Reactivity of Langerhans Cells after Application of Different Chemicals – An Ultrastructural Analysis

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Abstract: The goal of this study was to investigate changes in epidermal Langerhans cells after application of different chemicals (acetone, 60% alcohol, 5% nickel sulphate, iodisole, and 0.1% 2,4-dinitrochlorobenzene) on the skin of volunteers. The skin of eight volunteers was treated with acetone, 60% alcohol, iodisol, 5% nickel sulphate, and 0.1% 2,4-dinitrochlorobenzene (DNCB). After application of DNCB, Langerhans cells (LCs) showed increased accumulation of Birbeck granules (Bgs). Alcohol and nickel sulphate caused alternative changes, mainly cytoplasmic vacuolation, in LCs. Nickel sulphate was even responsible for the disappearance of dendrites. Both chemicals have cytotoxic effects on LCs: cytoplasmic organelles and Bgs disappear and subsequently, the antigen-presenting activity of epidermal LCs is inhibited. We did not found any morphological changes in LCs after application of acetone.

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Introduction

Langerhans cells (LCs) are migrating dendritic cells found in the suprabasal part of the epidermis. As the only cells of the epidermis, in which enzyme ATPase is coupled to the cell membrane, they are identified by detection of ATPase activity (Hanau et al., 1986) or detection with S-100 protein. High expression of CD1a and MHC class I and II molecules shows that LCs are prominent antigen-presenting cells of the skin immune system. Antigens incorporated in cells are degraded by proteolytic enzymes and with adhering MHC class II molecules are presented to T lymphocytes (Brodsky and Guagliardi, 1991; Cohen and Katz, 1992). MHC class I molecules take part in presentation of endogenous antigens such as tumour antigens (Moulon et al., 1993). LCs show common features of macrophages and dendritic cells, including expression CD 1a, complement receptors, and Fc immunoglobulin receptors (Teunissen, 1993; Katou et al., 2000).

At the ultrastructural level, LCs are characterized by the indented nucleus and clear cytoplasm that contains mitochondria, the Golgi complex, microtubules, microfilaments, and lysosomes, while tonofilaments and desmosomes are missing. The absence of tonofilaments and desmosomes is suggestive of the cell migration capacity. These cells originate from bone marrow, and arrive in the skin via blood capillaries. After completing their mission, they migrate towards the regional lymph node.

The most typical organelles of LCs are Bgs (Birbecket al., 1961). They appear in the cytoplasm of LCs as rod-shaped bodies with a central zipper-like structure or tennis-racket-shaped bodies with a vesicular dilation at one end. Recent studies have shown that Bgs take part in transfer of molecules entering the cell via receptor-mediated endocytosis and represent a dynamic structure. Nevertheless, the origin of Bgs and their function remain controversial (Rizova et al., 1999). LCs undergo radical functional changes during their life (Hanau et al., 1988; Bucana et al., 1992; Kolde, 1994). They migrate from the skin to regional lymph nodes where present antigens with adhering MHC class II molecules to T lymphocytes. While migrating, they become mature dendritic cells characterized by lower amounts of Bgs in the cytoplasm with subsequent higher expression of MHC class II molecules on the plasma membrane. These data are based mostly on observations of antigen-presenting LCs in mice or guinea pigs in which LCs were isolated from skin, lymph nodes, or spleen. Similar data are difficult to obtain on human epidermal LCs.

The goal of this study was to investigate changes in epidermal LCs after application of various chemicals (acetone, 60% alcohol, 5% nickel sulphate, iodisole, and 0.1% 2,4-dinitrochlorobenzene) on the skin of volunteers.

Material and Methods

The left forearm of each of eight volunteers was treated once with 0.2 ml of 60% alcohol, iodisol, 5% nickel sulphate and 0.1% 2,4-dinitrochlorobenzene (DNCB). Skin biopsy specimens were taken from a depth of 2 mm with a skin punch 3 mm in diameter, 10 and 30 minutes after application.

The specimens were fixed with 2.5% glutaraldehyde (0.1 M cacodylate buffer, pH=7.2) for 2 hours at 4 °C. After fixation in 1% osmic acid (0.1 M cacodylate buffer, pH=7.2) for 2 hours at 4 °C, the tissue was dehydrated in graded ethanol series and embedded in EPON 812.

Semithin sections were stained with 1% toluidine blue for investigation under a light microscope. Routine electron microscopy was done on ultrathin sections stained with uranyl acetate and lead citrate. 30 LCs were carried out from each biopsy specimens. The ultrathin 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

The ultrastructural changes in the cytoplasm of LCs and their dendrites after treatment with acetone, 60% alcohol, iodisol, 5% nickel sulphate and 0.1% 2,4-dinitrochlorobenzene (DNCB) were compared with LCs from untreated skin.

Ultrastructure of Langerhans cells of untreated skin

LCs had a lobular indented nucleus and clear cytoplasm containing mitochondria, lysosomes, and endoplasmic reticulum. Isolated rod-shaped and scarce tennis-racket-shaped Birbeck granules were present in the cytoplasm (Figure 1). The same features were observed in dendrites of these cells.

Morphological changes in Langerhans cells observed within 10 minutes after application of the following substances:

Acetone Marked morphological changes were not recorded either in cell bodies or in their dendrites. LCs showed practically the same ultrastructure as those of untreated skin.

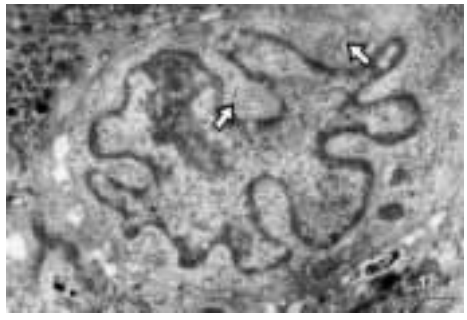

Figure 1 – The Langerhans cell of the untreated skin with the isolated rod-shaped Bgs (arrows). Bar is 750 nm.

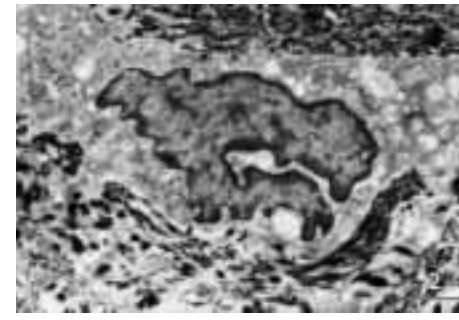

Figure 2 – Vacuolation of the cytoplasm of both the bodies and dendrites was observed in Langerhans cells 10 minutes after 60% alcohol application. Bar is 1,670 nm.

60% alcohol Vacuolation of the cytoplasm of the bodies and dendrites was observed in LCs. Vacuoles were tiny and multiple. Isolated mitochondria and rod-shaped Bgs were present in the cytoplasm. The chromatin of the LCs was condensed at the nuclear periphery (Figure 2).

Iodisol LCs and their dendrites showed an accumulation of racket-shaped Bgs with residues of zipper-like striation (Figure 3), and multiple tiny vacuoles were also observed.

5% nickel sulphate The changes noticeable initially were the shrinkage of LCs bodies and dendrites. Cytoplasmic organelles including Bgs were rarely found in the cytoplasm of LCs (Figure 4).

0.1% DNCB Mitochondria and rod-shaped Bgs located near the nucleus and Golgi complex were found. The LCs contained multiple vesicles, vesicular structures with

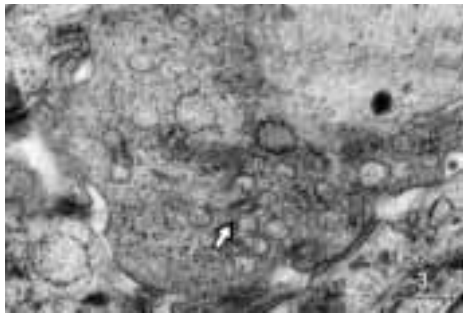

Figure 3 – Langerhans cells and their dendrites showed an accumulation of racket-shaped Bgs with residues of zipper-like striation (arrow) after 10 minutes iodisol application. Bar is 400 nm.

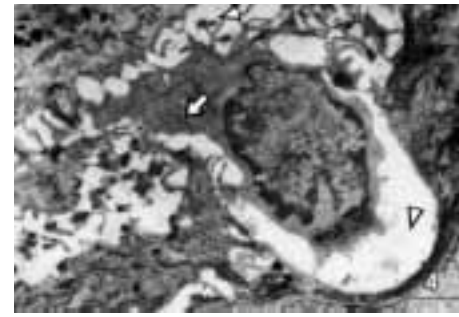

Figure 4 – The shrinkage of the Langerhans cells' body and the dendrite after 10 minutes 5% nickel sulphate application, enlarged intracellular spaces (arrow heads), rod-shaped Bgs (arrow). Bar is 1,670 nm.

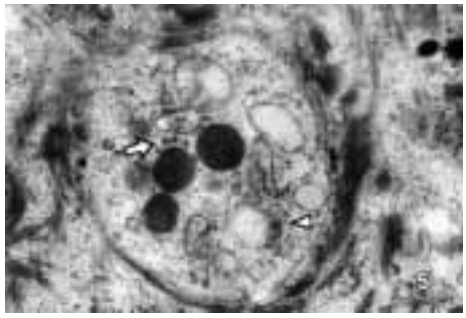

Figure 5 – Rod-shaped (arrow) Bgs and tennis-racked-shaped Bgs (arrow head) were found in dendrites of Langerhans cells10 minutes after DNCB application. Bar is 450 nm.

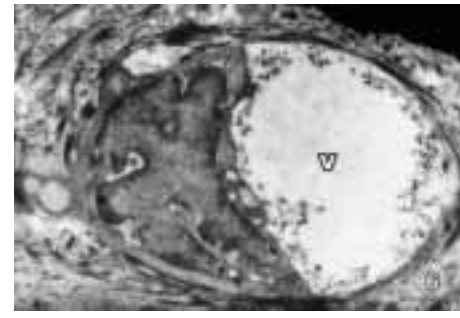

Figure 6 – The Langerhans cell 30 minutes after 60% alcohol application, with vacuolisation (V). Bar is 670 nm.

fine electron-dense content on the inner surface. Accumulation of mitochondria, vesicular structures, lysosomes, rod-shaped Bgs were found in dendrites of LCs (Figure 5).

Morphological changes in Langerhans cells were observed within 30 minutes after application of the following chemicals

Acetone LCs showed no ultrastructural changes.

60% alcohol Bgs were practically absent in LCs, and other cytoplasmic organelles were missing as well. Accumulated tiny vacuoles fused in large vacuoles occupied the cell; the cytoplasm of LCs was forming a thin band at the periphery only where the nucleus was forced in with evident chromatin condensation (Figure 6). Dendrites also were highly vacuolized, and showed thin bands of the cytoplasm at the periphery without the presence of cytoplasmic organelles (Figure 7).

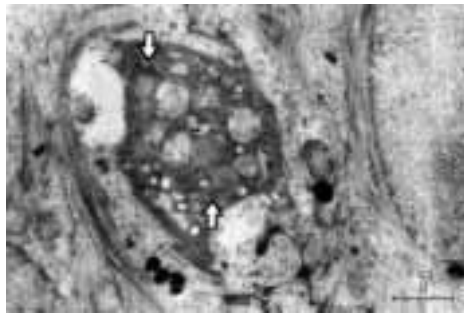

Figure 7 – Dendrite were vacuolized, 30 minutes after 60% alcohol application, the rod-shaped Bgs (arrows). Bar is 830 nm.

Figure 8 – The Langerhans cell 30 minutes after iodisol application. Bar is 400 nm.

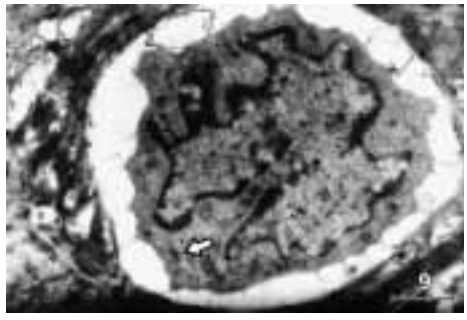

Figure 9 – The shrunk Langerhans cell without dendrites, rod-shaped Bgs (arrows). Bar is 830 nm.

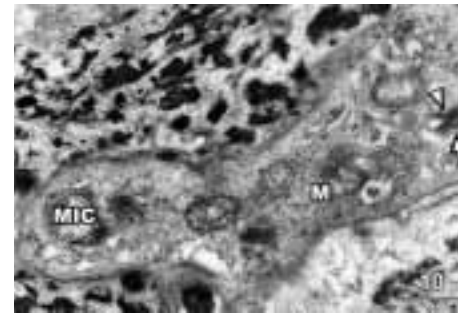

Figure 10 – Ten minutes after DNCB application the dendrite contained rod-shaped Bgs (arrow) and tennis-racked-shaped Bgs (arrow head), mitochondria (M), MIIC vesicular compartments (MIC). Bar is 830 nm.

Iodisol Cytoplasmic vacuolation continued in LCs, without condensation of chromatin in the nucleus. Tennis-racket-shaped Bgs with residues of zipper-like striation were still present but number were decreased (Figure 8).

5% nickel sulphate LCs were shrunk and without cellular organelles. The cytoplasm formed a thin ring around the nucleus and only isolated rod-shaped Bgs were found there. The most marked change in these cells was the loss of dendrites (Figure 9).

0.1% DNCB LCs showed typical accumulation of Bgs, mainly tennis-racket-shaped ones, and longer and branched dendrites. Pinocytotic vesicles, multiple mitochondria, multivesicular structures and endosomal structures with tiny vesicles inside were found in the cytoplasm of LCs. Dendrites of these cells contained Bgs of both types, mitochondria, pinocytotic vesicles and enlarged cisterns of rough cytoplasmic reticulum (Figure 10). Proliferation of LCs themselves was observed in the suprabasal part of the epidermis. Centrioles suggestive of the division of LCs in the epidermis were frequently present in the cells.

Discussion

In this study, we described ultrastructural changes in the bodies and dendrites of LCs after application of several different chemicals. We used DNCB since this agent is known to have a stimulation effect on the skin immune system. Since DNCB was also used in our previous study (Schramlová et al., 2007), it allowed comparison of results. Acetone was selected as a DNCB control, alcohol and iodisol served as examples of common disinfectants while nickel sulphate is a known allergenic agent.

Changes in the ultrastructure of LCs after DNCB application were found to be dynamic and consisted of 1) antigen internalization and processing in tennis-racket-shaped Bgs, and 2) expression of MHC class II molecules coupled with antigen fractions. Subsequently, Bgs disappeared and enlarged cisterns of the rough endoplasmic reticulum, multivesicular bodies, and vesicles became the dominant structures of LCs and their dendrites. These structures are part of MIIC compartments where MHC class II molecules accumulate. Ultrastructural sings of cellular activation were observed in increased number of mitochondria, lysosomes, endocytotic vacuoles of LCs, as also reported for other cells (Kleijmeer et al., 1994; Nesrin et al., 2005). Our findings are in agreement with those of other authors that described increased counts of Bgs in mice treated with different immunostimulants (Kolde and Knop, 1987). The presence of centrioles is also suggestive of division of cells directly in the epidermis as reported by some authors (Schuler et al., 1983).

We did not found any morphological changes in LCs after application of acetone. This means that acetone is suitable for use as a solvent of agents to be applied on the skin. Other authors have drawn the same conclusion (Kolde and Knop, 1987).

Formation of tiny vacuoles in the cytoplasm of LCs not accompanied by accumulation of the other cell organelles was observed after application of 60% alcohol. This alteration is probably due to alcohol toxicity. Reversibility of this reaction could not be assessed because of the shortness of the time intervals used.

Reaction of LCs to 5% nickel sulphate consisted of shrinkage of cells and dendrites, which was recorded in 10 minutes after application. These degenerative alterations of LCs were become more visible within 30 minutes. The cells were flattened and intracellular organelles completely disappeared. We propose that nickel sulphate harms LCs in such a way that these cells cannot pursue their antigen-presenting activity, which is rather surprising with reference to the notoriously known allergenicity of 5% nickel sulphate. Our findings support these studies, in which histochemical methods were used to show that contact allergens cause selective response in LCs (Katou et al., 2000).

It is known that the presence of LCs in the epidermis and their activity are influenced not only by chemical but also by physical factors such as UV radiation etc. (Dandie et al., 1998; Seité et al., 2003; Hussein et al., 2005). Cryotreatment also influences LCs' density and morphology, which lead to their shrinkage and to the disappearance of dendrites. These degenerative changes surely affect skin immunoreactivity (Horio et al., 1994).

The reaction of LCs to iodisol application was similar to observed reaction of DNCB or other immunostimulants (Kolde and Knop, 1987; Rizova et al., 1999). Accumulation of tennis-racket-shaped Bgs is suggestive of increased endocytotic activity. On the other hand, cytoplasmic vacuolation recall vacuolation observed as in the case of alcohol. One possible explanation consists in the composition of the agent used: iodine 450 mg, polyvinylpyrrolidone 2.55 g, and 95% ethylalcohol per 100 g iodisol. Whether the vacuolation is attributable to alcohol or to hypersensitivity to iodisol is difficult to say, since a similar reaction was observed with the use of DNCB at a toxic concentration (Kolde and Knop, 1987; Rustemeyer et al., 2003). Reaction of LCs to alcohol and nickel sulphate was characterized mainly by cytoplasmic vacuolation. Application of nickel sulphate led to the disappearance of dendrites. Both of these chemicals have a cytotoxic effect on LCs and consequently neither cytoplasmic organelles nor Bgs increase in numbers.

Conclusion

Our results indicate that acetone is more suitable for use, since it does not affect activity of LCs.

The skin is an important immune organ that can be influenced by external stimuli. Therefore, attention should be focused not only on the immunological responsiveness of epidermal LCs to various stimuli, but also on its morphological correlates. This means that further studies dealing with the morphology and function of Bgs are needed.

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