Comparison of the Expression of Langerin and 175 kD Mannose Receptor in Antigen-Presenting Cells in Normal Human Skin and Basal Cell Carcinoma

(Langerhans cell / Langerin / 175 kD mannose receptor / epidermis / basal cell carcinoma)

Z. PLZÁKOVÁ[1], M. CHOVANEC[1, 2], K. SMETANA, Jr.[1], J. PLZÁK[1, 2], J. ŠTORK[3], S. SAELAND[4]

[1]Charles University, 1st Faculty of Medicine, Institute of Anatomy, Prague, Czech Republic
[2]Charles University, 1st Faculty of Medicine, Department of OtorhinOlaryngology, Head and Neck Surgery, Prague, Czech Republic
[3]Charles University, 1st Faculty of Medicine, Department of Dermatovenerology, Prague, Czech Republic
[4]Laboratory for Immunological Research, Schering-Plough, Dardilly, France

Abstract. The presence of professional antigen-presenting cells in tumours can influence their further spreading. Location of cells exhibiting a specific marker of Langerhans cells – Langerin, and the 175 kD mannose receptor as a marker of dendritic cells of non-Langerhans type and macrophages, was studied using double staining in the normal human epidermis and in basal cell carcinomas. The Lagerin-positive cells strictly colonized the epidermis and no cells were found in the dermis, where 175 kD mannose receptor-exhibiting cells were present. Very rare elements in the epidermal/dermal interface were positive for both markers. A low incidence of Langerin-positive cells was found in tumours and 1/3 of studied carcinomas were even Langerhans cell-free. The extraepithelial presence of Langerin-positive cells forming contacts with dendrite-like protrusions of 175 kD mannose receptor-exhibiting cells was found in connective tissue surrounding the tumour epithelium and indicates possible cooperation of both elements.

Langerhans cells (LC) are members of the family of dendritic cells, a type of leukocytes that are specialized in antigen presentation. LC occur in epithelia, where they participate in the immune surveillance of the organism (for review see Plzák et al., 2003). It is known that mannosylated antigens are presented with significantly higher efficiency than when mannose- free (Tan et al., 1998). The endogenous tandem repeat C-type lectin 175 kD mannose receptor (MR), a main molecule recognizing this saccharidic motif, was established to participate in this process (for review see Martínez-Pomares and Gordon, 1999). However, although this lectin is generally expressed in many types of non-mature dendritic cells (DC), it is not present in LC (Mommaas et al., 1999). On the other hand, LC are able to discriminate mannosides (Mommaas et al., 1999). Discovery of another endogenous C-type lectin, Langerin, a specific marker of LC, helps to elucidate this problem. Langerin is able to recognize mannosides (Valladeau et al., 1999, 2000, 2002; Plzák et al., 2002). In company with another lectin, galectin-3, it is involved in the formation of Birbeck granules, organelles occurring selectively in LC (Smetana et al., 1999; Valladeau et al., 2000). Langerin seems to participate in the process of presentation of non-petide antigens, namely glycolipids, to T cells (Hunger et al., 2004; Mizumoto and Takashima, 2004). Local or distant progression of a malignant tumour is critically important for further destiny of patients. It is affected by many factors dependent on the biological behaviour of tumour cells. However, the question of failure of the immune surveillance, where antigen presentation is a key component, must also be assessed. Comparing the occurrence of LC in the normal epidermis and basal as well as squamous cell carcinomas demonstrated a very low incidence of these cells in basal cell carcinomas (BCC) (Sudo et al., 1987; Schreiner et al., 1995).

The present study compares the presence of cells exhibiting Langerin and MR, which occur in non-Langerhans DC and macrophages, in the normal human epidermis and BCC. The main emphasis is stressed on the tracking of cells exhibiting both lectins, i.e. Langerin and MR, and an ectopic localization of Langerin and MR.

Received April 26, 2004. Accepted May 5, 2004.

This study was supported by the Ministry of Education, Youth and Sport of the Czech Republic, project No. MSM111100005, and the Grant Agency of the Czech Republic, projects No. 304/03/P027 and No. 304/04/0171.

Corresponding author: Karel Smetana, Jr., Charles University, 1st Faculty of Medicine, Institute of Anatomy, U Nemocnice 3, 128 00 Prague 2, Czech Republic.

Abbreviations: BCC – basal cell carcinoma, DC – dendritic cell(s), LC – Langerhans cell(s), MR – 175 kD mannose receptor.

Material and Methods

Five samples of normal human skin (5 donors) were obtained from the Department of Esthetic Surgery, 3rd Faculty of Medicine, Charles University, the samples of BCC (12 donors) from the Departments of Otorhinlaryngology, Head and Neck Surgery and Dermatoverology, 1st Faculty of Medicine, Charles University in Prague after informed consent of donors.

The tissue samples were frozen in liquid nitrogen using the cryoprotective agent Tissue-Tek (Sakura, Zoeterwoude, the Netherlands). The cryocut frozen sections were treated by Triton X-100 (Sigma-Aldrich, Prague, Czech Republic) and fixed with paraformaldehyde (2% w/v) in phosphate-buffered saline (pH 7.2). Langerin was detected using DCGM4 monoclonal antibody (Valladeau et al., 1999) and MR by goat polyclonal antibody (TNO-PG, Leiden, the Netherlands) as described (Smetana et al., 2004). Specificity of the immunohistochemical reaction was validated by using isotype and polyclonal antibody against antigen not occurring in DC to exclude non-specific binding of antibody to Fc receptors. FITC-labelled swine anti-mouse immunoglobulins (AlSeVa, Prague, Czech Republic) and TRITC-labelled donkey anti-goat immunoglobulins (Santa Cruz, Santa Cruz, CA) were employed as second-step reagents. The specimens were mounted with Vectashield (Vector Laboratories, Burlingame, CA) and

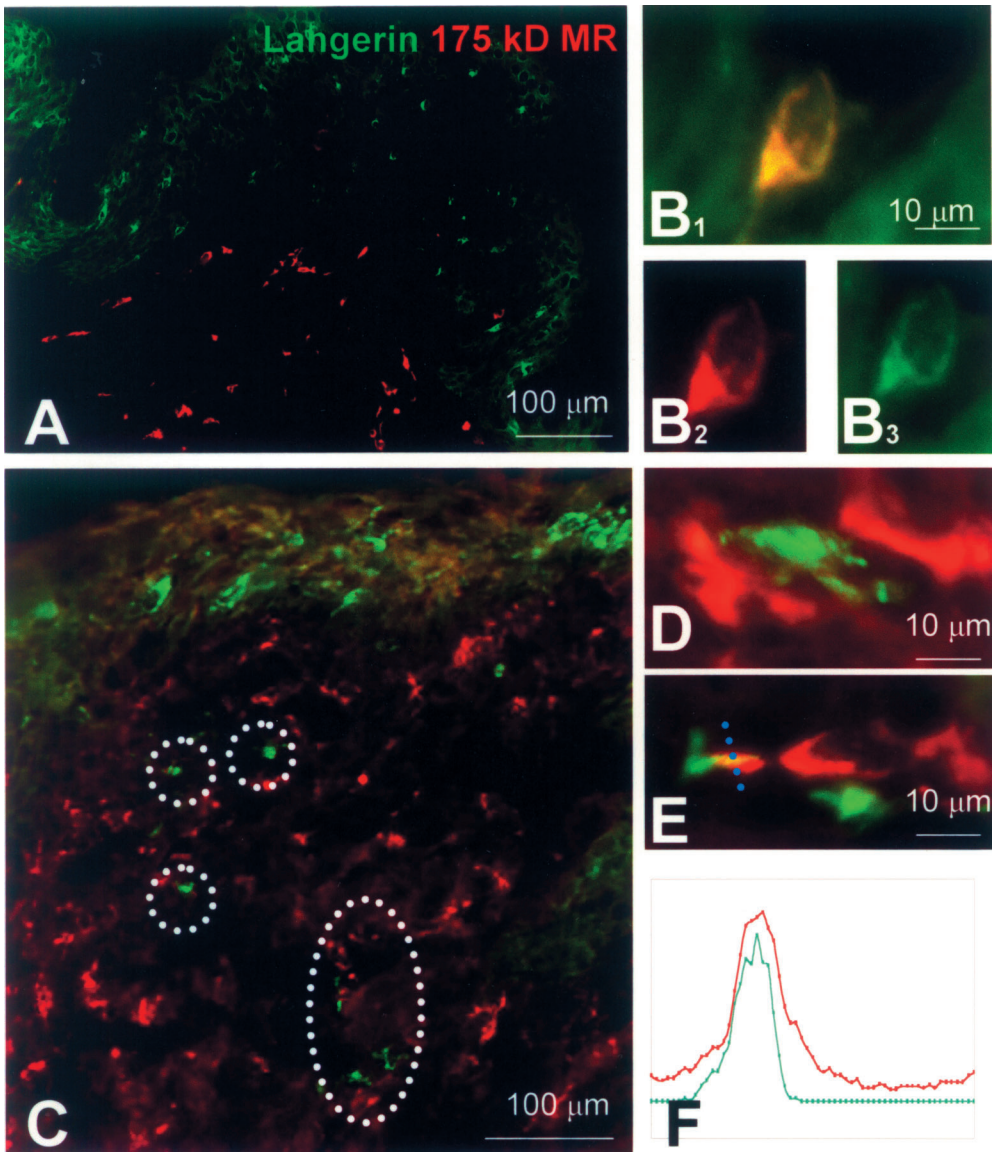

Fig. 1. Detection of Langerin (green signal) and the 175 kD mannose receptor (MR) (red signal) in the normal epidermis (A, B) and in basal cell carcinoma (C-E). Ectopic Langerin-positive cells are marked by a white dashed line. The presence of both cell populations is restricted to defined compartments, Langerin-positive cells to the epithelium and 175 kD mannose receptor-containing cells to the dermis (A). Rare cells exhibited co-expression of both markers (B_1, yellow shift). The intimate contact of dendrite-like protrusions of cells exhibiting Langerin (green signal) and 175 kD MR (red signal) is demonstrated by the yellow signal (E) and verified by measurements of the fluorescence profile (F) at the site of the blue dashed line (E). Bar is 100 µm.

observed in an Optiphot-2 microscope (Nikon, Prague, Czech Republic) equipped with a CCD camera and computer-assisted image analysis system (LUCIA) (Laboratory Imaging, Prague, Czech Republic).

Results and Discussion

Langerin-positive DC were exclusively observed in the epidermis and cells expressing MR in the dermis (Fig. 1A). Only two cells that were positive for both markers were found (Figs. $1B_{1-3}$). Interestingly, these cells were located in the epidermal/dermal interface and it was not possible to precisely determine without further labelling if they were located in the epidermis or in the dermis. The precursors of LC are formed in bone marrow, transported to the dermis where they leave blood vessels, and further migrate through basal lamina to the epidermis. After contact with antigen they migrate out of the epidermis and are transported to draining lymph nodes via lymphatic vessels (for review see Plzák et al., 2003). Finding these double positive cells in the above-described location could represent a checkpoint for the change of phenotype conditioned by the migration of DC into/out the epithelium. However, this phenomenon was very rare in our material, and without an *in vitro* model its interpretation remains speculative.

Studying the Langerin-expressing cells in BCC, the positive cells were found in the majority of tumours (2/3 of samples studied). Similarly to earlier published data, the incidence of LC in the tumour tissue was reduced (Sudo et al., 1987; Schreiner et al., 1995). In contrast to normal skin, no cell positive for both markers, i.e. for Langerin and MR, was found. MR-exhibiting cells (DC, macrophages) were observed in the dermal stromal tissue and no cells were detected in the tumour. One third of studied tumours presented Langerin-positive elements outside the tumour epithelium, frequently a long distance from the epithelial tissue (Fig. 1C). These cells were present in the vicinity of the cells expressing MR (Fig. 1D). The finding of very intimate contacts of dendrite-like structures of Langerin-positive elements with cells exhibiting MR was supported by the shift of red/green signal to yellow (Fig. 1E) and by the measurements of the fluorescence profile (Fig. E).

In conclusion, cells exhibiting both the Langerin and MR lectins were rarely found in the dermal/epidermal interface. A lower incidence of Langerin-positive LC was observed in BCC as compared to the normal epidermis. An atypical location of these elements outside epithelial tissue was observed in the stroma of BCC, where these cells were in contact with elements expressing MR.

Acknowledgements

The authors are grateful to Eva Vancová for excellent technical assistance.

References

Hunger, R., E., Sieling, P., A., Ochoa, M., T., Sugaya, M., Burdick, A., E., Rea, T., H., Brennan, P., J., Belisle, J., T., Blauvelt, A., Porcelli, S., A., Modlin, R., L. (2004) Langerhans cells utilize CD1a and langerin to efficiently present nonpeptide antigens to T cells. *J. Clin. Invest.* **113,** 701-708.

Marínez-Pomares, L., Gordon, S. (1999) The mannose receptor and its role in antigen presentation. *Immunologist* **7,** 119-123.

Mizumoto, N., Takashima, A. (2004) CD1a and langerin: acting as more than Langerhans cell markers. *J. Clin. Invest*. **113,** 658-660.

Mommaas, A., M., Mulder, A., A., Jordens, R., Out, C., Tan, M., C., A., A., Cresswell, P., Kluin, P., M., Koning, F. (1999) Human epidermal Langerhans cells lack functional mannose receptors and fully developed endosomal/lysosomal compartment for loading of HLA class II molecules. *Eur. J. Immunol.* **29,** 571-580.

Plzák, J., Holíková, Z., Dvořánková, B., Smetana, K., Jr., Betka, J., Hercogová, J., Saeland, S., Bovin, N., V., Gabius, H.-J. (2002) Analysis of binding of mannosides in relation to Langerin (CD207) in Langerhans cells of normal and transformed epithelia. *Histochem. J.* **34,** 247-253.

Plzák, J., Holíková, Z., Smetana, K., Jr., Riedel, F., Betka, J. (2003) The role of dendritic cells in the pharynx. *Eur. Arch. Otorhinolaryngol.* **260,** 266-272.

Schreiner, T., U., Lischka, G., Schaumburg-Lever, G. (1995) Langerhans cells in skin tumours. *Arch. Dermatol.* **131,** 1463.

Smetana, K., Jr., Holíková, Z., Klubal, R., Bovin, N., V., Dvořánková, B., Bartůňková, J., Liu, F.-T., Gabius, H.-J. (1999) Coexpression of binding sites for A (B) histo-blood group trisaccharides with galectin-3 and Lag antigen in human langerhans cells. *J. Leukocyte Biol.* **66,** 644-649.

Smetana, K., Jr., Měřička, O., Saeland, S., Homolka, J., Brabec, J., Gabius, H.-J. (2004) Diagnostic relevance of Langerin detection in cells from bronchoalveolar lavage of patients with pulmonary Langerhans cell histiocytosis, sarcoidosis and idiopathic pulmonary fibrosis. *Virchows Arch.* **444,** 171-174.

Sudo, S., Saito, A., Morohashi, M. (1987) Immunohistochemical study of Langerhans cells in skin tumours. *Dermatologica* **174,** 76-83.

Tan, M., C., A., A., Jordens, R., Geluk, A., Roep, B., O., Ottenhoff, T., Drijfhout, J., W., Koning, F. (1998) Strongly increased efficiency of altered peptide ligands by mannosylation. *Int. Immunol*. **10,** 1299-1304.

Valladeau, J., Duvert-Frances, V., Pin, J.-J., Dezutter-Dambuyant, C., Vincent, C., Massacrier, C., Vincent, J., Yoneda, K., Banchereau, J., Caux, C., Davoust, J., Saeland, S. (1999) The monoclonal antibody DCGM4 recognizes Langerin, a protein specific for langerhans cells, and is rapidly internalized from the cell surface. *Eur. J. Immunol.* **29,** 2695-2704.

Valladeau, J., Ravel, O., Dezutter-Dambuyant, C., Moore, K., Kleijmeer, Liu, Y., Duvert-Frances, V., Vincent, C., Schmitt, D., Davoust, J., Caux, C., Lebecque, S., Saeland, S. (2000) Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. *Immunity* **12,** 71-81.

Valladeau, J., Clair-Moninot, V., Dezutter-Dambuyant, C., Pin, J.-J., Kissenpfenig, A., Mattéi, M.-G., Ait-Yahia, S., Bates, E., E., M., Malissen, B., Koch, F., Fossiez, F., Romani, N., Lebecque, S., Saeland, S. (2002) Identification of mouse Langerin/CD207 in Langerhans cells and some dendritic cells of lymphoid tissues. *J. Immunol.* **168,** 782-792.