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Transgenic Expression of Cyclin D1 in Thymic Epithelial Precursors Promotes Epithelial and T Cell Development

David B. Klug, Elizabeth Crouch, Carla Carter, Lezlee Coghlan, Claudio J. Conti, and Ellen R. Richie

We previously reported that precursors within the keratin (K) 8+5+ thymic epithelial cell (TEC) subset generate the major cortical K8+5− TEC population in a process dependent on T lineage commitment. This report demonstrates that expression of a cyclin D1 transgene in K8+5+ TECs expands this subset and promotes TEC and thymocyte development. Cyclin D1 transgene expression is not sufficient to induce TEC differentiation in the absence of T lineage-committed thymocytes because TECs from both hCD3ε transgenic and hCD3ε/cyclin D1 double transgenic mice remain blocked at the K8+5+ maturation stage. However, enforced cyclin D1 expression does expand the developmental window during which K8+5+ cells can differentiate in response to normal hematopoietic precursors. Thus, enhancement of thymic function may be achieved by manipulating the growth and/or survival of TEC precursors within the K8+5+ subset.

Materials and Methods

Mice

C57BL/6J, hCD3ε transgenic, RAG-1−/−, and Nu/J mice were purchased from The Jackson Laboratory Animal Resource Unit, Bar Harbor, ME. The cyclin D1 transgenic line was produced as previously described (15).

Abs and lectin

Rabbit antisera specific for mouse K5 or K14 were developed as described by Roop et al. (16) and obtained from Covance Research (Richmond, CA). Troma-1, a mAb that recognizes K8, was kindly provided by Dr. Rolf Kemler (Max-Planck-Institut für Immunobiologie, Freiburg, Germany) (17). Anti-Cd4 (RM4-5) conjugated with PE, anti-CD8 (53-6.7) conjugated with FITC and biotinylated anti-CD3 (145-2C11), anti-CD69 (H1.2F3), anti-CD25 (7D4), and anti-CD44 (IM7) were obtained from PharMingen (San Diego, CA). Fluorochrome-conjugated anti-Ig second step reagents were purchased from Jackson ImmunoResearch (West Grove, PA). Binding of biotinylated Abs was detected by allophycocyanin-conjugated streptavidin (APC-SA, Molecular Probes, Eugene, OR). Biotinylated UEA-1 and FITC-conjugated streptavidin were obtained from Vector Laboratories (Burlingame, CA).

Immunohistology

Serial frozen sections (5 μm) from 6- to 9-wk-old mice were air dried for 30 min before acetone fixation. Thin sections were blocked with normal serum and, if necessary, an avidin-biotin blocking kit (Vector Laboratories) and subsequently incubated with optimal dilutions of primary Abs for at least 1 h at 25°C before washing and incubation with appropriate fluorochrome-conjugated secondary reagents. Controls included slides incubated with nonimmune species matched Ig or isotype-matched mouse Ig. For double staining, the sections were incubated simultaneously with primary Abs from different species. Microscopic analysis was performed with an Olympus ProVis AX70 microscope (Olympus, Melville, NY).

Flow cytometry

For three-color immunofluorescence analysis, cells in HBSS containing 1% BSA and 0.1% sodium azide were incubated with directly conjugated or biotinylated Abs on ice for 30 min followed by three washes. Binding of biotinylated Ab was detected with APC-SA. The cells were fixed in 1% paraformaldehyde before analysis. For determination of DN subsets, thymocytes were stained with a mixture of FITC-conjugated Abs to lineage markers CD4, CD8, CD3, B220, CD11b, and Gr-1 as well as with anti-CD44-PE and anti-CD25-biotin (PharMingen). After washing, the cells were incubated with APC-SA. FITC-negative cells were selected by electronic gating and analyzed for CD44 and CD25 expression. Cells were analyzed with a Coulter Epics Elite flow cytometer (Miami, FL) equipped with an argon laser (488 nm) for FITC and PE excitation and a helium-neon laser (633 nm) for APC-SA excitation. Data were collected on 10–20 × 10^6 viable cells (or 10^5 for DN analysis) using a four-decade log amplifier and were stored in list mode for subsequent analysis using Coulter Elite Software.

Transplantation of thymic grafts

Thymi from adult hCD3ε transgenic or hCD3ε/cyclin D1 double transgenic mice were grafted under the kidney capsule of anesthetized adult nude recipients. A small incision was made in the peritoneal cavity, and the left kidney was exposed. With an i.v. cannula, two thymic lobes from individual donors were positioned under the kidney capsule. The wound was closed with wound clips.

5-Bromo-2′ deoxyuridine (BrdU) and TUNEL labeling

Mice were injected i.p. with 1 mg BrdU in HBSS, and thymi were obtained 1 h later. To detect BrdU incorporation, deparaffinized thymic sections were incubated in 1 N HCl for 20 min at room temperature. After washing in 0.1% albumin, Tris-buffered saline, the sections were incubated with mouse anti-BrdU (B-D Sciences, San Jose, CA) for 1 h at room temperature followed by incubation with HRP-conjugated anti-mouse-IgG (Jackson ImmunoResearch), and the sections were developed with 3,3′-diaminobenzidine. To detect TUNEL-positive cells, 5-μm frozen sections were processed using an In Situ cell death detection kit (Boehringer Mannheim) per manufacturer’s instruction. Positive control slides were incubated in 10 μg/ml DNase (Sigma) in 4.2 mM MgCl2, 150 mM NaCl in H2O, pH 5.0, for 20 min at 37°C. Negative control slides were incubated in reaction mixture without TdT.

Results

The thymic epithelial compartment is expanded in cyclin D1 transgenic mice

Expression of a cyclin D1 transgene targeted to epithelial cells by a K5 promoter induces a mild hyperplastic phenotype in the skin (15). However, the prominent phenotype in transgenic mice is severe thymic hyperplasia that is readily apparent by 4 weeks of age and ultimately results in premature death by 5–6 mo due to cardiorespiratory failure (15). Fig. 1A shows that thymic weight in cyclin D1 transgenic mice exceeds that of nontransgenic littermates by 4 wk of age and continues to increase with age. Histological analysis revealed that gross thymic architecture is preserved in hyperplastic cyclin D1 transgenic thymi (Fig. 1B). The
transgenic thymi contain well-organized cortical and medullary regions, although the medullary areas appear somewhat dispersed compared with nontransgenic thymi. Furthermore, both previously described medullary subsets (i.e., stellate K8\(^+\)18\(^-\)K5\(^+\)K14\(^+\)UEA-1\(^-\) and globular K8\(^+\)18\(^-\)K5\(^+\)K14\(^-\)UEA-1\(^-\)\)) are present in transgenic as well as nontransgenic littermates (Fig. 1B and data not shown) (10). The cortex in cyclin D1 transgenic and nontransgenic mice consists of a predominant K8\(^+\)18\(^-\)K5\(^+\)K14\(^+\)UEA-1\(^-\) subset (hereafter referred to as K8\(^+\)5\(^+\)). In normal mice, a minor K8\(^+\)18\(^-\)K5\(^+\)K14\(^-\)UEA-1\(^-\) subset (hereafter referred to as K8\(^+\)5\(^+\)) is scattered throughout the cortex and concentrated at the corticomedullary junction. However, in cyclin D1 transgenic mice, there is a notable expansion of the K8\(^+\)5\(^+\) TEC subset. Because TEC progenitors reside within the K8\(^+\)5\(^+\) subset (10), the disproportionate expansion of K8\(^+\)5\(^+\) cells is likely to be responsible for the profound thymic hyperplasia observed in cyclin D1 transgenic mice.

**T cell development is normal, but thymocyte cellularity is increased in cyclin D1 transgenic mice**

Expansion of the thymic epithelial compartment in cyclin D1 transgenic mice supports an extensive increase in thymocyte cellularity compared with nontransgenic littermates (Fig. 2A). However, the thymic epithelial hyperplasia does not adversely affect intrathymic T cell development. As shown in Fig. 2B, there was a normal distribution of the major thymocyte subsets defined by CD4 and CD8 coreceptor expression in cyclin D1 transgenic mice. Similarly, transgene expression did not alter the percentage of CD4\(^-\)8\(^-\) precursors defined by CD25 and CD44 expression (Fig. 2C). Although the relative frequency of thymocyte subsets in cyclin D1 transgenic mice was apparently normal, the absolute number of thymocytes in each subset was greatly increased compared with age-matched littermates (Tables I and II). Interestingly, the number of splenic T cells increased ∼1.5 fold and the T:B cell ratio was reversed in 8- to 12-wk-old cyclin D1 transgenic mice (Table III). The minimal elevation in splenic T cell number contrasts with the extensive (∼11-fold) increase in thymocyte cellularity but is consistent with earlier studies demonstrating the existence of a strict homeostatic mechanism controlling the size of the peripheral T cell population (18, 19).

To determine whether the cyclin D1 transgene modified positive and/or negative selection, cyclin D1 transgenic mice were crossed with mice containing a transgenic TCR specific for the H-Y Ag presented by H-2D\(^b\) MHC molecules. The cyclin D1 transgene conferred a hyperplastic phenotype when coexpressed with the H-Y TCR transgene (data not shown). However, as shown in Fig. 3, the cyclin D1 transgene did not interfere with the enhanced positive selection of CD4\(^+\)8\(^-\) thymocytes typically observed in H-Y TCR transgenic H-2D\(^b\) females (20). Furthermore, cyclin D1 transgenic thymi support efficient negative selection. Thus, despite an increase in thymocyte cellularity due to expression of the cyclin D1 transgene, the majority of thymocytes in male H-Y TCR/cyclin

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**Table I. Cellularity of thymocyte subsets in cyclin D1 transgenic and nontransgenic mice**

<table>
<thead>
<tr>
<th>Age (wk)</th>
<th>No. of Mice</th>
<th>CD4(^+)8(^-)</th>
<th>CD4(^+)8(^+)</th>
<th>CD4(^-)8(^-)</th>
<th>CD4(^-)8(^+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontransgenic</td>
<td>4–6</td>
<td>6</td>
<td>0.4 ± 0.1</td>
<td>9.6 ± 1.9</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>Cyclin D1 transgenic</td>
<td>4–6</td>
<td>7</td>
<td>1.2 ± 0.1</td>
<td>27.3 ± 3.3</td>
<td>4.2 ± 0.6</td>
</tr>
<tr>
<td>Nontransgenic</td>
<td>8–12</td>
<td>10</td>
<td>0.3 ± 0.06</td>
<td>6.6 ± 0.8</td>
<td>1.5 ± 0.6</td>
</tr>
<tr>
<td>Cyclin D1 transgenic</td>
<td>8–12</td>
<td>8</td>
<td>3.04 ± 0.5</td>
<td>67.2 ± 8.2</td>
<td>12.3 ± 2.4</td>
</tr>
</tbody>
</table>

\(^{a}\) Mean ± SEM.
Table II. Comparison of CD4<sup>+</sup> CD8<sup>+</sup> CD3<sup>+</sup> subsets in cyclin D1 transgenic and nontransgenic mice

<table>
<thead>
<tr>
<th>Age (wk)</th>
<th>No. of Mice</th>
<th>CD4&lt;sup&gt;+&lt;/sup&gt; CD25&lt;sup&gt;-&lt;/sup&gt;</th>
<th>CD4&lt;sup&gt;+&lt;/sup&gt; CD25&lt;sup&gt;+&lt;/sup&gt;</th>
<th>CD4&lt;sup&gt;+&lt;/sup&gt; CD25&lt;sup&gt;-&lt;/sup&gt;</th>
<th>CD4&lt;sup&gt;+&lt;/sup&gt; CD25&lt;sup&gt;+&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontransgenic</td>
<td>4–6</td>
<td>3</td>
<td>1.7 ± 0.3</td>
<td>4.8 ± 1.4</td>
<td>14.8 ± 3.1</td>
</tr>
<tr>
<td>D1 transgenic</td>
<td>4–6</td>
<td>3</td>
<td>6.5 ± 2.1</td>
<td>12.7 ± 1.6</td>
<td>50.7 ± 7.0</td>
</tr>
<tr>
<td>Nontransgenic</td>
<td>8–12</td>
<td>8</td>
<td>2.5 ± 0.8</td>
<td>4.9 ± 0.9</td>
<td>8.8 ± 1.5</td>
</tr>
<tr>
<td>D1 transgenic</td>
<td>8–12</td>
<td>6</td>
<td>15.2 ± 5.8</td>
<td>54.1 ± 7.4</td>
<td>80. ± 15.5</td>
</tr>
</tbody>
</table>

* Mean ± SEM.

D1 double transgenic thymi express a CD4<sup>+</sup>8<sup>+</sup> phenotype indicative of an active negative selection process (Fig. 3). Taken together, these results demonstrate that the expanded TEC compartment in cyclin D1 transgenic mice is capable of supporting normal thymocyte development and selection.

Enforced cyclin D1 expression in K8<sup>+</sup> TEC precursors is not sufficient to induce TEC differentiation in the absence of T cell lineage commitment

As a consequence of the hCD3e transgene-induced block in thymocyte development, hCD3e transgenic mice contain severely hypoplastic thymi with prominent cysts and a poorly organized epithelium consisting predominantly of K8<sup>+</sup> TECs (10, 11). To determine whether enforced cyclin D1 expression would reverse thymic hypoplasia and promote TEC differentiation, the cyclin D1 transgene was crossed onto the hCD3e transgenic background. Gross examination revealed that cyclin D1 expression induced only slight enlargement of the thymus, although the hCD3e/cyclin D1 double transgenic thymus tended to be less cystic than age-matched hCD3e single transgenic thymi (data not shown). Fig. 4A shows that enforced cyclin D1 expression induced an increase in the frequency of DNA-synthesizing cells detected by BrdU incorporation. The increase in cycling cells was accompanied by an increased frequency of apoptotic cells in double transgenic thymi, suggesting that enforced expression of cyclin D1 couples cell cycle progression and cell death pathways (21, 22). Moreover, the cyclin D1 transgene failed to induce substantial differentiation of the TEC compartment (Fig. 4B). Thus, in contrast to the Rag-1<sup>-/-</sup> cortex which consists primarily of K8<sup>+</sup>5<sup>+</sup> TECs, the K8<sup>+</sup>5<sup>+</sup> subset predominates in both hCD3e and hCD3e/cyclin D1 thymi. Importantly, thymocytes from hCD3e/cyclin D1 double transgenic mice remain blocked at the CD4<sup>+</sup>8<sup>+</sup>44<sup>+</sup>25<sup>-</sup> stage (data not shown) indicating that expression of the cyclin D1 transgene in TECs does not abrogate the T cell developmental block imposed by the hCD3e transgene. Taken together, these data underscore the fact that TEC differentiation from a K8<sup>+</sup>5<sup>+</sup> stage to a K8<sup>+</sup>5<sup>+</sup> stage is dependent on T cell lineage commitment and development beyond the CD4<sup>+</sup>8<sup>+</sup>44<sup>+</sup>25<sup>-</sup> stage (10).

Table III. Splenic cellularity in cyclin D1 transgenic and nontransgenic mice

<table>
<thead>
<tr>
<th>Age (wk)</th>
<th>No. of Splenocytes ($\times 10^7$)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>n&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Ratio T:B</th>
<th>n&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontransgenic</td>
<td>4–6</td>
<td>6.5 ± 0.7</td>
<td>7</td>
<td>0.53</td>
</tr>
<tr>
<td>D1 transgenic</td>
<td>4–6</td>
<td>8.5 ± 1.5</td>
<td>4</td>
<td>0.69</td>
</tr>
<tr>
<td>Nontransgenic</td>
<td>8–12</td>
<td>6.0 ± 0.4</td>
<td>7</td>
<td>0.56</td>
</tr>
<tr>
<td>D1 transgenic</td>
<td>8–12</td>
<td>10.0 ± 1.1</td>
<td>9</td>
<td>1.23</td>
</tr>
</tbody>
</table>

* Mean ± SEM.

<sup>a</sup> Number of mice analyzed.

Enforced cyclin D1 expression in hCD3e TECs expands the developmental window for induction of normal thymic histogenesis

Previous studies have shown that reconstitution of hCD3e transgenic mice with nontransgenic bone marrow cells can restore thymic architecture, intrathymic T cell maturation and peripheral T cell function, but only if normal hemopoietic progenitors are transplanted within a narrow developmental period (up to 8 days after birth) (13, 14). Attempts to reconstitute adult hCD3e transgenic mice with normal bone marrow progenitors failed to restore normal thymic architecture and resulted in aberrant intrathymic T cell development, the accumulation of activated peripheral T cells and the induction of IBD (13, 23). To determine whether expression of the cyclin D1 transgene affects the developmental window within which the hCD3e thymic epithelium is responsive to induction of differentiation by normal hemopoietic progenitors, thymi from adult hCD3e or hCD3e/cyclin D1 transgenic mice were transplanted under the kidney capsule of athymic nude recipients (Fig. 5A).

Each of seven nude mice that received adult hCD3e thymic transplants developed a wasting syndrome associated with severe diarrhea and colonic enlargement typical of IBD (Fig. 5B). None of these mice survived beyond 12 wk after receiving a hCD3e thymic graft. Histological analysis revealed marked alterations in the large intestine including severe mucosal thickening, distorted crypt architecture, infiltration of the mucosa and submucosa, with a mixed inflammatory exudate and formation of abscesses and granulomatous lesions (Fig. 5C). These changes were generally restricted to the large intestine although inflammatory changes were also noted in the small intestine of mice that developed IBD within 8 wk after transplantation. In contrast, recipients of adult hCD3e/cyclin D1 transgenic thymic grafts remained healthy with no overt symptoms of IBD. Histological evaluation as late as 5 mo
after transplantation revealed minimal alteration in the gastrointestinal tract. There was no apparent thickening of the mucosa, although moderate mononuclear infiltration of the submucosa was noted in some animals. These minor alterations were not restricted to the large intestine but were observed throughout the entire gastrointestinal tract and seemed to regress in older animals.

Fig. 6 shows that adult hCD3e transgenic thymic grafts recovered from nude recipients retained an abnormal thymic architecture reflecting an aberrant epithelial compartment composed predominantly of K8+5− TECs. Consistent with previous reports, the hCD3e transgenic thymic microenvironment generated peripheral T cells that displayed an activation phenotype. Thus, as shown in Fig. 7A, CD4+ lymph node T cells recovered from nude mice 8–12 wk after transplantation of hCD3e transgenic thymic grafts expressed diminished levels of CD62L and elevated levels of CD44, CD25, and CD69. In striking contrast, the hCD3e/cyclin D1 thymi recovered from nude recipients revealed restoration of apparently normal thymic architecture, including clearly distinguishable cortical and medullary regions containing numerous thymocytes (Fig. 6). Staining for keratin expression demonstrated that, similar to the pattern in normal adult thymus, the reconstituted adult hCD3e/cyclin D1 thymic cortex consisted predominantly of K8+5− TECs, and the medullary regions contained the previously described subsets (10). Furthermore, generation of differentiated cortical and medullary epithelia in the hCD3e/cyclin D1 thymi was accompanied by the development of normal relative frequencies of DN, DP, and SP thymocyte subsets (Fig. 7B). Consistent with these findings, Fig. 7A shows that the majority of peripheral T cells recovered 5 mo after transplantation of adult hCD3e/cyclin D1 thymi expressed a naive rather than an activated phenotype.

**Discussion**

Inductive interactions between thymocytes and TECs are required for the maturation of both cell types (1–3). Although numerous investigations have provided insight into the intricacies of T cell developmental progression, the processes governing TEC development generally remain obscure. Using a combination of keratin expression patterns, surface markers, and lectin binding to identify TEC subsets and determine their lineage relationships, we previously identified a K8+5− subset that contains precursors that generate the major cortical K8+5− population in a process dependent on T cell lineage commitment (10). The present report extends the analysis of TEC development by examining the consequences of expressing a cyclin D1 transgene in K8+5− TECs. Four major findings have emerged from this investigation: 1) expression of a
cyclin D1 transgene in K8\(^{+}\)K5\(^{+}\) TEC precursors results in expansion of both cortical and medullary TEC subsets; 2) expansion of the thymic epithelial compartment is accompanied by increased production of phenotypically and functionally normal thymocytes; 3) transgenic expression of cyclin D1 in K8\(^{+}\)K5\(^{+}\) TECs does not induce their differentiation to the K8\(^{+}\)K5\(^{-}\) stage in the absence of T lineage-committed precursors, reaffirming that TEC maturation depends on inductive interactions with thymocytes; and 4) enforced expression of cyclin D1 in K8\(^{+}\)K5\(^{+}\) TECs expands the developmental window during which TEC progenitors are capable of responding to thymocyte-derived inductive signals that promote normal thymic development.

Although both cortical and medullary TECs were greatly expanded in cyclin D1 transgenic thymi, there was a disproportionate

FIGURE 5. Enforced cyclin D1 expression in hCD3\(\varepsilon\) TECs inhibits the development of IBD in nude recipients of adult hCD3\(\varepsilon\)/cyclin D1 transgenic (TG) thymic grafts. A. The representative thymic graft from a hCD3\(\varepsilon\)/cyclin D1 double transgenic donor (right) shows gross evidence of reconstitution and expansion compared with a representative thymic graft from a hCD3\(\varepsilon\) transgenic donor (arrow). B. The representative colon obtained from a nude recipient of a hCD3\(\varepsilon\) transgenic thymus (above) is greatly enlarged compared with the colon from a nude recipient of a hCD3\(\varepsilon\)/cyclin D1 double transgenic thymus. C. Histological analysis of colons from nude recipients of hCD3\(\varepsilon\) vs hCD3\(\varepsilon\)/cyclin D1 transgenic thymic grafts. Tissue sections of colon were analyzed by hematoxylin and eosin staining.

FIGURE 6. Enforced cyclin D1 expression in hCD3\(\varepsilon\)/cyclin D1 thymic grafts permits reconstitution of thymic architecture and development of cortical and medullary TEC subsets. Frozen 5-\(\mu\)m thymic sections obtained from hCD3\(\varepsilon\) transgenic or hCD3\(\varepsilon\)/cyclin D1 double transgenic (TG) thymic grafts recovered 10 wk after transplantation under the kidney capsule of nude recipients. The sections were stained with hematoxylin and eosin (H&E) for analysis of thymic architecture or with Abs to K8 and K5 followed by appropriate second-step reagents as previously described.

FIGURE 7. Phenotypic analysis of T cells recovered after grafting hCD3\(\varepsilon\) transgenic or hCD3\(\varepsilon\)/cyclin D1 double transgenic thymi under the kidney capsule of nude recipients. A. Flow cytometric analysis of activation markers on CD4\(^{+}\) lymph node cells from nude recipients of hCD3\(\varepsilon\) transgenic (○) or hCD3\(\varepsilon\)/cyclin D1 double transgenic (○) thymic grafts. Peripheral lymph node cells were stained with anti-CD8-FITC, anti-CD4-PE, and biotinylated Ab to either CD25, CD44, CD62L, or CD69. Binding of biotinylated Abs was detected with APC-SA. An electronic gate was set to analyze the expression of activation markers on the CD4\(^{+}\)8\(^{-}\) subset. B. Flow cytometric analysis of thymocytes from a hCD3\(\varepsilon\)/cyclin D1 double transgenic thymic graft recovered 12 wk after transplantation.
increase in the K8 \(^{5+}\) subset that is typically concentrated at the corticomedullary junction. The K8 \(^{5+}\) population is likely to be a heterogeneous population consisting of epithelial cells at various maturation stages each of which may be differentially affected by enforced cyclin D1 expression. In the skin, the basal epithelial population contains stem cells, characterized by a high self-renewal capacity, and their immediate progeny, transit amplifying cells, that produce daughter keratinocytes which undergo terminal differentiation (24–26). Whereas epidermal stem cells divide infrequently in vivo, the transit amplifying subset contains a relatively high percentage of dividing cells. By analogy, the K8 \(^{5+}\) TEC subset may consist of stem cell progenitors as well as transit amplifying-like cells that differentiate to produce cortical and medullary TECs. Expression of the cyclin D1 transgene in K8 \(^{5+}\) TECs could expand the epithelial compartment by promoting stem cell proliferation and/or lowering a signaling threshold required for differentiation of stem cells to transit amplifying-like cells. Alternatively, or in addition, transit amplifying-like cells may undergo additional rounds of replication before differentiating or become refractory to growth-inhibitory signals in the cyclin D1 transgenic mice. Either scenario is consistent with the well-recognized role that cyclin D1 plays in regulating G1 progression in epithelial cells by activating cyclin-dependent kinases that phosphorylate retinoblastoma (Rb) family proteins (reviewed in Ref. 27). Cyclin D1 also can act as a transcriptional regulator independently of its ability to inactivate Rb. For example, cyclin D1 activates the estrogen receptor in a ligand-independent manner via recruitment of transcriptional coactivators (28).

Regardless of the mechanism that governs the disproportionately increase in K8 \(^{5+}\) TECs, it is important to note that cyclin D1 transgene expression alone is not sufficient to support the differentiation of K8 \(^{5+}\) progenitors to K8 \(^{5+}\) progeny. These data suggest that TEC differentiation requires inducible signals from T cell lineage committed thymocytes. Thus, TECs from hCD3\( ^{e}\)/cyclin D1 double transgenic mice remain blocked at the K8 \(^{5+}\) developmental stage resembling the predominant TEC subset in hCD3\( ^{e}\) transgenic mice (10). TECs in the double transgenic mice fail to undergo normal differentiation and expansion despite the increase in DNA synthesizing cells that occurs as a consequence of cyclin D1 expression. This apparent dichotomy may be explained by the finding that the cyclin D1 transgene also increases the fraction of apoptotic cells in double transgenic thymi. Previous studies have shown that overexpression of cyclin D1 can induce apoptosis in various cell types (21, 22). Thus, during normal thymic development, thymocytes and TEC interactions may regulate the TEC developmental program by activating epithelial proliferation via a cyclin D1-dependent pathway and simultaneously inducing intracellular signals that block a potentially apoptotic pathway.

Although enforced cyclin D1 expression is not permissive for TEC maturation when thymocyte development is blocked at the CD4\(^^{8}\) CD8\(^{44} CD25\) stage, the cyclin D1 transgene does expand the developmental window during which K8 \(^{5+}\) progenitors respond to hematopoietic-derived signals that induce TEC differentiation and normal thymic histogenesis. Transplantation of adult hCD3\( ^{e}\) transgenic thymi under the kidney capsule of nude recipients not only failed to restore TEC differentiation but also resulted in the generation of aberrantly activated peripheral T cells associated with development of fatal IBD. In contrast, adult hCD3\( ^{e}\)/cyclin D1 transgenic thymic transplants were capable of supporting TEC development and thymocyte differentiation. Moreover, the peripheral T cell compartment in recipients of adult hCD3\( ^{e}\)/cyclin D1 thymic transplants did not display activation markers and the mice did not succumb to IBD. It is not clear why induction of TEC differen-

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