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Research article

Zipper-like series of desmosomes supported by subplasmalemmal actin belts in thymic epithelial reticular cells in the rat

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SUMMARY

Remodeling of epithelial tissues requires coordinated cell migration. Most of the mechanisms regulating desmosome assembly and stability in migrating epithelial cells are still unknown. The actin cytoskeleton is a significant component of desmosome assembly and maturation. The association of the actin cytoskeleton with adherens junctions requires additional ultrastructural investigations. A transmission electron microscopic study was performed on five samples of rat thymus. Interepithelial series of desmosomes up to 6.5 μm length were found as were composite series of junctions (tight, adherens, and desmosomes). As a particular feature, subplasmalemmal belts of microfilaments, apparently of actin, ran adjacent to the inner dense plaques of desmosomes, passing beneath and at a distance to the tight junctions. They were not found beneath the adherens junctions. The series of desmosomes were termed zipper-like desmosomes (ZLDs), and were either complete, or imperfect. Terminal imperfect or incomplete desmosomes were found at the ends of the zipper-like series. Alpha-smooth muscle actin immune labeling on six other samples of rat thymus was strongly suggestive of the existence of subplasmalemmal actin belts in the epithelial reticular system. Further studies are needed to establish the exact role of the ZLDs during processes of epithelial remodeling.

1. Introduction

Four major categories of intercellular junctions are distinguished: gap junctions (nexus), tight junctions (TJ; *zonulae* or *fasciae adherentes*), adherens junctions (*zonula adhaerens, fascia adhaerens, puncta adhaerentia*) and desmosomes (*maculae adhaerentes*) (Franke et al., 2009). However, continuing expansion of knowledge on the diversity of adherens junctional structures indicates that it may still be too premature to close the textbook on cell-cell junctions (Pieperhoff et al., 2010).

Desmosomes are intercellular junctions, the primary function of which is strong adhesion (hyperadhesion) (Garrod, 2010; Thomason et al., 2010). They connect intermediate filaments to the cell surface and thus mediate hyperadhesion (Beaudry et al., 2010; Kottke et al., 2006).

The characteristic structure of desmosomes has been recognized since the early days of electron microscopy (Thomason et al., 2010). Desmosomes are known: (a) to occur in various tissues subjected to significant mechanical stress (epithelial cells, cardiomyocytes), or not (thymic epithelial reticular cells); (b) to be associated with intermediate filaments (Franke et al., 2009; Kottke et al., 2006).

Although desmosomes function as robust adhesive structures, they are also subject to dynamic regulation and undergo continual turnover (Kottke et al., 2006). Adhesive junction assembly and stability are tightly controlled processes and there must be mechanisms to allow cells to move with respect to their neighbors during normal physiologic processes (Roberts et al., 2011). Mechanical anchoring of actomyosin networks at adherens junctions is essential for force production during cell morphogenesis (Rauzi et al., 2010).

The thymus is a central lymphoid organ in which bone marrow-derived T cell precursors undergo a complex process of maturation (Linhares-Lacerda et al., 2010). Thymic epithelium is organized in a highly connected three-dimensional (3D) network through which thymocytes interact with the thymic microenvironment in a defined spatial order, and differentiate (Guillemot et al., 2001; Linhares-Lacerda et al., 2010). The molecular mechanisms underlying this organization are still unknown (Guillemot et al., 2001).

It was hypothesized that the morphology of the thymic epithelial reticular (TER) network could relate to a peculiar ultrastructure

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that supports the epithelial remodeling processes. A qualitative transmission electron microscopy (TEM) study was designed to evaluate the interepithelial junctions in the TER network.

2. Materials and methods

Bioptic samples of thymuses were obtained from six Wistar rats (100–150 g, 6 months). After preanaesthesia with ether, the animals were euthanatized as described elsewhere (Rusu et al., 2012a, 2012b). All procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health, and "Carol Davila" University of Medicine and Pharmacy Bioethics Committee approved the protocols. The *EC Directive 86/609/EEC for animal experiments* and the *Uniform Requirements for manuscripts submitted to Biomedical journals* were followed accordingly. Samples of thymuses were formalin-fixed and paraffin-embedded. The samples were sectioned at 3 μm, and stained with hematoxylin eosin to appreciate the general histology of tissues.

2.1. Immunohistochemistry

Immunohistochemistry for alpha-smooth muscle actin (α-SMA) (1:100, BIOCARE MEDICAL CME 305 A,B, clone C04018, Concord, CA, USA) was performed on 3 μm thick sections from 10% formalin fixed paraffin-embedded specimens. The sections were deparaffinized in "Slide bright" and a descending series of alcohol rinses, and then rehydrated in distilled water. Endogenous peroxidase was blocked with 3% H_2O_2 5 min at room temperature. For protein blocking, the sections were incubated for 5–10 min at room temperature with a background sniper (Biocare Medical, Concord, CA, USA). Then the sections were incubated with the primary antibodies for 30 min at room temperature, followed by 30 min incubation with a polymer at the same temperature (MACH 4 detection system, Biocare Medical, Concord, CA). The next steps consisted of: incubation for 5 min at room temperature with 3,3′-diaminobenzidine (DAB, Biocare Medical, Concord, CA); counterstaining with hematoxylin.

Microscopic slides were analyzed and digital micrographs were taken and scaled using a ZEISS working station consisting of an AxioImager M1 microscope with an AxioCam HRc camera, and a digital image processing software AxioVision.

2.2. Transmission electron microscopy

For the transmission electron microscopy (TEM) study, Wistar male rats were used (five specimens, 280–300 g, 12 months). The animals were euthanized and thymic samples were dissected out. Small tissue fragments were processed for TEM as previously described (Rusu et al., 2012b). The grids were examined using a Philips electron microscope EM 208S (acceleration voltage of 80 kV); then snapshots were taken using a video camera Veleta and the iTEM Olympus Soft Imaging System.

3. Results

3.1. Immunohistochemistry

α-SMA positive epithelial cells were consistently identified on samples (Fig. 1), mostly, but not exclusively, in cortical areas. Immune positive labeling was eccentric in the cells of the TER network (arrows, Fig. 1), giving a "*honeycomb*" appearance. Positive α-SMA labeling of pericytes in the thymic microvessels was observed, and it was considered positive internal control.

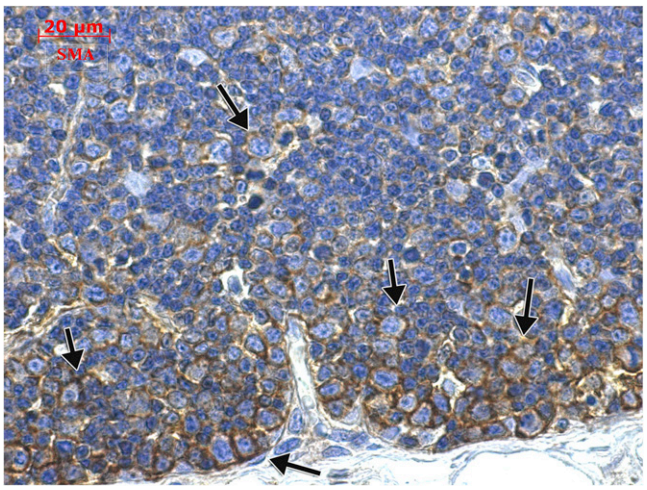

Fig. 1. Rat thymus, immune labeling with anti-α-SMA antibodies. The thymic lobules present a marked, but not exclusive, peripheral labeling, which determines a "*honeycomb*" appearance. The positive immune labeling of the reticular epithelial cells is rather peripheral (subplasmalemmal) than pan-cytoplasmic (arrows).

3.2. Electron microscopy

On ultrathin cuts, several series of spot-like desmosomes were found between thymic epithelial cells. These consisted of up to 17 spot-like desmosomes, with a maximum length of 6.5 μm (Fig. 2A). Due to their general appearance, these desmosome series were termed "*zipper-like desmosomes*" (ZLDs).

The usual structure of desmosomes was clearly identified on cuts; the outer and inner dense plaques were accurately identified on the cytoplasmic side of the plasma membrane (Fig. 2).

In both cells united by such *maculae adhaerentes* well-defined bundles of microfilaments, seemingly of actin, were located on the cytoplasmic side of the inner dense plaques and successively united them (arrowheads in Fig. 2B). In TEM these belts of microfilaments (BmFs) seemed exclusively associated with the ZLDs. Intermediate filaments and ribosomes were observed beneath the BmFs (Fig. 2C).

There were instances in which successive series of desmosomes were observed between adjacent epithelial cells (Fig. 3). In such instances, morphological differences were noted: (a) there were a series of morphologically complete desmosomes, the ZLDs (white arrowheads in Fig. 3A); (b) there were imperfect ZLDs, in which the BmFs and the adhesion plaques were poorly defined (black arrows in Fig. 2B, black arrows in Fig. 3A); (c) there were series of desmosomes in which dense BmFs were aligned beneath almost continuous outer adhesion plaques (white arrows in Fig. 3A).

Composite series of junctions were also found between the thymic epithelial cells, built up by tight and adherens junctions, and desmosomes (Fig. 4). The BmFs were associated with desmosomes (white arrowheads in Fig. 4), passing beneath, but at a distance to the tight junctions (black arrow in Fig. 4), and lacking at the *zonulae adhaerentes* level (white arrow in Fig. 4).

Imperfections in the ends of the zipper-like series of desmosomes were noted: BmFs continued beyond the terminal desmosome of the series, the outer dense plaques appeared disorganized, or in one of the two cells joined by ZLDs the adhesion plaques were lacking. These imperfections were regarded with caution due to the general three-dimensional architecture of the tissues explored on the ultrathin bi-dimensional cuts.

Successive desmosomes presenting well-defined outer dense plaques, but lacking the inner plaques, were also found. These desmosomes associated BmFs on the cytoplasmic side of the outer dense plaques (Fig. 5). The BmFs were continuous (black arrowheads in Fig. 5) between successive outer dense plaques. Beneath