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RESEARCH ARTICLE

Endoneurial-CD34 positive cells define an intermediate layer in human digital Pacinian corpuscles

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ABSTRACT

The endoneurial and/or perineurial origin of the outer core; i.e. the concentric and continuous lamellae located outside the complex formed by the axon and the Schwann-related cells, in human Pacinian corpuscles is still debated. Here we used immunohistochemistry coupled with a battery of antibodies to investigate the expression of perineurial (Glucose transporter 1 and epithelial membrane antigen) or endoneurial (CD34 antigen) markers in human digital Pacinian corpuscles. CD34 immunoreactivity was restricted to one layer immediately outside the inner core, whereas the proper outer core displayed antigens typical of the perineurial cells. These results demonstrate an intermediate endoneurial layer that divides the Pacinian corpuscles into two distinct compartments: the avascular inner neural compartment (formed by the axon and the Schwann-related cells that form the inner core), and the outer non-neural compartment (formed by the outer core). The functional relevance of these findings, if any, remains to be clarified.

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1. Introduction

The cutaneous Pacinian corpuscles are specialized and complex mechanosensory formations classically described to have a single central axon, periaxonic Schwan-like cells forming the so-called inner core (diversely arranged in the preterminal, terminal and ultraterminal segments of the corpuscle), and endo-perineurial cells arranged in a multilayered concentric fashion to form the outer core and the capsule (Bell et al., 1994; Zelená, 1994; Malinovský, 1996; Zimmerman et al., 2014). These main corpuscular constituents; i.e. axon, inner core and outer core-capsule, are continuous with structures of the nerves supplying them and share their immunohistochemical profiles (Vega et al., 1996, 2009). Pacinian corpuscles work as low-threshold rapidly-adapting mechanoreceptors connected to Aβ sensory nerve fibers (Johnson, 2001; Roudaut et al., 2012; Fleming and Luo, 2013).

The origin and phenotype of the periaxonic cells in Pacinian corpuscles have been linked to Schwann-like and endoneurial-perineurial cells (Saxod, 1996; see also Vega et al., 1996). Nevertheless, the precise contributions of the endoneurium and the perineurium still remain a matter of controversy (see for references Vega et al., 1996, 2009). At present, the availability of specific markers for both of these nerve seats (Weiss and Nickoloff, 1993; Khalifa et al., 2000; Hirose et al., 2003; Richard et al., 2014) and presumably of their corpuscular counterparts too, might contribute to solving this problem.

Thus, the present study was designed to investigate the occurrence and distribution of specific perineurial (epithelial membrane antigen-EMA-, and glucose transporter 1-Glut1-) and endoneurial (CD34) antigens in human cutaneous Pacinian corpuscles, using immunohistochemistry, and therefore contribute to establishing the actual filiation of the outer core cells.

2. Material and methods

Skin samples were obtained from the palmar aspect of the distal phalanx of the first finger of the right hand. The fingers were taken from autopsies of cadavers ranging 12–72 years old (n = 11). Peripheral nerve samples were obtained for comparative purposes from cervical lymphadenectomy without malignancy (60 years old), from Morton neuroma (54 years old), from a parotid Warthin

Abbreviations: EMA, epithelial membrane antigen; Glut1, glucose transporter 1; NSE, neuron specific enolase; NFP, neurofilament.

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tumor (85 years old) and from the cutaneous nerves comprised in the analyzed sections. Samples were fixed in 4% formaldehyde (neutral buffered, Sigma-Aldrich™) for 24–48 h, dehydrated and routinely embedded in paraffin (automated with Leica™ ASP6025, Leica Biosystems™, Wetzlar, Germany). All tissues employed in this study were obtained in compliance with Spanish laws, following the guidelines of the Helsinki Declaration (World Medical Association, 2013). The pieces were cut into 3 μm and 8 μm thick sections perpendicular to the skin surface and mounted on gelatin-coated microscope slides. The presence of Pacinian corpuscles was determined using hematoxylin-eosin staining.

Deparaffinized and rehydrated sections were processed for indirect inmunohistochemistry using a Leica Bond™ Max automated stainer, and the Leica Bond™ Polymer Refine Detection Kit (Leica Biosystems™) following manufacturer instructions. The following primary antibodies were used in order to reveal the main constituents of the Pacinian corpuscle: anti-CD34 (prediluted mouse, clone QB-END/10, Master Diagnóstica™; Granada, Spain), anti-S100 protein (prediluted rabbit polyclonal, Novocastra™, Wetzlar, Germany), anti-EMA (prediluted mouse monoclonal, clone GP1.4, Novocastra™, Wetzlar, Germany), anti-Glut1 (prediluted rabbit polyclonal, Cell Marque™, Rocklin, CA, USA), anti-neuron specific enolase (NSE, prediluted mouse monoclonal, clone E27, Master Diagnóstica™; Granada, Spain) and anti-neurofilament proteins (prediluted mouse monoclonal, clone 2F11, Biocare Medical™, Concord, CA, USA). Indirect immunohistochemistry had several negative and positive controls included as well as the internal positive and negative controls.

Double immunohistochemistry was performed in sequence using the same method: a complete staining process was applied to the first antibody, revealed with diaminobenzidine (Leica Bond™ Polymer Refine Detection Kit, Leica Biosystems™); then another complete process was performed with the second antibody, revealed with Fast Red. All the reagents were from the Leica Bond™ Polymer Refine Red Detection Kit, Leica Biosystems™.

Furthermore, double immunofluoresence was carried out to investigate the corpuscular distribution of CD34, S100 protein and vimentin. In deparaffinized and rehydrated sections the non-specific binding was reduced (incubation for 30 min with a solution of 5% bovine serum albumin in tris-buffer saline -TBS, pH 7.4-). The sections were then incubated overnight, at 4 °C in a humid chamber, with a 1:1 v/v mixture of anti-CD34 (Master Diagnóstica™) and anti-S100 protein (Dako, Glostrup, Denmark; rabbit polyclonal antibody, diluted 1:1000), or anti-CD34 (Master Diagnóstica™) and anti-vimentin (Santa Cruz Biotechnology™, Santa Cruz, CA, USA, diluted 1:200). After rinsing, the sections were incubated for 1 hour with Alexa fluor 488-conjugated goat anti-rabbit IgG (Serotec™, Oxford, UK, diluted 1:1000), then rinsed again and incubated for another hour with Cy 3-conjugated donkey anti-mouse antibody (Jackson-ImmunoResearch™, Baltimore, MD, USA, diluted 1:50). Both steps were performed at room temperature in a dark humid chamber. Sections were then washed and mounted with Fluoromount Gold. Finally, to ascertain structural details, sections were counterstained with DAPI (10 ng/ml) to label the nuclei. Triple staining was detected using a Leica DMR-XA automatic fluorescence microscope coupled with a Leica Confocal Software, version 2.5 (Leica Microsystems, Heidelberg GmbH, Germany) and the images captured were processed using the software ImageJ version 1.43 g Master Biophotonics Facility, Mac Master University Ontario (www.macbiophotonics.ca). For control purposes, representative sections were processed in the same way as described above using non-immune rabbit or mouse sera instead of the primary antibodies or omitting the primary antibodies in the incubation.

3. Results

All the investigated antigens were detected with the antibodies used in human Pacinian corpuscles. Specific immunoreactivity for NSE and NFP was detected in the central axon (data not shown), whereas S100 protein immunostaining selectively labelled the Schwann-like cells forming the inner core. EMA immunoreactivity was observed mainly in the outer compartment of the outer core but faintly in the inner portion, while Glut1 immunostaining labelled similarly the entire outer core. Regarding CD34, all the Pacinian corpuscles displayed CD34 immunoreactivity in a distinct immunoreactive flat layer disposed outside the inner core (Fig. 1). This finding was constant and was independent of the age and gender of the subjects. However, in the Pacinian corpuscles from the younger individuals the CD34-positive layer was apparently complete and continuous while in the older ones it had a less-defined contour (Fig. 1).

To confirm the precise localization of the CD34-posive layer within the Pacinian corpuscles, double immunohistochemistry and double immunofluorescence were performed. As expected, the

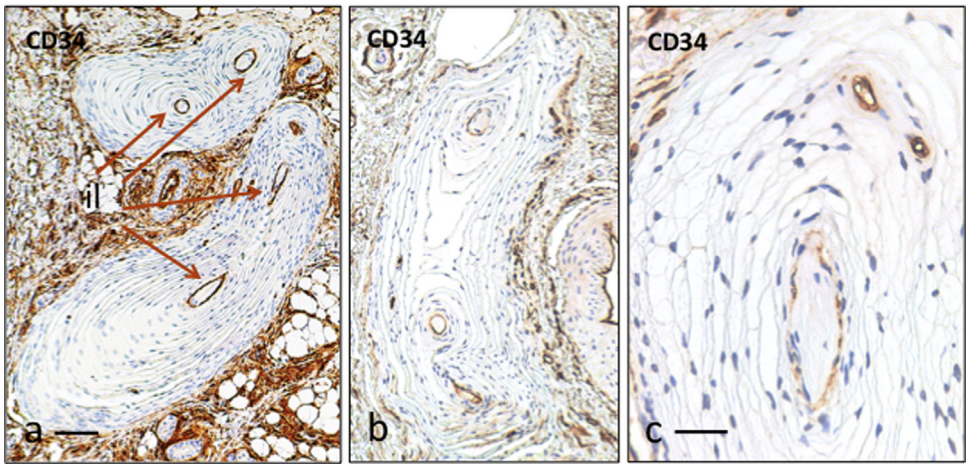

Fig. 1. Human cutaneous Pacinian corpuscles labeled with anti-CD34. Objective 10× (scale bar 100 μm) (a and b) and 20× (scale bar 40 μm) (c). A single cell-thick distinct layer is apparent surrounding the inner core. There are various structures reactive with CD34 like vascular endothelia and numerous stromal cells surrounding the corpuscle. Note the differences in thickness and sharpness of the CD34+ layer between a young individual (12 years old) (a) and an older one (54 years old) (b and c). Arrows indicate the intermediate layer (il).

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