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LANGERHANS CELLS OF THE SKIN IN LYMPHOID ORGANS: AN IMMUNOHISTOCHEMICAL STUDY

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ABSTRACT:

Purpose: Migration from sites of antigen uptake to lymphoid organs is crucial for the generation of immune responses by dendritic cells. We investigated the human epidermal Langerhans cells in their migratory pathway, from the epidermis to the draining lymph nodes (LNs) and/or other secondary lymphoid organs, by immunohistochemical methods, presenting the intensity of immunoreactive cells with Langerhans cells (LCs) specific antibodies.

Materials and Methods: Migrated LCs from the epidermis were measured as a function of the frequency of CD1a/HLA-DR and S100 positive LCs were found in epidermal sheets prepared from

punch biopsies of the normal skin sites of healthy humans. For this purpose, we investigated 6 healthy tissue samples of each lymphoid organ biopsy with these antigens. Moreover, we used L1 MoAb to distinguish macrophages from LCs or their derivatives. We examined normal samples from subjects who presented at the Pathology Department of Gazi University Medical Hospital, Turkey. Standard immunohistochemical methods were used to detect the presence of antigens in formalin fixed tissue specimens. The intensity of the immunoperoxidase reaction was classified under a light microscope (Leica DM 4000B).

Results: We noted CD1a-, S-100-, and HLA-DR-positive LCs in the epidermis and S-100/HLA-DR-positive LCs in the dermis and around its vessels. These were shaped like dendritic cells. We also saw these S-100/HLA-DR immunoreactive cells in the LNs, spleen, and thymus by discriminating them from the L1-positive macrophages. These positive cells were very close to the blood vessels.

Conclusion: These observations support the concept that Langerhans cells deliver antigen peptides to regional lymph nodes/thymus and spleen, to the former via lymph vessels and to the latter via arterial circulation, and CD1a, S-100, and HLA-DR were suitable markers for the detection of Langerhans cells in different lymphoid tissues except langerin.

Key Words: Langerhans Cells, Migration, Immunohistochemistry, Lymphoid Organs, Suitable Markers

LENFOİT ORGANLARDA DERİ'NİN LANGERHANS HÜCRELERİ: BİR İMMUNOHİSTOKİMYASAL ÇALISMA

ÖZ:

Amaç: Dendritik hücreler tarafından antijenlerin tanınmasının hemen ardından bu hücrelerin lenfoit organlara göç etmeleri immün yanıtın baslayabilmesi için son derce önemlidir.. Biz de çalısmamızda immünohistokimyasal olarak Langerhans hücrelerine özel antibodiler kullanarak, derinin bu özel hücrelerini antijenle tanısdıkları epidermisden ikincil lenfoit organlara doğru olan olası göç yolunugöstermeye çalıstık.

Gereç ve Yöntem : Sağlıklı insanlardan alınan iğne biyopsi örneklerinde CD1a/HLA-DR, S100 antibodileri ile positif LH'ni deri ve ikincil lenfoit organlarda çalısabilmek için her dokudan 6 örnegi, Gazi Üniversitesi Patoji bölümünden elde ettik. Makrofajları LH'nden ayırd edebilmek için makrofajlara özel L1 MoAb'sini kullandık. Formalin ile tespit edilmis ve parafine gömülmüs dokularda standart immunohistokimyasal yöntemler ile bu antibodileri gösterdik. Reaksiyonu Leica DM 4000B foto ısık mikroskobunda görüntüledik.

Bulgular: CD1a, S-100 ve HLA-DR-positif LH'ni epidermisde ve S-100/HLA-DR-positif LN'ni de dermis ve dermisin damarları çevresinde gözlemledik. Bunlar sekil olarak dendritik hücrelere benzeyen hücrelerdi. Bu S-100/HLA-DR immunoreaktif hücreleri aynı zamanda lenf düğümlerinde, dalakta ve timusda da gözlemledik ve bu hücrelerin L1 positif macrofajlardan ayırımına vardık. Bu pozitif hücrelerin kan damarları ile de son derce yakın iliskide bulunması ve endotel hücreleri aralarına doğru sokulmus olmaları onların göç eden hücreler olabileceği kanısını uyandırdı.

Sonuç: Bu gözlemler LH'nin antijenleri ikincil lenfoit organlara lenf damarlarının yanısıra kan damarları ile de götürebiliceğinin göstergesi olabilir ve Langerin'in yanisira/olmadigi durumlarda CD1a, S-100 ve HLA-DR ikincil lenfoit organlarda LH'ni göstermeye uygun antibodiler/isaretleyicilerdir.

Anahtar Kelimeler: Langerhans Hücreleri, Göç, İmmunohistokimya, Lenfoit Organlar, Uygun İsaretleyiciler

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INTRODUCTION

The monocyte-derived cell lineage contains remarkably heterogeneous subpopulations, including classical phagocytic macrophages and various antigen-presenting dendritic cells (DCs).[1,2] DCs have been identified within the interstitial space of most human tissues although notable exceptions are the absence of DCs in the cornea and central nervous system.[3,4,5] Within tissues, DCs exist as trace populations and may be identified by the combination of DC morphology and immunohistochemical labeling to demonstrate the expression of high levels of HLA-DR, CD1a (on Langerhans cells in the epidermis) and S100, and the absence of other lineage markers.[4,5,6,7] DCs migrate to the secondary lymphoid tissues via the afferent lymphatics as veiled cells, so called because of their characteristic sheet-like lamellipodia. Cannulation of dermal afferent lymphatic vessels in human subjects has demonstrated an increase in CD1a+ DCs leaving the skin following exposure to contact sensitizers.[5,8] There is also evidence from animal transplantation models that solid tissue DCs may migrate via the blood to the spleen.[5,9] The mechanisms of homing to the LNs and spleen are not fully understood but recent evidence suggests that expression of certain isoforms of the hyaluronic acid receptor (CD44) may be important.[5,10] In LNs, DCs reside within the T cell paracortical regions as interdigitating DCs (IDCs), whilst in the spleen they are located in the marginal zones at the periphery of the periarterial sheaths.[3,5]

Langerhans cells (LCs) were first described in 1868 by the investigator whose name they now bear, using a gold chloride staining technique that identified a unique nonpigmented dendritic appearing cell in the epidermis.[11,12] These cells are also found in lymph nodes and the thymus and in limited numbers in the oral mucosa, esophagus, main bronchi, and distal colon. LCs are thought to be bone marrow-derived from CD34+ precursor cells and to represent part of the DC system.[3,11,13]

These cells are potent antigen-presenting cells and, after antigenic stimulation, have the capacity to migrate from the epidermis via afferent lymphatics to the paracortical areas of lymph nodes, where they apparently complete the process of maturation to DCs, including interdigitating reticulum cells.[3,11] Antigenic infectious agents including vaccines induce pro-inflammatory cytokines (e.g., TNF-α). These cytokines promote Langerhans DC maturation in lymphoid organs where they home to the T cell rich area.[14,15] Langerhans DCs undergo phenotypic and functional changes during their maturation and migration. These cells, which are now loaded with antigenic peptides on MHC class II, down-regulate CD1a, CCR6, and E-cadherin, and lose the capacity to capture foreign antigens.[14-16] Mature DCs are an end stage of differentiation, and they cannot be converted into either macrophages or lymphocytes.

LCs have been shown to be the only cells in the epidermis to express MHC class II molecules under normal conditions;[17,18,19] upon antigen capture, LCs migrate to regional lymph nodes and are highly efficient at presenting MHC class II–restricted peptides to T cells.[20,21] These T cells then migrate back to the skin to participate in cutaneous inflammatory responses. These observations suggest that LCs are an important component of the innate immune response in the skin, functioning to initiate adaptive T cell responses locally. Two other features of LCs are intriguing in the context of their antigen-presenting role. First, LCs were shown to express high levels of CD1a, [21] ..a member of the family of group 1 CD1 proteins (CD1a, CD1b, and CD1c), which share the capacity to present microbial lipid antigens to T cells.[22] Second, LCs are the only cells known to express langerin (CD207), a C-type lectin that is sufficient to induce the formation of Birbeck granules, pentilaminar endosomal structures specific to LCs. These unique features of LCs suggested that they may be specialized by their expression of langerin to capture particular antigens as they enter the epidermal layers, and that at least some of these antigens may be presented to CD1a-restricted T cells.[22] In the current study, we examined the possible distribution of these important fighter cells of the skin in different lymphoid organs by using monoclonal antibodies known to be specific and/or expressed by the Langerhans cells. Most of the studies in the literature were performed in mice or guinea pigs. Similar data are difficult to obtain on human epidermal Langerhans cells. Therefore, we aimed to investigate expression of CD1a, S 100, and HLA-DR antibodies on human skin and other lymphoid organs and to distinguish these cells from the macrophages by applying L1 antigen.

MATERIALS AND METHODS

Specimens and tissue preparation methods

A variety of biopsy specimens were obtained from the routine pathology files after approval from Gazi University Ethics Committee was obtained. We studied normal samples from patients who presented to the Pathology Department of Gazi University Medical Hospital, Turkey, between April 2000 and June 2005. A total of 6 tissue samples of each organ (skin, lymph node, thymus, and spleen) were evaluated. This material had been fixed in buffered formalin (pH 7.4) and embedded in paraffin. The specimens included only histologically normal tissues. Serial tissue sections were cut at 4-6 μm on a microtome (Leica SM 2000, Germany) and stretched by water flotation on polylysine-coated glass slide.

Antibodies and staining procedure

The slides were de-waxed. Following dehydration through a descending ethanol series, endogenous enzymes were blocked using 1.2% hydrogen peroxide. In order to increase the immunoreactivity of formalin-fixed paraffin wax sections, an enzyme step is necessary for CD1a as EDTA and L1 as citrate buffer, but is not necessary for HLA-DR and S100 antibody. Slides were therefore incubated in 0.1% EDTA buffer (Cat # AP-9004-500, Lot# 9004 E211, Neomarkers, USA) and citrate buffer (Cat # AP-903-500, Lot# 9003C 411A, Neomarkers, USA) for 10 min at 37 °C. Following a phosphate buffer wash slides were blocked using normal goat serum prior to the application of a 1:100 concentration of mouse monoclonal primary antibodies (CD1a Ab-4, Lot# 1354 5010 MS-1354-S; L1 Cell Adhesion molecule Ab-1, Lot# 770 P203 MS-770-P; HLA-DR Ab-1, MS-133-PO and S100 Ab-1, Lot# 296 R209 MS-206-R7, Neomarkers, USA). Two phosphate buffer rinses preceded secondary antibody application (Anti-polyvalent HRP kit TP-060-HL, Neomarkers, USA) for 30 min. Epitopes were identified using the avidin biotin complex (ABC) method and visualized with the use of AEC (Lot# ANA 41118 TA-125-HA, Neomarkers, USA). Control slides were prepared using the same method omitting either primary antibody. Slides were counterstained with Mayer Hematoxylin, and mounted with Aqueous Mount (Cat # AML 060 Lot # 7265, ScyTek, USA). The intensity of the immunoperoxidase reaction was classified, investigated, and photographed with a Leica DMC 4500 photolight microscope.

RESULTS

S-100 reactive cells were observed all through the skin layers, especially in the stratum spinosum. These cells were star-shaped and had cytoplasmic extensions. Reactivity was very strong in both the nucleus and cytoplasm. Some S-100 positive cells in the stratum basale were observed to move across the papillary dermis. S-100 antibody, which is specific for LCs and interdigitating cells, was also reactive to some cells in the dermis. Moreover, their relation with blood vessels was obvious. These S-100 reactive cells that moved across the epidermis to the papillary dermis and afterwards to the dermal venules were evaluated as LCs – the immunofighters of the skin (Fig. 2).

HLA-DR antibody, which is specific to monocytes, macrophages, dendritic cells, and to LCs of the skin, gave a reaction in the skin that is similar to that of S-100's. Reactivity of epithelial cells was mild when compared to S-100 reactive cells. Those epidermal cells did not include macrophages, monocytes, or DCs and so were evaluated as LCs of the skin. The reactive cell population seen in the papillary dermis and their reactivity were larger and stronger than that in the epidermis. S-100 immunoreactive LCs in the papillary dermis that were still migrating were also immunoreactive for HLA-DR. However, their reaction in the papillary dermis was denser because it includes an HLA-DR positive reaction of dermal macrophages and monocytes with migrating LCs (Fig. 4).

On the other hand, in normal human skin tissue biopsies we observed CD1a immunoreactivity only in the epidermis. This antibody, which is specific for DCs and LCs, gave a reaction similar to that of skin LCs for S-100. CD1a immunoreactive cells were seen all through the epithelium with their cytoplasmic extensions. The absence of reactive cells in the papillary dermis is explained by the idea that this protein is expressed by the LCs that are not immunologically adequate yet. (As LCs differentiate Birbeck granules disappear and afterwards LCs lose their reactivity to CD1a). Although this differentiation could occur any time during the migration of LCs to the lymph node, still some LCs positive with CD1a may or may not be seen in lymph nodes. Although it is not well known at which stage of migration differentiation takes place, in our study the presence of CD1a negative cells in the papillary dermis may be a sign that differentiation of LCs is completed before these cells leave the epithelium (Fig 1).

L1 antibody, which is used to separate the LCs from macrophages, was applied to all tissues (but not shown in figures of all groups), and is reactive to macrophages that are found in the papillary dermis. We observed that epithelial cells in nor-

mal skin biopsies are negative for L1 except some keratinocytes, and positive macrophage cells are found in the papillary dermis, adjacent to the stratum basale, and this showed us that macrophages are waiting for LCs that are stimulated by an antigene and soon become nearer (Fig 3).

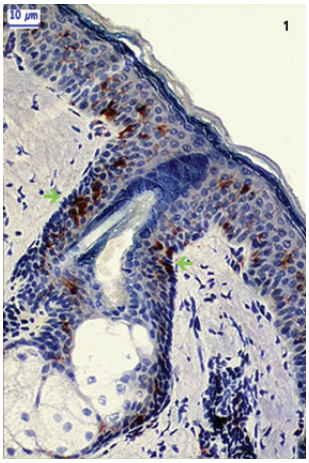

Figure 1. CD1a immunoreactive cells are seen (→) all through the epithelium but not in the dermis. (Immunoperoxidase, Hematoxylin)

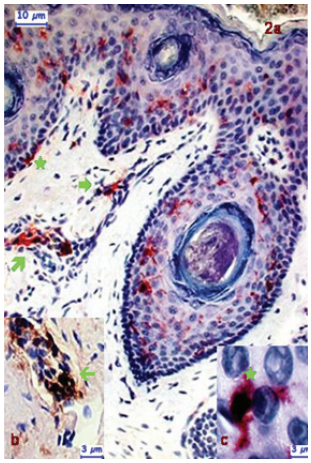

Figure 2. S100 positive cells in the stratum basale (☼), stratum spinosum (2c) and in the dermis (→) close to the dermal vessels (2b). (Immunoperoxidase, Hematoxylin)

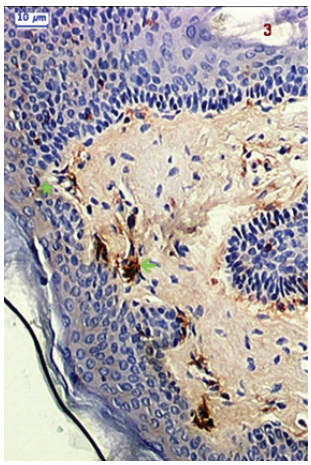

Figure 3. L1 positive cells of papillary dermis (→) and keratinocytes of epithelium (☼). (Immunoperoxidase, Hematoxylin)

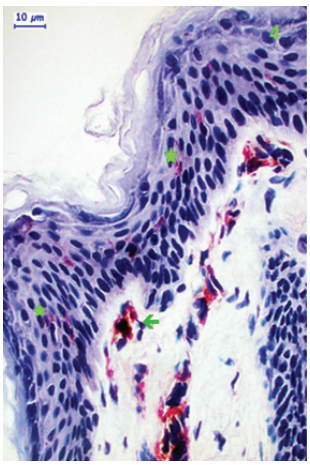

Figure 4. HLA-DR reactivity is seen in epithelial cells (☼) and in papillary dermis (→). (Immunoperoxidase, Hematoxylin)

In our immunostaining studies with S-100, HLA-DR, CD1a, and L1 proteins in lymph nodes, we found that LCs are found in the lymph nodes. We observed strong S-100 reactivity in the subcapsular sinus and in the walls of arterioles and cells nearby (Fig 7). This showed us that LCs reach the lymph nodes by blood flow and during this period they are S-100 reactive. We also observed that veiled cells at the subcapsular sinus, which are thought to be LCs, are strongly S100 reactive. In the arterial wall, some cells, which are not endothelial cells, were S-100 positive (Fig 7). Because of this, we thought that LCs could also migrate to lymph nodes via the arteries. HLA-DR and CD1a reactivity were similar in many ways (Fig 5, 6). DCs gave a strong and diffuse reaction with these proteins. As LCs reach the lymph node, they have probably completed their differentiation and are CD1a negative; in our immunostaining with this antibody the reaction seemed to be less diffuse but much stronger when compared to HLA-DR. CD1a reactive cells were observed both in lymphoid follicles and in the connective tissue of septa (Fig 6). At low magnifications reactions through the tissue were clearly visualized. The most diffuse immunoreactivity was observed by the HLA-DR monoclonal antibody, which is known to be reacted with lots of cells (monocytes, macrophages, LCs, DCs), and reactivity was seen mostly in lymphoid follicles' germinal centers (Fig 5b). On the other hand, S-100 reactive cells were seen separately and only a few in number through the LN (Fig 7, 8) and L1 immunostaining, which was performed to separate macrophages from LCs, gave the least diffuse reaction after the S100 (Fig 9). L1 reactive cells were seen mostly in the germinal center of the follicle. Because of this we assumed that most of the S-100 and HLA-DR positive cells are either LCs or DCs.

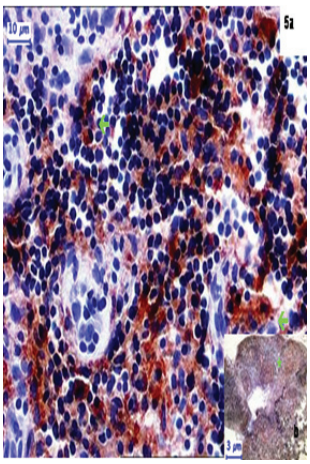

Figure 5. HLA-DR immunoreactive cells (→) of lymph node (5a) and diffuse immunoreactivity of HLA-DR in germinal center is seen (5b). (Immunoperoxidase, Hematoxylin)

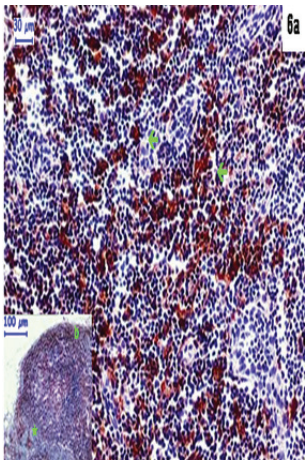

Figure 6. CD1a reactive cells are seen both in lymphoid follicles (→) (6a) and in connective tissue (☼) (6b). (Immunoperoxidase, Hematoxylin)

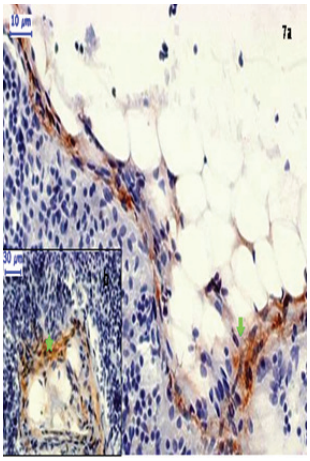

Figure 7. LN, S100 reactive cells are seen subcapsular sinus as veiled cells (→) (7a) and in thick arterial wall (☼) (7b). (Immunoperoxidase, Hematoxylin)

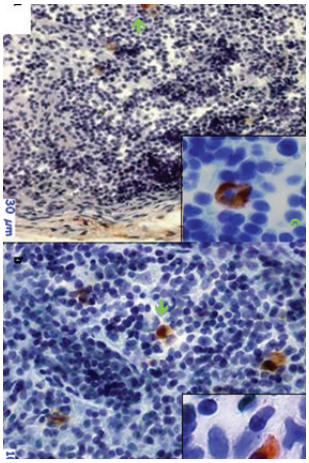

Figure 8. LN, Only a few S100 reactive cells are seen in lymph nodes paracortical region. (Immunoperoxidase, Hematoxylin)

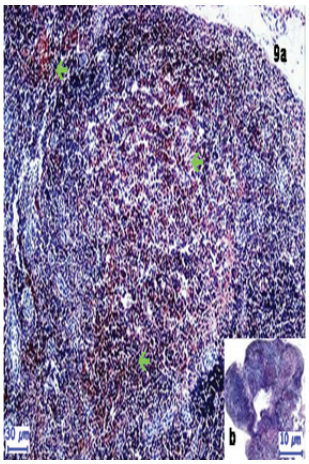

Figure 9. L1 reactive cells are seen mostly in germinal center of LN ([) (9a) and the staining pattern of antibody seen in LN (9b). (Immunoperoxidase, Hematoxylin)

S100 reactivity in the thymus was also highly limited. However, some cells with cytoplasmic extensions that are found in the capsule and inside the trabecules are evaluated as LCs that have just come to the thymus. With S100, which is specific to interdigitating cells and LCs, interdigitating cells in the medulla of the thymus showed a positive reaction (Fig 10). However, reactive cells were rare in the medulla. In the large picture with small magnification, we saw that the extensioned reactive cells had spread out from trabeculae to the cortex and afterwards to the medulla. Therefore, because of this we considered that these S-100 positive cells might be LCs (Fig 10). However, CD1a reactivity in human thymus tissue was more diffuse. Moreover, those positive cells are thought to be interdigitating cells that are found especially in the medulla and give a strong reaction. CD1a positive cells in the capsule had probably come to the thymus via the arterial blood circulation but had not differentiated to LCs yet (small picture). These cells are evaluated as cells that have affinity to enter the thymus parenchyma with trabeculae and are regarded as LCs (Fig 11).

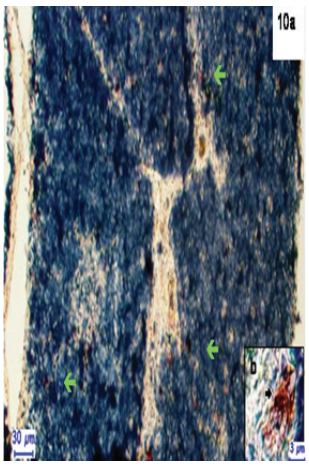

Figure 10. S100 reactive cells are seen in trabeculae ([) (10b) and parenchyma (10a) of the thymus ([). (Immunoperoxidase, Hematoxylin)

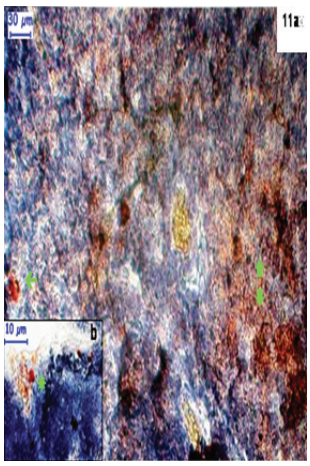

Figure 11. CD1a immunostained cells are seen in capsula (☼) (11b) and medullary region of human thymus (→,↕) (11a). (Immunoperoxidase, Hematoxylin)

The most diffuse reactivity in the thymus was that with HLA-DR and this result correlates with the fact that the most diffuse group of cells of the thymus expresses this antibody. Mature macrophages in the cortex, again macrophages and DCs in the medulla, lesser monocytes, and the possible existence of LCs may be responsible for this positivity. Some of the reactive cells were macrophages with their huge contours and some cells attracted attention due to their cytoplasmic extensions. There was no reaction in endothelial cells; on the other hand, macrophage or monocyte-like cells in the arterial wall were HLA-DR positive. Cells with small amounts of the cytoplasm adjacent to the arterial wall showed a strong HLA-DR reaction and they are evaluated as T-lymphocytes (Fig 12).

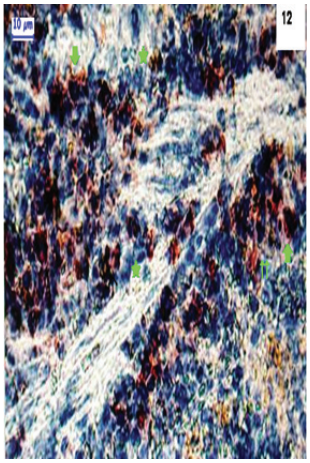

Figure 12. HLA-DR reactivity of thymus. Macrophages with their huge contours (→), cells with cytoplasmic extensions (↑↑) and thymocytes (→) are seen. Endothelial cells are immunonegative with HLA-DR (☼). (Immunoperoxidase, Hematoxylin)

In the spleen, CD1a immunopositive dendritic cells were seen in clusters in the periphery of the white pulp, whereas they were small in number beside the peripheral artery. Positive cell clusters were also observed in spleen cords. We thought that these reactive cells may be Dcs or LCs in cords of red pulp, which have many cell types like reticular cells, plasma cells, macrophages, and cells of the circulating blood in the cords of red pulp. Positive cells found around the central artery at PALS, which are adjacent to T lymphocytes, are evaluated as antigen presenting DCs (Fig 13). HLA-DR reactivity in the spleen's red pulp was limited to clusters of reactive cells. There was no reaction in the white pulp. In addition, we did not observe any positiveness in the sinusoids (Fig 14). Reactive cells are assumed to be the macrophages, monocytes, or DCs of the pulp cords. Reactive macrophages were seen also in the wall of the macrophage sheathed arterioles in red pulp. Also in the marginal

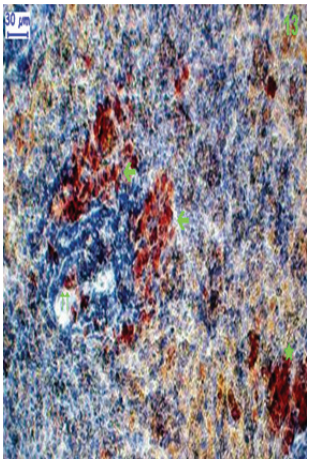

Figure 13. CD1a immunostaining in spleen. Reactive cell clusters are in periphery of white pulp (→) and in red pulp (☼). Some reactive cells are also seen in PALS (↑↑). (Immunoperoxidase, Hematoxylin)

Figure 14. HLA-DR reactivity of spleen. Reactive cells are seen in the periphery of white pulp, around the macrophage sheathed arterioles in red pulp and in marginal zones (→). (Immunoperoxidase, Hematoxylin)

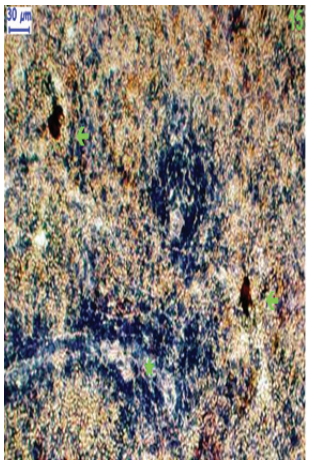

Figure 15. Spleen, S100 immunoreactive cells are seen adjacent to blood vessels (☼) and in red pulp (→). (Immunoperoxidase, Hematoxylin)

zone HLA-DR positive macrophage clusters were seen. Some of the reactive cells in the pulp cords are thought to be either reticular cells or LCs (Fig 14). In this tissue, we observed S100 reactive cells in the red pulp and corona of lymphoid follicles. Although there was no reaction at the germinal centers of the white pulp and PALS, we saw huge cells in the red pulp and appraised them as reticular cells or LCs (Fig 15).

DISCUSSION

Although initially thought to be related to the neural system, LCs are now known to express MHC class II molecules and represent one of the most potent antigen-presenting cells in the body.[23,24] These cells are part of the DC system, with the important function of immune surveillance, i.e. for the detection of foreign antigens entering the body through the skin.[11]

Epidermal LCs express high levels of MHC class II molecules and the CD1 complex, and are positive for S-100. [24-31] CD1a is a protein of 43 to 49 kDa expressed on DCs and cortical thymocytes CD1a staining has been shown to be useful in the differentiation of LCs from interdigitating cells. It has also proved useful for phenotyping LC histiocytosis. Cells reactive for CD1a demonstrated membrane accentuation with weak cytoplasmic reactivity, frequently with focal localized Golgi-type cytoplasmic staining.[32]

In the dermis, the Langerhans precursors are strongly HLA-DR positive, and are relatively non-phagocytic, "indeterminate cells." CD1a expression is not pronounced in the precursors and is not significantly expressed until the cells become differentiated.[33,37] It is shown in many studies that epidermis LCs are positive with CD1a.[24-27] LCs that are characterized by the existence of Birbeck granules are expressed with CD1a before being differentiated to DCs. However, these cells, which complete their differentiation to DCs and lose their granule structure in their cytoplasm, are CD1a negative.[28,38-40] In our study we aimed to differentiate these cells, which can be defined as LCs due to their S100/HLD-DR positive characteristic and due to its positive reaction with CD1a in the skin but negative reaction with other lymphoid organs, from the macrophages by applying L1 antibody to the investigated tissues and so strengthen the diagnosis of LCs. In conformity with the literature, we monitored CD1a positive cells in all layers of the epidermis with their cytoplasmic extensions. Some positive cells were found in the stratum basale but no reactive cells were observed in the papillary dermis. The lack of positive cells in the papillary dermis made us think that the LCs, known as migrating to the dermis from the epithelium, may complete their differentiation to DCs before they leave the epidermis.

It is well known from immunohistochemical studies that S-100 protein appears beyond nervous tissues, such as in certain epithelia, melanocytes, myoepithelial cells, IDCs, and LCs.[22] An immunohistochemical study based on polyclonal antibodies indicated that the α subunit of S-100 protein might occur in macrophages41 and studies on S-100 protein demonstrated diffuse nuclear and cytoplasmic staining. It has been reported that most DR-positive DCs were likewise negative for L1, and the same was true for Kupffer cells in normal livers. FDCs and tingible body macrophages in germinal centers were generally L1-negative as well.[22] In the literature, studies in which S100 protein was examined in splenic tissue stated that DCs, interdigitating cells, nerve fibers, and endothelium cells react positively with this protein. Researchers observed reactive nerve fibers in the hilus and in connective tissue neighboring main blood vessels[2,7] They also determined that DCs, secondary lymphoid follicles, and endothelium cells in PALS are reactive with S100. However, we did not observe such a reaction with follicular DCs, endothelium cells, or PALS. These structures were completely S100 negative. We only observed a medium to high reaction in DCs in red pulp cord. On the other hand, S100 positive cells in the thymus are defined as IDCs (interdigitating dendritic cells) in some mammals including humans. It is stated that these reactive cells are more intensely observed in the thymus medulla. Positive reacting cells are observed also in the cortex but it is stated with studies that their number is smaller compared to reactive cells in the medulla.

In our S100 antibody application in human tissue, we defined reactive cells as almost equally distributed IDCs similar to those of other researchers, but more in the medulla. These cells were very close to blood vessels in the trabeculae and had clear cytoplasmic extensions. It seemed as if they were leaving the trabeculae and being distributed in the cortex and then the medulla. In studies in the lymph nodes and skin, S100 protein is defined in paracortical zone of lymph node and in both the epidermis and dermis in the skin. In our study, an S100 reaction in the lymph node is observed, particularly in interdigitating cells of the paracortical zone. The reaction in dendritic reticular cells is determined also in follicles, similar to the reports in the literature. A strong reaction is observed in cytoplasmic extensions of these cells. In our study, a strong reaction of S100 positive cells in the epidermis is seen. Cytoplasmic

extensions and star-like shapes of these cells were clear. It was even observed in some zones that some of these reactive cells cross the basal membrane and pass to the dermis. It was also considerable that reactive cells in the dermis were again quite strongly positive, but even though their cytoplasmic extensions were clear the star-like shapes in the epidermis were disappearing. These reactive cells were observed very close to all vessel structures in the dermis. This made us think that reactive cells can use both lymphatics and the arteries for migration.

Identified and named by Bodmer in 1977, HLA-DR molecules fall under the category of MHC II molecules and regulate immune response.[42, 43] They are expressed primarily in antigen presenting cells such as B lymphocytes, monocytes, macrophages, thymic epithelial cells, and activated T lymphocytes. There is experimental evidence that MHC II molecules are expressed from the LCs of the epidermis also. As reported by many investigators in the early nineties, LCs carry receptors with high affinity for IgE (FC(RI); furthermore, they express CD1a and S100 proteins also but not L1.[44] Studies stated that HLA-DR protein generally reacts positively at ECs, DCs, MCs, lymphocytes, active T lymphocytes, and thymic nurse cells. The researchers who limited the reactivity in the spleen with IDCs report HLA-DR reactivity of active T cells, cortical cells and nurse cells, with CDs more in the thymus. Lymph node HLA-DR positive cells are defined as ICs similar to the ones in the spleen. HLA-DR positive cells of the skin are LCs and macrophages. In our study, we observed HLA-DR immunoreactivity in the thymus, similar to the literature, but more in active T lymphocytes and DCs close to the vessel wall. Moreover, in the spleen, a reaction was observed in IDCs of red pulp while there was no reaction in the white pulp. There were clusters of denser immunoreactivity in some zones. We defined it as zones where a few HLA-DR immunopositive cells gather. Positive cells of the lymph node were again at the level of IDCs, in conformity with the literature. HLA-DR positive cells of the skin were determined as LCs and macrophages in the epithelium and papillary dermis. The closeness of reactive cells to the vessels led to think that they might have a 'travel plan'.

The L1 antigen, which is a calcium-binding, heat-stable, multichain myelomonocytic protein of about 36 kD, however, was found to be mainly confined to reactive histiocytes (infiltrating macrophages)[17, 21]. The fact that L1 normally circulates in small amounts was reflected by a diffuse extracellular L1 positivity that was often seen in tissue directly fixed with ethanol, especially adjacent to vessels and in subepithelial zones.17,21 This feature was less apparent in formalin fixed material, obviously because of antigenic masking as described previously for other extracellular antigens. We performed our experiment on formalin fixed tissues and we saw positively reactive monocyte derived macrophages in the skin and other lymphoid tissues. Our aim was to distinguish these monocytes from LCs. Endothelium was likewise generally negative; occasional positivity might be ascribed to uptake of circulating L1[17,21]. In our study, we aimed to apply L1 monoclonal antibody to the skin and lymphoid organs together with other antibodies and consequently determine the macrophages in the follicles and distinguish these macrophages from possible LCs. We evaluated S100/HLA-DR/CD1a positive but L1 negative cells in the skin and S100/HLA-DR positive and CD1a/L1 negative cells in other lymphoid organs, as LCs and/or their derivates.

In agreement with the literature, we determined L1 reactivity as negative in the epidermis of normal human skin.17,21 However, macrophages in the papillary dermis reacted positively with L1. HLA-DR positive cells in the papillary dermis of the skin were denser with respect to L1 positive cells. If some of the HLA-DR positive cells were macrophages, then the others were probably migrated LCs. Especially in lymph nodes, L1 protein and positive macrophages were sporadically scattered. Macrophage distribution in other lymphoid organs was also limited as in the lymph nodes and particular to the zone it exists in as in PALS[17,20,21]. Thus, we thought that other HLA-DR/S100 positive and CD1a negative cells when the macrophage distribution is so limited are probably LCs.

In conclusion, while a reaction is observed with almost all antibodies in the skin, the most diffuse reaction is determined with HLA-DR and CD1a in lymphoid organs. Although S100 is the most powerful and diffuse reaction in the skin, its manifestation in other lymphoid organs is even rarer. S100 also strongly reacted in cells named "veiled" and that exist in subcapsular sinuses of lymph nodes and that are considered migrated LCs. Non-endothelium positive cells are determined in the artery wall. As a result, it is supposed that LCs and their derivates can migrate to other lymphoid organs by arteries/veins or lymphatic vessels and S100/HLA-DR and CD1a are the most suitable antibodies to show this distribution. Moreover, L1 enabled us to differentiate macrophages from these cells.

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REFERENCES

1. Tew JG, Thorbecke J, Steinman RM. Dendritic cells in the immune response: characteristics and recommended nomenclature. J. Reticuloendothelial Soc 1982; 31: 371-380.

2. Brandtzaeg P. Immunohistochemical identification of macrophages and various dendritic cells in paraffin-embedded biopsy material. APMIS Suppl 1988; 2:136-59.

3. Steinman RM. The dendritic cell system and its role in immunogenicity. Annu Rev Immunol 1991; 9:271-296.

4. Pavli P. Distribution of human colonic dendritic cells and macrophages. Clin Exp Immunol 1996; 104: 124-32.

5. S. Satthaporn OE. Scientific Review, Dendritic cells (I) J.R. Coll. Surg. Edinb 2001; 46: 9-20.

6. Takahashi K et al. Immunohistochemical localization and distribution of S-100 proteins in the human lymphoreticular system. Am J Pathol 1984; 116: 497-503.

7. Hart DN and McKenzie JL. Interstitial dendritic cells. Int Rev Immunol 1990; 6: 127-38.

8. Brand CU et al. Studies on Langerhans cell phenotype in human afferent skin lymph from allergic contact dermatitis. Adv Exp Med Biol 1995; 378: 523-5.

9. Larsen CP, Morris PJ and Austyn JM. Donor dendritic leukocytes migrate from cardiac allografts into recipients' spleens. Transplant Proc 1990; 22: 1943-4.

10. Girolomoni G and Ricciardi-Castagnoli P. Dendritic cells hold promise for immunotherapy. Immunol Today 1997; 18: 102-4.

11. Geraldine SP, Mark AL, Fumio M, Shigeko Y, Jonathan WS, Jack LP. Langerhans Cell Histocytosis: Immunohistochemical Expression of Fascin, a Dendritic Cell Marker, American Journal of Clinical Pathology 2002; 188; 3 (Page number not for citation purposes).

12. Langerhans P. Uber die nerven der menschlichen haut. Virchows Arch (Pathol Anat)1868; 44:325-337.

13. Katz SI, Tamaki K, Sachs DH. Epidermal Langerhans cells are derived from cells originating in bone marrow. Nature 1979; 282:324-326.

14. Cella M, Sallusto F, Lanzavecchia A: Origin, maturation and antigen presenting function of dendritic cells. Curr Opin Immunol 1997; 9:10-16.

15. Mansour M, Ronald L. Dendritic cells: In the forefront of immunopathogenesis and vaccine development – A review, Journal of Immune Based Therapies and Vaccines 2004; 2:1 (Page number not for citation purposes).

16. Mohmadzadeh M, Berard F, Essert G, Chaloini C, Pulendran B, Davoust J, Palucka K, Banchereau J. IL-15 skews monocytes differentiation into dendritic cells with features of Langerhans cells. J Exp Med 2001; 194:1013-1019.

17. Anderson KB, Sletten K, Berntzen HB, Fagerhol MK, Dale I, Brandtzaeg P, Jellum E. Leukocyte L1 protein and the cystic fibrosis antigen. Nature 1988; 332: 688.

18. Teunissen MB. Dynamic nature and function of epidermal Langerhans cells in vivo and in vitro: A review, with emphasis on human Langerhans cells. Histochem J 1992; 24:697-716.

19. Alavaikko M, Rinne A, Jarvinen M, Hopsu-Havu VKR, Levine AM, Meyer PR, Lukes RJ. Damage to secondary lymphoid follicles in AIDS-related persistent generalized lymphadenopathy, as revealed by the behaviour of dendritic reticulum cells possessing immunoreactive acid cysteine-proteinaze inhibitor. Virchows Arch B 1986; 50: 299-311.

20. Brandtzaeg P, Rognum TO. Evaluation of tissue preparation methods and paired immunofluorescence staining of immunocytochemistry of lymphomas. Histochem J 1983; 15:655-689.

21. Brandtzaeg P, Dale I, Fagerhol MK. Distribution of a formalin-resistant myelomonocytic antigen (L1) in human tissues. 1. Comparison with other leukocyte markers by paired immunofluorescence and immunoenzyme staining. Am J Clin Pathol 1987; 87: 681-699.

22. Brandtzaeg P, Jones DB, Flavell DJ, Fagerhol MK. Monoclonal antibody to a human monocyte marker detects the formalin-resistant myelomonocytic L1 antigen. J Clin Pathol In press 1988.

23. Steinman RM. The dendritic cell system and its role in immunogenicity. Annu Rev Immunol 1991; 9:271-296.

24. Romani N, Lenz A, Glassel H, et al. Cultured human Langerhans cells resemble lymphoid dendritic cells in phenotype and function. J Invest Dermatol 1989; 93:600-609.

25. Witmer-Pack MD, Olivier W, Valinsky J, et al. Granulocyte/macrophage colony-stimulating factor is essential for the viability and function of cultured murine epidermal Langerhans cells. J Exp Med 1987; 166:1484-1498.

26. Fithian E, Kung P, Goldstein G, et al. Reactivity of Langerhans cells with hybridoma antibody. Proc Natl Acad Sci USA 1981; 78:2541-2544.

27. Teunissen MB, Wormmeester J, Krieg SR, et al. Human epidermal Langerhans cells undergo profound morphologic and phenotypical changes during in-vitro culture. J Invest Dermatol 1990; 94:166-173.

28. McDermott R, Ziylan U, Spehner D, et al. Birbeck granules are subdomains of endosomal recycling compartment in human epidermal Langerhans cells, which form where Langerin accumulates. Mol Biol Cell 2002; 13:317-335.

29. Albertson DG, Fishpool R, Sherrington P, Nacheva E, Milstein C. Sensitive and high resolution in situ hybridization to human chromosomes using biotin labelled probes: assignment of the human thymocyte CD1 antigen genes to chromosome 1. EMBO J 1988; 7:2801-5.

30. Calabi F, Milstein C: A novel family of human major histocompatibility complex-related genes not mapping to chromosome 6. Nature 1986; 323:540-3.

31. Beckman EM, Melian A, Behar SM, Sieling PA, Chatterjee D, Furlong ST, Matsumoto R, Rosat JP, Modlin RL, Porcelli SA. CD1c restricts responses of mycobacteria-specific T cells. Evidence for antigen presentation by a second member of the human CD1 family. J Immunol 1996; 157:2795-803.

32. Alan N Elias, Vandana S Nanda and Ronald J Barr, CD1a expression in psoriatic skin following treatment with propylthiouracil, an antithyroid thioureylene, BMC Dermatology 2003; 3:1471-5945.

33. Breathnach AS: Aspects of epidermal ultrastructure. J Invest Dermatol 1975; 65:2-15

34. Lenz A, Heine M, Schuler G, Romani N. Human and murine dermis contain dendritic cells. Isolation by means of a novel method and phenotypical and functional characterization. J Clin Invest 1993; 92:2587-96.

35. Tamura M, Matsuura B, Miyauchi S, Onji M. Dendritic cells produce interleukin-12 in hyperthyroid mice. Eur J Endocrinol 1999; 141:625-9.

36 . Czernielewski J, Vaigot P, Prunieras M. Epidermal Langerhans cells – a cycling cell population. J Invest Dermatol 1985; 84:424-6.

37. Kanitakis J, Hoyo E, Perrin C, Schmitt D: Electronmicroscopic observation of a human epidermal Langerhans cell in mitosis. J Dermatol 1993; 20:35-9.

38. Cao X, Sugita M, Van Der Wel N, Lai J, Rogers RA, Peters PJ, Brenner MB. CD1 molecules efficiently present antigen in immature dendritic cells and traffic independently of MHC class II during dendritic cell maturation. J Immunol 2002; 169:4770-4777.

39. Kawano T, Cui J, KoezukaY, et al. CD1d-restricted and TCR-mediated activation of va14 NKT cells by glycosylceramides. Science 1997; 278:1626-1629.

40. Larsen CP, Morris PJ, Austyn JM. Donor dendritic leukocytes migrate from cardiac allografts into recipients' spleens. Transplant Proc 1990; 22:1943-4.

41. Tanaka Y. Immunocytochemical study of human lymphoid tissues with monoclonal antibodies against S-100 protein subunits. Virchows Arch [A] 1986; 410:125–132.

42. Zhao MT. HLA Typing: principle and application. Shanghai: Shanghai Sci-Tech Press; 1984; 2-25.

43. Gong LF. Basic immunology. Wuhan: Hubei Sci-Tech Press; 1998; 33-38.

44. Hughes RG, Norval M, Howie SEM. Expression of major histocompatibility class II antigens by Langerhans' cells in cervical intraepithelial Neoplasia, J Clin Pathol 1988; 41:253-259.