Stepwise development of thymic microenvironments in vivo is regulated by thymocyte subsets

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SUMMARY

T-cell development is under the tight control of thymic microenvironments. Conversely, the integrity of thymic microenvironments depends on the physical presence of developing thymocytes, a phenomenon designated as ‘thymic crosstalk’. We now show, using three types of immunodeficient mice, i.e. CD3ε transgenic mice, RAGnull mice and RAGnull-bone-marrow-transplanted CD3ε transgenic mice, that the control point in lymphoid development where triple negative (CD3ε-,CD4-,CD8-) thymocytes progress from CD44+CD25- towards CD44-CD25+, influences the development of epithelial cells, critically inducing the extra, third dimension in the organization of the epithelial cells in the cortex. This tertiary configuration of the thymic epithelium is a typical feature for the thymus, enabling lymphostromal interaction during T-cell development. Crosstalk signals at this control point also induce the formation of thymic nurse cells. Moreover, our data indicate that establishment of a thymic cortex is a prerequisite for the development of the thymic medulla. Thus, differentiating thymocytes regulate the morphogenesis of thymic microenvironments in a stepwise fashion.

Abbreviations: RAGnull, mice deficient for the recombinase activation gene; tgr26, transgenic mice expressing the human CD3ε gene; TN, triple negative (CD3ε-,CD4-,CD8-) thymocyte; DP, double positive (CD4+,CD8+) thymocyte; SP, single positive (CD4+, resp. CD8+) thymocyte; TCR, T-cell receptor; MHC, major histocompatibility complex

Key words: Thymus, Microenvironment, Crosstalk, Epithelium, Lymphostromal interaction, Mouse, RAGnull

INTRODUCTION

The thymus is a primary lymphoid organ crucially involved in the differentiation of T lymphocytes. Its stroma creates several distinct microenvironments, which control defined steps in T-cell development. Thymic microenvironments are composed of a network of epithelial reticular cells, present in both the cortex and medulla (van Ewijk, 1988, 1991). Non-epithelial stromal cells, such as fibroblasts, macrophages and interdigitating reticular cells are integrated in this network (Boyd et al., 1993). In adult mice, progenitor T cells derived from the bone marrow enter the thymic stroma at the border between cortex and medulla and migrate, while differentiating, through distinct microenvironments in the subcapsular cortex, the deep cortex, the corticomedullary junction and the medulla (Kydewski et al., 1987; van Ewijk, 1991; Anderson et al., 1996). During T-cell development, thymocytes interact with thymic stromal cells within these microenvironments. Lymphostromal interaction induces proliferation and ensures that a T-cell repertoire is selected, reactive to foreign epitopes but tolerant to self epitopes.

It has been shown, on the basis of avidity of the interaction between T-cell receptors (TCR) on the surface of thymocytes and peptide-MHC complexes expressed on stromal cells, that the fate of the developing thymocytes is determined by the thymic stroma (Ashton-Rickardt and Tonegawa, 1994). Thus, epithelial cells in the cortex mediate positive selection (Anderson et al., 1993; Bevan, 1997; de Koning et al., 1997; Jameson and Bevan 1998; reviewed by Laufer et al., 1999), whereas interdigitating cells and epithelial cells in the medulla mediate negative selection of developing T cells (Sprent et al., 1988; Lo et al., 1997; Laufer et al., 1999). The net balance between these two processes apparently determines the thymic output of selected T cells (Anderson et al., 1998).

Because of its embryological origin, the thymus differs from secondary lymphoid organs. While secondary lymphoid organs are mesodermal in origin, the thymus is ectodermal and endodermal in origin, explaining the epithelial nature of thymic stromal cells (Owen and Jenkinson, 1984). Importantly, the organization of the epithelial cells in the thymus differs completely from that in other (non-lymphoid) organs. Rather than forming a sheet of cells positioned on a basement membrane, thymic epithelial cells form a three-dimensional (3-D) sponge-like meshwork of elongated cytoplasmic extensions,
Here, we identify subsets of developing T cells involved in the establishment of thymic microenvironments in the cortex and the medulla. By transplanting tge26 mice with RAGnull bone marrow, we show that progression in T-cell development from TN CD44+CD25+ towards TN CD44-CD25+ stimulates the conversion of 2-D organized epithelial sheets into a 3-D organized epithelial network. Subsequent transplantation of these (RAGnull→tge26) chimeric mice with wild-type bone marrow cells enables normal thymopoiesis and is paralleled by correct organization of medullary microenvironments. Our data indicate that maturing thymocytes control the development of thymic stroma in a stepwise fashion, resulting first in the induction of cortical microenvironments, followed by the establishment of medullary microenvironments.

MATERIALS AND METHODS

Mice
tge26 mice (Wang et al., 1995) were maintained through sib breeding in the animal facility of the Beth Israel Hospital. RAG-2null mice were purchased from Jackson Laboratories (Bar Harbor, Maine).

Antibodies
All antibodies used in the present study are indicated in Table I.

Bone marrow transplantation
Neonatal tge26 mice (2 to 4 days post partum) were injected intraperitoneally with 5×10^5 bone marrow cells derived from adult RAGnull mice in a volume of 50 μl. No precondition treatment was performed to the neonatal recipients. 5-10 weeks after the neonatal bone marrow transplantation, these mice were killed for analysis, or used for subsequent transplantation with wild-type bone marrow cells. Bone marrow transplantation to adult tge26 mice was performed as described (Holländer et al., 1995a). Basically, the adult tge26 mice (8-10 weeks old), which were injected with RAGnull bone marrow cells at birth, were pretreated with superlethal irradiation (650 rad + 450 rad). 10^7 wild-type bone marrow cells treated with two rounds of anti-Thy-1.2 + complements were injected into the tail vein of these recipient mice. Such mice are defined as WT→(R→tge26). These mice were analyzed 6-10 weeks after the second bone marrow transplantation.

Flow cytometric analysis
Single-cell suspensions of thymocytes were obtained by mechanical disruption of the thymus. Cells were first preblocked with purified anti-mouse Fc receptor antibody and then stained for three-color flow cytometric analysis as described previously (Holländer et al., 1995a).

Microscopy
For histology, and transmission and scanning electron microscopy.

Table 1. Antibodies used

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Target</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Ra3-6B2</td>
<td>B20</td>
<td>Coffman and Weisman (1981)</td>
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<tr>
<td>7D4</td>
<td>CD25</td>
<td>Malek et al. (1983)</td>
</tr>
<tr>
<td>IM7</td>
<td>CD44</td>
<td>Lesley and Trowbridge (1982)</td>
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<td>DS-1</td>
<td>IgM</td>
<td>Steckmann et al. (1984)</td>
</tr>
<tr>
<td>ER-TR 4</td>
<td>cortical epithelial cells</td>
<td>Van Vliet et al. (1994)</td>
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<tr>
<td>ER-TR 5</td>
<td>medullary epithelial cells</td>
<td>Van Vliet et al. (1984)</td>
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<tr>
<td>145-2C11</td>
<td>CD3e</td>
<td>Leo et al. (1987)</td>
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<td>H7S-597</td>
<td>TCRβ</td>
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<tr>
<td>53-2, 1</td>
<td>Thy-1, 2</td>
<td>Ledbetter and Herzenberg (1979)</td>
</tr>
<tr>
<td>RM4-5</td>
<td>CD4</td>
<td>Frederickson and Bash (1987)</td>
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<tr>
<td>53-6, 7</td>
<td>CD8</td>
<td>Ledbetter and Herzenberg (1979)</td>
</tr>
<tr>
<td>2-4G2</td>
<td>FcR</td>
<td>Unkless (1979)</td>
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mice were fixed by total body perfusion, as described previously (van Ewijk, 1988). Fixed tissues were further processed according to standard technical procedures.

**Immunohistology**

For immunohistology, tissues were immersed in OCT compound and snapfrozen in liquid nitrogen. 5 μm frozen sections were cut using a Leitz cryostat. Sections were collected on gelatin-precoated microscope slides, and stored at room temperature in a desiccator, for not longer than 3 days. Sections were stained with rat monoclonal antibodies (Table 1) and incubated with a rabbit anti-rat horse radish peroxidase conjugate (DAKO). To prevent background staining, all antibodies were optimally titrated. The conjugate was visualized by incubation with a solution of 1% diaminobenzidine.

**RESULTS**

**Thymic microenvironments in tge26 tg and RAGnull mice differ in architecture**

The thymus of young adult tge26 mice is very small in size, containing around 10^6 lymphoid cells, with no clear distinction between cortex and medulla (Fig. 1A). Interestingly, several large cysts are present within the thymic stroma (Fig. 1A,B). Epithelial cells are palely stained and do not display the characteristic network configuration observed in the thymus of wild-type mice (Fig. 1A, inset).

At the ultrastructural level, epithelial cells lack the typical long cytoplasmic extensions (data not shown) and form many thymic cysts. Such cysts contain at least three types of classical epithelial cells also found in other tissues (Fig. 1B), i.e. (1) cells with microvilli (as in the gastrointestinal tract), (2) mucin-secreting goblet cells (as in the gastrointestinal tract and in the respiratory tract), and (3) cells expressing cilia (as in the respiratory tract). Lymphoid cells in the tge26 thymus are generally small in size; they show an electron-dense nucleus while vacuoles are frequently found in the cytoplasm (data not shown). These features indicate a state of apoptosis, most probably caused by overexpression of the CD3ε gene, early in T-cell development (Wang et al., 1995).

Compared to tge26 thymi, cellularity in the RAGnull thymus has increased to 3-5×10^6 thymocytes. Lymphoid cells are generally medium-sized and actively proliferating. Furthermore, the RAGnull thymus is well vascularized and contains many capillaries (Fig. 1C). Transmission electron microscopy reveals a network of epithelial cells with extended cytoplasmic processes, interconnected through desmosomes (Fig. 1D). Most strikingly, we found a high frequency of thymic nurse cells (TNC) in the thymus of RAGnull mice, frequently associated with capillaries (Fig. 1C,D). Transmission electron microscopy reveals a network of epithelial cells with extended cytoplasmic processes, interconnected through desmosomes (Fig. 1D). Most strikingly, we found a high frequency of thymic nurse cells (TNC) in the thymus of RAGnull mice, frequently associated with capillaries (Fig. 1D). 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Type 2 chimeric mice were created by transplantation of normal bone marrow cells into type 1 chimeric mice in adult life, and are designated as WT→(R→tg26). If crosstalk operates at the level of defined thymocyte subsets, we reasoned that the type 1 chimeric mice should develop thymic microenvironments identical to those found in RAGnull mice, whereas the double-transplanted tg26 mouse should reveal a thymic phenotype comparable to normal mice.

Flow cytometric analysis of the development of thymocytes in type 1 chimeric mice is presented in Fig. 2A. Most thymocytes in control tg26 mice are Thy-1⁺ CD44⁺CD25⁻ and express B220 and IgM. In contrast, lymphocytes in the RAGnull thymus are all Thy-1⁺ and have reached to the CD44⁺CD25⁺ stage, where further maturation is blocked (Holländer et al., 1995a). Injection of tg26 mice with bone marrow cells from RAGnull mice leads to a moderate increase in cellularity (to 2.5×10⁶ cells/thymus), and a dramatic change in thymocyte phenotype. More than 90% of the cells are now Thy-1⁺, and a large majority of these cells has differentiated to the TN CD44⁺CD25⁺ phenotype, identical to that of thymocytes in RAGnull mice. Moreover, the frequency of B220⁺ cells in the tg26 thymus has decreased close to the levels found in wild-type thymi.

Immunohistological analysis of the thymus of RAGnull→tg26 mice reveals an organization of thymic stromal cells comparable to that found in the thymus of RAGnull mice (see Holländer et al., 1995a). We present here the microscopic data from one type 1 mouse, representative of the series of 21 successfully transplanted mice, showing that the two thymic lobes were differentially reconstituted.

The small right lobe (Fig. 3) was not reconstituted, and showed a stromal organization comparable to the thymus of untransplanted tg26 mice (see Holländer et al., 1995). In contrast, the medium-sized left lobe was reconstituted with donor-derived thymocytes, and displayed a stromal architecture similar to that in RAGnull mice (see van Ewijk et al., 1999).

The unreconstituted right lobe (Fig. 3A-C) shows large cysts, and the cortical epithelial cells, defined by the antibody ER-TR4, have changed their orientation. While in the normal thymus cortical epithelial cells are oriented perpendicularly towards the capsule (van Vliet et al., 1984), thus guiding migration of developing thymocytes towards the medulla, thymic epithelial cells in the unreconstituted lobe are aligned parallel to the capsule. Moreover, a well-defined medulla is lacking and ER-TR5⁺ medullary epithelial cells are found scattered throughout the thymus (Fig. 3B). The unreconstituted lobe was further characterized by a high frequency of ER-TR7⁺ fibroblasts (Fig. 3C). Within this lobe, B lymphocytes occurred at high frequency, localized especially in close proximity to thymic fibroblasts (Fig. 3D).

The left thymic lobe of the bone marrow transplanted tg26 mouse demonstrates in contrast a well-developed cortical reticulum, displaying many round to oval ER-TR4⁺ structures (Fig. 4A,B), comparable in size to TNCs found in RAGnull mice (see also Fig. 1D,E). Although small patches of ER-TR5⁺ cells are present, well-defined medullas are still lacking (Fig. 3D). Large cysts are absent and the frequency of B220⁺ cells has decreased to that found in the RAGnull thymus (Fig. 4D). The many capillaries running through the thymic stroma show that there is increased angiogenesis after reconstitution (Fig. 4A,C).

The observed discrepancies between the stromal architecture of the left and right thymic lobes indicate that, under the present experimental conditions, only a limited number of T-cell precursors repopulated the thymus, giving rise to a progeny of cells arrested at the CD44⁺CD25⁺ stage. Importantly, progression to this stage in T-cell development rescues the 3-D organization of the thymic reticulum, reduces the frequency of fibroblasts and induces a high frequency of thymic nurse cells.

Injection of RAGnull→tg26 chimeras with normal bone marrow leads to a complete restoration of the architecture of the thymic stroma

Injection of adult RAGnull→tg26 chimeras with bone marrow cells derived from normal mice restores, as expected, T-cell development completely, with thymic cellularity dramatically increasing to 1.1×10⁸ cells. Phenotypical analysis with antibodies directed to CD4, CD8 and TCRαβ shows similar patterns between the WT→(R→tg26) mice and untransplanted wild-type controls (Fig. 2B). Normal numbers of mature T cells are also present in peripheral lymphoid organs of double-transplanted mice (data not shown).

Immunohistological analysis of the thymus of double-transplanted mice shows a normal distribution of CD3⁺ thymocytes (Fig. 5A) and a complete restoration of cortical thymic microenvironments (Fig. 5B-D). In contrast to the...
thymus in RAG→tg26 chimeras, the thymus in the double-transplanted tg26 mice now also reveals expansion of the ER-TR5+ medullary epithelial cells, which have regrouped into discrete medullary areas (Fig. 5C).

Importantly, a difference in reconstitution was observed between the present double-transplanted mice and the single bone marrow transplanted adult tg26 mice, reported earlier (Holländer et al., 1995b). In the latter mice, T-cell development remained abnormal, and these mice developed a severe colitis, leading to death of the mice 5-8 weeks after bone marrow transplantation. In contrast, in the present experiments, none of seven double-transplanted mice analyzed showed any sign of colitis or other defects (data not shown).

**DISCUSSION**

Early stages in T-cell development are defined by absence of CD3, CD4 and CD8 (triple negative [TN] thymocytes). TN thymocytes can be further subdivided into four differentiation stages defined by antibodies directed to the markers CD44 (PgP-1) and CD25 (the α chain of the IL2 receptor). Thus, TN thymocytes progress subsequently from TN CD44+CD25−, TN CD44+CD25+, TN CD44+CD25+ to TN CD44+CD25−. Productive rearrangement of the TCRβ locus occurs in the TN CD44+CD25+ stage (Fehling and von Boehmer, 1997), immediately leading to the TN CD44+CD25− stage. Thymocytes in this stage of development are precursors for
CD4+, CD8+ (double positive, DP) thymocytes, which in turn develop in mature CD4+ or CD8+ (single positive, SP) thymocytes.

The two immunodeficient mouse strains used in the present study have a block in T-cell development within the window of TN thymocytes. While in tge26 mice, due to the overexpression of the human CD3ε gene, T-cell development is blocked at the TN CD44+/CD25− stage, the null mutation in the Rag locus results in a slightly later arrest in T-cell development, phenotypically defined as: TN CD44−CD25+. Apparently, this small phenotypical shift in differentiation stage has a major influence on the composition and organization of the thymic stroma. Thus, a blockade in lymphoid development at the level of TN CD44+/CD25− leads to loss of the typical organization of the epithelial cells. In the normal thymus, epithelial reticular cells are oriented perpendicular to the thymic capsule, promoting cell traffic from cortex to medulla. By contrast, in tge26 mice, epithelial cells are positioned parallel to the thymic capsule, impairing proper cell traffic through the thymus. Furthermore, the 3-D orientation of the epithelial network is replaced by a 2-D orientation in these mice, resulting in the formation of 3-D structured epithelial network, the 2-D oriented epithelium is replaced by 3-D structured epithelial network.

The induction of a high frequency of TNC in the thymus of RAGnull→tge26 mice is unexpected. Although precursors of TNC might be present in the thymus of untransplanted tge26 mice, we were unable to detect their presence, because of the low frequency of thymocytes and due to lack of a specific marker for TNC. TNC were initially described in vitro, as lymphoepithelial complexes (Wekerle et al., 1980), and their existence was later confirmed in situ (van Ewijk, 1988). The function of TNC remains to be determined; they have been implicated in the induction of thymocyte proliferation (Gao et al., 1993), in positive selection (Pezzano et al., 1996) and in the induction of apoptosis (Aguilar et al., 1997). A recent ultrastructural study shows that the thymus contains at least three different types of TNC, each with different functions (Brelinska and Warchol, 1997). The TNC in RAGnull mice resemble the described type 1 TNC, which are indeed thought to be involved in the early expansion of T-cell progenitors, since many TNC complexes in RAGnull contain dividing lymphoid cells. Proliferating cells in TNC probably represent TN thymocytes, shifting from CD44+/CD25− to CD44−CD25+. This latter subpopulation is subject to β selection (Fehling and von Boehmer, 1997), which raises the possibility that type 1 TNC control this crucial checkpoint in thymopoiesis.

The tge26 thymus is characterized by a high frequency of B lymphocytes (Takoro et al., 1998). These cells predominantly develop within the thymus and co-localize with thymic fibroblasts rather than with thymic epithelial cells. The
capacity of the thymic microenvironment to support B lymphopoiesis has also been demonstrated in recent in vitro experiments, using deoxyguanosin-treated fetal thymic lobes seeded with fetal-liver-derived B-cell progenitors. These thymic lobes show considerable growth and maturation of B lymphocytes. However, as in the tge26 thymus, B lymphocytes prefer localization in areas where fibroblasts are predominantly present. Moreover, B lymphocytes crosstalk to fibroblasts and stimulate their expansion. In contrast, seeding of de-Guo lobes with T-cell progenitors restores thymopoiesis, induces the 3-D organization of the epithelial network and reduces the frequency of thymic fibroblasts (W. v. E., W. T. V. Germeraad, H. Kawamoto and Y. Katsura, unpublished data). Likewise, injection of tge26 mice with RAGnull bone marrow decreases both the frequency of B lymphocytes and the frequency of fibroblasts in the thymus. It seems likely therefore that, under experimental conditions where T-cell development is promoted in the thymic microenvironment, B lymphopoiesis decreases, resulting in diminished crosstalk to thymic fibroblasts.

Our experiments further indicate that generation of a regular cortex is a prerequisite for the development of the medulla. In earlier experiments (Holländer et al., 1995a), we observed that bone marrow transplantation of adult tge26 mice with wild-type bone marrow did not lead to a complete restoration of T-cell development. The stromal architecture of the thymus in these transplanted mice remained disturbed. As a consequence, T-cell selection remained abnormal and the mice died after several weeks from severe colitis (Holländer et al., 1995b). In contrast, our present data show that sequential transplantation of tge26 mice with RAGnull bone marrow at neonatal age, followed by bone marrow transplantation with wild-type cells at adult age, results in normal T-cell development. Thus, thymic epithelial cells in the tge26 mutant mice are susceptible to crosstalk signals and can create a regular thymic architecture. Our experiments clearly demonstrate that these signals are provided by thymocytes beyond the TN CD44+CD25+ developmental stage and, more precisely, that TN CD44+CD25+ T-cell precursors present in the RAGnull (but not the tge26) mice can mediate this inductive event for cortical epithelial cells. Moreover, these experiments also demonstrate that this induction of a regular cortex occurs during a developmentally restricted ‘window’ ranging from day 14.5 in gestation to the first days after birth (this paper: S. Zuklys, B. W., G. H., unpublished results). Preconditioning’ of the cortical stroma by transplantation of RAGnull bone marrow cells is a prerequisite for a successful second transplantation with wild-type bone marrow at adult age. Apparently, T-cell progenitors in the normal bone marrow inoculate are able to migrate in this ‘cortex-only’ thymus, and are allowed to differentiate into TCRβ-CD3-expressing DP thymocytes. In turn, these more mature thymocytes crosstalk to medullary epithelial cells, leading to reconstitution of medullary microenvironments (van Ewijk et al., 1994). At this stage, after complete repair of thymic microenvironments in both cortex and medulla, thymopoiesis results in the appearance of SP thymocytes. This corticomedullary interdependence was also noted in normal mice during ontogeny, where the thymic cortex (as defined by the presence of an ER-TR4+ epithelial network) develops prior to the medulla (van Vliet et al., 1985).

So far, we have shown that plasticity of the thymic epithelial stroma is crucially important in the induction of thymic microenvironments. A logical question is whether the capacity to remodel is maintained throughout life. Analysis of the thymus in older mice indicates that the organization of medullary epithelial cells is affected from 6 months of age, while the cortical stroma is disrupted from 12 months of age (Hirokawa et al., 1994; Takeoka et al., 1996). These defects are, however, not a permanent feature of the old thymus. Unexpectedly, orchidectomy in mice even at an age of 24 months results in restored thymopoiesis, paralleled with complete restoration of cortical and medullary microenvironments (R. Boyd, personal communication), indicating that the plasticity of the thymic stroma is indeed maintained throughout life.

The cellular events regulating the induction of a normal cortical architecture have not yet been elucidated. At present, it is still unclear whether crosstalk between developing T cells and thymic epithelial cells induces proliferation and differentiation of epithelial cells, or more repositioning of epithelial cells already differentiated into cells with a cortical and medullary phenotype, respectively. In this regard, two findings are of interest. First, a subpopulation of embryonic and neonatal thymic epithelial cells concomitantly express both cortical and medullary markers (Röpke et al., 1995). Such cells also exist at low frequency in the adult thymus and it has been postulated that these cells form a pool of thymic epithelial precursors. Second, Penit et al. (1996) have shown that, in RAGnull mice transplanted with normal bone marrow, cortical rather than medullary epithelial cells start to proliferate, while medullary cells are found 10-15 days after bone marrow transfer. This observation is consistent with the notion that medullary epithelial cells could differentiate from epithelial precursor cells in the cortex. In line with this observation is a recent report by Klug et al. (1998) indicating the presence of epithelial cell precursors at the corticomedullary junction.

The cellular events regulating the induction of a normal cortex are presently a focus of attention. Wnt-mediated signals are likely involved because Wnt proteins regulate proliferation, differentiation and cell fate of epithelial cells (Cadigan and Nusse, 1997). Moreover, Wnt proteins are expressed in a differential pattern by thymocytes at various maturational stages during intrathymic ontogeny. Recently performed co-cultures with thymic epithelial cell lines indicate that, upon lymphostromal interaction, the Wnt signaling pathway is activated, in turn leading to upregulation of Fz (Frizzled) members on epithelial cells (G. H., G. Balciunaite and S. Zuklys, unpublished data). Furthermore, angiogenesis is induced in tge26 tg mice transplanted with RAGnull bone marrow cells, and angiopoietin was recently found to be differentially expressed in RAG thymi compared to unmanipulated tge26 thymic tissue (G. H. and S. Zuklys, unpublished data). In line with this notion, we have found that, using in vitro cultured fetal thymic lobes, the oxygen concentration in the culture system critically regulates the induction of a 3-D organized thymic stroma in the thymic lobes (W. T. V. Germeraad, H. Kawamoto, Y. F. Jiang, Y. Katsura and W. v. E., unpublished data). Thus, increased blood supply to the reconstituted tge26 thymus may be reflective of a role for oxygen and nutrients in the recovery of thymic microenvironments.

Taken together, our data indicate that developing thymocytes
play an important role in the functional organization of thymic microenvironments. As illustrated in Fig. 6, crosstalk between thymocytes and epithelial cells regulates the creation of thymic microenvironments in a stepwise fashion. Thus, early developing TN T cell precursors able to progress to the stage of TN CD44+/CD25- (transfer with RAG null bone marrow) restores epithelial microenvironments in the cortex, but not in the medulla, creating a ‘cortex only’ thymus. In contrast, progression of T-cell development to the stage of late double positive, or mature single positive thymocytes (induced by a second transplantation with wild-type bone marrow) induces full restoration of epithelial microenvironments in the medulla.

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