Crosstalk in the mouse thymus

Willem van Ewijk, Elisabeth W. Shores and Alfred Singer

The development of mature T cells within the thymus is dependent upon intact cortical and medullary microenvironments. In turn, thymic microenvironments themselves are dependent on lymphoid cells to maintain their integrity. Here, Willem van Ewijk and colleagues discuss experiments that have established the phenomenon of 'crosstalk' within the mouse thymus and suggest a mechanism whereby lymphoid and stromal cells influence each other in a consecutive manner during T-cell development.

Prothymocytes, upon entering the thymus, communicate with a variety of nonlymphoid (stromal) cell types during their development[1–3]. The sequential interaction of thymocytes with bone-marrow-derived macrophages, cortical epithelial cells and interdigitating reticulum cells (dendritic cells) leads to the proliferation, maturation and selection of the developing T cells. The sequence of these lympho–stromal interactions reflects an intrathymic migration route, where prothymocytes travel from the cortico–medullary junction through the thymic parenchyma towards the outer (subcapsular) cortex. From there, differentiating thymocytes move down the cortex to enter the thymic medulla as mature T cells. The orientation of cortical epithelial cells[4], and the recently observed flow of intrathymic fluid (P. Nieuwenhuis, pers. commun.), both support such a migration pathway.

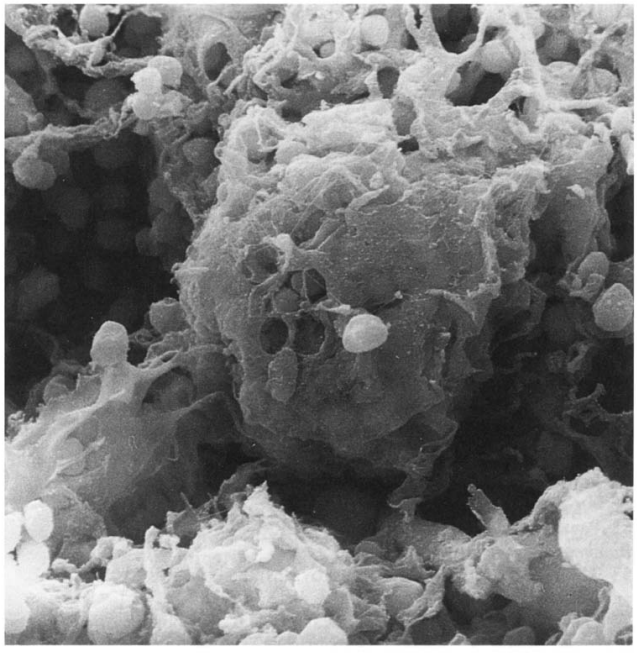

Fig. 1. *Scanning electron microscopy (×1000) of an in vivo equivalent of a 'thymic nurse cell'. These lympho-epithelial complexes are found in the subcapsular cortex of the mouse thymus.*

During migration, thymocytes acquire receptors for growth factors, begin expression of adhesion molecules and co-receptors, such as CD4 and CD8, and finally develop a T-cell receptor (TCR)/CD3 complex at the cell surface[5]. Most importantly, the T-cell repertoire is *shaped* during differentiation, such that T cells with high affinity for self major histocompatibility complex (MHC) molecules, or self peptides presented by these molecules, are clonally eliminated[6]. By contrast, T cells with affinity for foreign peptides presented by self MHC molecules are positively selected[7,8].

Experimental models showing crosstalk of stromal cells to developing T cells

It is generally accepted that the thymic stroma plays a key role in the development and differentiation of T cells. This lympho–stromal interaction can be visualized by scanning electron microscopy, particularly in the outer cortex of the thymus[9]. For example, Fig. 1 shows the presence of lympho-epithelial complexes, which are comparable with 'thymic nurse cells', in the subcapsular cortex. It is possible to isolate these complexes, as well as complexes of thymic macrophages and thymocytes, from the thymus. An analysis of their cellular composition has added support to the idea that developing cells migrate up and down the cortical thymic parenchyma[10].

In addition to morphological analyses, at least three different experimental approaches have provided an insight into the cell types and molecules that are involved in thymic lympho–stromal interactions. Kruisbeek and colleagues were the first to demonstrate, by *in vivo* antibody treatment, the role of MHC class I (Ref. 11) and class II (Ref. 12) determinants on stromal cells in the positive selection of $CD8^+$ and $CD4^+$ T cells, respectively. However, these experiments were unable to define the nature of the MHC^+ cell that mediated positive selection, since MHC molecules are expressed on cortical and medullary epithelial cells, as well as on interdigitating reticulum cells and a few B lymphocytes at the cortico–medullary junction[9,13]. Furthermore, the thymic stroma itself is extremely heterogeneous, as revealed by a panel of newly developed monoclonal antibodies (mAbs)[3,14].

A second approach to studying the role of stromal cells in lympho–stromal interactions has involved the

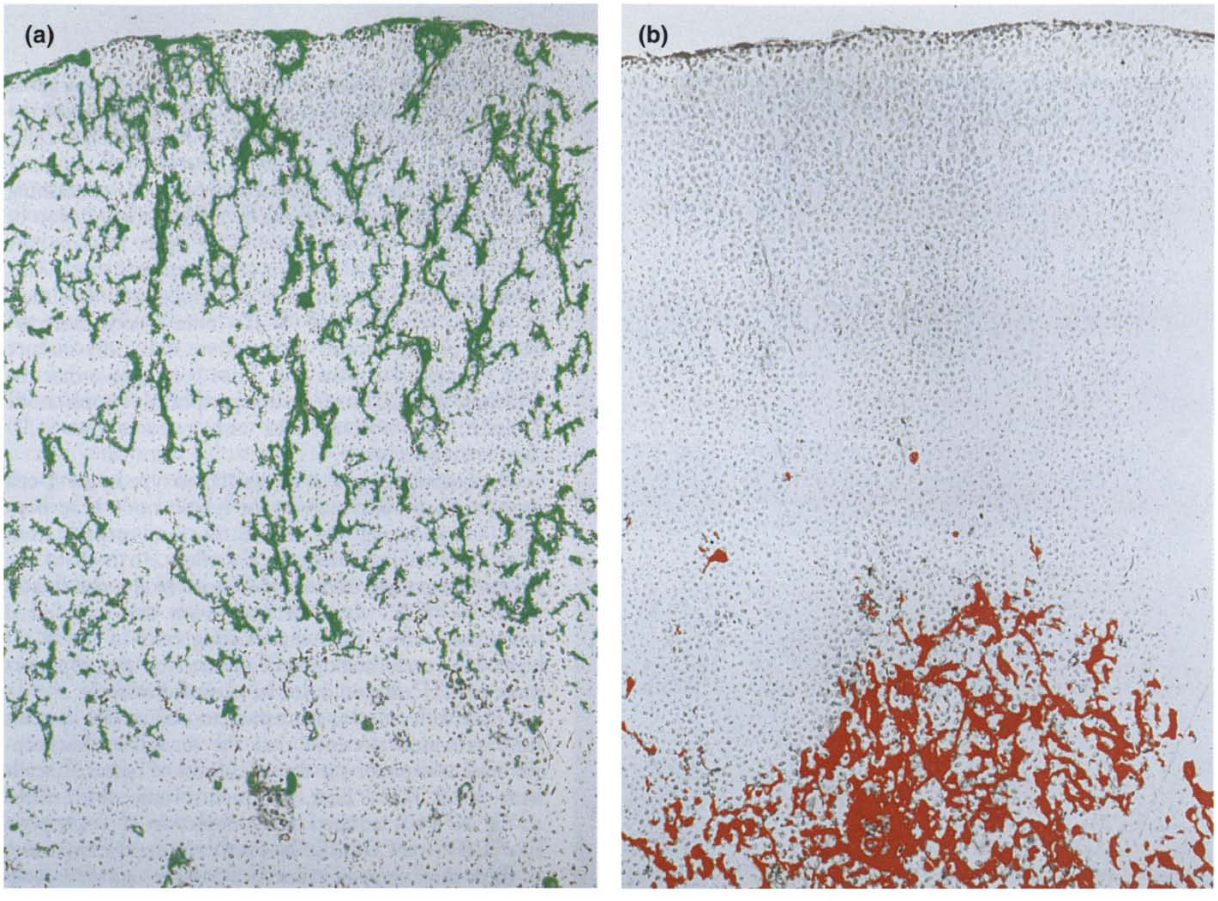

Fig. 2. *Stromal cells in the thymus of normal mice: (a) cortical epithelium (shown in green) detected with the ER-TR4 monoclonal antibody; (b) medullary epithelium (shown in red) detected with the ER-TR5 monoclonal antibody.*

isolation of stromal cell populations[15–17], and the cloning of stromal cell lines[1,18], in order to study their contribution in *in vitro* or *in vivo* models of T-cell differentiation. Most importantly, it has been shown recently that cloned epithelial cell lines are able to influence positive selection after being placed in the intact thymus[19,20]. However, progress in this particular field of research is relatively slow, and one of the reasons for this is that prolonged cell culture of thymic epithelial cell lines is technically difficult[15–17]. Not only do these cells not readily proliferate, but they also tend to lose their original phenotype and functional characteristics during prolonged culture. There are two possible reasons for this problem: (1) that intrinsic microenvironmental defects occur when stromal cells are put into a monolayer culture (e.g. through the loss of cell–cell contact or three-dimensional architecture); and (2) that extrinsic defects hamper the outgrowth and phenotypic stability of cultured thymic stromal cells (e.g. through the loss of lympho–stromal interaction or thymocyte-derived cytokines).

A third approach to 'manipulate' cell-surface determinants on stromal cells in order to study their contribution to T-cell differentiation is based on gene-knockout experiments. Such studies have demonstrated that mice deficient for MHC class I genes show a strong reduction in the number of mature $CD8^+$ T cells, whereas mice deficient for MHC class II genes are devoid of mature $CD4^+$ T cells[20,21]. Taken together, all three experimental systems show clearly that T cells are dependent on intact thymic microenvironments for their maturation.

Experimental models showing crosstalk of developing T cells to stromal cells

Surprisingly, not only are T cells dependant on microenvironments for development, but microenvironments themselves are dependent on the presence of lymphoid cells to differentiate and to maintain their integrity. The suggestion that the differentiation of thymic stroma does not occur independently of thymocyte differentiation came initially from studies in which mice were treated with cyclosporin A (CsA) (Refs 23,24) or total lymphoid irradiation (TLI) (Ref. 25). Both types of treatment resulted in a marked reduction in the number of thymic medullary epithelial cells, which reappeared after cessation of treatment. However, it was not certain whether the effects of CsA or TLI on medullary thymic epithelium were primary effects of the treatments or secondary consequences of the effects on thymocyte development.

The first compelling demonstration that differentiation of medullary thymic stroma was dependent upon lymphocytes came from experiments in severe combined immunodeficiency (SCID) mice[26]. SCID mice have a genetic defect that is limited to lymphocytes and

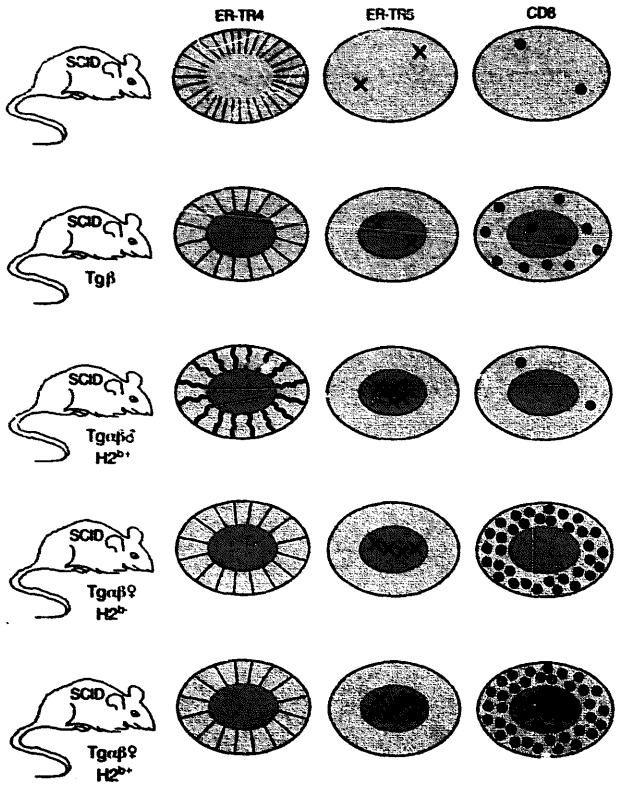

Fig. 3. *Schematic representation of the thymus in the progeny of severe combined immunodeficiency (SCID) mice crossed with mice transgenic for the T-cell receptor β chain (Tgβ) or the α and β chains (Tgαβ). The TCR transgene recognizes the HY male antigen in the context of major histocompatibility complex (MHC) class I H-2^b, such that cells expressing this TCR are deleted in males but are positively selected in females (see Ref. 31 for review). Antibody ER-TR4 was used to determine the relative development of the cortical epithelium: thickened red lines indicate a condensed epithelial stroma (the highly cortical nature of the SCID mouse thymus is indicated by extra lines). Antibody ER-TR5 was used to determine the relative development of the medullary epithelium: red crosses in the central region indicate degree of development (the collapse of the medulla in SCID mice is indicated by the lack of a central ER-TR5$^+$ region). The relative number and location of CD8$^+$ T cells are indicated by blue dots. Positive selection of CD8$^+$ T cells is histologically manifested by the filling up of the medulla with these cells.*

that results in a failure of T and B cells to express clonotypic receptor proteins. The thymus of SCID mice is largely cortical, and the majority of stromal cells are recognized by a mAb (ER-TR4) that is specific for cortical epithelial reticular cells[27]. Furthermore, the thymic medulla of SCID mice is disorganized, with only a few scattered cells that are recognized by a mAb (ER-TR5) to thymic medullary epithelium[27]. The organization of cortical and medullary epithelial cells in a normal mouse thymus is shown in Fig. 2.

Recently, the thymuses of lymphocyte-deficient *RAG-1*- and *RAG-2*-knockout mice have also been found to have structural defects similar to those found in the thymus of SCID mice (W. van Ewijk, F.W. Alt and D. Baltimore, unpublished). Significantly, it was shown that the failure of medullary thymic epithelial cells to mature and organize in SCID mice was not the result of an intrinsic defect in SCID epithelial cells, since they could be induced to mature and organize normally by infusion of bone-marrow cells containing normal thymocyte precursors[26]. Indeed, the infusion of normal bone marrow into SCID mice restores the architecture of both thymic compartments, and leads to the complete development of cortices and medullas. In these reconstituted SCID mice, bone-marrow-derived prothymocytes differentiate into fully mature TCR$^+$ T cells. Furthermore, it has been shown that the infusion of SCID mice with peripheral lymph node T cells from normal mice also restores the SCID thymic architecture[28].

The question arises as to which cell type in the T-cell inocula from bone marrow or lymph node is responsible for inducing the maturation and organization of SCID medullary thymic epithelium. In both of the studies described above, the most likely candidates were the TCR$^+$ cells derived from the transferred inoculum, rather than the existing cells in the SCID host. However, hemopoietically-derived macrophages or dendritic cells could not be excluded entirely.

Three different experimental animal model systems have provided evidence that the medullary microenvironment is under the direct control of mature T cells bearing αβ TCRs. First, treatment of mice with mAbs to the αβ TCR resulted in the depletion of single positive (CD4$^+$ or CD8$^+$) cells. Furthermore, the thymuses of these mice showed reduced medullas, with few medullary epithelial cells (W. van Ewijk and W. Born, unpublished). Second, mice homozygous for a disrupted TCR α gene showed a complete absence of mature CD4$^+$ and CD8$^+$ T cells[29]. In addition, no defined medullas were observed in the thymus. Since these mice developed normal numbers of T cells expressing the γδ TCR, it was concluded that the composition of the thymic stroma is exclusively regulated by αβ TCR$^+$ T cells. Third, formal proof of the involvement of αβ TCR$^+$ T cells has been provided recently by experiments in which maturation of the thymic medulla was assessed in SCID mice crossed to TCR-transgenic mice (E.W. Shores, W. van Ewijk and A. Singer, unpublished). In these experiments (Fig. 3), two types of TCR-transgenic mice were crossed to SCID mice: one type was transgenic for the TCR α and β chains; the other type was transgenic for only the TCR β chain[30,31]. It was found that introduction of the α and β TCR transgenes into SCID mice leads to the surface expression of complete CD3/TCR αβ complexes. By contrast, introduction of only the β chain into SCID mice results in the surface expression of incomplete TCR complexes, primarily comprising TCR β chains. Surprisingly, analysis of the thymic stroma in both these mice revealed reorganization of the ER-TR5$^+$ medullary epithelial cells *only* in crosses of SCID mice with αβ TCR-transgenic mice, but not in crosses between SCID mice and β TCR-transgenic mice. Clearly, these experiments show that the integrity of the medullary microenvironment is dependent on the presence of T cells expressing complete CD3/TCR αβ complexes. However, the state of differentiation of

these T cells remains to be established, since they could be either mature single-positive T cells or double-positive T cells expressing the CD3/TCR complex at the cell surface. Nevertheless, these observations indicate that thymocytes need to be stimulated *via* CD3/TCR complexes in order to provide the inductive signal for maturation of the medullary environment.

At present, the exact nature of the effect of maturing T cells on medullary epithelium is unclear. It is possible that cell–cell contact stimulates the outgrowth of medullary epithelial cells. Alternatively, locally secreted cytokines derived from T cells may influence the proliferation and differentiation of thymic epithelial cells[24]. The latter idea is supported by experiments in which fetal thymic organ cultures (FTOC) were maintained in the presence of exogenously added cytokines. In these studies, it was found that interleukin 2 (IL-2) strongly induced expansion of ER-TR5$^+$ stromal cells in the cultured thymic lobes (P. Brekelmans, M. De Smedt, P. van Soest, J. Plum and W. van Ewijk, unpublished).

Do γδ T cells also contribute to the expansion of the medullary environment? The answer to this question is presently still unclear. The experiments described above, in which knockout mice for the TCR α gene showed disrupted development of the medulla, but normal levels of γδ T cells, does not favor a role for these T cells. Similarly, knockout mice that lack the TCR δ gene develop a normal medulla (R. Boyd, pers. commun.). By contrast, mice transgenic for TCR $V_\gamma 1.1$, $J_\gamma 4$, $C_\gamma 4$ do show hyperplasia of the (ER-TR5$^+$) medullary epithelial cells[32].

Conclusions

The following hypothesis regarding the relevance of lympho–stromal interaction during T-cell development can be suggested: (1) early in ontogeny, the thymic microenvironment is largely cortical in nature, as observed by the presence of ER-TR4$^+$ cells[33]; (2) prothymocytes enter this environment and require cortical stromal elements for the initial steps of their differentiation. As T-cell differentiation proceeds, the CD4, CD8, CD3/TCR complex and other relevant molecules are expressed at the cell surface; (3) these maturing cells then stimulate the expansion of the medullary epithelium (ER-TR5 expression follows ER-TR4 expression in ontogeny; Ref. 33); and (4) the organized medullary epithelium, in turn, allows thymocytes to mature to virgin T cells, to accumulate in the medulla and to exit to the peripheral immune system. It is possible that chemotactic factors secreted by the epithelial cells regulate these phenomena.

Thus, lympho–stromal interactions in the thymus represent a *symbiotic* relationship, whereby T cells depend upon an intact thymic stroma for their maturation and, in turn, the integrity of the stroma depends on the presence of developing T cells. Indeed, this latter notion may explain why isolated thymic stromal cells are difficult to maintain *in vitro*.

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