Microsc. Microanal. 8 (Suppl. 2), 2002
DOI 10.1017.S1431927602106878

Distribution of Keratin Intermediate Filaments in Cultured Thymic Epithelial Cells (TEC) is Dependent Upon Growth Medium Calcium Content

S. S. Sands*, W. D. Meek*, J. Hayashi**, and R. J. Ketchum*

* Oklahoma State University Center for Health Sciences (OSU-CHS), Tulsa, OK 74107
** University of Maryland School of Pharmacy, Baltimore, MD 21201

Isolation and culture of TEC involves conventional primary tissue culture techniques combined with growth factor/hormonal supplementation and intermittent chelation therapy to reduce fibroblast overgrowth. It is known that primary cultures of murine keratinocytes are highly responsive to medium calcium concentrations, with low calcium levels supporting proliferation and effortless passaging [1]. Medium with calcium levels higher than approximately 0.09 mM initiate terminal differentiation and decrease cell proliferation [2]. Often the cells are so adherent that subsequent trypsinization leads to cell damage/death. TEC lines (TE-R 2.5 and R-TNC.1) were established from primary rat thymic cultures and characterized using a panel of human, rat, and mouse monoclonal antibodies [3, 4]. We established an immortalized TEC line derived from the LDA rat, an F1 cross between the Lewis (major histocompatibility complex [MHC] haplotype $Rt1^l$) rat and the Dark Agouti (MHC haplotype $Rt1^a$) rat. Our purpose for this was the constant demand for TEC that could be manipulated *in vitro* and subsequently transplanted intrathymically to induce donor specific unresponsiveness.

Thymi are harvested from 4-5 day old neonates, dissected free of fat and debris, and finely minced with scissors. TEC are isolated with a modified enzymatic technique [5] using collagenase (1 mg/mL at 37° C for 1 hour) and cultured in WAJC404A medium containing cholera toxin 20 ng/mL, dexamethasone 10 nM, epidermal growth factor 10 ng/mL, insulin 10 µg/mL, transferrin 10 µg/mL, 2% supplemental calf serum, 2.5% DMEM, and 1% antibiotic solution [6]. The most noteworthy aspect of maintenance of this cell type is the reduction of medium calcium concentration to .0925 mM using WAJC404A medium. TEC grown in this medium are readily passaged via light trypsinization (.05%). Trypsinization at higher concentrations (0.25%) leads to lifting of cell sheets, cellular damage in the form of blebs, and cellular necrosis. TEC cultured in low calcium medium display a round to spindle shaped morphology with intercellular spacing even at confluence and dense keratin patterns with some net-like arrangements (FIG 1). TEC cultured in relatively high calcium growth medium (3.7 mM) form a senescent cobblestone-like confluent monolayer that is impervious to light trypsinization (.05%) and display keratin intermediate filaments concentrated near the cell membrane (arrows, FIG 2). At any time increasing medium calcium content will induce terminal differentiation and stimulate a morphological change into the cobblestone-like monolayer of TEC (FIG 2) in which cells are joined by desmosomes and contain numerous cytoplasmic keratin filament bundles (arrows, FIGS 3 and 4).

This study suggests that desmosome formation results from increased medium calcium concentrations and may be responsible for terminal differentiation of keratinocytes, including TEC, exposed to high-calcium growth medium. This would further explain the strong intercellular and cell/cell substrate adherence observed in high-calcium conditions. These findings may be important considerations in the application of cultured TEC as an *in vitro* or *ex vivo* model of positive/negative lymphocyte clonal selection, maturation, and differentiation. It may be helpful to maintain cultured TEC in an undifferentiated state using low-calcium growth medium, inducing terminal differentiation by exposing them to high-calcium growth medium as required by specific experimental protocols [7].

References

[1] P.G. Sacks, et al. In Vitro Cell. & Dev. Bio. 21 (1985) 99-107.
[2] D.S. Rosenthal, et al. Cell Growth and Diff. 2 (1991) 107-113.
[3] R. V. Rouse, et al. J Histochem Cytochem. 36 (1988) 1511-1517.
[4] M. Colic, et al. Thymus. 12 (2) (1988, 1989) 123-30.
[5] A. Piltch, et al. In Vitro Cell. & Dev. Bio. 24 (1988) 289-93.
[6] W. L. McKeehan, et al. Cancer Res. 44 (1984) 1998-2010.
[7] This research is supported by an OSU-CHS intramural grant to Dr. Robert J. Ketchum.

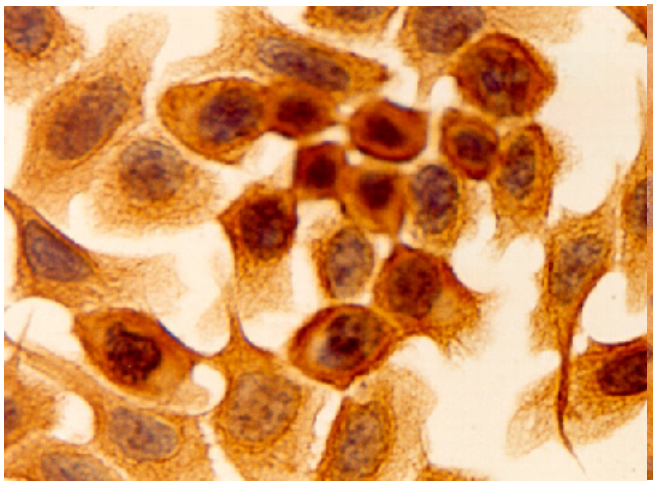

FIG 1. Low calcium growth medium (WAJC404A) cultured TEC. ABC-DAB immunoperoxidase technique with an anti-cytokeratin primary antibody. 250 X

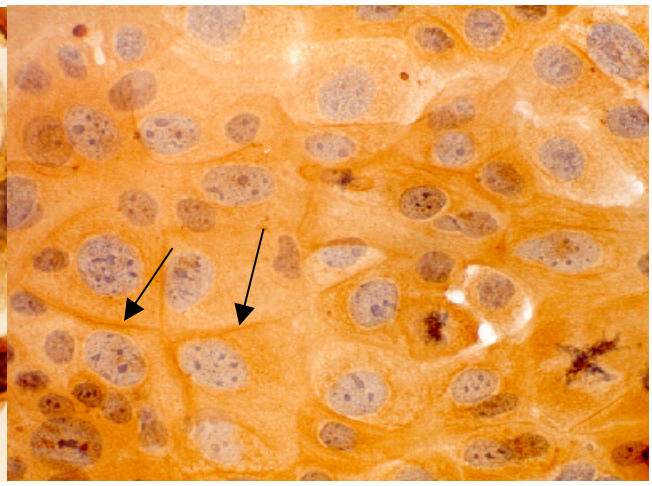

FIG 2. High calcium growth medium cultured TEC. ABC-DAB immunoperoxidase technique with an anti-cytokeratin primary antibody. 250 X

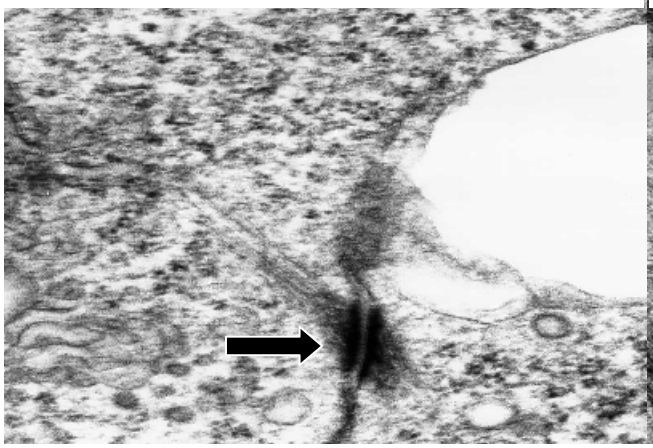

FIG 3. Electron micrograph of TEC demonstrating desmosome type junctions with adjoining filaments. 15,000 X

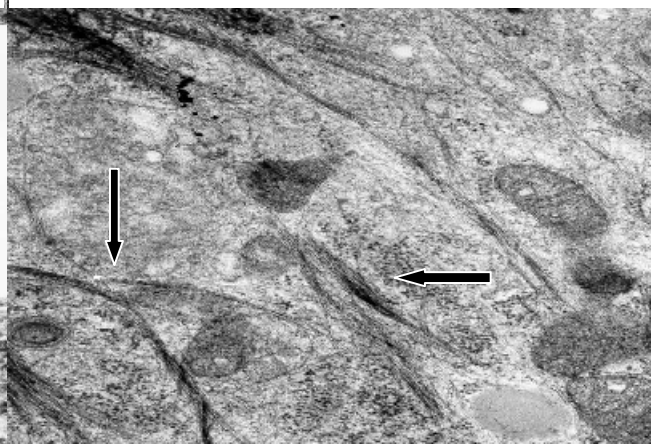

FIG 4. Electron micrograph of TEC demonstrating intracytoplasmic keratin intermediate filament bundles. 10,000 X

https://doi.org/10.1017/S1431927602106878 Published online by Cambridge University Press