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A Function for IL-7R for CD4⁺CD25⁺Foxp3⁺ T Regulatory Cells

Allison L. Bayer, Joon Youb Lee, Anabel de la Barrera, Charles D. Surh, and Thomas R. Malek

The IL-2/IL-2R interaction is important for development and peripheral homeostasis of T regulatory (Treg) cells. IL-2- and IL-2R-deficient mice are not completely devoid of Foxp3⁺ cells, but rather lack population of mature CD4⁺CD25⁺Foxp3⁺ T cells and contain few immature CD4⁺CD25⁺Foxp3low T cells. Interestingly, common γ chain (γc) knockout mice have been shown to have a near complete absence of Foxp3⁺ Treg cells, including the immature CD25⁺Foxp3low subset. Therefore, other γc-cytokine(s) must be critically important during thymic development of CD4⁺CD25⁺Foxp3⁺ Treg cells apart from the IL-2. The present study was undertaken to determine whether the γc-cytokines IL-7 or IL-15 normally contribute to expression of Foxp3 and Treg cell production. These studies revealed that mice double deficient in IL-2Rβ and IL-7Rα contained a striking lack in the CD4⁺Foxp3⁺ population and the Treg cell defect recapitulated the γc knockout mice. In the absence of IL-7R signaling, IL-15/IL-15R interaction is dispensable for the production of CD4⁺CD25⁺Foxp3⁺ Treg cells, indicating that normal thymic Treg cell production likely depends on signaling through both IL-2 and IL-7 receptors. Selective thymic reconstitution of IL-2Rβ in mice double deficient in IL-2Rβ and IL-7Rα established that IL-2Rβ is dominant and sufficient to restore production of Treg cells. Furthermore, the survival of peripheral CD4⁺Foxp3⁺ Foxp3/foxp3 mice appears to depend upon IL-7R signaling. Collectively, these data indicate that IL-7R signaling contributes to Treg cell development and peripheral homeostasis.


Naturally occurring CD4⁺CD25⁺Foxp3⁺ regulatory T cells (Treg) develop in the thymus as a fully functional distinct subset of CD4⁺ T cells that migrate to the periphery to actively suppress autoreactive T cells that escaped thymic negative selection. Along with Foxp3, expression of IL-2R represents one of the better-characterized molecules of Treg cells. Furthermore, the IL-2R is functionally important for Treg cells as IL-2- and IL-2R-deficient mice develop rapid lethal autoimmunity due to impaired production of these cells (1). The IL-2/IL-2R interaction, primarily through STAT5, contributes to thymic Treg cell development and their homeostasis in peripheral immune tissues (2–5). An important consequence of the IL-2/IL-2R interaction in Treg cells is to maintain optimal expression of Foxp3 and CD25. The Foxp3 gene contains consensus-binding sequences for STAT5 that are required for transcription, indicating an important role STAT5 in Foxp3 expression (6, 7). Furthermore, IL-2 was shown to regulate Foxp3 expression in both mouse and human CD4⁺CD25⁺ regulatory cells through STAT5-dependent signals revealing the critical link between IL-2 and STAT5 signaling in the maintenance of Foxp3-expressing regulatory cells (6–8). Mice deficient in IL-2Rβ, IL-2Rα, or IL-2 are not devoid of Foxp3⁺ T cells, but rather contains a reduced number of immature CD4⁺CD25⁺Foxp3low T cells. Although these immature Foxp3lowCD4⁺ T cells in IL-2β⁻/⁻ mice exhibit some suppressor function in vitro, they do not suppress autoreactive T cells in vivo and are found at very low numbers in the periphery of IL-2β⁻/⁻ mice that were rendered autoimmune-free through adoptive transfer of a low number of wild-type (WT) Treg cells (2, 9). Furthermore, when endogenous Foxp3 is attenuated in Treg cells, mice succumb to lethal autoimmunity (10). These results taken together are consistent with an intrinsic defect in IL-2/IL-2R-deficient Foxp3lowCD4⁺ T cells.

Induction of Foxp3 is a hallmark of commitment to Treg cell lineage (11). Therefore, the signals leading to Foxp3 expression in the thymus represent key steps in development of this T cell subset. In this regard, common γ chain (γc)⁻/⁻ mice exhibit a much more striking defect in the development of Foxp3⁺ cells than IL-2⁻ or IL-2R-deficient mice, with a near complete absence of these cells in both the thymus and periphery (3, 5). Therefore, another γc-cytokine along with IL-2 functions during thymic development of Treg cells. Because the development of natural Treg cells depends on signaling through STAT5 (3, 6, 12, 13), in the current study we assessed whether IL-7 or IL-15, which like IL-2 also activates STAT5 (14, 15), contributes to expression of Foxp3 and Treg cell production. By evaluating mice that do not respond to IL-2, we uncovered a role for IL-7R signaling for Treg cell development and peripheral homeostasis that is usually dominated by IL-2.

Materials and Methods

Mice

C57BL/6, B6129SF2/S, IL-15Rα⁻/⁻, and γc⁻/⁻ mice were obtained from The Jackson Laboratory. The thymic-targeted transgenic WT IL-2Rβ⁺ mice expressed in IL-2Rβ⁻/⁻ mice (designated 2Rβ⁰/TG in this study; thymic

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3 Abbreviations used in this paper: Treg, T regulatory cell; DKO, double knockout; SP, single positive; DP, double positive; TG, thymic transgene; TSLP, thymic stromal lymphopoietin; WT, wild type; γc, common γ chain.
transgene (TG)) and the thymic-targeted WT IL-7Rα expressed in IL-7Rα−/− mice (designated 7Rα−/− in this study) on the C57BL/6 genetic background have been previously described (16, 17). These mice, along with IL-7Rα−/− mice, were maintained in the breeding colony at the University of Miami. Double deficient IL-7 and IL-15 (double knockout (DKO)IL-7/IL-15) mice on the C57BL/6 genetic background have been previously described (18) and were bred and maintained at The Scripps Research Institute. Thymic transgenic mice expressing WT IL-2Rα and/or IL-7Rα on the double deficient IL-2Rα−/− and IL-7Rα−/− (DKO2Rα/7Rα) mice that were used as breeding pairs to generate and maintain the following lines of mice: DKO 2Rα/7Rα, 2Rα/7Rα TG DKO2Rα/7Rα, and 7Rα−/− DKO2Rα/7Rα. Protocols were approved by the Institutional Animal and Use Committee at the University of Miami and at The Scripps Research Institute.

**Reagents**

Pacific Blue-conjugated CD4, PerCP-conjugated CD4 and CD8, and PE-Cy7-conjugated CD25 were purchased from BD Biosciences. Biotin-conjugated anti-CD3ε and anti-CD127, FITC-conjugated anti-CD25, Cy5-conjugated anti-CD8, and the Foxp3 staining kit were purchased from eBioscience. Pacific Blue-conjugated streptavidin was purchased from Molecular Probes.

**Experimental animals**

C57BL/6, DKO2Rα/7Rα, 2Rα/7Rα DKO2Rα/7Rα, and 7Rα−/− DKO2Rα/7Rα mice were sacrificed 3–15 wk of age. The B6129S2/S and IL-15Rα−/− or 7Rα−/− mice were sacrificed at 7–12 or 6 wk of age, respectively. Inguinal, axillary, brachial, and cervical lymph nodes were collected at the time of sacrifice along with the spleen and thymus. Tissues were made into single-cell suspensions and subjected to FACS analysis.

**FACS analysis**

For four-color analysis, cells were incubated for 20 min at 4°C with Pacific Blue-CD4, PerCP-CD8, and PE-Cy7-CD25 followed by two washes with HBSS containing 0.2% BSA and 150 mM NaN₃. The PE-Foxp3 staining was performed according to the manufacturer’s kit instructions (eBioscience). FACS analysis was performed as previously described (19) using a Becton Dickinson LSRII and Diva software. The total number of events collected for analysis was between 500,000 and 1,000,000.

**FIGURE 1.** Individual contribution of IL-15R or IL-7R signaling in Treg cell thymic development and peripheral homeostasis. Shown are representative FACS dot plots for Foxp3 and CD25 expression after gating on CD4+ CD8− SP thymocytes (A) or CD4+ splenocytes (B) from the indicated mice. C, Percentage of Foxp3 and CD25 cells among the gated SP CD4 thymocytes (left panels) or CD4 splenocytes (right panels) and (D) number of CD4+ Foxp3+ cells per 10⁶ thymocytes (left panels) or splenocytes (right panels) in B6129S2/S and IL-15Rα−/− mice (top panels) or B6 and IL-7Rα−/− (bottom panels). Data are means ± SEM for five to eight mice per group.
FIGURE 2. Thymic development and peripheral homeostasis. Thymocytes and splenocytes from individual C57BL/6 (B6), IL-2Rβ−/−, DKO2R/−/−, γc−/−, 7Rα+/−DKO2R/−/−, 2Rβ−/−DKO2R/−/−, and 2Rβ+/−7Rα+/−DKO2R/−/− mice were subjected to FACS analysis. Shown are representative dot plots for CD4 and CD8 staining. The percent positive staining is indicated in each quadrant.

Table I. Cellular composition of thymic and splenic lymphoid compartments

<table>
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<tr>
<th></th>
<th>Thymus Cellularity (×10^6)</th>
<th>Thymus DP (%)</th>
<th>Thymus Double Negative (%)</th>
<th>Thymus SP CD8 (%)</th>
<th>Thymus SP CD4 (%)</th>
<th>Spleen Cellularity (×10^6)</th>
<th>Spleen CD8 (%)</th>
<th>Spleen CD4 (%)</th>
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<td>B6129SF2/S</td>
<td>56.0 ± 9.5</td>
<td>78.5 ± 2.3</td>
<td>4.2 ± 0.2</td>
<td>3.7 ± 0.3</td>
<td>13.8 ± 1.9</td>
<td>55.6 ± 9.7</td>
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<td>70.7 ± 3.0</td>
<td>4.5 ± 0.5</td>
<td>2.3 ± 0.1</td>
<td>12.4 ± 2.6</td>
<td>76.5 ± 6.0a</td>
<td>4.3 ± 0.3a</td>
<td>23.1 ± 1.5</td>
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<td>B6</td>
<td>81.2 ± 6.1</td>
<td>81.2 ± 1.7</td>
<td>3.9 ± 0.6</td>
<td>3.1 ± 0.4</td>
<td>11.5 ± 1.3</td>
<td>70.8 ± 5.8</td>
<td>11.3 ± 0.5</td>
<td>18.4 ± 0.5</td>
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<td>46.3 ± 3.4</td>
<td>74.3 ± 2.8</td>
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<td>3.5 ± 1.3</td>
<td>15.5 ± 2.2</td>
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<td>71.5 ± 3.4</td>
<td>6.2 ± 1.3</td>
<td>4.0 ± 1.9</td>
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<td>2Rβ−/−DKO2R/−/−</td>
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<td>2Rβ+/−7Rα+/−DKO2R/−/−</td>
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<td>34.4 ± 13.5</td>
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* Denotes p < 0.05 compared with B6129SF2/S.
* Denotes p < 0.05 compared with B6.

Results

Mice doubly deficient in IL-2Rβ and IL-7Rα lack CD4+ Foxp3+ Treg cells

Past work revealed that IL-2Rβ−/− mice contain CD4+ Foxp3+ T cells, but when compared with WT mice, the number of these cells was reduced 2-fold in the thymus and 4-fold in peripheral immune tissues, with a 2-fold lower level of intracellular Foxp3 protein [Ref. 2 and see Fig. 4]. Initially, we investigated whether other IL-2-independent STAT5-activating γc-dependent cytokines, i.e., IL-7 and IL-15, individually contribute to the development and homeostasis of natural Treg cells. The IL-15α−/− mice have largely normal production and maintenance of CD4+ CD25+ Foxp3high Treg cells (Fig. 1A, C, and D). Although not significantly different, we observed that the thymus of IL-15α−/− mice contained a somewhat lower number of CD4+ Foxp3+ cells. The spleen contained a percentage and number of CD4+ CD25+ Foxp3+ T cells comparable to littermate control B6129SF2/S mice (Fig. 1A, C and D). IL-15α−/− mice did not exhibit any signs of autoimmunity associated with IL-2/IL-2R-deficiency (data not shown).

IL-7Rα-deficient mice have a severe block in T cell development, such that thymic cellularity is reduced 10- to 20-fold, but those cells present usually progress through all major thymic stages (Fig. 2 and Table I). Reduced thymic number is largely due to a developmental arrested at the pre-T cell stage (20), leading to a peripheral immune compartment that is severely lymphopenic due to low number of T and B cells. Sometimes the arrest at the pre-T cell stage in IL-7Rα−/− mice prevented further T cell development and these mice (three of eight) were excluded from this analysis. Nevertheless, IL-7Rα−/− mice have a normal fraction of CD4+ CD25+ Foxp3+ Treg in both the thymus and spleen when compared with WT B6 mice, although IL-7Rα−/− mice contain a higher fraction of CD4+ CD25+ Foxp3+ thymocytes (Fig. 1, B and C). Despite the severely reduced thymic and splenic cellularity, the number of CD4+ Foxp3+ thymocytes or splenocytes was largely comparable to B6 mice when normalized to 1 × 10^8 cells/tissue (Fig. 1D, left bottom panel). Although severe lymphopenia has been associated with immune dysregulation and autoimmune diseases (21–23), IL-7Rα−/− mice were overtly healthy. In total, these findings and similar analysis of IL-7−/−, IL-15−/−, IL-7R−/−, and IL-15Rα−/− mice (3, 24, 25) indicate that thymic development and peripheral homeostasis of CD4+ Foxp3+ Treg cells do not require either of these cytokines when there is IL-2R signaling.

The presence of immature CD4+ Foxp3low cells in IL-2Rβ−/− mice, i.e., CD4+ T cells with ~2- to 4-fold lower level of Foxp3...
Figure 3. Absence of CD4⁺ Foxp3⁺ Treg cells in mice doubly deficient in IL-2Rβ and IL-7Rα. A, Thymus and spleen of C57BL/6 (B6) and IL-7Rα⁻/⁻ mice were subjected to FACS analysis for the expression of IL-7Rα (CD127). Shown is representative dot plot for Foxp3 and CD127 staining of gated SP CD4 thymocytes or CD4 splenocytes. B, Thymocytes (left panels) and splenocytes (right panel) from indicated mice were subjected to FACS analysis. Shown are representative FACS dot plots for Foxp3 and CD25 expression after gating on SP CD4 thymocytes or CD4 splenocytes. Number in the upper quadrants indicates percentage of Foxp3⁺ cells, while number in parentheses in the upper right quadrants indicates the mean fluorescent intensity of Foxp3⁺ cells. In the thymus (C) or spleen (D), the percentage of Foxp3 and CD25 among the gated SP CD4 thymocytes or CD4 splenocytes (top panels), respectively, or number of CD4⁺Foxp3⁻/⁺/⁻ cells (bottom panels). *, p < 0.001; and #, p < 0.05 and was calculated by one-way ANOVA followed by Newman-Keuls multiple comparison test.

Based on the mean fluorescence intensity, [Fig. 3B and Ref. 2], indicates that development of these cells is independent of both IL-2 and IL-15, as signaling by these cytokines depends upon IL-2Rβ. Furthermore, in WT B6 mice, a small but detectable population of thymic CD4⁺Foxp3⁺ Treg cells expresses low levels of IL-7Rα (CD127), and the majority of periphery Treg cells express similarly low levels of IL-7Rα (Fig. 3A). Detection of such low levels of IL-7Rα is specific as IL-7Rα mice do not exhibit severe autoimmunity due to impaired T cell development, both types of mice contained peripheral T cells that consisted of reduced CD8⁺ T cells, but CD4⁺ T cells with an activated phenotype (Table I and data not shown). Collectively, these data indicate that the lack of Foxp3⁺ T cells in all major thymic subsets (Table I and Fig. 2). The peripheral compartment in DKO2Rβ/7Rα mice contained predominantly CD4 T cells (Table I and Fig. 2), probably, because CD8 T cells survive poorly with a combined loss of IL-7 and IL-15. Importantly, DKO2Rβ/7Rα mice contained a striking defect in the CD4⁺Foxp3⁺ cells with near complete absence in the thymus and spleen (Fig. 3, B–D). Based on their relative abundance, WT B6 mice contained 6- and 14-fold more CD4⁺Foxp3⁺ thymocytes and splenocytes, respectively, than the DKO2Rβ/7Rα mice (Fig. 3, C and D). This defect in CD4⁺Foxp3⁺ cells in DKO2Rβ/7Rα mice was much more striking than found in IL-2Rβ⁻/⁻ mice [Fig. 3B and Ref. 2]. The number of CD4⁺Foxp3⁺ cells in the thymus of DKO2Rβ/7Rα was slightly lower and not statistically significant when compared with that in γc⁻/⁻ mice (Fig. 3, B and C). Although DKO2Rβ/7Rα and γc⁻/⁻ mice do not exhibit severe autoimmunity due to impaired T cell development, both types of mice contained peripheral T cells that consisted of reduced CD8⁺ T cells, but CD4⁺ T cells with an activated phenotype (Table I and data not shown).
mice have comparable number of immature CD4^{+}Foxp3^{+} T cells (2, 5) and that the IL-15/IL-15R interaction is not essential for T_{reg} cell thymic development or peripheral homeostasis [this report and Ref. 3], these data suggest that IL-2 is sufficient to drive full expression of Foxp3 and CD25 on T_{reg} cells.

**Thymic-targeted IL-2Rβ expression is sufficient to restore the production of CD4^{+}CD25^{high}Foxp3^{high} T_{reg} cells in DKO^{IL-7R-/7Rα} mice**

To further explore the role of IL-7 and IL-2 signaling in T_{reg} cell development, we examined the production of CD4^{+}Foxp3^{+} T cells in the thymus of DKO^{IL-7R-/7Rα} mice that were reconstituted singly with thymic-targeted transgenic IL-2Rβ or IL-7Rα and dually with both transgenes, using previously developed transgenic mice. Thymic-directed IL-2Rβ (2R^{TG}) has been shown to restore T_{reg} production and prevent autoimmunity when expressed in IL-2Rβ^{−/−} mice (2, 9, 16). Thymic-directed IL-7Rα (7R^{TG}) expression has been shown to promote mainstream T cell development when expressed in IL-7Rα^{−/−} mice (17, 28), while peripheral T cells do not obviously express or functionally respond to IL-7 (29).

In either thymic transgenic DKO^{2Rβ/7Rα} mice, T cell development was largely normal with only minimal alterations in the major thymic subsets (Table I and Fig. 2). Previous work from our laboratory demonstrated that 2R^{TG} in IL-2Rβ^{−/−} mice was detected at high levels in double positive (DP) CD4^{+}CD8^{−}Foxp3^{+} expressing thymocytes with very little detectable CD122 expression in the single positive (SP) CD4^{+}CD8^{−} Foxp3^{+} expressing cells (2). The 7R^{TG} expression alone or in combination with 2R^{TG} in DKO^{2Rβ/7Rα} mice (7R^{TG}DKO^{2Rβ/7Rα} or 2R^{TG}/7Rα^{TG}DKO^{2Rβ/7Rα}, respectively) also resulted in low CD127 expression being largely restricted to DP Foxp3^{+} thymocytes, with essentially none on SP CD4^{+}CD8^{−} Foxp3^{+} expressing cells (Fig. 5A). With respect to T_{reg} cell development, either 7R^{TG} or 2R^{TG} thymic-directed transgene led to the production of Foxp3^{+}-expressing CD4^{+} T cells (Fig. 5, B and C). With respect to T_{reg} cell thymic development, 7R^{TG}DKO^{2Rβ/7Rα} mice contained a population of immature CD4^{+}CD25^{−}Foxp3^{high} thymocytes as seen in IL-2Rβ^{−/−} mice, with a decreased frequency of Foxp3^{+}-expressing CD4^{+}CD8^{−} SP thymocytes (Fig. 5B, C, and D). Furthermore, these immature Foxp3^{high} T cells in 7R^{TG}DKO^{2Rβ/7Rα} mice did not suppress autoimmunity (data not shown). These data indicate that IL-7R signaling is not sufficient to fully support production of mature CD4^{+}CD25^{−}Foxp3^{high} T_{reg} cells. In contrast, expression of the 2R^{TG} in DKO^{2Rβ/7Rα} mice (2R^{TG}DKO^{2Rβ/7Rα} or both transgenes in DKO^{2Rβ/7Rα} mice (2R^{TG}7Rα^{TG}DKO^{2Rβ/7Rα}) reconstituted a normal population of mature CD4^{+}CD25^{+}Foxp3^{high} thymocytes (Fig. 5B, C, and D) and these mice did not show signs of autoimmunity (data not shown). Furthermore, the expression of both 2R^{TG} and 7R^{TG} in the DKO^{2Rβ/7Rα} mice resulted in ~2.6-fold more Foxp3^{+}-expressing SP CD4 thymocytes. This increase is likely due to the broader expression on IL-2Rβ and IL-7Rα in the thymus of these DKO mice, which favored a greater production of these cells. Importantly, the data from 2R^{TG}DKO^{2Rβ/7Rα} mice indicate that IL-2R signaling is sufficient to restore T_{reg} cell development and function leading to a normal population of mature CD4^{+}CD25^{+}Foxp3^{high} T_{reg} cells (2, 9, 30).

**A role for IL-7 in peripheral T_{reg} cell homeostasis**

Past work showed that IL-2Rβ is expressed at nearly undetectable levels by peripheral CD4^{+}Foxp3^{+} T_{reg} cells within IL-2Rβ^{−/−} mice containing the thymic-directed 2R^{TG} (2). This minimal IL-2Rβ did not support IL-2-dependent T_{reg} cell activity when

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**FIGURE 4.** Presence of CD4^{+}Foxp3^{+} T_{reg} cells in the absence of IL-7 and IL-15 double deficient mice. (A) Thymus and (B) lymph node cells from individual C57BL/6 (B6) and DKO^{IL-7R-/IL-15} mice were subjected to FACS analysis. Shown are representative FACS dot plot for CD4 and Foxp3 expression after gating on CD3^{+} cells (left panels) or CD25 and Foxp3 expression after gating on CD3^{+}CD4^{+} cells (right panels). Data are representative of two to five individual mice.

γc^{−/−} mice is due to impaired signaling through both IL-2Rβ and IL-7Rα-chains.

**IL-2 and IL-7 are essential for CD4^{+}CD25^{−}Foxp3^{+} T_{reg} cell production**

Because IL-2Rβ-chain is shared by both IL-2 and IL-15 receptors, the defect in CD4^{+}Foxp3^{+} cells in DKO^{2Rβ/7Rα} and γc^{−/−} mice might reflect the absence of IL-2, IL-7, and IL-15 or a loss in IL-7 and either IL-2 or IL-15. This possibility was explored by examining whether the thymus and lymph nodes from mice doubly deficient in IL-7 and IL-15, i.e., DKO^{IL-7R-/IL-15} mice, resembled that from DKO^{2Rβ/7Rα} and γc^{−/−} mice. However, even though thymic cellularity was reduced, DKO^{IL-7R-/IL-15} mice have a proportion of thymic CD4^{+}CD25^{−}Foxp3^{+} T cells comparable to WT B6 mice, while the periphery contains a higher fraction of CD4^{+}CD25^{+}Foxp3^{+} T_{reg} cells when compared with WT mice (Fig. 4, A and B). Thus, in the absence of IL-7R signaling, IL-15 is dispensable for production and maintenance of CD4^{+}Foxp3^{+} T cells. Given that IL-2- and IL-2R-deficient...
directly evaluated in several assays in vivo and in vitro, but resulted in weak transient IL-2-dependent STAT5 activation. Thus, peripheral Treg cell function and homeostasis can be maintained in the periphery in an IL-2-independent fashion or with minimal IL-2-dependent STAT5 activation. These peripheral Treg cells with impaired IL-2R\(^{+}\) signaling exhibited diminished proliferation and turnover. Thus, these homeostatic properties more closely resembled naïve conventional CD4 T cells, which are known to depend in part on IL-7 (20). Thus, we investigated whether IL-7R signaling might account for peripheral T\(_{reg}\) cells in 2R\(\beta\)\(^{+}\) mice.

In a manner similar to 2R\(\beta\)\(^{TG}\), IL-7R expression is not detected on peripheral T cells within IL-7R\(\alpha\)\(^{-/-}\) mice containing the thymic-directed 7R\(\alpha\)\(^{TG}\) and peripheral T cells from these mice are functionally impaired when stimulated with IL-7 in vitro and in vivo, but IL-7 also induced weak and transient STAT5 activation (29). Therefore, we examined whether lowering IL-7R signaling affected the survival of IL-2R-defective peripheral T\(_{reg}\) cells by
enumerating the relative abundance of CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>high</sup> T cells in the spleens of 2R<sup>α<sup>+/+</sup></sup>IL-2R<sup>±/−</sup>TGDKO<sup>±/−</sup>/7R<sup>−/−</sup> and 2R<sup>β<sup>−/−</sup></sup>DKO<sup>β<sup>−/−</sup></sup>/7R<sup>−/−</sup> mice. The periphery of these transgenic DKO<sup>β<sup>−/−</sup></sup>/7R<sup>−/−</sup> mice was largely normal except that these mice contained a higher percentage of CD4 T cells (Table I and Fig. 2), which may be due to the broader activity of these transgenes in the thymus favoring greater production of these cells. In both mice, the proportion of Treg cells was essentially identical with that found within the spleen of WT B6 mice (Fig. 6, A and B). Thus, these data suggest peripheral Treg cell homeostasis can be independent of IL-2 or IL-7 or that the remaining weak STAT5 activation is sufficient in the context of TCR or other signals.

Immature CD4<sup>+</sup>CD25<sup>−</sup>Foxp3<sup>low</sup> cells are readily found in IL-2R<sup>±/−</sup> mice and develop due to thymic IL-7R signaling, as evident through evaluation of 7R<sup>−/−</sup>DKO<sup>β<sup>−/−</sup></sup>/7R<sup>−/−</sup> mice (Fig. 5). Thus, we also examined the periphery of 7R<sup>−/−</sup>DKO<sup>β<sup>−/−</sup></sup>/7R<sup>−/−</sup> mice for CD4<sup>+</sup>Foxp3<sup>+</sup> T cells. In marked contrast to 2R<sup>β<sup>−/−</sup></sup>TGDKO<sup>−/−</sup>/7R<sup>−/−</sup> mice, the spleen of 7R<sup>−/−</sup>DKO<sup>β<sup>−/−</sup></sup>/7R<sup>−/−</sup> mice contained very few CD4<sup>+</sup>Foxp3<sup>+</sup> cells, but rather contained a relatively small population of immature CD4<sup>+</sup>Foxp3<sup>+</sup> T cells (Fig. 6C). Based on relative abundance, the spleen of 7R<sup>−/−</sup>DKO<sup>β<sup>−/−</sup></sup>/7R<sup>−/−</sup> mice have 11- and 2.4-fold fewer cells in the spleen than WT and IL-2R<sup>±/−</sup> mice, respectively (Fig. 6C). This decrease was also similar in the lymph nodes (Fig. 6C). Unlike

**FIGURE 6.** IL-7 in peripheral T<sub>reg</sub> cell homeostasis. A, Representative FACS dot plots for Foxp3 and CD25 expression after gating on CD4<sup>+</sup> splenocytes. B, The percentage of Foxp3 and CD25 among the gated CD4 cells from spleen and lymph nodes. C, Number of CD4<sup>+</sup>Foxp3<sup>+</sup> per 1 × 10<sup>6</sup> cells from each tissue. Data in B and C are means ± SEM for four to eight mice per group. †, p < 0.001 compared with B6 and was calculated by one-way ANOVA followed by Newman-Keuls multiple comparison test. ‡, p < 0.05 and was calculated by unpaired t test.
peripheral immature CD4⁺Foxp3low T cells from IL-2Rβ⁻/⁻ mice, which can respond to IL-7, peripheral cells in 7Rα⁻/⁻DKO2Rβ⁻/⁺ mice are genetically non-responsive to IL-2 and IL-15 and only minimally responsive to IL-7. Thus, the lower fraction of peripheral CD4⁺ Foxp3low T cells when compared with CD4⁺ Foxp3low T cells in IL-2Rβ⁻/⁻ mice (2) suggests that IL-7/IL-7R may contribute to survival of the peripheral immature Foxp3low T cells.

**Discussion**

We have described two roles for IL-7R during naturally occurring CD4⁺Foxp3⁺ Treg cell development. One function of IL-7R is during thymic development that was uncovered by analyzing DKO2Rβ⁻/⁺ mice, which recapitulated the phenotype of γc⁻/⁻ mice. This study revealed a relatively normal production of Treg cells by DKO2Rβ⁻/⁺ mice, which strongly suggests that IL-15R signaling is dispensable in the absence of IL-7R and that IL-7 and IL-2 are the only γc-dependent cytokines that normally function during Treg cell development. Furthermore, expression of either 2RβTg or 7RαTg in DKO2Rβ⁻/⁺ mice established that IL-2R signaling is dominant and sufficient for development of Treg cells and that the CD4⁺ CD25⁺Foxp3low thymic phenotype is a consequence of IL-7R signaling, accounting for these cells in IL-2Rβ⁻/⁻ mice. We currently do not know how IL-7 promotes development of these Foxp3low thymocytes. Normal Treg cell development was rescued in IL-2Rβ⁻/⁻ mice after expression of a transgenic chimeric receptor linking IL-2 binding to IL-7R signaling but not reciprocally after linking IL-7 binding to IL-2R signaling (28). This finding suggests IL-7 is not available in the niche where IL-2 normally functions. Therefore, we favor the notion that IL-7 promotes Foxp3 expression at point in thymic development distinct from where IL-2 functions. We speculate that the IL-7R signal is delivered early, perhaps during pro/pre-T cell development where IL-7R normally functions (Fig. 7), only to be revealed later by another developmental signal, because IL-7R-dependent Foxp3low cells in IL-2Rβ⁻/⁻ mice are first readily found at the CD4⁺ CD8⁻ stage of thymic development (2). Furthermore, the data from the 2RβTg/DKO2Rβ⁻/⁺ mice along with our past work support that the essential role for IL-2R resides later during the DP and/or SP stage (Fig. 7).

IL-7 and thymic stromal lymphopoietin (TSLP) both use IL-7Rα as a subunit and activate the transcription factor STAT5 (31). Therefore, the defect in IL-7Rα potentially reflects impairment due to both of these cytokines. For example, IL-7Rα deficiency results in greater block in T and B cells compartments compared with IL-7 knockout mice. Furthermore, mice deficient in both γc and TSLP receptors have a greater lymphoid defect than γc-deficient mice (32). In regards to Treg cell development, TSLP has been shown to play a role in the differentiation and maturation of Foxp3-expressing cells in the human and murine thymus (31, 33, 34). However, this function of TSLP is indirect in that it acts to condition dendritic cells to express CD80 and CD86, which in turn contributes to the maturation of Treg cells (34). Thus, the detection of normal proportion of mature Treg cells in IL-7Rα⁻/⁻ mice indicates that the activity of TSLP is not essential for Treg cell production. Furthermore, the γc-chain is a subunit of the IL-7R and not the TSLPR, yet both DKO2Rβ⁻/⁺ mice and γc-deficient mice do not produce Treg cells. This loss in Treg cells is consistent with IL-7, rather than TSLP, as a critical cytokine along with IL-2 for the normal development of Treg cells. Nevertheless, we cannot completely exclude the possible contribution by TSLP in promoting Treg cell development.

The absence of either IL-15 or IL-15Rα alone does not result in a major defect in Treg cell development [Ref. 3 and this study]. We and others (2, 5) have shown that mice deficient in IL-2, IL-2Rα, or IL-2β have a similar 2-fold reduction in thymic Foxp3-expressing CD4⁺ T cells. These findings, therefore, support the notion that this reduction in Foxp3⁺ thymocytes is accounted for by the lack of IL-2/IL-2R function. However, Burchill et al. (3) have suggested a role for IL-15, which also depends upon IL-2Rβ signal transduction, during Treg cell development because a greater reduction in CD4⁺ Foxp3⁺ thymic cells was noted in IL-2Rβ⁻/⁻ mice.
mice when compared with IL-2/−/− mice. We do not know the reason for these differences, but the absence of both IL-2Rβ and IL-7Rα leads to an essentially complete absence of thymic Foxp3 expressing thymocytes, which is much more striking than the relatively low proportion of Foxp3+ cells detected in IL-2Rβ/−/− mice by Burchill and colleagues. Furthermore, the presence of CD4+ Foxp3+ T cells in mice deficient in both IL-7 and IL-15 is consistent with no critical role for IL-15 in Treg cell development. Overall, these findings support a model where IL-2 and IL-7 are IL-7R dependent (36). Thus, stromal cell-derived IL-7 might aid Treg cell conventional T cells in the periphery is very transient and Ag-independent cytokines that normally function during thymic development of Treg cells. The other function of IL-7Rα may be to promote the survival of peripheral Foxp3+ T cells, which was uncovered only after analysis of the periphery of 7RαTKO/2RβKO/−/− mice. In this situation, only immature CD4+ CD25+ Foxp3low T cells develop due to thymic IL-7R expression. The peripheral Foxp3low cells do not respond to IL-2 but generate a weak IL-7 signal through minimal expression of transgenic IL-7R and cannot prevent autoimmunity. These Foxp3low T cells behave identically to the immature CD4+ Foxp3low cells in IL-2Rβ−/− mice, which express WT endogenous IL-7R, except their prevalence in the periphery was much lower, raising a potential role for IL-7R in their survival. However, the normal frequency of peripheral Treg cells in IL-7Rα/−/− mice is consistent with IL-2 dominantly controlling peripheral Treg cell homeostasis. Quite remarkably, functional peripheral Treg cells with impaired IL-2R and IL-7R signaling were found at a normal frequency in 2RβTKO/7RαTKO/−/− mice. This finding raises the possibility that the increased, albeit it minimal, STAT5 activation between 7Rα/−/− and 2Rβ/−/− mice is in that the latter mice is an IL-2 signal given leading to production of CD4+ CD25+ Foxp3high Treg cells that control autointolerance. Whether this productive developmental signal is favorable for peripheral homeostasis independent of IL-2 and IL-7 remains to be determined.

Our study suggests that this may indeed be an important line of future investigation as polymorphism in the IL-7Rα gene, that likely lead to reduced IL-7R signaling, is an autoimmune susceptibility gene for multiple sclerosis and type 1 diabetes (37–40).

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Disclosures

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References


These data, our past work, and that of others support a dominant role for IL-2 during thymic Treg cell development and peripheral homeostasis (2, 4, 5). Thus, what might be the biological relevance of IL-7 in regulating Treg cells? One possibility is to lower the requirement for IL-2R signaling. This might be particularly relevant during thymic development if the IL-7R-dependent Foxp3low stage precedes the IL-2R-dependent stage as immature Foxp3low thymocytes should require less IL-2 signaling to drive normal Foxp3 levels than cells that have not yet expressed Foxp3. As IL-2 producing cells are limited within the thymus (35), increasing signal sensitivity to IL-2 may assure optimal number of Treg cell over a long time period. Another possibility is that IL-2 production by conventional T cells in the periphery is very transient and Age-dependent (36). Thus, stromal cell-derived IL-7 might aid Treg cell homeostasis at times when IL-2 is unavailable. Although Treg cell production occurs in the experimental setting of IL-7−/− or IL-7-deficiency [this study and Ref. 3], impaired T cell development makes autoimmunity less likely. In the real world of diminishing thymic output with age and infection, IL-7 may be a more important factor preventing autoimmune disease at the level of Treg cells.


