Growth of Epithelial Cells in the Thymic Medulla Is Under the Control of Mature T Cells

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Summary

Epithelial cells in the thymic medulla are conspicuous in normal adult mice, but sparse in the early fetal thymus and the thymus of adult T cell–deficient SCID mice. To examine whether growth of medullary epithelial cells (MEC) depends upon local contact with mature T cells, we used the finding that the SCID thymus is unusually permeable to mature T cells entering from the bloodstream. When SCID mice received multiple injections of mature lymph node T cells from birth, the thymus accumulated large numbers of mature TCR⁺ T cells of resting phenotype, but contained virtually no immature (CD4⁺8⁺) cells. The injected T cells localized in the medullary region of the thymus and led to marked regeneration of MEC. These and other data suggest that the growth of MEC is under the control of mature T cells. Placing MEC under T cell control might be a device for regulating the size and integrity of the medulla, especially during the phase of rapid thymic growth. Maintaining the cellular components of the medulla in proper balance could be critical for ensuring efficient self tolerance induction.

In a recent study we demonstrated that the epithelial component of the thymic medulla consists of at least two phenotypically distinct subsets of cells (1). One subset of medullary epithelial cells (MEC) expresses a high density of typical MHC (H-2) class I and II molecules and stains with the fucose-specific lectin *Ulex europeus* agglutinin-1 (UEA-1) (2). These MHC⁺ UEA-1⁺ cells show little or no expression of I-O molecules, a class of atypical nonpolymorphic MHC-encoded class II molecules present on B cells (3). The other subset of MEC is largely MHC⁻ UEA-1⁻, but shows high I-O expression. This applies in adults: in the fetal thymus, the majority of MEC express both markers.

MEC are prominent in the normal adult thymus, but are sparse in the early fetal thymus (1, 4) and also in the atrophic thymus of T cell–deficient SCID mice (5). Since TCR⁺ cells are rare in the early fetal thymus and are almost undetectable in the SCID thymus, growth of MEC might require the presence of TCR⁺ cells. In support of this idea it has been shown that restoring TCR⁺ cells to the SCID thymus by reconstituting the host with nondefective stem cells leads to regeneration of MEC (5).

The apparent capacity of TCR⁺ cells to promote the growth of MEC could be an indirect consequence of positive and/or negative selection of thymocytes. Conversely, epithelial cell growth might simply require contact with mature T cells, as most of the T cells in the normal medulla are mature rather than immature. To investigate this second possibility, we took advantage of the observation that, unlike the normal thymus (6), the SCID thymus is permeable to circulating mature T cells. As documented in this paper, injecting SCID mice intravenously with purified mature resting T cells leads to a considerable accumulation of these cells in the host thymus. Significantly, T cell migration to the SCID thymus results in substantial regeneration of each of the two subsets of MEC. The data thus support the notion that growth of MEC is under the control of mature T cells.

Materials and Methods

Mice. C57BL/6-Thy-1.1 (B6.PL), BALB/c, (BALB/c × B6.PL)F₁, and C.B-17 SCID mice were obtained from the Scripps Research Institute.

Reagents. The origin of the following mAbs has been described elsewhere (6–9): 6C3 (anti-cortical epithelial cells), J11d (anti-HSA), H57-597 (anti-αβ TCR), 19E12 (anti-Thy-1.1), YTS-169 (anti-CD8), GK1.5 (anti-CD4), MEL-14 (anti–L-selectin), and 23G2 (anti-CD45RB). We also used mAb 1.M.7.8.1 (anti-CD44) (10), affinity-purified I-O (Oβ)-specific K507 Ab (3), and lectin UEA-1 (anti-MEC) (2).

Reconstitution of SCID Mice. For single injections of T cells, nylon-wool (NW)-purified LN T cells were injected intravenously into young adult SCID mice. For multiple T cell injections, neonatal SCID mice received 2 × 10⁷ NW T cells at birth followed by three weekly injections of 2–5 × 10⁷ cells intraperitoneally, and then a final intravenous injection of 4 × 10⁷ cells. The mice were analyzed 1 wk after the last injection. For BM reconstitution, adult SCID mice were exposed to 200 rad and then injected with T-depleted (1) BALB/c bone marrow cells intravenously.

Immunohistology. As described previously (1), dried and acetone-fixed cryostat sections were incubated with optimal dilutions of biotinylated Ab (or lectin) followed, after washing, with horse-radish peroxidase-conjugated streptavidin (HRP-SA) (1). For mAb 6C3, sections were incubated with culture supernatant followed by biotinylated anti–rat IgG (1) followed by HRP-SA. Sections were then incubated with the substrate 3-amino-9-ethylcarbazole, and mounted after counterstaining with Meyer's hematoxylin.

FACS® Analysis. As described elsewhere (9), cell suspensions were incubated with biotinylated H57-597 or 19E12 mAb followed by FITC-conjugated streptavidin (Jackson ImmunoResearch Laboratories, West Grove, Pa) plus PE-labeled GK1.5 and propidium iodide (2 μg/ml). Anti-FcR mAb was used to block background staining. To detect activation markers, three-color staining was performed as follows: cells were stained with one of the rat mAbs (I.M.7.8.1, 23G2, or Mel-14) followed by FITC anti–rat IgG, rat serum, biotinylated H57-597, and R613-streptavidin (Gibco BRL, Gaithersburg, MD) plus PE-conjugated GK-1.5. Cells were analyzed on a FACScan® flow cytometer (Becton Dickinson & Co., Mountain View, CA) (9).

Results

To test whether mature T cells control the growth of MEC, we constructed a SCID thymus containing mature but not immature T cells. This approach relies on the finding that mature T cells enter the SCID thymus after intravenous injection. T cell homing to the SCID thymus has not been reported previously and is documented below.

Migration of Mature T Cells to the SCID Thymus. We showed previously that homing of mature T cells to the normal adult thymus after intravenous injection is very limited and is restricted to activated T cells (6). To study T cell homing to the SCID thymus, C.B-17 (Thy-1.2) SCID mice were injected intravenously with a dose of 4 × 10^7 resting T cells (NW-passed LN T cells) taken from Thy-1-marked (BALB/c × B6.PL)F_1 (Thy-1.2 × Thy-1.1) mice. As shown in Table 1, Exp. 1, migration of these mature T cells to the host thymus was clearly significant. Thus, when the host thymus was removed 1 d later for FACS® analysis, about 0.9% of unseparated thymocytes expressed the Thy 1 marker of the donor cells. By contrast, T cell homing to the thymus of control normal BALB/c mice given the same dose of T cells was almost undetectable (0.02%) (Table 1, Expt. 1 and Fig. 1 a).

T cell homing to C.B-17 SCID mice was also demonstrable by injecting H-2-identical normal BALB/c T cells. Since the normal SCID thymus is devoid of TCR$^+$ cells (<0.05%; Fig. 2 c) (tested in six mice), the presence of donor T cells in the host thymus could be assessed simply by enumerating TCR$^+$ cells. As shown in Table 1, Expts. 2 and 3, and Fig. 1 e, unseparated thymocytes from SCID mice injected with 4 × 10^7 BALB/c T cells 1 d before contained about 1% TCR$^+$ cells.

In sections, the TCR$^+$ cells in the thymus of T cell–injected SCID mice were easily detectable and formed focal accumulations in the pseudo-medullary regions (Fig. 1 f). TCR staining of the thymus of uninjected SCID mice was negative (Fig. 1 d). For control BALB/c mice injected with Thy-1-marked T cells, sections of the host thymus revealed only very occasional donor Thy-1$^+$ cells (Fig. 1 b).

The phenotype of the thymic immigrants in SCID mice

Table 1. *Homing of Mature T Cells to the Thymus of SCID Mice after Intravenous Injection*

Exp.	No. of cells injected intravenously	Donor → host	Days post-injection	Donor cells detected by expression of:	Mean percent donor cells in Thymus (cell number)	Spleen
	Single injection into adults					
1	4 × 10^7 LN T (NW)	(BALB/c × B6.PL)F_1 → BALB/c	1	Thy-1.1	0.02 (0.06 × 10^4)*	3.1
	4 × 10^7 LN T (NW)	(BALB/c × B6.PL)F_1 → SCID	1	Thy-1.1	0.86 (1.72 × 10^4)	25.3
2	4 × 10^7 LN T (NW)	BALB/c → SCID	1	$\alpha\beta$ TCR	1.45	18.4
3	4 × 10^7 LN T (NW)	BALB/c → SCID	1	$\alpha\beta$ TCR	1.29	16.3
	1 × 10^7 LN T (NW)	BALB/c → SCID	1	$\alpha\beta$ TCR	0.41	1.7
	Multiple injections from birth					
4	220 × 10^6 LN T (NW)	BALB/c → SCID	7	$\alpha\beta$ TCR	63.6	47.3
5	220 × 10^6 LN T (NW)	BALB/c → SCID	7	$\alpha\beta$ TCR	55.9	27.9

T cells were injected intravenously as described in Materials and Methods. The host mice were killed 1–7 d after the last injection of T cells. Single cell suspension from thymus and spleen were stained for either Thy-1.1 or $\alpha\beta$ TCR and analyzed on a FACScan® (see Materials and Methods). The very low level of nonspecific staining, determined in each experiment by analyzing an age-matched uninjected mouse, has been subtracted from the data shown. All experiments were performed with groups comprising two to three mice. Each mouse was analyzed individually.
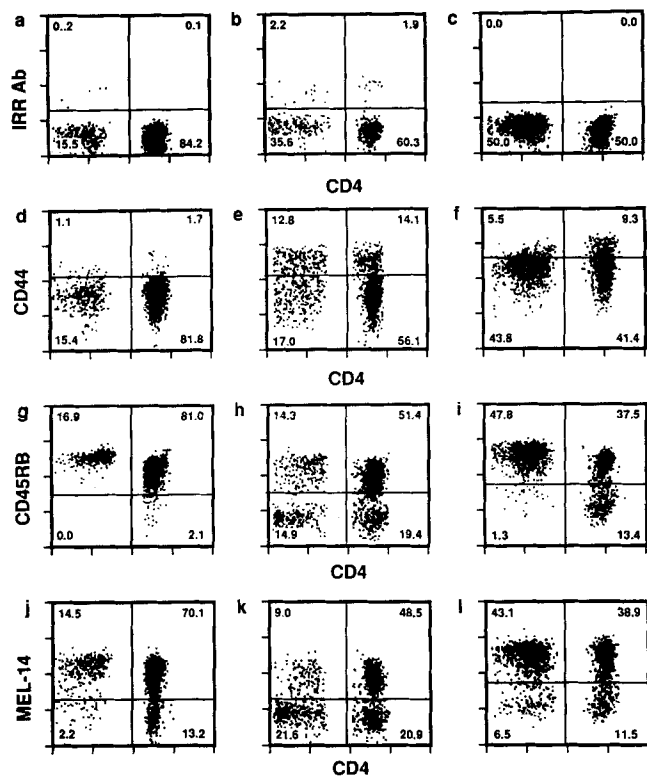* Mean number of donor cells detected; the total number of donor cells that reached the thymus is shown in parentheses. Thymocytes from BALB/c mice were treated with Jlld mAb + C before staining to remove immature T cells and thus enrich for the (mature) donor cells.
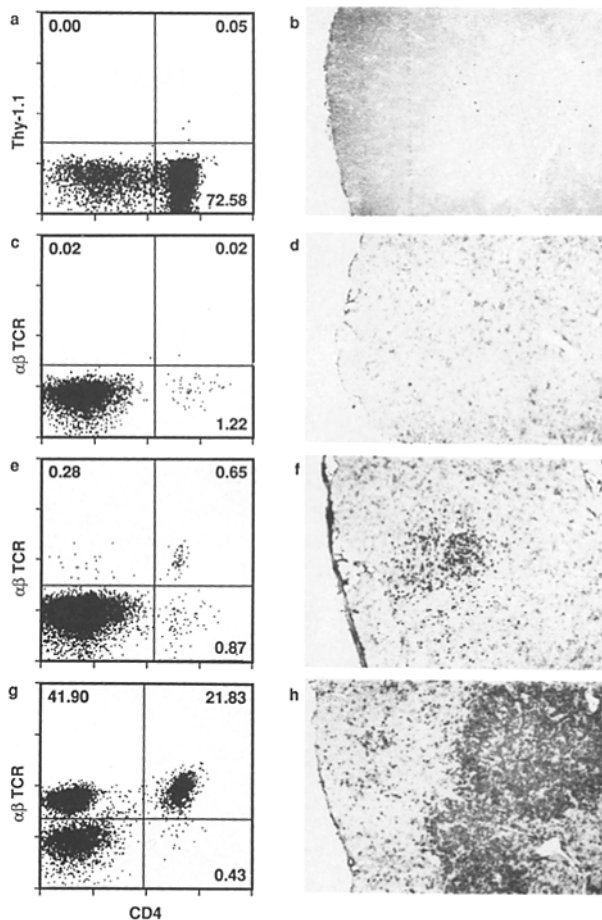
Figure 1. Detection of intravenously injected donor T cells in the thymus of normal vs. SCID mice by FACS® analysis and staining of cryostat sections. (a and b) Thymus of BALB/c mouse (Thy-1.2) injected 1 d before with 4 × 10⁷ NW-passed (BALB/c × B6.PL)F₁ (Thy-1.2 × Thy-1.1) LN cells stained for Thy-1.1⁺ cells. Donor cells are virtually undetectable by either FACS® analysis of Jlld⁻ thymocyte suspensions (background staining has not been subtracted) or by staining cryostat sections. Note that the scattered granular cells in the medulla are cells with endogenous peroxidase activity. (c and d) Thymus of uninjected SCID mouse stained for αβ TCR. No staining is evident except for some weak diffuse background staining in sections. (e and f) αβ TCR staining of thymus of SCID mouse injected 1 d before with 4 × 10⁷ NW-passed BALB/c LN cells. TCR⁺ cells are detectable in cell suspensions and, in sections, form focal clusters. (g and h) αβ TCR staining of thymus of SCID mouse given multiple injections totaling 2.2 × 10⁸ NW-passed BALB/c LN T cells from birth. TCR⁺ cells comprise a conspicuously high percentage of (unseparated) SCID thymocytes and, in sections, are mostly localized in the central (medullary) area of the thymus. All sections were counterstained with hematoxylin and photographed at ×100.

Figure 2. Surface phenotype of TCR⁺ cells recovered from thymus of SCID mice given either single or multiple injections of BALB/c T cells. Two experiments are shown. In the first experiment, the host mice received a single intravenous dose of 4 × 10⁷ NW-passed LN T cells: the LN (a, d, g, and j) and thymuses (b, e, h, and k) were removed from the hosts 1 d later and, using three-color staining, the TCR⁺ cells were stained for expression of CD4 vs. Pgp-1 (CD44), CD45RB or MEL-14, using an irrelevant (IRR) mAb as a negative control. In the second experiment, the host mice received multiple injections of BALB/c NW-passed LN T cells from birth. TCR⁺ cells from thymus (c, f, i, and l) were then stained for CD4 vs. the above markers. For each experiment, cell suspensions were prepared from two to three mice/group and pooled for staining. The slight difference in the intensity of staining in the two experiments reflects that the experiments were not assayed on the same day.

injected with BALB/c T cells is shown in Fig. 2. For CD4⁺ cells (detected by three-color staining), it is evident that most of the donor TCR⁺ cells that reached the thymus (Fig. 2, b, e, h, and k), as well as LN (Fig. 2, a, d, g, and j), of the host were Pgp-1ˡᵒ, CD45RBʰⁱ, MEL-14ʰⁱ, and thus displayed a virgin/resting phenotype. For CD8⁺ cells, the thymus migrants included both resting T cells and memory/activated (Pgp-1ʰⁱ, CD45RBˡᵒ, and MEL-14ˡᵒ) cells.

The above data refer to T cell migration to the SCID thymus

measured within 1 d of T cell injection. In the experiments shown in Table 1, Expts. 4 and 5, four C.B-17 SCID mice were given weekly injections of NW-passed BALB/c LN T cells from birth. Each mouse received a total of around 2 × 10⁸ T cells. At 5 wk of age, the thymus was moderately enlarged and contained 5 × 10⁶ cells (relative to 10⁶ cells in uninjected SCID mice). Suspensions of these thymuses consisted predominantly of TCR⁺ cells (55–65%) (Fig. 1 g). The remaining TCR⁻ cells were CD4⁻8⁻ and were presumably of SCID origin. Immature CD4⁺8⁺ TCRˡᵒ cells were very rare (<2%) (not shown). With regard to the TCR⁺ cells, three-color staining revealed that most of these cells expressed a resting phenotype (Fig. 2, c, f, i, and l); this applied to both CD4⁺ and CD8⁺ cells. It is interesting that CD8⁺ cells were often the major subset (50–70%) of the TCR⁺ cells in the thymus (Figs. 1 g and 2 c). In sections, TCR⁺ cells were largely restricted to the central (medullary) region of the thymus with only scattered TCR⁺ cells in the cortex (Fig. 1 h).
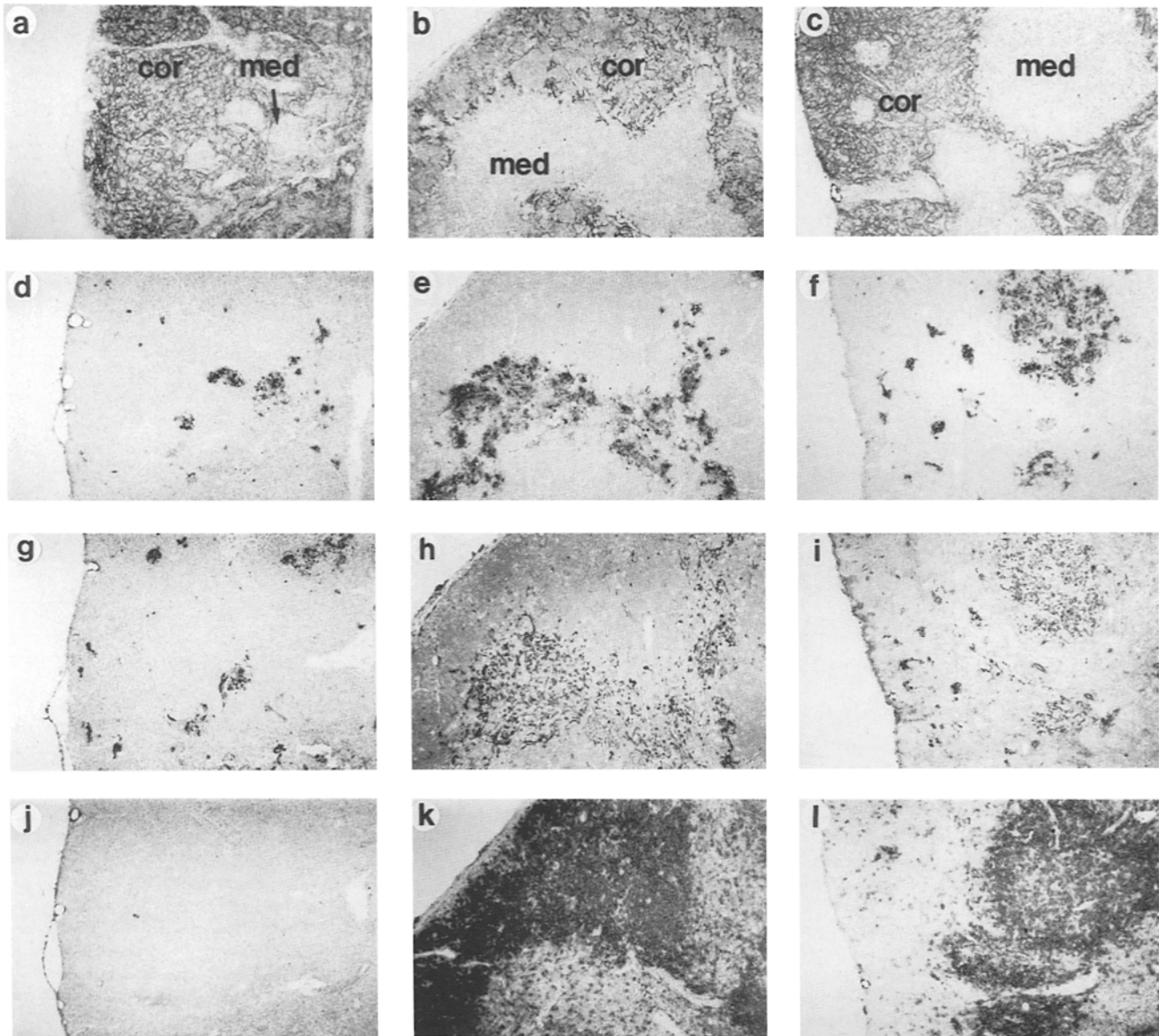
Figure 3. T cell control of the growth/expansion of medullary epithelial cells in SCID mice. Serial sections were stained; sections were lightly counterstained with hematoxylin. (*a*) 6C3 expression in normal SCID thymus. There is strong reticular staining of epithelial cells in the collapsed cortex. Unstained (medullary) areas are sparse. (*b*) 6C3 expression in the thymus of SCID mouse reconstituted with normal BALB/c BM. Staining pattern resembles the normal thymus (1) with reticular staining of cortical epithelium and large unstained areas of medulla. (*c*) 6C3 expression in thymus of SCID mouse given multiple injections of BALB/c LN T cells. Cortex is condensed and shows strong staining. Unstained areas of medulla are prominent. (*d*) UEA-1 expression in normal SCID thymus. Only sparse aggregates of stained cells are evident. Stained areas correspond to the areas of 6C3⁻ medulla (compare with *a*). (*e*) UEA-1 expression in thymus of BM → SCID mouse. Staining of the medulla is much more extensive than in the normal SCID thymus (compare with *d*). (*f*) UEA-1 staining of LN T → SCID mice. As in BM → SCID mice, staining of the medulla is much more extensive than in the normal SCID thymus. (*g*) I-O expression in normal SCID thymus. Only sparse aggregates of stained cells are evident. (*h*) I-O expression in thymus of BM → SCID mouse. There is extensive staining of the medulla. (*i*) I-O expression in thymus of LN T → SCID mouse. There is extensive staining of the medulla. (*j*) CD8 expression in normal SCID thymus. No staining is evident. (*k*) CD8 expression in thymus of BM → SCID mouse. There is strong staining of the cortex (presumably of CD4⁺8⁺ cells), and only scattered staining of the cortex (presumably of CD4⁻8⁺ cells). (*l*) CD8 expression in thymus of LN → SCID mouse. In marked contrast to BM → SCID mice, there is only scattered staining of the cortex (presumably indicating a paucity of CD4⁺8⁺ cells) and strong staining of the medulla (immigrant CD4⁻8⁺ cells). All sections were photographed at ×100.

The above findings document that giving multiple intravenous injections of mature T cells to SCID mice generates a thymus containing only mature TCR⁺ T cells with virtually no immature CD4⁺8⁺ cells. The histology of the medullary region of thymus is discussed below.

Histology of the Thymus of T Cell–injected SCID Mice. As mentioned earlier, the thymus of normal adult SCID mice is very small and contains only around 10^6 thymocytes, nearly all of which are CD4⁻8⁻TCR⁻. Shores et al. (5) reported that the SCID thymus consists largely of ER-TR4⁺

cortical epithelial cells (CEC) with very few ER-TR5$^+$ MEC (5). Staining with other reagents specific for CEC vs. MEC is shown in Fig. 3, a, d, and g. It can be seen that staining with the CEC-specific mAb, 6C3, extends throughout most of the SCID thymus with only small central areas of 6C3$^-$ tissue. These 6C3$^-$ pseudo-medullary areas contain only sparse collections of UEA-1$^+$ (Fig. 3 d) and I-O$^+$ (Fig. 3 g) MEC.

The thymus of SCID mice reconstituted with nondefective BALB/c BM cells contains high numbers of typical TCR$^+$ thymocytes (>10^8). In sections, the thymus has a prominent 6C3$^-$ medulla (Fig. 3 b) with large collections of UEA-1$^+$ cells (Fig. 3 e) and I-O$^+$ (Fig. 3 h) cells. Similar regeneration of MEC in BM-injected SCID mice was reported previously for ER-TR5 expression (5).

As mentioned earlier, the thymus of SCID mice given multiple injections of mature T cells from birth is moderately enlarged. In sections, the thymus contains a large 6C3$^-$ medulla (Fig. 3 c) and thus superficially resembles the thymus of stem cell-injected SCID mice. Staining for CD8 expression, however, reveals a striking difference. Thus, whereas CD8 expression in the thymus of stem cell-injected SCID mice is most prominent in the cortex (Fig. 3 k) (indicative of immature CD4$^+$8$^+$ cells), CD8$^+$ cells in the thymus of T cell-injected mice are packed in the medulla with only scattered cells in the cortex (Fig. 3 l). This finding complements the data on TCR expression (Fig. 1 h), and indicates that the thymus immigrants in T cell-injected SCID mice localize almost exclusively in the medulla. The important finding is that migration of these mature T cells to the medulla causes a marked regeneration of MEC. Thus, in contrast to the normal SCID thymus, the thymus of T cell-injected SCID mice contains dense accumulations of UEA-1$^+$ cells (Fig. 3 f) and I-O$^+$ cells (Fig. 3 i).

Discussion

This paper makes two points. First, the atrophic thymus of SCID mice is unusually permeable to T cells entering from the circulation. Second, migration of mature T cells to the thymus causes regeneration of MEC.

The capacity of mature resting-phenotype T cells to migrate to the SCID thymus is surprising because homing of resting T cells to the thymus of normal adult mice is virtually undetectable (6). The possibility that T cell entry to the SCID thymus is simply a reflection of thymic atrophy is unlikely because inducing atrophy of the normal thymus with steroids does not enhance T cell homing to the thymus (6). With regard to other possibilities, it is interesting that the failure of resting T cells to migrate to the normal thymus does not apply in the neonatal period (Surh, C., J. Sprent, and S. Webb, manuscript in preparation). Indeed, in terms of permeability to circulating T cells, the thymus of adult SCID mice closely resembles the normal neonatal thymus. These data raise the possibility that the thymus of neonatal

mice and adult SCID mice share certain vascular addressins (11) that are not represented in the thymus of normal adult mice. Recognition of these addressins might be essential for resting T cells to enter the thymus. This question is currently under investigation.

To achieve prominent T cell reconstitution of the SCID thymus, it was necessary to give multiple injections of mature T cells from birth. This regime produced a thymus containing a high proportion of mature TCR$^+$ T cells (55–65%), but virtually no immature CD4$^+$8$^+$TCR^{lo} cells. The immigrant TCR$^+$ T cells were excluded from the cortex and localized almost exclusively in the medulla. The key finding was that restoring mature T cells to the medulla caused a marked regeneration of MEC. This applied to both the UEA-1$^+$ and I-O$^+$ subsets of MEC. In contrast to the severe atrophy of MEC in the normal SCID thymus, the distribution of MEC in the thymus of T cell–reconstituted SCID mice was extensive and quite similar to the thymus of BM-reconstituted mice. This finding provides strong support for the idea that growth of MEC in the normal thymus is under the control of mature T cells.

The notion that growth of MEC depends on contact with mature T cells is in agreement with the finding that selective elimination of mature T cells from the thymus by treatment with cyclosporine or total lymphoid irradiation leads to disappearance of the medulla and dramatic atrophy of MEC (12, 13). In these two situations, the thymus consists almost entirely of immature CD4$^+$8$^+$ cells. The possibility that immature T cells contribute to the growth of MEC thus seems unlikely. Whether γ-δ TCR$^+$ cells play a role in MEC growth is unknown, although it is worth noting that cyclosporine treatment does not eliminate mature γ-δ^+ T cells from the thymus (14). γ-δ^+ T cells were undetectable in the T cell–reconstituted thymuses used in this paper (not shown).

How mature T cells control the growth/expansion of MEC is obscure. The class of T cells involved (CD4$^+$ or CD8$^+$ cells) has not been studied, and it is unclear whether T cells act by making direct contact with epithelial cells or cause local release of growth-promoting cytokines. Further experiments will be needed to resolve these issues. It will be especially important to establish why MEC are under T cell control. What evolutionary pressures could have shaped this dependency? Prevention of traffic congestion in the medulla is a possibility. Since T cells leave the thymus via the medulla, efficient release of T cells from the growing thymus presumably depends on the medulla undergoing expansion to accommodate the increasing numbers of T cells reaching the medulla from the cortex. Failure of the medulla to grow in parallel with the cortex might lead to T cell congestion in the medulla, and thereby compromise T cell exit to the periphery. Such congestion might also impair T cell contact with blood-borne APC in the medulla, and thereby interfere with tolerance induction. Placing the growth of stromal cells in the medulla under T cell control would avoid these potential problems.

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