Immunogold detection of CD3 and CD4 antigens in patients with Mycosis fungoides

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Received February 13, 2006

In this study, we analyzed the distribution of CD3 and CD4 antigens at the ultrastructural level in tissue samples from mycosis fungoides patients using double-immunogold labeling. We observed clusters composed of CD3 and CD4 antigens on the plasma membrane and intracellulary. There were also clusters only of one type of the antigen and we could observe more often CD4 than CD3. Labeling of CD3 and CD4 was not found in control cells incubated with non-immune serum. In conclusion, our ultrastructural studies not only visualized pattern distribution and relationship between CD3 and CD4 antigens but might also suggest that the type and form of distribution provides new clues to their possible translocation in mycosis fungoides cells.

Key words: double-gold labeling, ultrastructure, mycosis fungoides, CD3 and CD4 receptors

Mycosis fungoides is classifyied in the European Organization for Reserch and Treatment of Cancer (EORTC) as well as in World Healt Organization (WHO) classification as primary cutaneous lymphoma (CTCL) [1]. Cutaneous T-cell lymphomas are differentiated group of lymphoproliferative disorders characterized by presenting clonal T-cells in the skin. Clinical presentation and histopathology findings usually are insufficient to make diagnosis. Additional methods are needed to diagnose CTCL. These include immunohistochemistry, immunocytochemistry and molecular methods. The most common form of primary cutaneous T-cell lymphomas is mycosis fungoides, seen usually between 30 and 60 years, although there were cases reported in infants and people older than 80. It is the rare type of lymphoma and its cause is unknown. There are some theories on etiology of mycosis fungoides including genetics, exposure to chemicals, viral infections, and chronic lymphocyte stimulation but none of these factors have yet been confirmed [2]. Mycosis fungoides is characterized by lymphoproliferative growth of T cells with CD3 and CD4 phenotype. Mycosis fungoides biopsy shows atypical malignant CD4+ lymphocytes which frequently have not expression of certain T cell surface markers, such as CD7 and CD26 but the loss of CD2, CD3, CD5 antigens can be also observed [3, 4]. This disease is typically slowly progressive and chronic which develops over many years, usually begins as an eczematous reaction. The skin in this disorder is affected by flat patches, thin plaques and tumors [5]. In the tumor stage it becomes invasive and infiltrates the neighbouring organs. It can spread to lymph nodes or to other organs, such as the spleen, lungs gastrointestinal system, liver or brain. In the literature there are many clinical and histological variants of mycosis fungoides described [6–9]. A skin biopsy is often required to confirm the diagnosis of this disease because it not only can look similar to eczema but also as psoriasis and/or other inflammatory conditions. Treatment of mycosis fungoides is only temporarily effective, and may need to be continued for a long time. The purpose of this study was to investigate the expression of CD3 and CD4 antigens on lymphocytes in patients with mycosis fungoides by using immunogold method at the ultrastructural level.

Material and methods

Tissue material taken from 7 patients with mycosis fungoides in plaque stage was used in this study. Samples

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were fixed with 4% paraformaldehyde in PBS for 1 h at 4 °C. For conventional electron microscopy to estimate the morphology, cells were fixed with 3.6% glutaraldehyde in phosphate buffer, postfixed in OsO_4 in the same buffer and after dehydratation with alcohol embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate.

To demonstrate CD3 and CD4 antigens at the ultrastructural level double-immunogold labeling was used. After fixation samples were washed overnight in PBS at 4 °C. Dehydration was performed in an ascending series of ethanol. Tissue was than embedded in LR White. Sections (60 nm thick) were cut and placed on nickel grids (Sigma) and were incubated with RTU-CD3 antibody (Novocastra). After incubation with primary antibodies for 30 min, the grids were rinsed in PBS. Then were exposed to biotinylated rabbit anti-mouse immunoglobulins (DAKO) diluted 1:100 and again washed in PBS. Afterwards, the grids were transferred onto drops of a 1:50 dilution of 10 nm gold particles conjugated to streptavidin (Sigma) and incubated for 30 min. After the grids were dried, the procedure was repeated on the other side of the grids. The sections were then incubated with RTU-CD4 antibody (Novocastra) and 20 nm gold/streptavidin particles (SPI Supplies). All incubation steps were performed at room temp. Control specimens were incubated with nonimmune antiserum (normal mouse serum, DAKO). The preparations were examined using a transmission electron microscope JEM 100 CX (JEOL, Tokyo, Japan) at 80 kV.

Results

The expression of CD3 and CD4 antigens in samples from patients with mycosis fungoides using immunogold method was estimated. We found positive expression of these antigens in ultrathin section in all studied samples. Morphological analysis at the ultrastructural level showed cells with big and abnormally convoluted nuclei. The nucleus with rather prominent nucleoli was occupying nearly whole area of the cell and often had cerebriform shape. The cytoplasm was seen as a narrow rim around the nucleus (Fig. 1). The accumulation of CD3 and CD4 antigens at the ultrastructural level using double-immunogold labeling was observed mainly on the plasma membrane and intracellulary in T cells (Fig. 2–4). We found rather clusters of CD3 and CD4 antigens on the surface of the cells than pattern of distribution as single gold particles along the plasma membrane (Fig. 2a-b). The clusters were composed of both gold particles for CD3 and CD4 antigens (Fig. 3, 4) but there were also aggregates only composed of CD3 or more often of CD4 antigens (Fig. 2a–b).

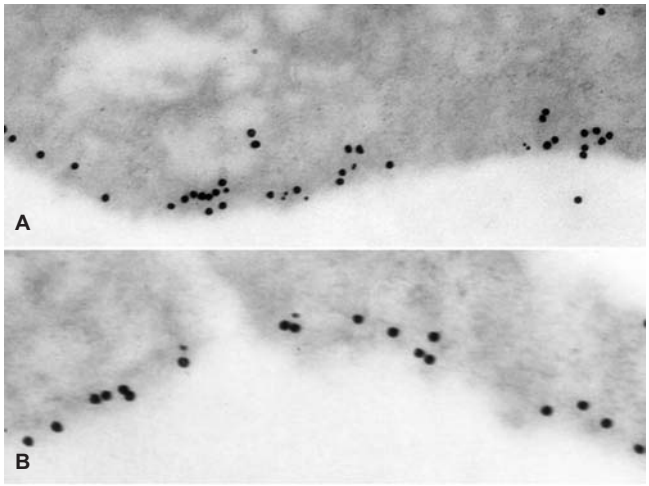

Figure 2. Mycosis fungoides with double gold labeling of CD3 and CD4 antigens. Clusters of both antigens and/or composed only of CD4 are seen on plasma membrane. Magnification a – x 42 000; b – x 50 000.

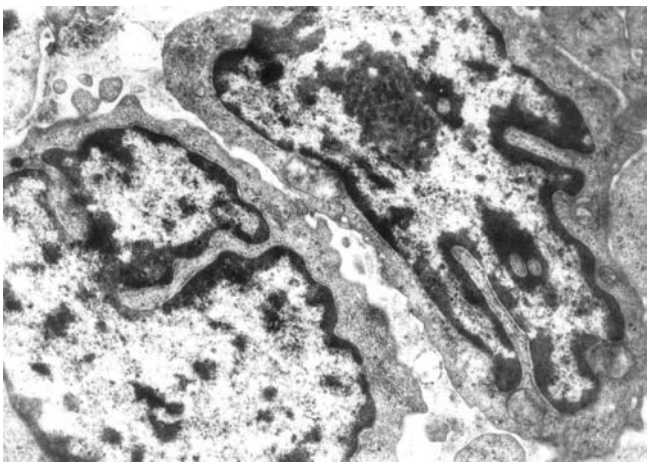

Figure 1. Electron micrograph of mycosis fungoides cells with characteristic cerebriform shape of nuclei. Magnification x 23 000.

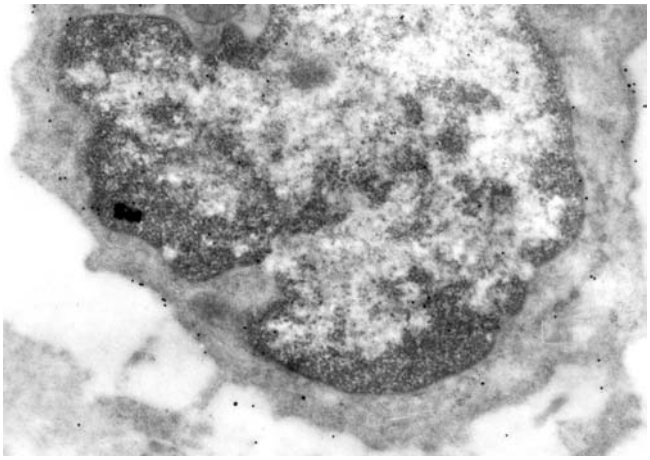

Figure 3. Electron micrograph of mycosis fungoides cell with expression of CD3 and CD4 antigens on the surface, cytoplasm and nucleus. Magnification x 27 000.

Immunogold labeling for both antigens was predominantly concentrated on and or close to the plasma membrane but there were gold particles scattered also throughout the cytoplasm and often seen even as clusters composed of both antigens. In our experiments we observed also accumulation of gold particles of both studied antigens even in nucleus (Fig. 3, 4). CD3 and CD4 labelling was not found in control cells incubated with non-immune serum (Fig. 5).

Discussion

In this study, we used immunogold electron microscopy to show expression of CD3 and CD4 antigens in tissue samples from patients with mycosis fungoides. Immunoelectron microscopic studies of these antigens revealed not only their labeling pattern throught the cells but also by using double-immunogold labeling we were able to conclude the relation in distribution between both studied antigens. There are yet not reports considering above mentioned problems. Instead, there are several papers describing molecular genetic, oncogenic defects and surface marker expresion abnormalities [5, 10–14]. Even the results on evidence that CD3 and CD4 complex interacts both physicall and functionally on T-cells were published [15–17]. Here, we demonstrated close localization of CD3, CD4, T cell-restricted antigens. Our data showed that under used conditions in mycosis fungoides there were labeled clusters often composed of both different size of gold particles localized not only on the surface and in cytoplasm of cells but also in the nucleus. Concluded on distribution of antigens esspecially in nucleus we suggest that there might be translocation of CD3 and CD4 clusters within mycosis fungoides T-cells but more studies is needed to confirmed it. The suggested process may be involved in pathogenesis of mycosis fungoides T-cells. The close localization of CD3 and CD4 antigen in clusters at the ultrastructural level shows that there must be some relation between both antigens. VIGNALI and co-workers showed that D3/D4 domains of CD4 may interact directly or indirectly with the TCR-CD3 complex and influence the signal transduction process [16]. There are more reports on association between CD3 receptor and CD4 molecule on T cells [18–21]. It is also worth noting that some of the clusters were composed of one type of the antigen and we could observe more often CD4 than CD3 antigen. Our results are similar to others in which the lower level of CD3 expression was observed [22, 23]. EDELMAN et al showed that diminished CD3 expression correlated with morphologic evidence of mycosis fungoides cells [22]. In conclusion, the ultrastructural approach enabled us not only to visualized pattern distribution and relationship between CD3 and CD4 antigens but we might suggest also that the type and form of distribution provides new clues to their possible translocation in mycosis fungoides cells. We are aware of the fact that these are very first observations and we ought to go in this direction using additional methods and samples.

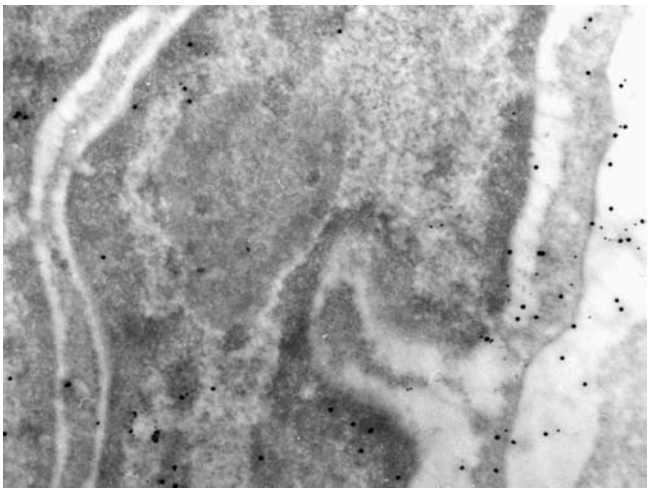

Figure 4. Mycosis fungoides cell with expression of CD3 and CD4 antigens. Cluster of antigens are seen on plasma membrane, cytoplasm and nucleus. Magnification x 32 000.

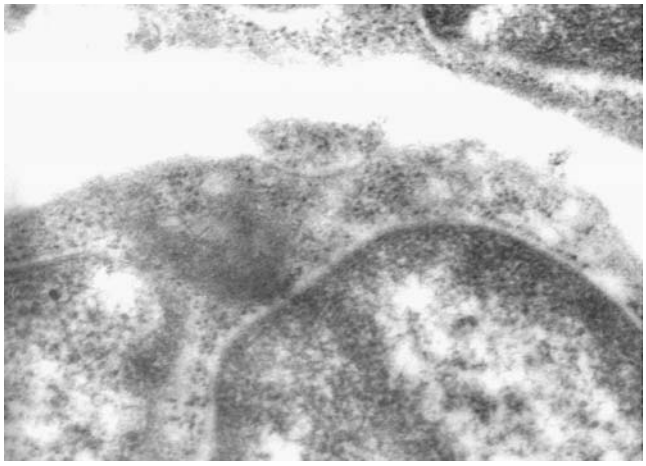

Figure 5. Electron micrograph of mycosis fungoides cell showing the control reaction with normal mouse serum instead of primary antibodies. Magnification x 30 000.

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