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SHORT COMMUNICATION

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LN-5 antibody against human macrophages cross-reacts with routinely processed human sebaceous glands

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Abstract: LN-5 monoclonal antibody against human macrophages was found to selectively stain human sebaceous glands in formalin-fixed, paraffin-embedded skin samples. Undifferentiated sebocyte progenitors were negative, and only sebocytes from the onset of their differentiation revealed positive cytoplasmic immunofluorescence. Since there are very few selective and easy-to-use markers of sebaceous glands, LN-5 antibody can offer a simple and relatively specific way to detect human sebocytes from the onset of their differentiation in routinely processed material, both freshly prepared and archival. *(Folia Histochemica et Cytobiologica 2012, Vol. 50, No. 2, 319–321)*

Key words: LN-5 antibody, sebaceous glands, human, immunofluorescence

Introduction

LN-5 is a mouse monoclonal antibody reactive with the cytoplasm of human macrophages and Langerhans cells in formalin-fixed, paraffin-embedded tissues [1]. The responsive antigenic epitope has not been characterized, since whole Ficoll-Hypaque-separated normal human peripheral blood mononuclear cells were used for immunization of mice. The antibody was employed to study distribution of macrophages in normal tissues [2] and in histiocytic lesions [3]. We have noticed that LN-5 intensely cross-reacts with differentiated sebocytes of sebaceous glands in formalin-fixed, paraffin-embedded human skin.

Material and methods

Human thin skin samples were fixed in 4% buffered formalin for 48 h, and routinely embedded in paraffin. Six μm sections were collected on poly L-lysine-coated glass slides, deparaffinized in xylene, rehydrated in ethanol, and washed in phosphate-buffered saline (PBS) (pH = 7.4). After pre-incubation with 5% normal goat serum for 40 min at room temperature, sections were incubated overnight with LN-5 (Leica Microsystems/Novocastra, Newcastle, UK, product code NCL-LN5) antibody diluted 1:50, in a humid chamber at room temperature. Next, sections were washed extensively in PBS and incubated for 90 min with goat anti-mouse Cy-3-conjugated antibodies (Jackson IR, West Grove, PA, USA, code no. 115-165-146) at a dilution of 1:400. Cell nuclei were counterstained with DAPI (Sigma, St Louis, MO, USA). Sections were washed three times in PBS and mounted in glycerol/PBS solution (pH = 8.6). Negative control was performed by omitting the primary monoclonal antibodies during the first incubation. Positive control was carried out on paraffin-embedded human lung. Sections were examined under an Olympus BX50 light/fluorescence microscope equipped with a DP-71 digital CCD camera (Olympus, Japan).

Results and discussion

In the lung, LN-5 stained alveolar macrophages (Figure 1). In the skin, LN-5 intensely cross-reacted with sebaceous glands. The immunofluorescence was confined to sebocytes that at least entered the differentiation pathway, since undifferentiated sebocyte progenitors located at the periphery of glandular acini were negative and the adjacent 'young' sebocytes displayed weak immunoreactivity (Figure 2). In sebocytes, the staining was cytoplasmic, with more in-

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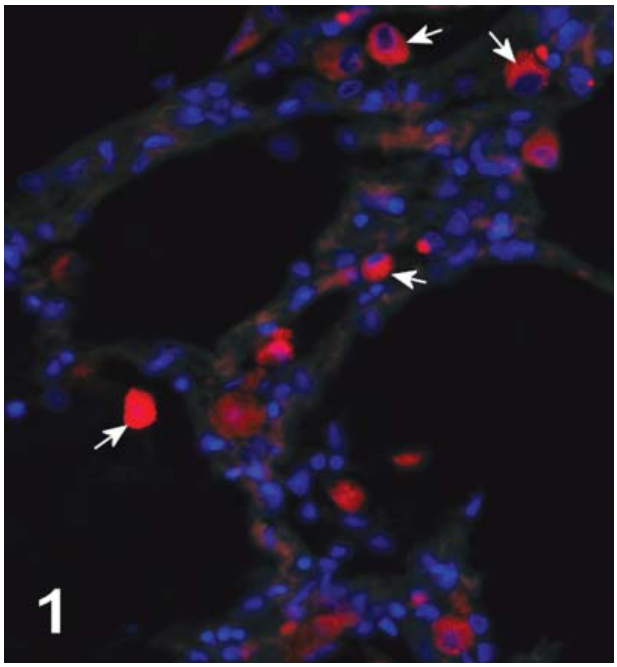

Figure 1. LN-5 positive alveolar macrophages in human lung (arrows). Cell nuclei (blue) counterstained with DAPI (magnification × 400)

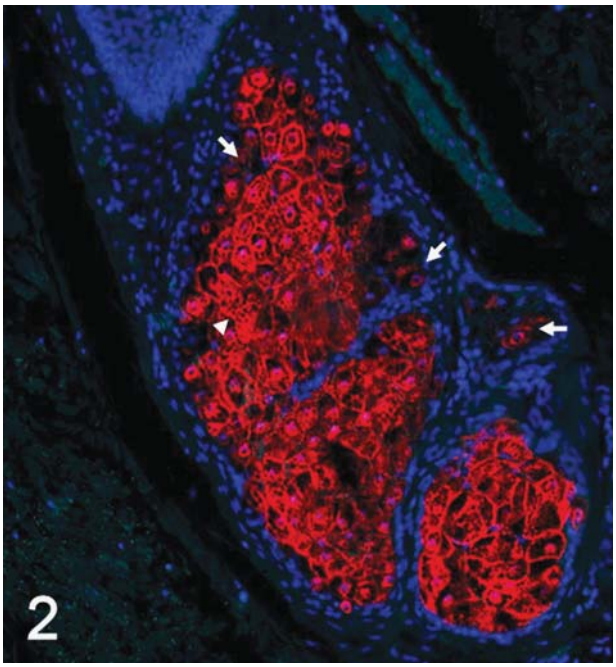

Figure 2. LN-5 positive human sebaceous gland. Peripherally located undifferentiated sebocyte progenitors are negative and 'young' sebocytes display weak immunofluorescence (arrows). 'Negatives' of sebum droplets are visible on the background of fluorescent cytoplasm (arrowhead). Cell nuclei (blue) counterstained with DAPI (magnification × 200)

tensely fluorescent subplasmalemmal and perinuclear areas and 'negatives' of sebum droplets visible between the fluorescent areas in the other areas of cytoplasm. Immunoreactive sebaceous glands were observed in skin sections irrespective of whether they had been previously subjected to high temperature antigen-unmasking treatment according to the manufacturer's instructions.

In their original paper, Bhoopat et al. [1] reported cross-reactions of LN-5 with human B lymphocytes, spermatogonia and chief cells of fundic glands. In the skin, they observed only immunopositive Langerhans cells, which suggests that they tested thick skin, lacking sebaceous glands. Because of the observed cross-reactivity, LN-5 could be regarded as a selective marker of sebocyte differentiation in routinely processed human skin.

A number of putative sebaceous gland markers, mostly involved in the sebocyte differentiation process, have been proposed in the literature. The markers include cytokeratins 4, 7, 13, 19 [4], peroxisome proliferator-activated receptors (PPARs) [5], OM-1 antigen [6], C/EBP transcription factors [7], melanocortin-5 receptor [8], Indian hedgehog and Gli1 [9]. However, all these markers (possibly with the exception of OM1 which is also expressed in ovarian carcinoma cells, but not in other normal tissues), are not specific for sebaceous glands, since they are present in different cell types. Moreover, they mostly require special immunohistochemical procedures and cannot be demonstrated in formalin-fixed, paraffin-embedded tissues.

Hence, LN-5 antibody could offer a simple and relatively specific way of detecting human sebocytes from the onset of their differentiation in routinely processed material, both freshly prepared and archival.

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