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Expression of the $\alpha\beta$ T-Cell Receptor is Necessary for the Generation of the Thymic Medulla

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The architecture of the thymus of mice that congenitally fail to express the $\alpha\beta$ T-cell receptor (TCR$\alpha\beta$) has been examined by immunohistology. In these mice, a defined mutation was introduced into the TCRα gene by homologous recombination. By using antibodies specific for cortical or medullary epithelium and for major histocompatibility complex antigens, the network of cortical epithelium in these mice was shown to be essentially unaltered in comparison with that of normal mice. In contrast, the thymic medulla was considerably reduced in size. This analysis shows that expression of the $\alpha\beta$ TCR but not the $\gamma\delta$ TCR is obligatory for establishing the thymic medulla and suggests that the growth of medullary epithelial cells may require contact with TCR$\alpha\beta$-expressing cells.

KEYWORDS: Thymus, medullary epithelial cells, $\alpha\beta$ T-cell receptor, TCRα-mutant mice.

INTRODUCTION

The thymic microenvironment is essential for the differentiation of T cells. Prothymocytes migrate from sites of haematopoiesis into the thymus and there undergo a series of differentiation and expansion events, during which they express either $\alpha\beta$ or $\gamma\delta$ forms of the TCR (reviewed by von Boehmer, 1988). TCR$\alpha\beta$-expressing thymocytes undergo negative selection to remove autoreactive TCRs and positive selection to generate MHC-restricted TCRs. Both epithelial and dendritic cells are mediators of the selection processes, with the epithelial cells being particularly important in positive selection (Benoist and Mathis, 1989; Vukmanovic et al., 1992).

The thymic lobules of normal mice are arranged into an outer cortex and an inner medulla. In contrast to the normal thymus, the network of medullary epithelial cells is poorly developed in scid mice (Shores et al., 1991; Surh et al., 1992). However, injection of mature T cells or transfer of normal bone marrow cells restores this network of epithelial cells (Shores et al., 1991; Surh et al., 1992). These observations suggest that mature T cells may be necessary for the development of the thymic medulla.

We have previously generated mice that fail to express the $\alpha\beta$ TCR by disrupting the TCRα chain by homologous recombination (Philpott et al., 1992). In these mice, thymocytes arrest at the double-positive (CD4+CD8+) stage of development and fail to mature into single positive (CD4+ or CD8+) thymocytes. We also observed that the thymic medulla, characterized by its less dense cellularity in histological preparations, was undetectable morphologically. However, the basis for the disruption of the cellular architcture of the medulla was not elucidated. To determine whether this loss extended to the network of medullary epithelial cells or other cells in the microenvironment, thymi from these mice were analyzed using a series of monoclonal antibodies specific for thymic epithelial subsets, and major histocompatibility complex antigens. In the present study, we show that there is a drastic reduction of medulla epithelial cells, suggesting that contact with TCR$\alpha\beta$ cells are necessary for the maintenance of the thymic medulla.

MATERIALS AND METHODS

Mice

Mice used in this study were congenitally deficient in mature $\alpha\beta$ TCR (TCRα−/−) expressing cells as

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described by Philpott et al. (1992). The control mice were wild type (WT) littermates expressing normal levels of the $\alpha\beta$ TCR.

Immunohistochemistry

Thymi were removed from 4–8-week-old mice, snap frozen in liquid nitrogen, and stored at −70°C. Five-micron frozen sections were cut from the tissue, air dried, fixed in acetone, and stained using the indirect immunoperoxidase method at room temperature. Slides were incubated with primary antibody for 1 hour and after washing in Tris-buffered saline (TBS), pH 7.6, were incubated for a further hour with biotinylated secondary antibody diluted in TBS containing 2% normal mouse serum. After washing for 20 min, the sections were incubated with avidinperoxidase (Sigma, UK) and visualized by adding diaminobenzidine (DAB, Sigma) (10 mg/15 ml with 12 μl of H_2O_2). Slides were counterstained with haematoxylin, dehydrated, and mounted using DPX mountant (BDH, UK). Positively staining cells were identified by the presence of a brown reaction product.

Reagents

The rat monoclonal antibodies (mAb) used in this study were HB-51, anti-K^b,D^b (Ozato and Sachs, 1981); M5/114, anti-Aβ^b (Bhattacharya et al., 1981); ER-TR5, anti-medullary epithelial cells, the gift of Dr. J. Owen (van Vliet et al., 1984); NLDC-145, anti-cortical epithelial cells and dendritic cells, the gift of Dr. D. Gray (Kraal et al., 1986); 4F1, anti-cortical and IVC4, anti-medullary epithelial cells (Kanariou et al., 1989). The polyclonal rabbit anti-keratin antibody was from Dakopatts (Denmark). The second-layer antibodies used were biotinylated sheep anti-rat Ig (Amersham, UK) and biotinylated mouse anti-rabbit Ig (Sigma).

RESULTS

Thymic Epithelial Staining

Generation of mAbs raised against thymic epithelium has revealed molecular heterogeneity within the thymus microenvironment (Ritter and Crispe, 1992). The mAbs NLDC-145 and 4F1 recognize cortical epithelium, and NLDC-145 also weakly reacts against dendritic cells (Kraal et al., 1986; Kanariou et al., 1989). In contrast, the mAbs ER-TR5 and IVC4 react specifically with medullary epithelium (van Vliet et al., 1984; Kanariou et al., 1989). Although both sets of anti-cortical and anti-medullary epithelium antibodies were used, only staining with NLDC-145 and ER-TR5 are shown. Staining with either NLDC-145 or 4F1 on normal sections of thymus revealed a typical network of cortical epithelial cells, which are characterized by long thin processes separated by densely packed thymocytes (Fig. 1a). In contrast, the network of medullary epithelial cells in a normal thymus, as revealed by the mAbs ER-TR5 and IVC4, possessed shorter processes that were surrounded by fewer lymphoid cells (Fig. 1c).

The thymic cortex of TCRα−/− mice showed no apparent differences in comparison with that of the normal cortex, using either NLDC-145 or 4F1 (Fig. 1b). In contrast, the mAbs ER-TR5 and IVC4 showed that the thymic medulla was considerably reduced in size in the TCRα−/− mice (Fig. 1d). Staining with the anti-keratin antibody revealed the total network of epithelial cells in the thymus and confirmed these findings in both the wild type and TCRα−/− mice (Figs. 1e and 1f). Macrophages were evenly distributed in the thymus of both control and TCRα−/− mice (data not shown).

MHC Antigen Expression

On the normal thymus MHC class II distribution in the cortex revealed a reticular network of epithelial cells, whereas the medulla showed confluent staining of MHC class II molecules (Fig. 2a). This pattern of staining was in agreement with that shown by Rouse et al. (1979). In the TCRα mutant mice, class II expression of the cortical epithelial cells was unaltered. However, only a small area of dense medullary class II staining was evident, underscoring the small size of the medulla in these mice (Fig. 2b). MHC class I staining produced a similar picture, although the overall expression is weaker, of dense staining in the medulla that was almost absent in the thymus from TCRα−/− mice (data not shown).

DISCUSSION

We have previously shown that the less dense cellularity characteristic of the thymic medulla was lacking in thymic sections from mice congenitally deficient in expression of the TCRα gene (Philpott et

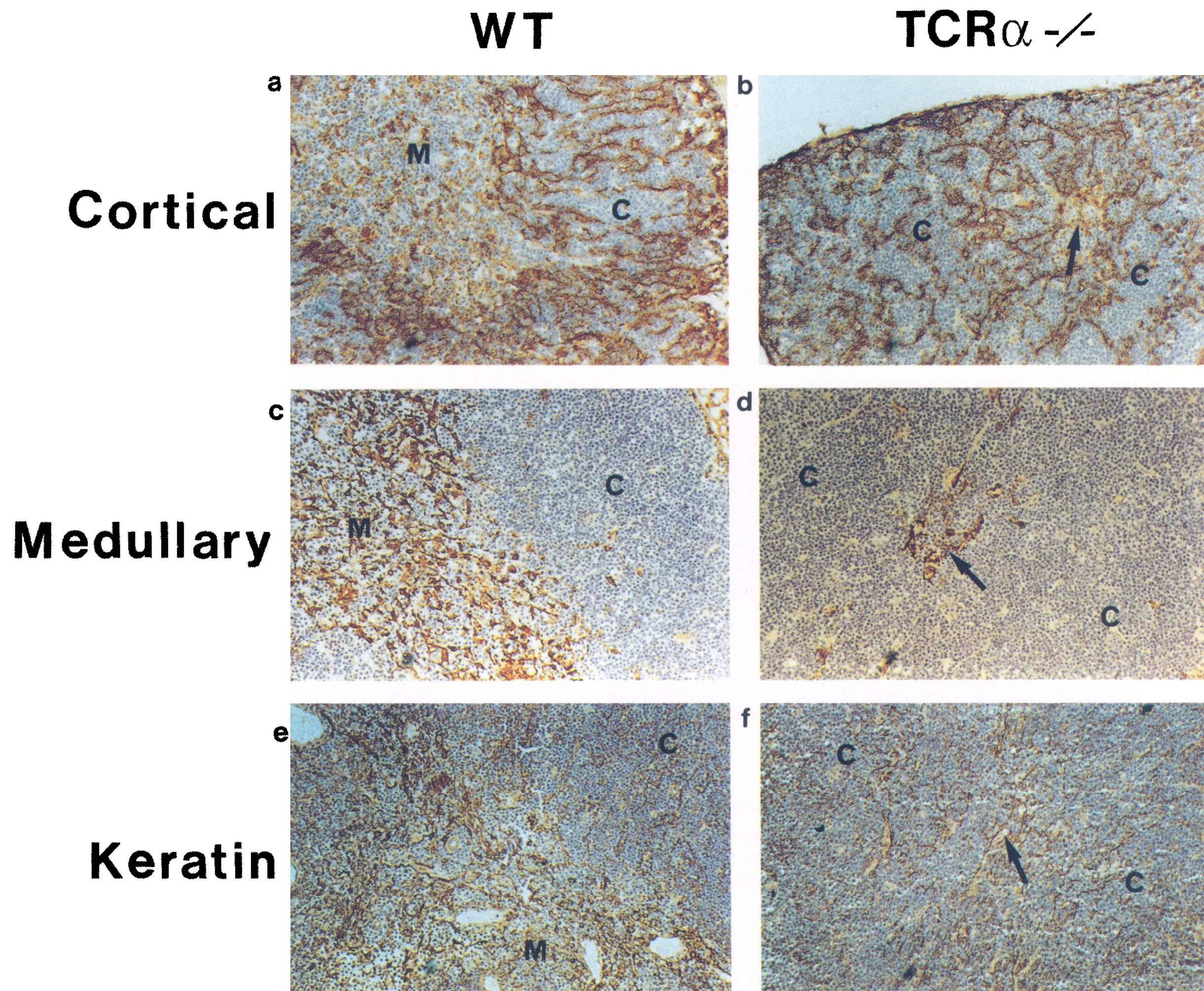

FIGURE 1. (See Colour Plate XIX at the back of this publication). Immunoperoxidase staining of cryostat serial section of wild type (WT) and TCRα-/- thymus with the anti-cortical epithelial marker NLDC-145 (sections a and b); the anti-medullary epithelial cell marker ER-TR5 (sections c and d); and anti-keratin antibody (sections e and f). The network of medullary epithelial cells is dramatically reduced in TCRα-/- mice, which is indicated by an arrow. C=cortex, M=medulla. Magnification ×320.

al., 1992). In the present study, we have extended these observation by showing that thymic medullary epithelial cells fail to mature due to the absence of TCR$\alpha\beta$-expressing cells. These observations are consistent with a number of previous results. Thus, the removal of mature thymocytes either by total lymphocyte irradiation or treatment with cyclosporin results in a marked decrease of medullary epithelial cells, whereas the cortex remains unchanged (Adkins et al., 1988; Kanariou et al., 1989). Removal of cyclosporin leads to rapid restoration of the medulla and mature thymocytes. Similarly, the medulla in the mice harboring the scid genetic defect is also reduced, but can be restored when these mice receive either T cells or bone marrow from normal mice (Shores et al., 1991; Surh et al., 1992). These observations strongly suggest that the development of medullary epithelial cells requires mature T cells.

A caveat to these experiments is that treatment with cyclosporin or total lymphocyte irradiation and the scid mutation may have more pleiotropic effects on the maturation of medullary epithelial cells. In contrast, the mice used in this study have a defined mutation only in the TCRα gene, demonstrating that the development of medullary epithelial cells is

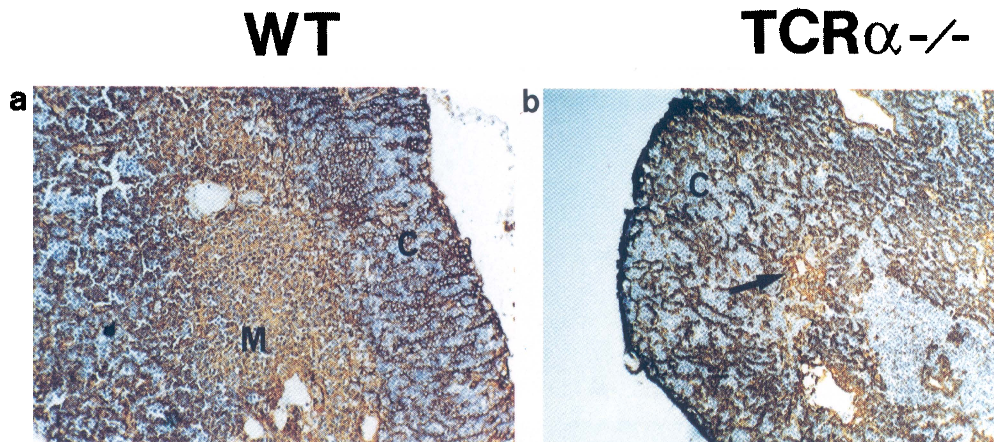

FIGURE 2. (See Colour Plate XX at the back of this publication). Immunoperoxidase staining of wild type (WT) (section a) and TCRα−/− (section b) mice for major histocompatibility class II antigens (using the antibody M5/114). The dense staining area representing the thymic medulla is reduced in TCRα−/− mice, which is indicated by an arrow. The class II negative region observed in the thymus of the TCRα−/− mice was also present in wild type mice. C=cortex, M=medulla. Magnification×320.

mediated either directly or indirectly by the TCRαβ chain. The maturation of the medulla does not appear to involve TCRγδ-expressing cells, as proposed by Shores et al. (1991), because these cells are unaffected in TCRα−/− mice (Philpott et al., 1992). Thus, if positive selection is necessary for maturation of thymic TCRγδ-expressing cells, the thymic medulla is unlikely to play a role.

How mature thymocytes mediate the development of medullary epithelial cells is not clear. One possibility is that the interaction of TCR with either MHC antigens or other molecules on thymic stromal cells may result in the production of cytokines that organize the architecture of the medullary network (Berrih et al., 1985; Mat et al., 1991). The stromal cell type involved in this interaction could be the cortical epithelium or the few epithelial cells present in the medulla of TCRα−/−mice (Fig. 1). These latter cells may also be the precursor for the medullary network. Alternatively, medullary epithelial progenitor cells may exist in normal numbers, but lack the epitope recognized by the antibodies used in this study. Contact of αβ TCR-expressing cells with cortical epithelium may induce differentiation of these progenitor cells.

The requirement for αβ TCR expression for generation of the thymic medulla provides an efficient mechanism for regulating the response of the thymus to throughput of T cells. Thus, the medulla, the site of mature αβ-expressing thymocytes, may expand in response to signals generated by the αβ TCR, thus preventing congestion of T cells in the medulla. In this way, the thymus can respond to the requirement for a varying output of mature T cells depending on immunological needs.

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