The ER-TR4 monoclonal antibody recognizes murine thymic epithelial cells (Type 1) and inhibits their capacity to interact with immature thymocytes: immuno-electron microscopic and functional studies

Abstract The thymic stroma is heterogeneous with regard to cellular morphology and cellular function. In this study, we employed the monoclonal antibody ER-TR4 to characterize stromal cells at the ultrastructural level. To identify the labelled cell type, we used two techniques: immunogold labelling on ultrathin frozen sections and immunoperoxidase staining on thick "vibratome" sections. ER-TR4 reacted with thymic Type 1 epithelial cells (according to our classification). A dense labelling appears in the cytoplasm of cortical cells using the two techniques. Immunogold labelling identified small cytoplasmic vesicles whereas the cytoplasm and the cell membrane seem to be labelled with the immunoperoxidase technique. ER-TR4 also identified isolated thymic nurse cells (TNC), and was observed in vitro to inhibit the capacity of some type 1 epithelial cells to establish interactions with immature thymocytes. This finding supports the hypothesis that the factor is involved in the formation of lymphoepithelial interactions within thymic nurse cells, and thus in the relations that immature thymocytes establish with the thymic microenvironment.

Introduction

The thymus plays a crucial role in the generation of T lymphocytes (for review see Fowlkes and Pardoll 1989; Van Ewijk 1991). Differentiating thymocytes interact with stromal cells and, as a consequence, are triggered to proliferate and to mature. It is commonly thought that these events are mediated via direct cell-cell contact between lymphoid and stromal cells and/or via the secretion of short-range operating factors (Carding et al. 1991; Deman et al. 1992; Duijvestijn and Hoefsmit 1981; Kendall and Ritter 1988; Marrack et al. 1988, Ramsdell and Fowlkes 1990; Stutman 1978; Van Ewijk 1989).

To understand the mechanisms underlying intrathymic lymphopoiesis, it is necessary to consider the complexity of the thymic microenvironment. Morphological studies have shown that the thymic stroma is composed of different cell types: (1) epithelial cells of which we have identified three types in the mouse thymus, and (2) two types of accessory cells originating in bone marrow (macrophages and interdigitating cells; for review see Nabarra and Andrianarison 1987, 1991).

In this report, we will consider the first type of epithelial cells (Type 1) observed in our classification, which corresponds to the “classical” epithelial cells described by numerous authors. Type 1 cells are observed in the cortex and a few in the medulla, whereas the two others (Type 2 and Type 3) are present only in the medulla (Nabarra and Andrianarison 1987). Type 1 epithelial cells are stellate in shape with numerous pseudopods extended between thymocytes forming a complex network. In addition to tonofilaments and desmosomes, these cells are characterized by the presence in the cytoplasm of a few "clear vacuoles" containing several dense granules.

A key question that arises is whether different stromal compartments control distinct events of intrathymic T cell differentiation. In a first approach, progress in this direction has been achieved by in vitro isolation of multicellular complexes from the thymus by enzymatic digestion and by sedimentation. Some of these complexes are identified as "thymic nurse cells" (TNC), i.e. epithelial cells enclosing intact lymphocytes (Andrews and Boyd 1985; Defresne et al. 1986, 1990; De Waal Malefijt et al. 1986; Hirokawa et al. 1986; Kyewski 1991; Kyewski and Kaplan 1982; Wekerle et al. 1980) and appear to play a role in T cell differentiation (De Waal Malefijt et al. 1986; Kyewski 1991). At the ultrastructural level, the thymic nurse cells show a characteristic cytoplasm of epithelial cells of Type I (De Waal Malefijt et al. 1986; Toussaint-Demylle et al. 1990).
Fig. 1a–c Immunoperoxidase labelling on sections of murine thymus.

a General aspect of the cortical zone showing labelling in the cytoplasm of epithelial cells (arrow). The slight retraction of the cells allows us better to observe the labelling on the cell surface membrane (arrowheads). Counterstained with uranyl acetate and lead citrate. ×6500; bar, 1 μm.

b Staining of a Type 1 epithelial cell in the cortical zone (the arrow indicates tonofilaments and “clear vacuoles”). A great part of the cytoplasm is labelled by immunoperoxidase fine granulation (arrowhead). The plan of section passes by a nucleus indentation (N). Counterstained with uranyl acetate and lead citrate. ×9000; bar, 1 μm.

c An epithelial cell is labelled (arrowhead) and the adjacent is not labelled (arrow). The processes of the labelled epithelial cell are extended between unlabelled cells. (N Nucleus). Counterstaining with uranyl acetate and lead citrate. ×9000; bar, 1 μm.