Short Communication

Human Epidermal Langerhans Cells Are Selectively Recognized by Galectin-3 but Not by Galectin-1

(Langerhans cell / galectin-1 / galectin-3 / keratinocyte / endogenous lectins / lectin histochemistry)

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Abstract. Langerhans cells are dendritic antigen-presenting cells residing predominantly in the epidermis. Since endogenous galactoside-binding lectins with the jelly-roll motif (galectins) are known to trigger cellular responses, including mediator release, we investigated by lectin histochemistry the cells' capacity to bind two common members of this family, i.e. galectin-1 and -3. Actually, surrounding keratinocytes express a high level of galectin-3, and these cells can be considered as donors of this lectin to Langerhans cells. Employing biotinylated galectin-1 and -3, and concomitantly an antibody against CD1a as a second marker, to visualize the position of Langerhans cells in the human epidermis, the expression of galectin-3-reactive glycoligands in contrast to the lack of binding of galectin-1 was observed. Although the functional consequences of this selectivity are unclear, these results reveal an example for differential cellular reactivity towards two related endogenous lectins.

Langerhans cells are dendritic professional antigen-presenting cells derived from the myeloid lineage, located predominantly in the epidermis (Maurer and Stingl 1999; Santiago-Schwarz, 1999). Although these cells give no signal of the presence of mRNA for galectin-3 in their cytoplasm (Wollenberg et al., 1993), they exhibit strong positivity in the Birbeck granules (Smetana et al., 1999). Evidently, Gal-3 is expressed by keratinocytes, except for the cells of the basal layer (Wollenberg et al., 1993; Konstantinov et al., 1994; Holíková et al., 1999; Smetana et al., 1999), rendering it likely that these cells are donors of Gal-3 for import into Langerhans cells (Wollenberg et al., 1993; Smetana et al., 1999). Gal-3 (M_r ranging from 29.000–35.000 depending on the animal species) is a member of the ß-galactoside-binding animal lectin family of the galectins. Gal-3 molecules have remarkable biological activities such as activation of macrophages, mediator release and participation in the control of cell proliferation or apoptosis (Gabius, 1997; Rabinovich, 1999). Thus, Gal-3 can belong to effectors responsible for modification of the activity of Langerhans cells by the nervous system and products released from the surrounding keratinocytes (Kimber et al., 1998; Misery, 1998; Kondo, 1999; Schmitt, 1999). One prerequisite for such a role is a selective binding of galectin-3 to this cell type. In this study we demonstrate the binding of Gal-3 to the epidermal Langerhans cells *in situ*, employing the technology of dual labeling colocalizing CD1a as a marker of Langerhans cells. The results were compared with the binding of galectin-1, a related but functionally different galectin.

Material and Methods

The skin samples of 3 donors were received from the Department of Esthetic Surgery of the 3rd Faculty of Medicine, Charles University in Prague. They were frozen in liquid nitrogen using Tissue-Tek™ (Miles Inc., Elkhart, IN) as a cryoprotective agent. The cryocut sections were fixed with 4% (w/v) paraformaldehyde in PBS (pH 7.2–7.4), and the procedure of simultaneous labeling (Froňková et al., 1999; Smetana et al., 1999) for CD1a (Immunotech, Prague, Czech Republic) and for

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Abbreviations: FITC – fluorescein isothiocyanate, Gal-1 – galectin-1, Gal-3 – galectin-3, PBS – phosphate-buffered saline, TRITC – tetramethylrhodamine isothiocyanate.

glycoligands reactive for Gal-1 or -3 using biotinylated galectins (Smetana et al., 1998) was used. The FITC-labeled swine anti-mouse antibody (SwAM-FITC, SEVAC, Prague, Czech Republic) and TRITC-labeled ExtrAvidine (Sigma, Prague, Czech Republic) diluted as recommended by suppliers were used as second-step reagents. The specimens were mounted in Vectashield (Vector Laboratories Inc., Burlingame, CA). Control experiments were performed by omission of the first-step antibody and galectins, by replacing the first-step antibody by mouse non-immune serum, and by addition of lactose as a competitive inhibitor to the incubation medium. The results of double-labeling experiments were verified using the computer-assisted image analysis system LUCIA G/F Magic (Laboratory Imaging, Prague, Czech Republic).

Results

The keratinocytes of every layer of human epidermis expressed Gal-1-reactive binding sites (in very low concentration) in contrast to the reactivity for Gal-3, which was observed strictly suprabasally (Table 1). The signal for Gal-3 binding to Langerhans cells was clearly visible when these cells were located in the basal layer of epidermis. The Gal-3 binding to suprabasally placed Langerhans cells was only weak, because they were masked by the presence of keratinocytes with extensive binding of Gal-3 (Table 1). However, the double-labeling procedure

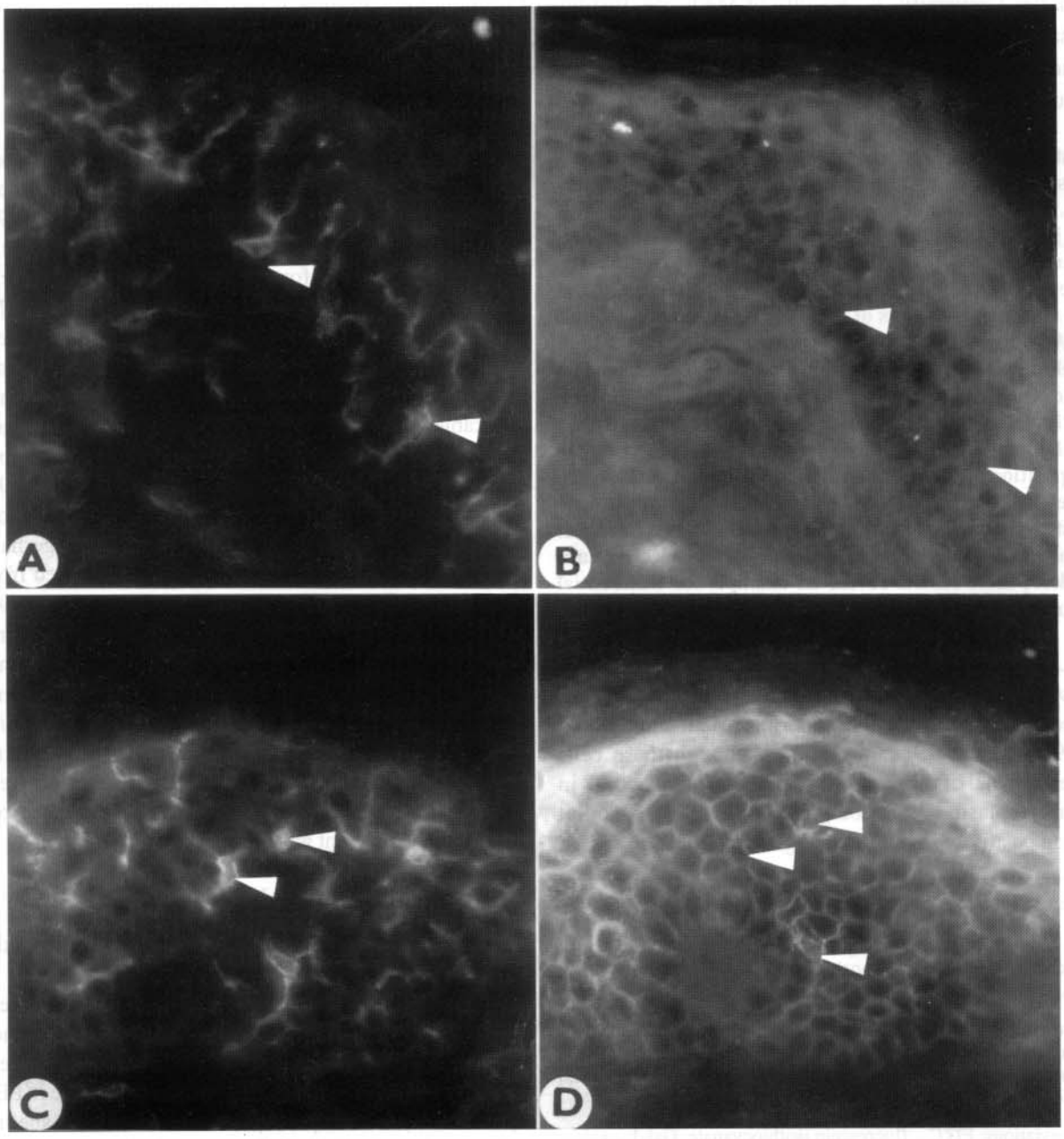

Fig. 1. Double labeling of sections of adult human skin for CD1a (A, C) and binding sites for galectin-1 (B) and galectin-3 (D). Position of Langerhans cells is indicated by arrowheads. Magnification 450x.