

We sought to determine whether intrathymic IL-7 was required and/or sufficient for generation of thymus-derived TCRγδ cells, and conversely, whether peripheral IL-7 was sufficient to support extrathympic TCRγδ cell development. Overall, we found that thymus-derived TCRγδ cells absolutely required intrathymic IL-7, but did not require additional IL-7 in peripheral tissues. In contrast, peripheral IL-7 was sufficient for development of extrathympically derived IEL. In addition, extrathympic IL-7 produced by radiation-resistant stromal cells was more potent than that produced by BM-derived cells in promoting generation of TCRγδ IEL.

Materials and Methods

Animals

C57BL/6-Ly5.2 (B6) mice were obtained from the National Cancer Institute. C57BL/6-TCRβ tm1Mom (TCRβ−/−) mice were obtained originally from Drs. Mombaerts and Toneyega at Massachusetts Institute of Technology (Cambridge, MA) (35) and were maintained in our facility on a C57BL/6-Ly5.2 background. IL-7−/−Ly5.1 mice were maintained on a C57BL/6 × 129 Ola hybrid background, as previously described (1). Mice were fed sterile food and water, and housed in specific pathogen-free conditions.

nTx and thymus grafting

Within 24 h after birth, TCRβ−/− (Ly5.2) and IL-7−/− (Ly5.1) mice were thymectomized using suction (nTx) (36). Immediately before weaning, nTx mice were grafted s.c. with three to four neonatal thymi from the alternate strain. Lymphocytes were isolated from host mice 4 to 10 wk later and examined by fluorescence flow cytometry.

BM chimeras

Donor BM cells were obtained from femurs and tibias of C57BL/6-Ly5.2 or IL-7−/− mice. Adult (7–12 wk old) C57BL/6-Ly5.2 and IL-7−/− mice were thymectomized using suction (36). One week later, mice were exposed to lethal doses of γ-irradiation (1100 rad) from a 137Cs source and injected i.v. with 1 × 105 anti-Thy-1 + C-depleted BM cells. Six to twelve weeks later, lymphocytes were isolated and analyzed by fluorescence flow cytometry.

Lymphocyte isolation

Thymus, spleen, lymph nodes, and Peyer’s patches were homogenized, and then passed through 100 µm NITEX nylon mesh (Tetko, Kansas City, MO) to remove connective tissue. Splenic RBCs were lysed via two sequential incubations in Tris-ammonium chloride (13 mM Tris, 135 mM NH4Cl, pH 7.2) for 4 min at 37°C. Before staining for flow cytometric analyses, splenocyte FeR were preblocked with affinity-purified mouse IgG (200 µg/ml) (Jackson ImmunoResearch, West Grove, PA). This was the minimum concentration necessary to saturate FeRs and eliminate nonspecific staining of splenocytes by fluorescent-conjugated mAb. The fluorescence intensity of some subsequently added mAb was reduced by pretreatment with the high concentration of mouse IgG.

IEL were isolated as previously described (17). Briefly, intestines were cut longitudinally, and then into 5-mm pieces, and washed three times with CMF (Ca2+, Mg2+-free HBSS with 1 mM HEPES, 2.5 mM NaHCO3, pH 7.3) containing 2% calf serum. Washed intestinal pieces were stirred at 37°C for 20 min in CMF containing 10% calf serum and 1 mM dithioerythritol. This step was repeated, and the supernatants of both treatments were combined and rapidly filtered through nylon wool. Cells in the filtrate were incubated at 37°C in HBSS with 5% calf serum for 60 min before centrifugation on a 44%/67.5% Percoll gradient. Viable cells at the interface were collected and prepared for flow cytometric analysis.

Flow cytometric analyses

The following mAbs were used: anti-CD3e biotin (500A2) or FITC (145-2C11), anti-TCRαβ FITC or PE (H57.597), anti-TCRγδ FITC or PE (GL5), anti-Thy-1.2 FITC or PE (53-2.1), anti-CD44 PE (IM7), anti-CD8α PE (53-6.7), anti-CD4 FITC (RM4-1) obtained from PharMingen (San Diego, CA), or CD4 PE (GK1.5) from Becton Dickinson Collaborative Technologies (Bedford, MA), rabbit anti-rat IgG biotin from Vector Laboratories (Burlingame, CA), anti-CD8a (3.168) FITC (37), anti-Ly-5.1 FITC or biotin, and anti-Ly-5.2 FITC or biotin (38). Anti-c-Kit (ACK2) (26) and anti-IL-7Rα (A7R34) (39) were generous gifts of S.-I. Nishikawa (Kyoto University, Japan). Biotin-conjugated Abs were detected with streptavidin-RED 670 (Life Technologies, Grand Island, NY). Relative fluorescence intensities were measured with a FACSscan (Becton Dickinson, San Jose, CA).

c-Kit and IL-7R were detected as previously described (17). Briefly, cells were incubated with ACK2 (IgG2b,κ) or A7R34 (IgG2b,κ) culture supernatant respectively, or an irrelevant rat IgG2b,κ (PharMingen), followed by biotinylated rabbit anti-rat IgG, which was detected with streptavidin-RED 670. Samples were blocked with a mixture of rat and hamster Ig (200 µg/ml) to saturate free lg binding sites before staining with FITC- and PE-conjugated Abs specific for the Abs of interest.

Statistical analyses

Statistical analyses were two-tailed Student’s t tests, or ANOVAs conducted using Instat Instant Biostatistics (GraphPad Software, San Diego, CA).

Results

Intrathymic IL-7 is required for development of thymus-derived TCRγδ cells

IL-7 is produced by thymus cortical epithelial cells (14), and some TCRγδ IEL develop from precursors present in newborn thymus (18, 19). To determine the role of intrathymic IL-7 in TCRγδ IEL development, neonatal TCRβ−/− thymi were grafted into nTx IL-7−/− hosts. Four to ten weeks after engraftment, TCRγδ IEL derived from both host BM (Ly5.2−) and the thymus graft (Ly5.2+) were present in small and large intestine (Fig. 1A). The presence of graft-derived TCRγδ IEL demonstrated that TCRγδ precursors present in neonatal TCRβ−/− thymus grafts developed normally within a thymus able to produce IL-7 (IL-7+ thymus), despite the absence of IL-7 production by intestinal epithelium or other host tissues. Our previous experiments that identified the thymus as a source of TCRγδ IEL (18) did not indicate at what point in development TCRγδ IEL exited the thymus and migrated to the intestine. The data presented in Figure 1A definitively showed that TCRγδ IEL exited the thymus subsequent to their IL-7-dependent stage of development. However, these experiments did not determine whether IEL left the thymus as mature T cells, or as immature precursors, both of which express IL-7Rα-chain (39, and see below).

Analysis of the thymocytes within TCRβ−/− grafts confirmed that IL-7−/− host BM-derived TCRγδ cells were produced as a result of exposure of T cell precursors to IL-7 within the graft. TCRβ−/− thymus grafts contained >97% IL-7−/− BM-derived cells, but in contrast to endogenous IL-7−/− thymi (1), contained large numbers of thymocytes (1.1 ± 0.8 × 106, n = 13) with normal CD4:CD8 ratios, and TCRγδ cells of both donor graft, and host BM origin (Fig. 1B). Thymi-1 TCRγδ cells were also present in the spleen of grafted IL-7−/− mice (Fig. 1B). Thus, in the absence of peripheral IL-7, T cell precursors in IL-7−/− BM migrated to IL-7+ thymi, expanded, productively rearranged TCR genes, and matured to T cells of both the TCRαβ and TCRγδ lineages. Since neonatal thymus contain T cell precursors from fetal liver (40), intrathymic IL-7 was sufficient for development of TCRγδ T cells from both fetal liver- and adult BM-derived progenitors. The presence of TCRγδ cells in the thymus graft and in the spleen suggested that at least some TCRγδ cells left the thymus as mature T cells.

To determine whether extrathympic IL-7 was equally effective in supporting TCRγδ IEL development, IL-7−/− thymi were grafted to nTx TCRβ−/− hosts. When lymphocytes were isolated 4 to 6 wk after grafting, there were no graft-derived TCRγδ cells in the intestinal epithelium (Fig. 2A), or in any other peripheral lymphoid
organ (see below). As expected in TCRβ−/− mice, host TCRγδ cells were present in IEL, spleen, lymph node, and Peyer’s patches. The absence of IL-7−/− graft-derived TCRαβ IEL prompted us to confirm that IL-7−/− thymus grafts had survived, contained IEL precursors at the time of grafting, and were capable of supporting T cell maturation. Thymus grafts were analyzed, and TCRβ−/− BM-derived cells (Ly5.2+) made up >83% of total thymocytes (2.2 ± 2 × 10^3, n = 5), most of which were TCRγδ CD3−CD4−CD8−B220−. A few single-positive (CD4 or CD8) TCRαβ cells also remained associated with the IL-7−/− thymus graft (data not shown). T cells in the peripheral lymphoid tissues of TCRβ−/− hosts were also examined for graft-derived cells. All TCRγδ cells were host BM derived (Ly5.2+) (data not shown). All thymus graft-derived (Ly5.1+) cells present in lymph nodes, spleen, small and large intestinal IEL, Peyer’s patches, and large intestine lymphoid aggregates were TCRαβ cells (Fig. 2B and data not shown). These data confirmed that IL-7−/− thymus grafts had been accepted, populated by BM-derived precursors, and supported development of TCRαβ cells, but not TCRγδ cells, thereby reiterating thymopoiesis of a true IL-7−/− animal. Since extrathymic IL-7 was not able to support development of TCRγδ cells, intrathymic IL-7 was not only sufficient, but absolutely necessary, for development of thymus-derived TCRγδ IEL.

**IL-7R and c-Kit expression on T lymphocyte progenitors in newborn thymus**

We previously demonstrated that TCRγδ IEL express c-Kit and proliferate in situ in response to SCF produced by intestinal epithelial cells (17). Recently, we also found that TCRγδ IEL failed to bind an Ab specific for the IL-7Rα-chain. In contrast, TCRγδ cells in spleen and lymph node lack c-Kit, but retained IL-7Rα (Fig. 3, and L. Puddington and K. Laky, unpublished results). This diametrical profile of growth-factor receptor expression suggested a dichotomy in requirements for IL-7 (and SCF) during thymic development of TCRγδ cells destined for different tissues. Thus, to identify potential tissue-specific TCRγδ cell precursors in the thymus, we compared the pattern of expression of c-Kit and IL-7Rα on mature TCRγδ cells and on T lymphocyte progenitors in newborn TCRβ−/− thymi (Fig. 3). Most CD3−CD4−CD8− (triple-negative (TN)) thymocytes express c-Kit (CD117) (41) and all express IL-7Rα-chain (CD127) (39), albeit at different levels for each TN subset. Interestingly, mature TCRγδ cells in the newborn thymus were essentially all IL-7Rα+, c-Kit−.

**Peripheral IL-7 supports extrathymic development of TCRγδ IEL**

To evaluate the ability of peripheral IL-7 to support extrathymic development of TCRγδ IEL, adult B6-Ly5.2 mice were thymectomized, lethally irradiated, and reconstituted with IL-7−/− (Ly5.1) BM cells (ATxBM). When IEL were isolated 6 to 9 wk later and analyzed for TCR expression, substantial percentages of TCRγδ IEL were present in both small and large intestine (mean 60 ± 25% and 49 ± 30%, respectively, n = 6) (Fig. 4, left panels). TCRγδ small intestinal IEL were CD8+, αβ+, and data not shown). These data confirmed that IL-7−/− thymus grafts had been accepted, populated by BM-derived precursors, and supported development of TCRαβ cells, but not TCRγδ cells, thereby reiterating thymopoiesis of a true IL-7−/− animal. Since extrathymic IL-7 was not able to support development of TCRγδ cells, intrathymic IL-7 was not only sufficient, but absolutely necessary, for development of thymus-derived TCRγδ IEL.

It has been argued that IEL in ATxBM mice result from expansion of either mature T cells remaining in T cell-depleted donor BM, or radioreistant host IEL (42). In B6 mice reconstituted with IL-7−/− BM, these points were invalid because there were no mature TCRγδ cells in IL-7−/− BM, and any remaining host (Ly5.2+) IEL were gated out before analysis of donor-derived (Ly5.1+) IEL. As a control for the presence of a thymic remnant, lymph node of ATxBM mice were analyzed and found to contain no TCRγδ cells, and <3% donor-derived TCRαβ cells. This was significantly less than the mean of 54% TCRαβ lymph node T cells present in sham-thymectomized mice reconstituted with the same BM inoculum (p < 0.0001).

Surprisingly, in the converse experiment when IL-7−/− mice were reconstituted with B6 BM, a small but reproducible percentage of donor BM-derived TCRγδ IEL were present in both small and large intestine (6 ± 3%, n = 11, and 6 ± 5%, n = 6, respectively) (Fig. 4, middle panels). Both the percentage (6- to 10-fold) and absolute number (>10-fold) of TCRγδ IEL were significantly
less than in B6 mice reconstituted with IL-7 \(^{-/-}\) BM (small intestine \(p < 0.0001\), large intestine \(p < 0.05\)), but TCR\(\gamma\delta\) IEL are normally completely absent from IL-7 \(^{-/-}\) mice (5). No thymic remnants were detected, and even if an IL-7 \(^{-/-}\) thymic remnant had been present, it would have been unable to support development of mature TCR\(\gamma\delta\) cells (Fig. 1A).

To rule out that irradiation of IL-7 \(^{-/-}\) hosts had altered cytokine production by intestinal epithelial cells, which then compensated for the absence of IL-7, IL-7 \(^{-/-}\) mice were thymectomized, irradiated, and reconstituted with syngeneic IL-7 \(^{-/-}\) BM. The number of IEL isolated 6 to 12 wk later did not differ significantly from the number of IEL isolated from either IL-7 \(^{-/-}\) mice reconstituted with B6 BM, or B6 mice reconstituted with IL-7 \(^{-/-}\) BM (\(p > 0.10\)). However, no TCR\(\gamma\delta\) cells were present in either small or large intestinal IEL (Fig. 4, right panels), spleen, or lymph node (data not shown). Thus, radiation-induced changes were not sufficient to reconstitute TCR\(\gamma\delta\) IEL development in ATxBM IL-7 \(^{-/-}\) hosts. These results suggested that BM-derived, extrathymic sources of IL-7 existed in these mice. The limited TCR\(\gamma\delta\) IEL development noted in B6 BM-reconstituted IL-7 \(^{-/-}\) mice also occurred when TCR\(\beta\) \(^{-/-}\) BM was used (data not shown), which indicated that TCR\(\alpha\beta\) IEL-derived IL-7 (15) had no role in TCR\(\gamma\delta\) IEL development. Experiments are in progress to determine which BM-derived lineages synthesized IL-7 that supported extrathymic TCR\(\gamma\delta\) IEL development in this model.

**Discussion**

Since all thymus graft-derived IEL were TCR\(^{+}\) cells, peripheral IL-7 was neither sufficient nor required for development of thymus-derived TCR\(\gamma\delta\) cells (Figs. 1A and 2A). Furthermore, the presence of TCR\(\gamma\delta\) cells within IL-7 \(^{-/-}\) thymus grafts (Fig. 2B) demonstrated that some TCR\(\gamma\delta\) cells rearranged and expressed TCR before exiting the thymus. Together with the c-Kit and IL-7R expression patterns observed on T cell precursors in thymus (Fig. 3), we propose the following developmental pathways for thymus-derived TCR\(\gamma\delta\) cells. One possibility was that TCR\(\gamma\delta\) IEL and splenocytes left IL-7 \(^{-/-}\) thymi as mature T cells (Fig. 5, pathway 1).
This is consistent with compromised TCRγ rearrangements in the thymus (6, 10, 11) and the lack of peripheral TCRγβ cells in IL-7Rα(−/−) animals (2–5). If an IL-7R signal is required for TCRγ rearrangement, that would explain why newborn IL-7(−/−) thymus grafts gave rise to only TCRγδ IEL, whereas newborn IL-7(+/+) thymus grafts yielded both TCRγδ and TCRγδβ IEL. In addition, the expression of IL-7R by TCRγδ cells in newborn thymus (both HSA high and HSA low subsets, data not shown) suggested that IL-7 could be required for the survival, expansion, or final maturation steps of immature TCRγδ thymocytes with productively rearranged TCR.

An alternative to IEL leaving the thymus as mature T cells, was that the TCRγδ cells in IL-7(+/+) thymi were destined to be splenic TCRγδ cells, and that thymus-derived IEL left the thymus as immature T cell precursors (Fig. 5, pathway 2). It is possible that cortical thymocytes in IL-7(−/+) thymus grafts initiated TCRγ rearrangement in response to intrathymic IL-7, migrated to the gut, became mature TCRγδ IEL, and expanded in response to SCF.

**FIGURE 3.** IL-7R and c-Kit expression on TN thymocytes and TCRγδ cells. Thymocytes from newborn TCRβ(−/−) mice were isolated, and analyzed by fluorescence flow cytometry using mAb against CD44, CD25, TCRγδ, IL-7Rα (CD127), or c-Kit (CD117). In newborn TCRβ(−/−) mice, 48% of thymocytes were TN, 7% CD44+CD25−, 7% CD44−CD25+, and 34% CD44+CD25+. Twenty-two percent of thymocytes were CD3+, 100% of which were TCRγδ+. Splenocytes and IEL were isolated from 4-wk-old TCRβ(−/−) mice and analyzed by fluorescence flow cytometry using mAb against CD3, TCRγδ, IL-7Rα, or c-Kit. In TCRβ(−/−) mice, CD3+ TCRγδ cells comprised 15% of the total lymphocyte population in the spleen and >90% of IEL. Cells were positively gated on the subsets indicated, and then analyzed for IL-7Rα-chain or c-Kit (shaded histograms), or the isotype-matched irrelevant Ab (open histograms). CD4+CD8+ thymocytes were negative for both receptors, as reported previously (27, 39).

**FIGURE 4.** Peripheral IL-7 was sufficient to support development of some extrathymic TCRγδ IEL. Adult Ly5.2(+) C57BL/6J and Ly5.1(+) IL-7(−/−) mice were thymectomized, lethally irradiated (1100 rad), and reconstituted with 1 × 107 anti-Thy-1 + C(−)-depleted BM cells (ATxBM) in the combination indicated. Six to twelve weeks later, small and large intestinal IEL were isolated, and analyzed by fluorescence flow cytometry using mAbs against Ly5.1, Ly5.2, CD3, TCRαβ, or TCRγδ. IL-7(−/−) BM-derived cells (Ly5.1(−/+)) were 70 to 94% of IEL in ATxB6 hosts (left panels), whereas B6 BM-derived cells (Ly5.2(−/+)) were 60 to 90% of IEL in ATxB6 hosts (middle panels). No Ly5 marker unique for the donor population was available for IL-7(−/−) BM into ATx IL-7(−/−), thus all IEL are shown (right panels). After gating on donor BM-derived cells, TCRαβ IEL were analyzed for TCRαβ and TCRγδ expression. The results presented are representative of 6 C57BL/6J mice reconstituted with IL-7(−/−) BM, 12 IL-7(−/−) mice reconstituted with C57BL/6J BM, and 10 IL-7(−/−) mice reconstituted with IL-7(−/−) BM.
produced by intestinal epithelial cells (17). Indeed, pro-T cells are known to respond to IL-7, and TCRγ and TCRβ gene rearrangements are found in pre-T cells during thymocyte development (33, 41, 43–45). Thymocytes in IL-7−/− thymus grafts would have been unable to initiate TCRγ rearrangements in the thymus, and given rise only to precursors to TCRαβ IEL. Exit of thymus-derived IEL precursors before TCR expression on the cell surface could also explain why MHC class I expression is required in the intestine for selection/selective expansion of CD8+ TCRαβ IEL (46), and why IEL expressing forbidden VB are not deleted during negative selection in the thymus (23).

De novo generation of TCRγδ IEL from IL-7−/− BM precursors in adult athymic B6 hosts (Fig. 4) strongly suggested that extrathymic TCRγδ rearrangements had taken place. IL-7 mRNA is expressed by intestinal epithelial cells (15), and RAG1 mRNA is expressed by a small population of Thy-1−/− CD45+ ε mRNA in the thymus (16). Thus, if signaling through IL-7R induces TCRγ rearrangement, then both the stimulus for rearrangement and the enzymatic machinery necessary to carry out TCR rearrangement were present within the intestine of ATxBM B6 mice. Moreover, IL-7 and/or SCF synthesized by intestinal epithelium could have stimulated expansion of TCRγδ T cells with productive rearrangements, resulting in the substantial numbers of IEL isolated (17, 31, 47).

In summary, TCRγδ IEL that matured extrathymically in BM chimeras required only peripheral IL-7, whereas thymic TCRγδ was necessary and sufficient to support development of TCRγδ cells derived from thymus precursors. These results are consistent with either a single lineage of T cell precursors in the BM that randomly seed the thymus and intestine, or two distinct lineages of T cells in the BM, whose precursors deliberately home to either the thymus or the gut, due to differential expression of homing receptors (discussed in Ref. 48). If two distinct lineages of T cells exist, then IEL precursors that home to the intestine are destined to become IEL before leaving the BM. An IEL commitment signal could have provided by BM stromal cells via direct cell-cell contact, or via cytokine(s). However, until such a commitment signal is identified, questions remain as to the mechanisms by which 1) the developmental pathways of intra- and extrathymic IEL diverge, 2) the developmental pathways of intrathymic IEL and peripheral T cells diverge, and 3) commitment to either the TCRαβ or TCRγδ lineage occurs during extrathymic IEL development.

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