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Critical Involvement of Tcf-1 in Expansion of Thymocytes

Marco W. Schilham, Anne Wilson, Petra Moerer, Barry J. Benaissa-Trouw, Ana Cumano, and Hans C. Clevers

T cell maturation in Tcf-1−/− mice deteriorates progressively and halts completely around 6 mo of age. During fetal development thymocyte subpopulations seem normal, although total cell numbers are lower. By 4 to 6 wk of age, obvious blockades in the differentiation of CD4−8− thymocytes are observed at two distinct stages (CD44+25+ and CD44−25−), both of which are normally characterized by extensive proliferation. This lack of thymocyte expansion and/or differentiation was also observed when Tcf-1−/− progenitor cells from the aorta-gonad-mesonephros region (embryonic day 11.5), fetal liver (embryonic day 12.5/14.5), and fetal bone marrow (embryonic day 18.5) were allowed to differentiate in normal thymic lobes (fetal thymic organ cultures) or were injected intrathymically into normal recipients. Despite these apparent defects in thymocyte differentiation and expansion, adult Tcf-1−/− mice are immunocompetent, as they generate virus neutralizing Abs at normal titers. Furthermore, their peripheral T cells have an activated phenotype (increased CD44 and decreased CD62L expression) and proliferate normally in response to Ag or mitogen, suggesting that these cells may have arisen from the early wave of development during embryogenesis and are either long lived or have subsequently been maintained by peripheral expansion. As Tcf-1 is a critical component in the Wnt/β-catenin signaling pathway, these data suggest that Wnt-like factors play a role in the expansion of double-negative thymocytes. The Journal of Immunology, 1998, 161: 3984–3991.

T he differentiation of hemopoietic stem cells into mature lymphocytes occurs throughout life and serves as an accessible model system to study differentiation. In the thymus, the various stages of T cell differentiation can be defined by the differential expression of numerous surface markers and intracellular genes (1, 2). As individual precursors progress along this developmental pathway two important processes occur. First, functional genes encoding Ag receptors (TCR) are assembled by rearrangement of germline gene segments for which the recombination activating genes Rag-1 and Rag-2 are essential (3–5). Second, extensive proliferation of selected precursor populations occurs at defined stages of the developmental pathway to generate a large repertoire of receptors (6). This latter process is thought to be driven by a complex combination of gene expression, soluble factors, and cell-cell interactions with components of the thymic microenvironment.

The murine thymus is seeded around embryonic days 10 to 11 (E10−11) by pluripotent progenitor cells that give rise to a wave of thymocytes and mature T cells, most of which have left the thymus 2 to 3 wk after birth (7). The fetal thymus increases exponentially in size from E11 to birth and up to 2 to 3 wk of age.

[ Abbreviations used in this paper: E, embryonic day; SP, single positive; DN, double negative; ISP, immature single positive; DP, double positive; FTTC, fetal thymic organ culture; PE, phycoerythrin; AGM, aorta-gonad-mesonephros; SFV, Semliki Forest virus. ]
stage and is thought to be mediated by signals through the pre-TCR complex (12). This complex, consisting of a functional TCR β-chain, the pTα molecule (22), and the CD3 chains, is expressed on immature thymocytes from DN3 through DN4, ISP to the DP stage (23, 24), where it is replaced by a mature TCR complex, containing a functional α-chain instead of pTα.

The ordered expression of all important genes in these differentiating cells is thought to be controlled through transcriptional regulation (25). The T cell-specific transcription factor, Tcf-1, is one factor implicated in this process. Tcf-1 is a T cell-specific DNA-binding nuclear protein that has been cloned and studied in our laboratory (26, 27). The DNA binding domain of Tcf-1 is a so-called HMG box, shared by many members of a recently identified gene family (28). Several members of this family have been shown to be involved in developmental processes in mammals (29–33). Although Tcf-1 expression is widely distributed in the embryo (34), its expression is confined to immature and mature T cells after birth. It is expressed in all thymocyte subpopulations, including the earliest, DN1, and represents the first definitive T-lineage marker (35, 36). Mice lacking a functional Tcf-1 gene develop normally, but their thymus start to age prematurely as a partial blockade in adult T cell development becomes gradually apparent after birth. This blockade occurs at the ISP stage when small, noncycling cells accumulate and the subsequent population of DP cells decreases dramatically in size, resulting in reduced numbers of total thymocytes. Although the thymus appears relatively normal until birth, fetal progenitors from various sources were found to be unable to develop properly upon transfer into normal fetal thymic organ cultures (FTOC) or adult thymus. The results show that Tcf-1−/− progenitors do not differentiate in normal numbers, probably because they fail to expand properly.

Materials and Methods

Mice

Mice were kept at the transgenic mouse facility of the central laboratory for experimental animals, University of Utrecht (Utrecht, The Netherlands). C57BL/6 (Charles River, Iffa Credo, France) and C57BL/Ly-5.1 (Ly-5.1; The Jackson Laboratory, Bar Harbor, ME) mice were purchased from PharMingen (San Diego, CA). CD4 (129-19), CD8 (53/6.7) (37), TCRβ (H-57/597) (38), CD25 (PC61), CD24 (MI/ 69), B220 (RA3-6B2), Mac-1 (M1/70.15), Gr-1 (RB6-8C5), and Ly-5.1 (A20) specific hybridomas were grown, and supernatant was collected and supplemented medium (Opti-MEM, Life Technologies) for 14 days. Lobes for Ly-5.1 were analyzed.

FACS sorting and DNA analysis

Individual thymi from normal or Tcf-1−/− mice were stained for four-color fluorescence with the following Abs: a mixture of FITC conjugates as described above to eliminate CD4+8+3′ aβ6+8β+ mature T cells, B cells granulocytes, and macrophages; CD44-PE, CD25-Red613 (Life Technologies, Grand Island, NY); and CD24-biotin followed by streptavidin-allophycocyanin (Molecular Probes, Leiden, The Netherlands). FACS sorting was performed using a FACStar+ (Becton Dickinson) by gating first on FITC− CD24+ cells (immature CD4+ CD8+ CD3−) and then for the four populations defined by CD44 and CD25 expression. Fifty thousand cells of each population were sorted from both normal and Tcf-1−/− thymi. Propidium iodide in Nonidet P-40 detergent was added to each sample, and DNA analysis was performed as previously described (35) on a FACSscan using the doublet discrimination module. Control populations were total thymus and lymph node.

Irradiation chimeras

Ly-5.1 mice were lethally irradiated (9.5 Gy), and within 24 h bone marrow cells (Ly-5.2) were injected i.v. as a cell suspension. All un.injected and 20 % of injected mice died after 12 to 18 days. Host-derived cells were distinguished by staining with an Ly-5.1-specific Ab (A20). Donor cells always contributed >90% of the myeloid cells in the blood after 3 wk.

Fetal thymic organ cultures

Thymic lobes were dissected from E14.5 or E15.5 Ly5.1 embryos and irradiated (30 Gy). Individual lobes were cultured for 48 h in hanging drop cultures (Terasaki plates) together with aorta-gonad-mesonephros (AGM) cells (E11.5, one embryo equivalent per lobe) or fetal liver cells (E14.5, 2 × 105/lobe). Subsequently, the lobes were transferred to floating filters (0.8 μm pore size; Nucleopore polycarbonate, Costar, Cambridge, MA) in supplemented medium (Opti-MEM, Life Technologies) for 14 days. Lobes were teased apart individually and stained with mAbs. Only cells negative for Ly-5.1 were analyzed.

Intrathymic injections

Ly-5.1 mice (2–4 mo old) were sublethally irradiated (7.5 Gy) and anesthetized. The thorax was opened, and fetal cell suspensions were injected into the thymus (4 × 106 to 1 × 107 cells/10 μl). The thorax was closed immediately, and thymocyte subpopulations were analyzed 3 wk later by flow cytometry.

Stimulation assays

Spleen cells were isolated, counted, and stimulated in 96-well plates (4 × 104, 2 × 105, and 1 × 106/well) with Con A (2.5 μg/ml). For stimulation with alloantigen, irradiated spleen cells (1 × 105/well) were added to the responder cells (6 × 105, 2 × 105, and 6 × 105/well). After 3 days, [3H]thymidine (1 μCi/well) was added, and cells were harvested 18 h later. For cytotoxicity assays, 1 × 106/ml spleen cells were stimulated with 5 × 106/ml stimulator cells (CBA) in 24-well plates. After 6 days, cells were harvested and counted, and cytotoxic activity was tested against 51Cr-labeled Con A blasts (C57BL/6, H-2b and CBA, H-2k) in a 4-h assay according to standard procedures.

Virus immunization

BALB/c mice, Tcf-1+/−, and Tcf-1−/− mice were bled (preimmune sera) and immunized with 1000 plaque-forming units avirulent Semliki Forest virus (SFV) (39) s.c.. Two weeks later, blood was collected again, and titers of virus-neutralizing Abs were determined by a virus neutralization assay (40). Titers of SFV-specific Abs of various subclasses were determined by ELISA (41).

Results

Rapid involution of the adult thymus with age

We have previously reported (35) that thymi of 2-mo-old Tcf-1 (exon VII)-deficient mice are very small compared with thymi of age-matched normal mice and have a partial blockade in T cell development at the ISP stage. However, the differences between Tcf-1−/− and normal control mice (Tcf-1+/− and Tcf-1+/+, phenotypic differences between heterozygote or wild-type mice were never observed) were less apparent when the mice were only 2 to 3 wk old. This observation raised the question of how thymopoiesis was affected at different ages. To investigate this, thymi from Tcf-1−/− embryos and mice of different ages were analyzed in more detail. The results show that the thymic phenotype, as defined by CD4 and CD8 expression, was similar in normal and Tcf-1−/− E18.5 embryos (Fig. 1). However, total numbers of thymocytes were about fourfold lower, in Tcf-1-deficient E18.5 embryos compared with controls (Fig. 1). This difference in total number of thymocytes was apparent as early as E15.5 (0.14 × 106
for control vs \(0.095 \times 10^6\) for Tcf-1\(^{-/-}\) thymus) and became greater with increasing age (Fig. 1). By day 10 after birth, there were changes in the distribution of CD4 and CD8 expression, most notably a decrease in the percentage of DP cells (Fig. 1). These effects became even more pronounced with age, such that 4- to 8-mo-old mice typically contain around \(1 \times 10^6\) thymocytes and are completely devoid of DP cells, indicating a total lack of ongoing differentiation. Immature DN cells were still present, as were a small number of mature SP (TCR\(^{+}\)) cells that probably represent the remnants of differentiation occurring at a younger age or may have re-entered the thymus from the periphery.

Absence of cycling cells in Tcf-1-deficient thymi

In Tcf-1\(^{-/-}\) mice DP thymocytes fail to develop from ISP thymocytes, which, unlike their counterparts in normal thymi, are not in cycle (35). As a consequence, there is a relative accumulation of ISPs in thymi of 1- to 2-mo-old mice, although absolute numbers are not very different from those in control mice (Fig. 1). The total disappearance of ISPs in Tcf-1\(^{-/-}\) mice by 6 mo after birth suggests that an even earlier block in T cell development may be present. Therefore, the DN compartment in young and old thymi (10–20%) of 6-mo-old mice, the subset DN3 was completely absent. The most mature DN subpopulation, DN4 (CD44\(^{+}\)/CD25\(^{+}\)), was still present at 1 mo of age but was missing at 6 mo of age (Fig. 2).

In Tcf-1\(^{-/-}\) mice, ISP thymocytes have been shown to be small, resting cells, in contrast to their counterparts in normal mice, which are predominantly in cycle (11, 42). To determine whether this lack of cycling cells in Tcf-1-deficient mice was already evident in the DN populations, we isolated each of the four subsets described above by FACS sorting and determined the DNA content by propidium iodide staining. In normal mice, the DN2 and DN4 populations contain 25 and 39% of cells in G2/S/M phase of the cell cycle, as previously reported (14, 43), whereas the remaining populations DN1 and DN3 have only around 8% of cycling cells (Fig. 3). By contrast, in the absence of Tcf-1 none of the DN subsets isolated had any significant proportion of cells in cycle. DN2 cells from Tcf-1\(^{-/-}\) mice could not even be analyzed because they were completely absent (Fig. 2), and only 3% of DN4 cells from Tcf-1\(^{-/-}\) mice were in G2/S/M phase compared with 39% in the same population isolated from normal mice (Fig. 3).

Taken together, these results show that within the DN compartment of Tcf-1\(^{-/-}\) thymus, subpopulations that normally contain cycling cells are either lacking (DN2 at all ages after birth, DN4 in older animals) or contain fewer cells in cycle (DN4). This suggests that an important aspect of the Tcf-1\(^{-/-}\) defect might be due to a lack of expansion. Differentiation could proceed normally but be accompanied by such a low rate of proliferation that the presence of cells in the proliferating subsets (DN2 and DN4) is not observed, while accumulation of cells in the other subsets (DN3 and DP) still occurs.

**FIGURE 1.** Thymi from Tcf-1\(^{-/-}\) mice contain fewer CD4\(^{+}\)/CD8\(^{+}\) DP cells and increased proportions of ISPs with increasing age. Thymi were isolated from E18.5, 10-day-old, 1-mo-old, 2-mo-old, and 6-mo-old Tcf-1\(^{-/-}\) mice as well as from wild-type mice. Cells were counted and stained with CD4\(^{+}\), CD8\(^{+}\), and TCR\(^{+}\)-specific Abs. Flow cytometry results for CD8 (horizontal) and CD4 (vertical) are shown. Percentages of CD8\(^{+}\) TCR\(^{+}\) ISP cells were determined, and absolute cell numbers were calculated. The total number of thymocytes (×10⁸), percentage of ISPs, the total number of ISPs (×10⁶), and SDs are given below each panel. Values were determined from individual mice or, for E18.5, per individual litter and expressed as cell number per thymus.
Lack of Tcf-1 affects lymphocytes and not stromal cells
The inability of Tcf-1$^{−/−}$ T cell precursors to differentiate into mature T cells could be due to an inherent defect in the hemopoietic precursors themselves or to defects in the thymic stroma or microenvironment. Therefore, Tcf-1-deficient bone marrow cells (Ly-5.2) were injected i.v. into lethally irradiated normal (C57BL/6.Ly-5.1) hosts, and the thymi of these chimeras were analyzed 2 mo after reconstitution. While control bone marrow fully reconstituted host thymi with both immature and mature T cells could be due to an inherent defect in the hemopoietic precursors themselves or to defects in the thymic stroma or microenvironment. Therefore, Tcf-1-deficient bone marrow cells (Ly-5.2) were injected i.v. into lethally irradiated normal (C57BL/6.Ly-5.1) hosts, and the thymi of these chimeras were analyzed 2 mo after reconstitution. While control bone marrow fully reconstituted host thymi with both immature and mature T cells, thymi reconstituted with Tcf-1-deficient bone marrow had 100-fold fewer cells, 90% of which were of host origin (Fig. 4, A and B). Similar results were obtained when Tcf-1$^{−/−}$ fetal liver cells were used as donor cells (data not shown). Reciprocal experiments in which normal bone marrow was injected into Tcf-1$^{−/−}$ hosts demonstrated that Tcf-1-deficient thymi are able to support normal T cell development (data not shown), as reconstitution occurred normally. Taken together, these experiments demonstrate that the Tcf-1$^{−/−}$ stromal environment is capable of supporting thymocyte differentiation and that the phenotype observed in Tcf-1$^{−/−}$ mice is due to a defect in the hemopoietic compartment.

Fetal progenitors do not yield mature T lymphocytes after transfer
As the expansion of Tcf-1$^{−/−}$ thymocytes appeared to be affected in adult mice more than in mutant embryos, the differentiative potential of fetal progenitors was investigated in transfer experiments. E18.5 fetal bone marrow cells or E12.5 fetal liver cells from Tcf-1-deficient or normal embryos were injected intrathymically into sublethally (7.5 Gy) irradiated Ly-5.1 hosts. While progenitors from both organs of normal embryos successfully reconstituted the thymus 3 wk after transfer, no thymocytes of donor origin were detected in mice reconstituted with Tcf-1-deficient precursors (Fig. 4, C–F).

Failure of the Tcf-1$^{−/−}$ fetal progenitors to develop could be due to incompatibility with the adult microenvironment. Therefore, fetal liver (E14.5) progenitor cells from Tcf-1$^{−/−}$ embryos were allowed to differentiate in a normal fetal environment in FTOC. Also under these conditions Tcf-1$^{−/−}$ cells were unable to differentiate into DP or SP thymocytes (Fig. 5). As the first progenitors that colonize the embryonic thymus are derived from the AGM region around E10–11, we investigated whether cells from the AGM of Tcf-1$^{−/−}$ embryos could give rise to T cells after transfer to a normal fetal environment in FTOC. It was observed that Tcf-1$^{−/−}$ cells did not differentiate into significant numbers of CD4$^+$CD8$^+$ DP cells, while control AGM did (Fig. 5). Some γδ T cells did arise in the lobes reconstituted with Tcf-1$^{−/−}$ AGM cells, confirming that the lobes were indeed seeded by progenitor cells. It has been reported that the development of γδ T cells is only moderately dependent on the presence of Tcf-1 (44). Together, these data indicate that although the fetal Tcf-1$^{−/−}$ thymus appears to contain reduced numbers of normal thymocytes, the observed defect in differentiation of Tcf-1$^{−/−}$ cells is present in the earliest precursor cells (AGM) of the mutant embryo.

Tcf-1$^{−/−}$ peripheral T cells function normally and express an activated phenotype
Despite the absence of Tcf-1, mature T cells accumulate in the peripheral lymphoid organs. Peripheral T lymphocyte numbers are about two- to threefold lower than those in control mice, but their level remains stable with increasing age (35). When analyzed phenotypically, Tcf-1-deficient mature T cells were shown to express...
markers typical of cells with an activated phenotype. Naive T cells recently produced by the thymus normally do not express CD44, while peripherally expanded activated T cells do (45). Analysis of lymph node cells of Tcf-12/2 mice revealed an increase in the proportion of CD44+ cells, most pronounced in the CD8+ subset (Fig. 6). This effect became more obvious with increasing age (data not shown), indicating a lack of CD8+ T cell production by the thymus. Correspondingly, a higher proportion of CD4+ T cells in Tcf-12/2 mice lack the surface marker CD62L (Mel-14; Fig. 6) that is normally expressed at high levels on most mature T cells and is only down-regulated on activated cells.

The impaired ability of Tcf-12/2 thymocytes to expand raised the question of whether mature Tcf-1-deficient T lymphocytes can proliferate normally in response to mitogenic or antigenic stimuli. To study whether activation and subsequent expansion of mature Tcf-1-deficient T cells was affected, splenocytes from normal or Tcf-1-deficient mice were stimulated with Con A. On a per T cell basis, Tcf-12/2 splenocytes proliferated in response to Con A (Fig. 7A) and alloantigen (Fig. 7B) to a level equivalent to that in normal splenocytes. In addition, the cytolytic activity of Tcf-12/2 alloantigen-specific CTLs at fixed E:T cell ratios was the same as that of normal or heterozygote mice (Fig. 7C). Finally, Thy cell function in Tcf-12/2 mice was normal, as determined in vivo by quantitation of circulating IgG Abs after infection with SFV. Tcf-12/2 mice produced high titers of virus neutralizing Abs, comparable to or higher than those obtained in heterozygous and BALB/c mice (Table I). Taken together, these results show that although the numbers of peripheral T cells in Tcf-12/2 mice are low, their responsiveness is normal.
The results described in this report extend our earlier observations on thymocyte differentiation in the absence of Tcf-1 (35). Tcf-1 appears to be required primarily for the expansion of thymocytes, because numbers of thymocytes are lower than those in control mice and because blockades in differentiation are at stages when proliferation normally occurs.

During thymocyte development there are two stages at which extensive proliferation is observed. The first occurs just before rearrangement of the TCRβ-chain at the DN2 (CD441251) stage, while the second occurs after surface expression of the pre-TCR (post-DN3) and requires a functional TCRβ-chain. This latter expansion phase involves the DN4 (CD442252) and ISP subsets that rapidly transit through to the DP stage, where TCRα is rearranged and expressed. From the experiments in this study it can be concluded that Tcf-1−/− thymocytes have an impaired capability to expand at both these stages.

The second blockade in differentiation cannot easily be explained by the absence of pre-TCR components, as pTα, CD3ε, and TCRβ were all expressed at relatively normal levels (data not shown). The first observed block in Tcf-1−/− thymocyte differentiation occurs just before or at the time at which TCRβ gene rearrangement commences (DN2). To our knowledge, this is the earliest block in T cell differentiation by mutation of a T cell-specific gene. The only other examples of such an early block are found in mice transgenic for the human CD3ε gene (46), which is

Table I. Titers of SFV-specific Abs in Tcf-1−/− mice

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<th>Mouse Strain</th>
<th>Neutralizing Serum Titer</th>
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<tr>
<td></td>
<td></td>
<td>IgM</td>
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<tr>
<td>Tcf-1+/+</td>
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<td>BALB/c</td>
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* Mice were immunized with 1000 plaque-forming units of an avirulent strain of SFV and bled 2 wk later. Mean neutralizing titers of serum were tested in a virus neutralization assay. Titers of SFV-specific Abs of various subclasses were determined by ELISA according to a method described for influenza virus-specific Abs.
copy number dependent and may be due to sequestration of downstream signaling molecules rather than to a direct effect of CD3, and in mice that are unable to signal via both the IL-7/IL-7R pathway and the c-kit/stem cell factor pathway (20). Earlier blocks in lymphocyte development have been described (47, 48), but these mutations result in the absence of all lymphocytes (T, B, and NK cells).

Although the blockades in differentiation of postnatal and adult thymocytes are very clear, a similar lack of differentiation was not very obvious in thymi of the developing Tcf-1(VII)−/− embryos or when the Tcf-1(VII)−/− thymi were cultured in FTOC (49). However, after transfer to a normal environment (in FTOC or after intrathymic injection), Tcf-1(VII)−/− progenitors do not differentiate as their control counterparts. The reason for this observation is unclear. One possibility is that after transfer, reconstitution depends on the expansion of fewer progenitors than when seeding occurs under physiologic conditions. A relative inability to expand of the Tcf-1(VII)−/− progenitors could become more obvious under such restrictive conditions. A comparable situation was recently observed (V. Korinek, N. Barker, E. van Donselaar, G. Huls, P. J. Peters, and H.C.C., unpublished observations).

From the described experiments it seems clear that the expansion of Tcf-1(VII)−/− thymocytes is affected. At the DN4/ISP stage, this appears to be due to a lack of cycling cells. At the DN2 stage it is not clear whether cell cycle progression or cell survival (or both) is impaired. It was recently reported that thymus-specific inactivation of the GTPase, Rho, affected development of thymocytes in a very similar way to that reported here (50), as both the CD25+ (DN2/3) as well as the DN4 subpopulations were affected. It was demonstrated that, on the one hand, while Rho is required for survival but not expansion of the DN2/3 thymocytes, it is required for cell cycle progression but not survival of the later DN4 cells, on the other hand (51).

Due to the lack of adult T lymphopoiesis in the absence of Tcf-1, the peripheral T cell compartment in adult Tcf-1(VII)−/− mice is derived mostly from fetal thymocytes. The number of T lymphocytes in lymphoid organs and blood of Tcf-1(VII)−/− mice is lower than that in normal mice, and the phenotype of these cells is generally that of activated T cells (CD8+CD44+ and CD4+Mel-14+). This is similar to the phenotype observed in the periphery of thymectomized mice (52), where the peripheral T cell pool appears to be maintained by peripheral expansion (45). Functionally, the peripheral T cells appear indistinguishable from normal T cells, as while seeding during maturation of thymocytes appears to be limited, expansion of mature Tcf-1(VII)−/− T lymphocytes in response to mitogenic/antigenic stimuli is unaffected. This suggests that the molecular processes required for proliferation of thymocytes and T lymphocytes are distinct, as previously reported (53).

Recently, it has been shown that Tcf-1 (and Lef-1) can associate with β-catenin (54–56). β-Catenin associates with the cadherin family of cellular adhesion molecules and provides the link between gap junctions and the actin cytoskeleton. A role for E-cadherin in thymus development has been suggested (57). Anti-E-cadherin Abs inhibit thymic reaggregation and interfere with the seeding and/or maturation of fetal liver derived progenitors in deoxyguanosine-treated thymic lobes. β-Catenin is also a key component of the Wnt/Wingless signaling pathway. In this function, β-catenin is present in the cytoplasm, usually in complex with the tumor suppressor protein adenomatous polyposis coli (58). Wnt/β-catenin/Tcf signaling occurs in systems as diverse as Drosophila segment polarity, Xenopus axis formation, and colon tumorigenesis (54–56, 58–61). Molecularly, the function of β-catenin in cellular adhesion resides in a different part of the protein than its function in Wnt signaling. In Drosophila it has been demonstrated that although cadherins associate with armadillo (the Drosophila homologue of β-catenin) from the same pool as that used in the wingless signaling pathway, wingless does not specify cell fate by modulating cell-cell adhesion (62). The specific defect in differentiation of thymocytes suggests a direct role of Tcf/Lef transcription factors downstream of β-catenin in a signaling cascade crucial for DN thymocyte expansion. Whether Tcf-1 exerts this role in transduction of signals from a Wnt-like factor or in cellular adhesion events (or both) remains uncertain at the present. Nevertheless, the present results raise the possibility that Wnt-like factors are required for normal thymopoiesis.


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