Klin. Biochem. Metab., 15 (36), 2007, No. 1, p. 14–20.

Ultrastructural analysis of the reactivity of human Langerhans cells at various time intervals after the administratioin of 2,4-dinitrochlorobenzene

Schramlová J.[1], Mardešičová L.[2], Barták P.[1], Vaněk M.[3]

[1]National Institute of Public Health, Prague, Czech Republic
[2]Department of Skin Surgery and Esthetic Dermatology, Institute for Postgradute Medical education, University Hospital, Prague, Czech Republic
[3]Mendel University of Agriculture and Forestry, Brno, Czech Republic

SUMMARY
Objective: The goals of this study were to assess morphologically the DNCB effect on Langerhans cells in the epidermis at different time intervals and to devise an in vivo test for characterization of immunoreactivity of Langerhans cells and thus of the whole skin.
Methods: Reactivity of Langerhans cells (LCs) at different time intervals after the application of 0.1 % 2,4--dinitrochlorobenzene (DNCB) on the skin of 20 volunteers was studied. Skin biopsy specimens were investigated 1, 3, 10, 30 minutes and 72 hours after DNCB application.
Results: The dendrites underwent similar morphological changes as the bodies of Langerhans cells and the ultrastructural composition of the former also reflected cell activation. Other investigators did not previously pay attention to the dendrites. Our findings confirm formation of Birbeck granules (Bgs) resulting from ligand-receptor mediated endocytosis, most evident in group II where LCs showed more rapid and more vigorous activation and Bgs connected to the plasma membrane were detected in both dendrites and cell bodies. Furthermore, Birbeck granule-like structures were found in group II LCs dendrites. They reflect enhanced reactivity of these cells that do not represent a different type of LCs. A majority of the intracellular MHC class II molecules were found in vesicular structures, the so-called MHC-II compartment (MIIC). Simultaneously with Bgs, MIIC compartments develop in the cytoplasm and are most abundant at the moment when LCs leave the epidermis.
Conclusions: We found that within 30 minutes after DNCB application, skin reactivity can be assessed, since at that interval the activation of Langerhans cells is fully completed. We suppose that the DNCB concentration used is sufficient for testing activation of Langerhans cells and at the same time no harm to the patient is to be expected. The use of biopsy needles of a small diameter is safe since the puncture affects only the epidermis and the upper layer of the corium and thus the use of local anaesthesia can be avoided.
Key words: human skin, Langerhans cells (LCs), Birbeck granules (Bgs), 2,4-dinitrochlorobenzene (DNCB).

SOUHRN
Schramlová J., Mardešičová L., Barták P., Vaněk M.: Ultrastructural analysis of the reactivity of human Langerhans cells at various time intervals after the administratioin of 2,4-dinitrochlorobenzene
Cíl studie: Cílem této studie bylo morfologické zhodnocení vlivu DNCB na Langerhansovy buňky v epidermis v různých časových obdobích a najít vhodný test *in vivo*, který by charakterizoval imunoreaktivitu LCs a tím i celé kůže.
Materiál a metody: Dvaceti dobrovolníkům byly aplikovány 0,2 ml DNCB (2,4-dinitrochlorobenzen) v acetonu na kůži levého předloktí. Kožní biopsie byly odebírány kožními průbojníky o průměru 3 mm do hloubky 2 mm v intervalech 1, 3, 10, 30 minut a 72 hodin po aplikaci.
Výsledky: Sledovali jsme nejen morfologické změny těl Langerhansových buněk (LCs), ale také jejich dendritů. Zjistili jsme, že dendrity těchto buněk procházejí obdobnými morfologickými změnami jako těla LCs a že jejich ultrastrukturální složení je rovněž obrazem aktivace buněk. Naše nálezy potvrzují vznik Birbeckových granul (Bgs) jako ligand receptor zprostředkovanou endocytózu, což bylo nejvíce patrné u skupiny II, kde aktivace LCs probíhala rychleji, bouřlivěji a Bgs byla zachycena ve spojení s plazmatickou membránou v dendritech i v těle buněk. Dále jsme prokázali Birbeckovým granulím podobné struktury, zachycené v dendritech LCs skupiny II. Jsou projevem zvýšené reaktivity těchto buněk a nikoli jiným typem LCs. Současně s objevením Bgs v cytoplazmě Langerhansových buněk vznikají v cytoplazmě MIIC kompartmenty, jejichž množství převládá v období, kdy Langerhansovy buňky opouštějí epidermis.
Závěr: Zjistili jsme, že za 30 minut po aplikaci 0,1% DNCB je možné posuzovat stupeň reaktivity kůže, protože 30 minut po aplikaci je aktivace LCs již plně rozvinuta. Domníváme se, že použitá koncentrace je dostatečná pro test vyvolávající aktivaci LCs a že přitom nemůže nepříznivě ovlivnit pacienta. Použití bioptických jehel malého průměru je šetrné, neboť jejich hloubka zasahuje jen epidermis a svrchní části koria, a není třeba užívat místní znecitlivění.
Klíčová slova: lidská kůže, Langerhansovy buňky (LCs), Birbeckova granula (Bgs), 2,4-dinitrochlorobenzen (DNBC).

Introduction

The skin does not only serve as a physical and mechanical barrier between the environment and the organism but it also plays an important role as an immune organ. Interactions between cell elements and humoral mediators such as cytokines released by keratinocytes [1] are involved in immune reactions of the skin. The most important cell elements taking part in these reactions are T lymphocytes, keratinocytes, and antigen-presenting cells. The

antigen-presenting cells process antigens and present the resultant immunogenic peptides with the adhering MHC class II molecules to T lymphocytes [2, 3, 4, 5].

Langerhans cells located in the suprabasal epidermal layer are most relevant from the point of view of the skin immune reaction. Langerhans cells account for 4% of the epidermal cell population and are part of the system of antigen-presenting cells. Langerhans cells together with other dendritic cells originate from the bone marrow. Circulating precursors of these cells can be detected in the peripheral blood, spleen, and skin [6].

Langerhans cells are the only cells of the epidermis that show high membrane ATPase activity [7]. They play an important role in antigen processing and presenting to T cells [8]. They have common features of macrophages and dendritic cells, including expression of high levels of MHC class II molecules. Langerhans cells also express CD 1a, complement receptors, and Fc immunoglobulin receptors [9].

Morphologically, dendritic epidermal Langerhans cells are characterized at the ultrastructural level by the cytoplasm organelles called Birbeck granules [10]. These granules are present in the cytoplasm as striped rod- or tennis-racket-shaped organelles. Birbeck granules only develop in the cytoplasm of those Langerhans cells that become residential cells of the epidermis [3]. Recent studies have shown that Bgs are involved in transfer of the molecules entering the cell by receptor mediated endocytosis and are dynamic structures. Nevertheless, the origin and function of Bgs remain unexplained [8, 11, 12, 13]. In vitro functional studies with fresh cultured Langerhans cells showed that they can process and present haptens, proteins, and tumor antigens with adhering MHC class II molecules to T lymphocytes [14, 15]. Intracellular distribution of MHC class II molecules was studied in antigen-presenting cells, including B lymphocytes, macrophages, and dendritic cells [16]. In these types of cells, most MHC class II molecules are located in lysosomes and vesicular structures called MIIC compartments. The same intracellular MIIC compartments were also described in Langerhans cells [16]. The effect of contact sensitization on LCs activation and MHC class II molecules expression has been frequently studied on murine models [17, 18]. Langerhans cells pick up antigens in the skin and then migrate into the regional lymph node to present them together with adhering MHC class II molecules to T lymphocytes that stimulate the cell and humoral responsiveness. During their migration and stimulation of T lymphocytes, Langerhans cells become mature dendritic cells characterized by the loss of ATPase activity [18] and Fc receptors and a decreased amount of Birbeck granules in the cytoplasm. Consequently, expression of MHC class II molecules increases and so the number of the vesicles of the MIIC compartments is increased. Langerhans cells undergo radical functional changes during their life [19].

The aims of this study were to assess morphologically the DNCB effect on Langerhans cells in the epidermis at different time and to devise an in vivo test for characterization of immunoreactivity of Langerhans cells and thus of whole skin.

Material and methods

Aliquots of 0.2 ml 0.1% DNCB in acetone were applied on the skin of the left forearms of 20 volunteers (11 females, 9 males, aged between 25 and 41 years). Skin biopsy specimens were taken with skin punches 3 mm in diameter, from a depth of 2 mm, at intervals of 1, 3, 10, 30 minutes and 72 days after DNCB application.

The biopsy specimens obtained were fixed in 0.1 M cacodylate-buffered 2.5% glutaraldehyde, pH = 7.2, for 2 hours at 4 °C. After postfixation in 0.1 M cacodylate-buffered 1% OsO_4, pH = 7.2, for 2 hours at 4 °C, the tissue was dehydrated and embedded in EPON 812.

Semithin sections were stained with 1% toluidine blue for general investigation under a light microscope. Ultrathin sections were contrasted with 2% uranyl acetate and Reynolds solution. The sections were investigated under a Jeol 100 CX II electron microscope.

Approval No 4509-3 of Ethical Commission of National Institute of Public Health.

Results

Reactivity to 0.1 % DNCB application was compared with that of the untreated skin and of the skin treated with acetone only.

Macroscopic findings

Some volunteers showed erythema within 30 minutes at the site of DNCB application. These volunteers were included in group II, while group I comprised those without any macroscopic sign of reactivity.

Electron microscopy

In the suprabasal layer of the epidermis of the untreated individuals and those treated with acetone only, Langerhans cells with indented nuclei and several unbranched dendrites were found. The cytoplasm of these cells contained only several mitochondria, endoplasmic reticulum, isolated lysosomes, and scattered coated vesicles. Rod-shaped Birbeck granules were either infrequent or even absent (Fig. 1). Dendrites of these cells were structurally simple without cytoplasmic organelles.

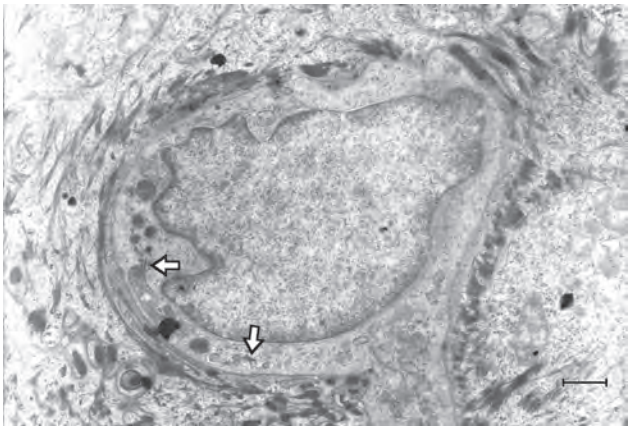

Fig. 1. The Langerhans cell with the rod-shaped Birbeck granules (arrows) of the untreated skin. Bar 1300 nm.

Group I

No substantial changes were detected in the cytoplasm of Langerhans cells (LCs) within one minute after DNCB application in comparison with control specimens. Clear morphological changes were observed three minutes after DNCB application: LCs showed signs of high metabolic activity. The amounts of mitochondria, coated pits, and coated vesicles increased, the Golgi complex and rough endoplasmic reticulum became more evident, and rod-shaped Birbeck granules (Bgs) appeared. Isolated rod-shaped Bgs connected to the plasma membrane were found in dendrites of these cells (Fig. 2).

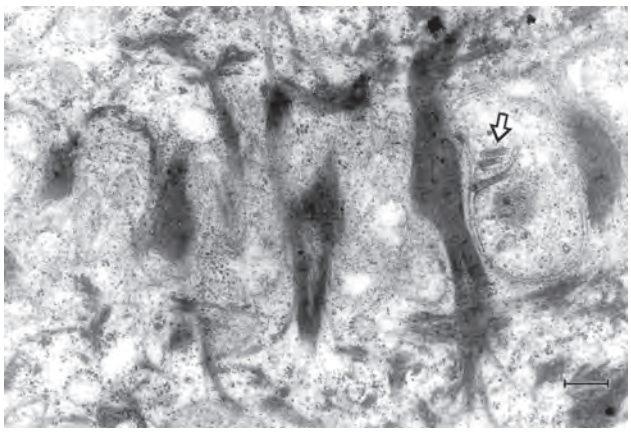

Fig. 2. The dendrite of the LCs with the rod-shaped Bgs connected to the plasma membrane (arrow) three minutes after DNCB application (group I). Bar 870 nm.

Ten minutes after the DNCB application, cells and nuclei were enlarged. The nuclei showed clear chromatin condensation near the nuclear membrane. Rod-shaped Bgs accumulated in the cell cytoplasm and were found in bundles near the nucleus and the Golgi complex. The cells comprised multiple vesicles, fine vesicular structures, electron-dense vesicles and endosome-like structures, possibly with inner vesicles. Mitochondria, vesicular structures, and both rod-shaped and tennis-racket-shaped Bgs accumulated in dendrites of these cells where enlarged cisterns of the rough endoplasmic reticulum were also found (Fig. 3).

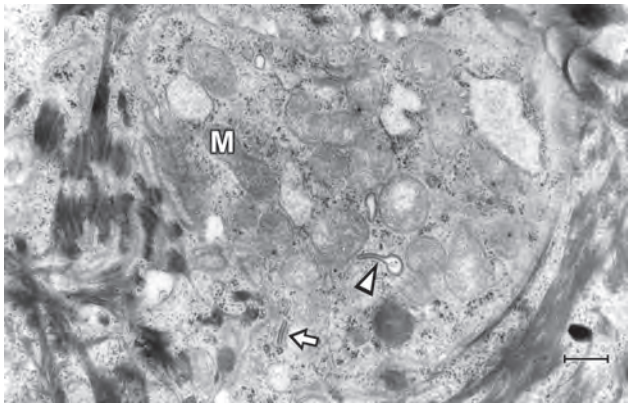

Fig. 3. 10 minutes after DNCB application the accumulation of the mitochondria (M), the rod-shaped (arrow) and the tennis-racket-shaped Bgs (arrow head) were found in the dendrite of the LCs (group I). Bar 870 nm.

Thirty minutes after DNCB application (volunteers in this group remained without erythema), not only prolonged and branched dendrites of LCs, but also more abundant LCs in the suprabasal layer of the epidermis were typically found. Both rod-shaped and tennis-racket-shaped Bgs, which prevailed, were found in the cell cytoplasm. Pinocytotic vesicles, multiple mitochondria, vesicles, multilamellar and endosomal structures with tiny vesicles inside were observed as well (Fig. 4 and 5). Centrioles were frequently present in Langerhans cells. Dendrites of these cells contained rod- and tennis-racket-shaped Birbeck granules, multilamellar bodies, mitochondria, pinocytotic vesicles and enlarged cisterns of the rough endoplasmic reticulum. Isolated rod-shaped Birbeck granules were present in the cytoplasm, sometimes plunged in the endocytic compartment (Fig. 6). This plunging was only observed within this period in - group I.

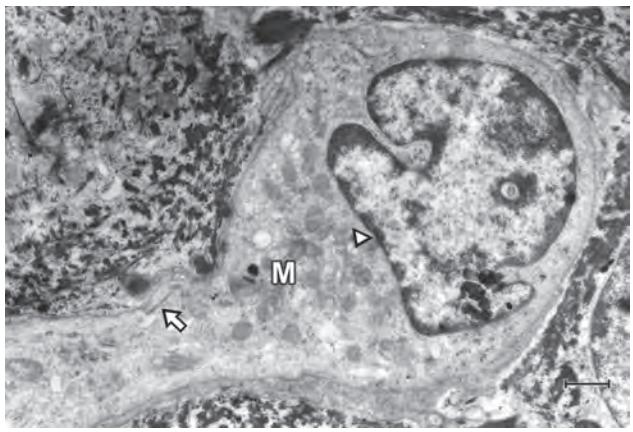

Fig. 4. The Langerhans cell thirty minutes after DNCB application: multiple mitochondria (M), the rod-shaped Bgs (arrow), the tennis--racket-shaped Bgs (arrow head) - group I. Bar 1400 nm.

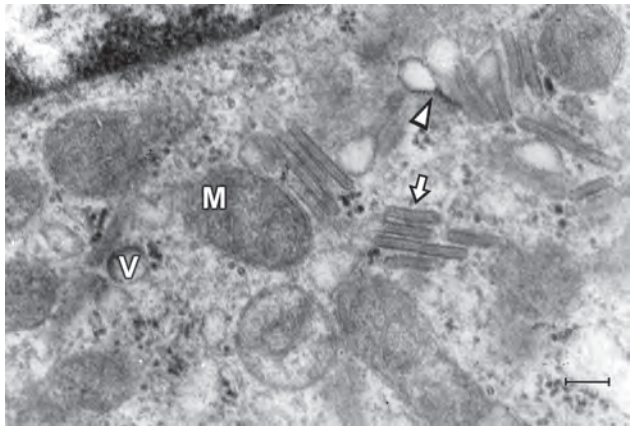

Fig. 5. The detail of cytoplasm of the Langerhans cell from the Fig. 4., mitochondria (M), the rod-shaped Bgs (arrow), the tennis-racket-shaped Bgs (arrow head), the vesicles (V). Bar 230 nm.

Seventy-two hours after DNCB application, Langerhans cells were observed in the suprabasal part of the epidermis, but were not metabolically active. Dendrites of these cells were clearly thinner.

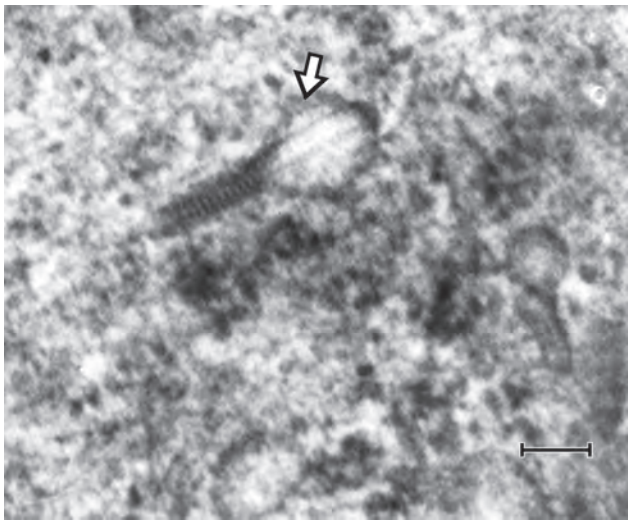

Fig. 6. The rod-shaped Bgs pluged in the endoplasmic compartment (arrow). Bar 173 nm.

Group II

This group included volunteers who had developed erythema within 30 minutes after 0.1% DNCB application.

In the first minute after the DNCB application, Langerhans cells exhibited enlarged indented nuclei and multiple mitochondria, vesicles, pinocytotic vesicles and isolated rod-shaped granules in their cytoplasm. Rod-shaped Bgs were also found in dendrites of these cells.

A further increase in the amount of cytoplasmic organelles was recorded within 3 minutes after DNCB application. Enlarged cisterns of the endoplasmic reticulum, lysosomes, multivesicular bodies, rod- and tennis-racket-shaped Bgs, and centrioles (Fig. 7) were found. Morphologically, LCs of group II at this time interval corresponded to those of group I as described 10 minutes after DNCB application.

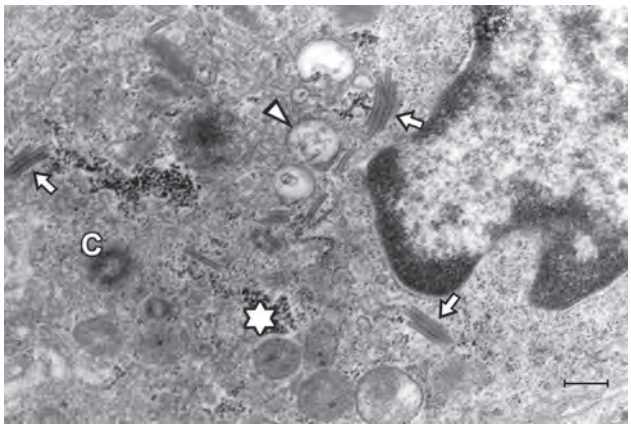

Fig. 7. The Langerhans cell 3 minutes after DNCB application, group II, the rod-shaped Bgs (arrows), the multivesicular bodies (arrow head), the centriole (C), glycogen (*). Bar 870 nm.

Ten minutes after the DNCB application, a rapid increase in the amount of all intracellular organelles was observed in LCs. The most striking features were the proliferation of tennis-racket-shaped Bgs and the location of rod-shaped Bgs close to the cell plasma membrane (Fig. 8). Dendrites of these cells were extremely long, branched, with multiple cytoplasmic organelles, among which rod-shaped Bgs in bundles predominated; tennis-racket-shaped Bgs were also present.

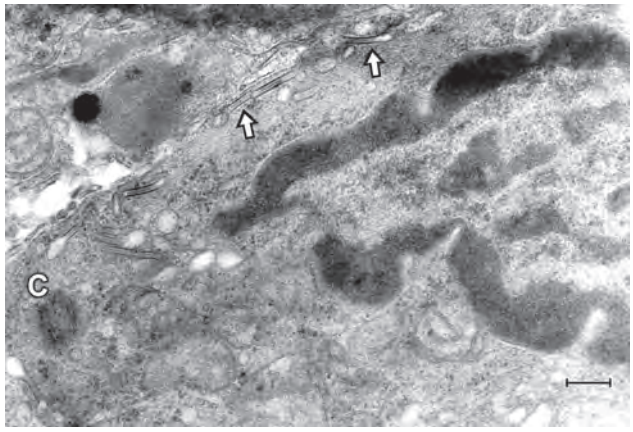

Fig. 8. The location of the rod-shaped Bgs close to the plasma membrane (arrows), the centriole (C), ten minutes after DNCB application – group II. Bar 476 nm.

Thirty minutes after the DNCB application, the abundant cytoplasmic organelles persisted in LCs (Fig. 9). A marked change was observed in the amount of predominant tennis-racket-shaped granules. They were twice as frequent as the rod-shaped granules. Rod-shaped granules plunged into the endosomal system were observed in the LCs bodies. Structures similar to Birbeck granules were detected in dendrites (Fig. 10). These structures were extremely long and almost always connected to the plasma membrane of the dendrite (see Fig. 10). LCs were found even in the dermis. These cells had no dendrites and existed in a reduced form only. Bgs, mostly plunged into endocytoplasmic compartments, were infrequent in their cytoplasm. Conversely, multivesicular and multilamellar bodies corresponding to MIIC compartments predominated among the cytoplasmic organelles.

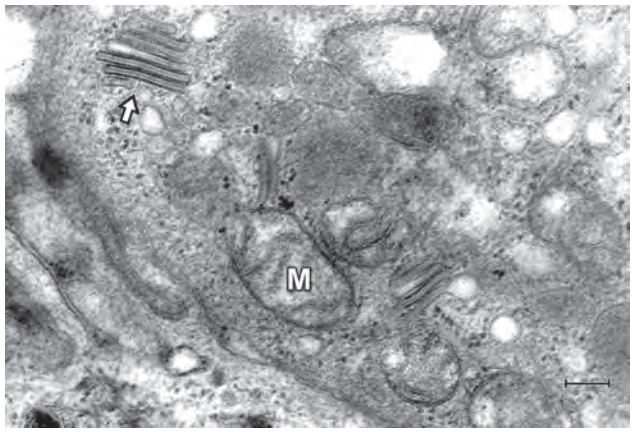

Fig. 9. The cytoplasm of the LCs thirty minutes after DNCB application, group II – the rod-shaped Bgs (arrow), mitochondria (M). Bar 440 nm.

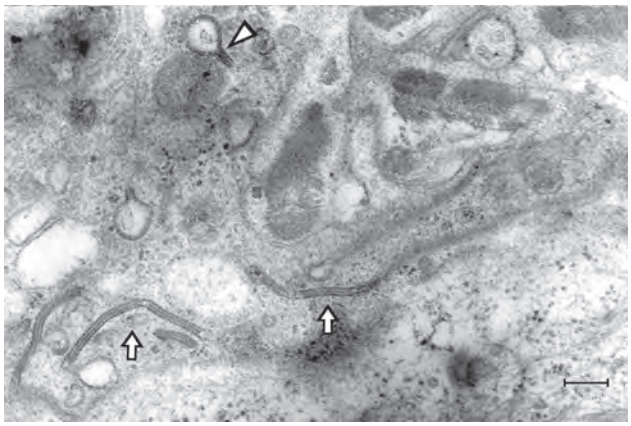

Fig. 10. Structures similar to the Bgs (arrows) were extremely long and almost connected to the plasma membrane of the dendrite, tennis-racked-shaped Bgs (arrow head), 30 minutes after application of DNCB – group II. Bar 476 nm.

Seventy-two hours after DNCB application, some cells still remained metabolically active; nevertheless, we also found cells corresponding morphologically to those of the untreated skin. These may have been cells that had newly migrated to the epidermis.

Discussion

In the present study, we focused on DNCB effects on Algerians cells and their dendrites at different time intervals from application in 20 volunteers. Data on antigen-presenting activity of Algerians cells are based mainly on experiments on mice and guinea pigs, or in vitro observation of reactivity of antigen-presenting cells isolated from the skin, lymph nodes and possibly spleen. Nevertheless, data on functional changes in Algerians cells from biopsy specimens of the human skin are difficult to obtain. Only a few studies are available, in which the skin specimens originating from plastic surgery of the breast or abdominal surgery were used. Attention has not been paid to the dendrite ultrastructure.

DNCB epicutaneous application caused erythema in some volunteers within 30 minutes while others did not show any macroscopic changes. DNCB caused morphological changes in Langerhans cells and their dendrites that could be recorded with the passage of time. Similar activation of Langerhans cells after 0.1 % DNCB application was described by Kolde [18] in guinea pigs with different genetically determined immune responses. In his study biopsy specimens were not taken earlier than one hour after DNCB application, and thus only differences in apposition of Langerhans cells and lymphocytes could be described, which is not true of reactivity development. By comparing findings at successive short time intervals after DNCB application, we could find evidence that Langerhans cells showed higher reactivity and higher proliferation of cytoplasmic organelles in group II (with erythema) compared to group I (without erythema). Within the first minutes after DNCB application, dominant cytoplasmic organelles of Langerhans cells were coated pits, coated vesicles, mitochondria, Golgi complex, and endoplasmic reticulum.

Immuno electron microscopic study of the location of MHC class II molecules allowed detection of immunopositivity initially in the Golgi complex, cisterns of the endoplasmic reticulum and vesicles with dense contents belonging to the compartments called MIIC [16]. Simultaneously with the presence of rod-shaped Birbeck granules, multivesicular bodies started to be evident close to both the nucleus and enlarged cisterns of the endoplamic reticulum. These multivesicular bodies are also part of the MIIC compartments [16]. The MIIC compartments were found to be the site where newly synthesized MHC II molecules waited to couple with peptides. Intracellular distribution of MHC-II molecules was studied intensively for B cells and macrophages. For both of these cell types, most intracellular MHC-II molecules were found in the vesicular structures called MHC-II compartments and were designated MIIC [16]. Langerhans cells contain typical MIIC compartments harboring MHC-II molecules; nevertheless, a relatively great part of MHC-II molecules were also present in the membranes of the rough endoplasmic reticulum. This explains why the cells and their dendrites showed enlarged cisterns of the rough endoplasmic reticulum as observed in this study. Langerhans cells of the epidermis are known to synthesize actively MHC-II molecules that accumulate in the MIIC compartments. If fresh Langerhans cells are cultured, synthesis of MHC-II molecules decreases while their expression on the cell surface increases. Conversely, in the epidermal Langerhans cells this synthesis increases while expression is zero. MHC-II molecules only migrate towards the plasma membrane at the moment when Langerhans cells leave the epidermis [8]. This is consistent with our findings: Langerhans cells in the epidermis contain multiple Birbeck granules. When located in the corium, the Langerhans cells almost never displayed Birbeck granules, while their cytoplasm contained large amounts of MIIC compartments located close to the cell plasma membrane.

Rod-shaped Birbeck granules appeared in group I within three minutes after DNCB application. In the cell body, no Birbeck granule was connected to the plasma membrane while in dendrites at this interval Birbeck granules were generally connected to the plasma membrane. We believe that Birbeck granules are early endocytotic structures since found in fusion with endosomal compartments. Supposedly, they may deliver exogenous material towards the conventional endocytotic pathway. Recent studies have shown that Birbeck granules take part in receptor mediated endocytosis [8], nevertheless, the origin of Birbeck granules and their actual function remain controversial [9]. We have found the rod-shaped Birbeck granules, probably resulting from the ligand-receptor mediated endocytosis, to appear first in the cytoplasm of Langerhans cells and their dendrites. The rod-shaped Birbeck granules soon took the tennis-racket shape to be most abundant in both groups 30 minutes after the DNCB application. In

group I, connection of Birbeck granules to the plasma membrane was observed in dendrites only. In group II, where activation was more rapid, Birbeck granules were either connected or were very close to the plasma membrane, even in the cell bodies. This is suggestive that the development of Birbeck granules is very rapid and can be noticed within the first minutes after only DNCB application. Later, Birbeck granules are not longer found close to the plasma membrane, but are located in the cell centers where they couple with endosomal compartments. Their development within the plasma membrane is confirmed indirectly by the loss of ATPase positivity as also observed by other authors [20].

Birbeck granule-like structures were observed in dendrites of these cells in group II at this time interval, almost always connected to the plasma membrane. Several studies showed that these structures result from increased activation of Langerhans cells due to application of contact-sensitizing haptens [21]. In this case, the presence of Birbeck granule-like structures seems to be suggestive of a hypersensitive reaction of Langerhans cells of group II. The presence of Birbeck granule-like structures was not evidenced in group I. The same Birbeck granule-like structures were also reported by others at the site of contact allergic dermatitis or after action of DNFB in mice [22, 23], in human leukemia cells incubated in the presence of thorium [24], and in platelets after EDTA treatment [25, 26]. All these examples suggest that structural changes follow the process of receptor-ligand interaction. Thus, interaction between the epidermal ligands and receptors of Langerhans cells may induce morphological modifications, particularly in susceptible individuals such as transformation of vacuolar or canalicular structures into Birbeck granule-like structures. This may result in an increased intake of exogenous antigens in the cell possibly leading to inadequate reactivity after their transformation and presentation to T cells.

In the present study, we focused not only on morphological changes of the Langerhans cell body but also on their dendrites, to which attention has not thus far been directed. We found that dendrites of these cells undergo similar morphological changes, as do Langerhans cells, and that their ultrastructural composition also reflects cell activation. Based on the study of the dendrite ultrastructure, we found Birbeck granules close to their plasma membrane in group I. Birbeck granule-like structures located in dendrites of Langerhans cells of group II seem to reflect increased activity of these cells.

The goal of our study was to create an intravital test indicative of skin immunoreactivity. We found that within 30 minutes after DNCB application, skin reactivity can be assessed, since at that interval the activation of Langerhans cells is fully completed. We suppose that the DNCB concentration used is sufficient for testing activation of Langerhans cells and at the same time no harm to the patient is to be expected. The use of biopsy needles of a small diameter is safe since the puncture affects only the epidermis and the upper layer of the corium and thus the use of local anaesthesia can be avoided.

References

1. **Katz, S. L.** The skin as an immunological organ: allergic contact dermatitis as a paradigm. *J. Dermatol.*, 1993, 20, p. 593–603.
2. **Friedmann, P. S.** The immunobiology of Langerhans cells. Immunol. *Today*, 1991, 2, p. 124–128.
3. **Wolf, K., Stingl, G.** The Langerhans cells. *J. Invest. Dermatol.*, 1983, 80, p. 17–21.
4. **Kimber, I., Cumberbatch, M.** Dendritic cells and cutaneous immune response to chemical allergens. *Toxicol. Appl. Pharmacol.*, 1992, 117, p. 137–146.
5. **Czernielewski, J. M.** *Early events in the induction phase of contact hypersensitivity.* In *In vitro skin toxicology* (Rougier A., Goldberg A., Maibach H., eds), 1994, vol. 10, NY: Mary Ann Liebert, p. 263–269.
6. **Mackensen, A., Herbst, B., Köhler, G., Wolff – Vorbeck, G., Rosenthal, F. M., Veelken, H., Kulmburg, P., Schaefer, H. E., Mertelsmann, R., Lindemann, A.** Delineation of the dendritic cell lineage by generating large numbers of Birbeck granule – positive Langerhans cells from human peripheral blood progenitor cells in vitro. *Blood*, 1996, 86, p. 2699–2707.
7. **Hanau, D., Fabre, M., Schmitt, D. A., Garaud, J.-C., Pauly, G., Cazenave, J.-P.** Appearance of Birbeck granule – like structures in anti – T6 antibody treated human epidermal Langerhans cells. *J. Invest. Dermatol.*, 1988, 90, p. 298–304.
8. **Rizova, H., Carayon, P., Barbier, A., Lacheretz, F., Dubertret, L., Michel, L.** Contact allergens but not irritants, alter receptor – mediated endocytosis by human epidermal Langerhans cells. *British J. Dermatol.*, 1999, 140, p. 200–209.
9. **Caux, C., Vanbervliet, B., Massacrier, C. et al.** CD34 hematopoietic progenitors from human cord blood differentiated along two independent dendritic cell pathways in response to GM-CSF+TNFa. *Exp. Med.*, 1996, 184, p. 695–706.
10. **Birbeck, M. S., Breathnach, A. S., Everall, J. D.** An electron microscope study of basal melanocytes and high level clear cells (Langerhans cells) in vitiligo. *J. Invest. Dermatol.*, 1961, 37, p. 51–64.
11. **Guagliardi, L. E., Koppelman, B., Blum, J. S. et al.** Co – localization of molecules involved in antigen processing and presentation in an early endocytic compartment. *Nature*, 1990, 343, p. 133–139.
12. **Hanau, D., Fabre, M., Schmittt, D. A. et al.** Human epidermal Langerhans cells internalize by receptor–mediated endocytosis T6 (CD1 NA1/34) surface antigen. Birbeck granules are involved in the intracellular traffic of the T6 antigen. *J. Invest. Dermatol.*, 1987a, 2, p. 172–177.
13. **Hanau, D., Fabre, M., Schmitt, D. A. et al.** Human epidermal Langerhans cells cointernalize by receptor–mediated endocytosis, nonclassical major histocompatibility complex I molecules (T6 antigens) and class II molecules (HLA–DR antigens). *Proc. Natl. Acad. Sci. USA*, 1987b, 84, p. 2902–2905.
14. **Cohen, P. J., Katz, S. I.** Cultured human Langerhans cells process and present intact protein antigens. *J. Invest. Dermatol.*, 1992, 99, p. 331–336.
15. **Moulon, C., Peguet-Navarro, J., Courtellemont, P. et al.** In vitro primary sensitization and restimulation of hapten – specific T cells by fresh and cultured human epidermal Langerhans cells. *Immunol.*, 1993, 80, p. 373–379.
16. **Kleijmeer, J. M., Oorschot, V. M. J., Geuze, H. J.** Human resident Langerhans cells display a lysosomal compartment enriched in MHC class II. *J. Invest. Dermatol.*, 1994, 103, p. 516–523.

17. **Becker, D., Mohamadzadeh, M., Reske, K., Knap, J.** Increased level of intracelullar MHC class II molecules in murine Langerhans cells following in vivo and in vitro administration of contact allergens. *J. Invest. Dermatol.*, 1992, 99, p. 545–549.
18. **Kolde, G.** Effect of immunological responsiveness on Langerhans cell behavior in contact sensitization. *Exp. Dermatol.*, 1994, 3, p. 269–275.
19. **Rizova, H., Carayon, P., Michel, J. P. et al.** Internalization of surface HLA – DR molecules by human epidermal LCs: analysis by flow cytometry and confocal microscopy. *Cell Biol. Toxicol.*, 1994, 10, p. 367–373.
20. **Hanau, D., Fabre, M., Stamf, J.-L., Grosshans, E., Benezra, C.** ATPase Langerhans cell staining: A technique allowing progression from light to electron microscope observation. *J. Invest. Dermatol.*, 1986, 86, p. 5–8.
21. **Elofsson, R., Anderson, A., Falck, B., Sjoborg, S.** Evidence for endocytotic mechanisms in the epidermal Langerhans cells. *Acta Dermatovener,* 1981, 99, p. 29–39.
22. **Romani, N., Lenz, A., Glassel, H., Stossel, H., Stanzl, U. et al.** Co – localization of molecules involved in antigen processing and presentation in an early endocytic compartment. Nature of Birbeck granule – like structures in murine T-lymphocytes and dendritic Thy-1 – positive epidermal cells by a T cell-activating anti-Th-1 monoclonal antibody. *J. Invest. Dermatol.*, 1990, 84, p. 327.
23. **Becker, D., Kolde, G., Reske, K., Knap, J.** An in vitro test for endocytotic activation of murine epidermal Langerhans cells under the influence of contact allergens. *Immunol. Methods*, 1994, 169, p. 195–204.
24. **Sanel, F. T., Serpick, A. A.** Plasmalemmal and subsurface complexes in human leukemic cells: membrane bounding by zipperlike junctions. *Science*, 1970, 168, p. 1458–1460.
25. **Hanau, D., Gacket, Ch., Schmitt, D. A., Ohlmann, P. et al.** Ultrastructural similarities between epidermal Langerhans cell Birbeck granules and the surface – connected canalicular system of EDTA – treated human blood platelets. *J. Invest. Dermatol.*, 1991, 97, p. 756–762.
26. **Seité, S., Zucchi, H., Moyal, D., Tison, S., Compan, D., Christiaens, F., Gueniche, A., Fourtanier, A.** Alterations in human epidermal Langerhans cells by ultraviolet radiation: quatitative and morphological study. *British Journal of Dermatology*, 2003, 148, p. 291–299.

Do redakce došlo 12. 6. 2006.

Adresa pro korespondenci:
RNDr. Jana Schramlová, CSc.
National Institute of Public Health
Šrobárova 48, 100 42 Prague 10
Czech Republic
e-mail: jschraml@szu.cz

Tematický plán kurzů Katedry klinické biochemie IPVZ pro období září – prosinec 2007 (část 1)

211001 Specializační kurz v klinické biochemii – 1. část

Určeno pro biochemiky-analytiky ve specializační přípravě, případně lékaře v přípravě k atestaci z klinické biochemie.
Program: Hormony, cytokiny. Žaludeční a duodenální šťáva, ascites, pot, žluč, moč. Vitaminy, porfyriny, bílkoviny, volné kyslíkové radikály, lipidy, organické kyseliny, ionty. Krev a krevní oběh. Ledviny, plíce, játra, srdce a kosterní svalstvo.
Místo konání: Praha 4, Budějovická 15
Termín: 3.–7. 9. 2007
Předpokládaná cena: 1500,- Kč
Vedoucí kurzu: doc. RNDr. P. Štern, CSc.
(e-mail: petr.stern@vfn.cz)

211002 Specializační kurz v klinické biochemii – 5. část

Určeno pro biochemiky-analytiky ve specializační přípravě, případně lékaře v přípravě k atestaci.
Program: Kmenové buňky krvetvorby, anémie, hematologické analyzátory, hemostáza, antitrombotická léčba, anomálie červené, bílé a destičkové řady, hodnocení nátěrů periferní krve, základy morfologie, stanovení železa. Imunohematologie erytrocytů, leukocytů a trombocytů; systémy krevních skupin, HLA systém, klinická hematologie, DNA techniky. Cytologie likvoru a likvorové proteiny, izoelektrická fokusace a diagnostika RS, vyšetření u pacienta v akutním stavu.
Místo konání: Praha 4, Budějovická 15
Termín: 10.–14. 9. 2007
Předpokládaná cena: 1500,- Kč
Vedoucí kurzu: doc. RNDr. P. Štern, CSc.
(e-mail: petr.stern@vfn.cz)

211003 Specializační kurz v klinické biochemii – 7. lékařská část

Určeno pro lékaře před atestací z klinické biochemie.
Program: Klinická farmakologie a toxikologie. Farmakokinetické monitorování terapie. Drogově závislý pacient. Stopové prvky. Analytická instrumentace v oblasti imunochemie a biochemie. Analýza nukleových kyselin. Klinická biochemie nádorového bujení. Základní statistické postupy s ohledem na použití v metrologii.
Místo konání: Praha 4, Budějovická 15
Termín: 1.–5. 10. 2007
Předpokládaná cena: 1500,- Kč
Vedoucí kurzu: prof. MUDr. A. Jabor, CSc.
(e-mail: antonin.jabor@ikem.cz)

211004 Specializační kurz v klinické biochemii - 2. část

Určeno pro biochemiky-analytiky ve specializační přípravě (absolventy 1. části kurzu), případně lékaře v přípravě k atestaci.
Program: Osmolalita a koloidně osmotický tlak, ABR a její poruchy, kyslíkové parametry, stopové prvky, enzymy. Patobiochemie aminokyselin, bílkovin, glykoproteinů, lipidů, sacharidů a hormonů. Monitorování kritických stavů. Struktura a funkce buněčných membrán, přenosové mechanismy a jejich základní principy. Štítná žláza a gastrointestinální trakt. Malignity. Akutní a chronické hepatitidy, hyperbilirubinémie, gravidita a vrozené vývojové vady, osteoformace a osteoresorpce, intoxikace a drogové závislosti.
Místo konání: Praha 4, Budějovická 15
Termín: 29. 10.–2. 11. 2007
Předpokládaná cena: 1500,- Kč
Vedoucí kurzu: doc. RNDr. P. Štern, CSc. (e-mail: petr.stern@vfn.cz)