ORIGINAL PAPER

The study of CD20 and CD45.Ro antibodies in the inflammatory infiltrate involved in acne and seborrheic dermatitis

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Abstract

The aim of our study was to evaluate the role of CD20 and CD45.Ro antibodies in acne and seborrheic dermatitis. A number of 20 patients with papular, pustular or nodular acne and another 20 patients with seborrheic dermatitis were available for our study. We removed bioptic material from all of them and we perform histochemical and immunohistochemical processing within the Laboratory of Histology, Histopathology and Immunohistochemistry of the University of Medicine and Pharmacy of Craiova. In acne, we could reveal a positive CD45.Ro immunomarking in rare lymphoid cells situated in the middle derma at a distance from the affected pilosebaceous follicle and in the inflammatory infiltrate subepidermically, and also a negative immunomarking in the inflammatory cells from the proximity of the affected pilosebaceous follicle. In patients with seborrheic dermatitis we noticed a positive immunomarking infiltrate of the papillary derma and a positive immunomarking of membrane for CD45.Ro in many lymphoid cells of the inflammatory infiltrate situated in the papillary derma predominantly disposed perivasculary. *Conclusions*. The absence of the cells marked with CD45.Ro in the proximity of the pilosebaceous follicle interested in acne excludes the direct participation of B- and T-lymphocytes in the perifollicular inflammatory process, though the T-lymphocytes can be revealed in a small number at a distance from the affected follicle. The inflammatory infiltrate from the seborrheic dermatitis proved to be rich in positive CD45.Ro cells and poorer in positive CD20 cells.

Keywords: acne, seborrheic dermatitis, immunohistochemistry, CD20, CD45.Ro.

Introduction

Acne vulgaris is a common disorder that can have a significant effect on patients' physical and psychological well-being [1]. The pathogenesis of acne is multifactorial, including microbiological, hormonal and immunological mechanisms [2, 3].

The primary event in inflammatory acne involves the disruption of the follicular epithelium and colonization of the follicles with *Propionibacterium acnes* (*P. acnes*). *P. acnes* induce inflammation in acne with release proinflammatory cytokines, which regulate the local immune response. The bacterium has been shown to have mitogenic as well as antigenic activity and the inflammatory infiltrate within early lesions is predominantly CD4+ T-cells [3–5].

Seborrheic dermatitis is a common inflammatory disorder of the skin, characterized by erythema covered with greasy scales and seen over areas rich in sebaceous glands. The etiopathogenesis of seborrheic dermatitis is still unknown. The organism *Malassezia furfur* or its yeast form, *Pityrosporum ovale*, plays an etiological role in seborrheic dermatitis [6, 7].

How *Malassezia* yeasts initiate the inflammation of seborrheic dermatitis is not clear. They or their products may cause inflammation by inducing cytokine production by keratinocytes or through involvement of Langerhans cells and T-lymphocyte activation [8].

The purpose of our study was to evaluate the involved of CD20 and CD45.Ro antibody in inflammatory infiltrate in acne and seborrheic dermatitis.

Material and methods

A number of 20 patients with papular, pustular or nodular acne, and another 20 patients with seborrheic dermatitis were available for our study. We removed bioptic material from all of them and the pathologic product come from recent, untreated lesions exclusively placed at the level of the posterior thorax. As to perform the histopathologic exam, the pieces were sent to the Laboratory of Pathologic Anatomy of Craiova. First, they have been processed by usual histopathologic technique of wax embedding until the stage of wax blocks.

Usual staining were then performed and both the histochemical within the Laboratory of Histology, Histopathology and Immunohistochemistry of the University of Medicine and Pharmacy of Craiova.

We used one of the immunohistochemical methods based on soluble immunoenzymatic complexes called LSAB/HRP (Labelled Streptavidin Biotin). DAKO LSAB 2 System HRP (Universal DAKO Labeled Streptavidin Biotin 2 System Horseradish Peroxidase) kit was also used.

LSAB method (with Streptavidin-Biotin) is also called ABC (Avidin-Biotin Complex) where avidin is substituted for streptavidin and it has a basis the direct conjugation of streptavidin to enzymatic molecules.

Streptavidin is a tetrameric analogue of avidin with a molecular weight of 60 kD; it was extracted from *Streptomyces avidinii* bacteria, which can very clearly bind the biotin molecules. This relationship is theoretically ten times greater than that of the antibodies for their antigens, thus leading to a specific, intense detection and an increase of antigen-antibody bindings.

The result of the immunohistochemical reactions consists in visualizing the examined antigens by means of DAB chromogene determining a brown precipitate at their level (cell nucleus stained light blue with Hematoxylin).

For the immunohistochemical study, we used concentrated antibodies made by DAKO Cytomation, Denmark, whose dilution and pre-treatments performed by us, are presented in Table 1. We mention that, in order to get the optimal dilution, the antibodies were diluted in PBS–solution–azyde in the moment of using.

Table 1 – ***Antibodies used, clone, dilution and pre-treatment cycles***

Antibody	Clone	Dilution	Pre-treatment	Incubation time
CD20	L 26	1:150	5 cycles MW in citrate buffer	30 min. TA
CD45.Ro	UCHL1	1:200	5 cycles MW in citrate buffer	30 min. TA

Results

In our study, the histologic exam of acne lesions shows many local changes corresponding to the clinical elementary lesions specific for that disease. However, phenomena of hiperproliferation and cell retention (microcomedones) accompanied by sebaceous glands hypertrophy and decrease of the infundibular sizes took place at the level of the pilosebaceous follicle. Papules and pustules follow the polimorphonuclears storing into the follicular infundibulum and into the perifollicular derma; they also came from the dermic inflammatory infiltrate storing and the nodules appear because of expelling into the derma the strong irritate material contained into the comedones (Figure 1).

Histopathologic changes of the seborrheic dermatitis varies according to the age of the disease, such as: in the recent lesions of the disease, a perivascular lympho-histocytary superficial infiltrate is present, light to moderate spongiosis, ortho- and parakeratosic plugged on the top of the follicular orifices (Figure 2). In the chronic stages of the seborrheic dermatitis, many capillaries and venules expanded in the superficial plexus are present; often they are difficult to differ from the psoriatic lesions [9].

As concerning the inflammatory infiltrate in acne, we could reveal a positive CD45.Ro immunomarking in rare lymphoid cells situated in the middle derma at a distance from the affected pilosebaceous follicle (Figure 3), and in the inflammatory infiltrate subepidermically, and also a negative immunomarking in the inflammatory cells from the proximity of the affected pilosebaceous follicle (Figure 4).

In the patients with seborrheic dermatitis we noticed a positive immunomarking infiltrate of the papillary derma (Figures 5 and 6), and a positive immunomarking of membrane for CD 45.Ro in many lymphoid cells of the inflammatory infiltrate situated in the papillary derma predominantly disposed perivasculary (Figures 7 and 8). We noticed rare T-lymphocytes at the intraepidermic level.

Discussions

Since the very beginning, we should mention that the histopathologic and the immunohistochemical exams in acne and seborrheic dermatitis do not constitute routine investigations to establish the diagnosis for those diseases. In most of the cases, the diagnosis of acne is based on the clinical aspect, both the histologic and the immunohistochemical exams were necessary just for researching the etiopathogenetic mechanisms involved in [10–12].

As concerning the seborrheic dermatitis, the histologic exam is also very rarely necessary but it is useful just when the cutaneous eruption looks like psoriasis, a subacute eczema, a manifestation of the atopic dermatitis etc. Immunohistochemical exam in the seborrheic dermatitis is also important for the scientific contribution, which the local immunologic disorders bring into the attempt of clearing up the pathogeny of that disease [13, 14]. Though there are just a few immunohistochemical studies upon acne or seborrheic dermatitis published up to date, this method brings a huge contribution in revealing the pathogenetic mechanisms specific for those diseases [15, 16].

Up to the present, there was no immunohisto-chemical study published in our country, where CD20 and CD45.Ro lymphocytary antigens were revealed, so that our work represented a national premiere. CD20 (L26) antigen is a transmembranar unglycosilated protein of 33–37 kD, which especially marks B-lymphocytes, the cells of the germinative centre and those of the cloak area. CD20 does not mark B-lymphocytes precursors, neither hystiocytes nor plasmocytes [17].

CD20 oligomeres form a Ca^{2*} channel at the level of the cell membrane and they have a part in regulating the local immune response during B-lymphocytes activation. Anti L26 antibody is very useful beside the CLA (the common leukocytary antigen) to make differences between lymphomas and unlymphoid tumors. CD20 epitope is acquired during the pre-B stage of cell maturation and it remains there all along the maturation [4, 18].

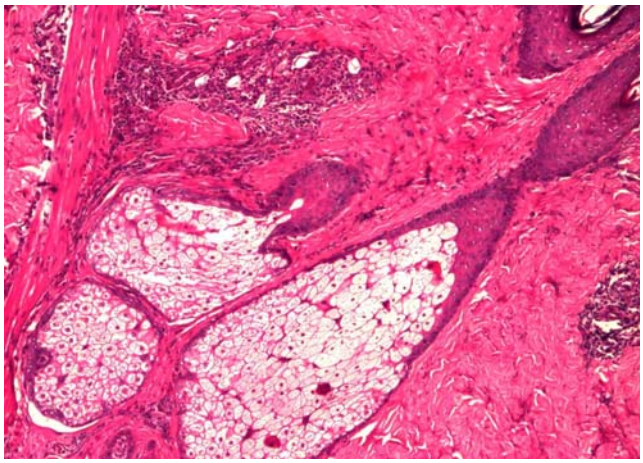

Figure 1 – ***Acne: inflammatory infiltrate with PMN at the levels of sebaceous glands (HE, ×4)***

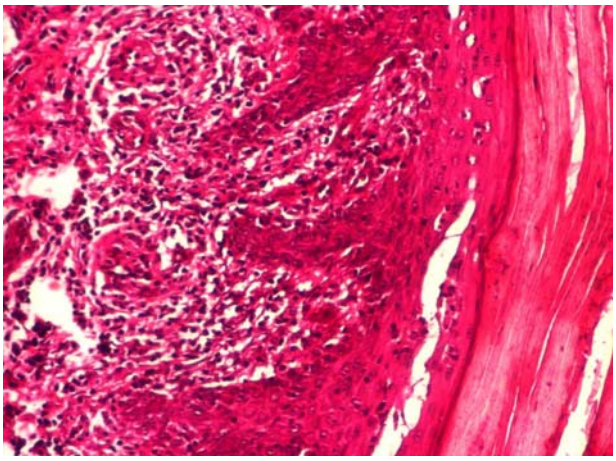

Figure 2 – ***Seborrheic dermatitis: hyperkeratosis, agranulosis, spongiosis with intraepidermical microvesicula, inflammatory infiltrate in superficial dermis (HE, ×10)***

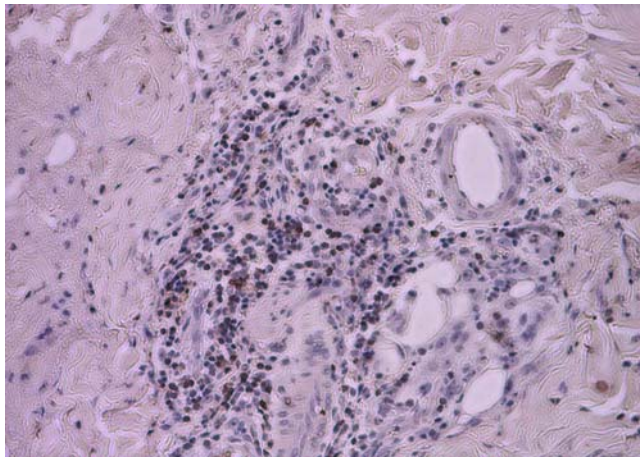

Figure 3 – ***Acne: (+) immunomarking for CD45.Ro in rare lymphoid cells at a distance from pilosebaceous follicle, ×10***

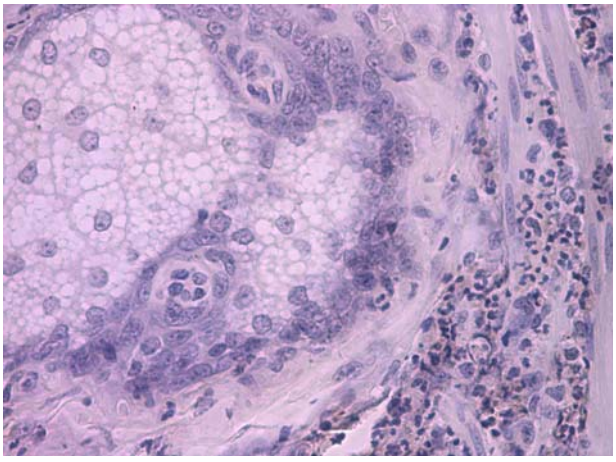

Figure 4 – ***Acne: (-) immunomarking for CD45.Ro in lymphoid cells of inflammatory infiltrate from the proximity of the pilosebaceous follicle, ×20***

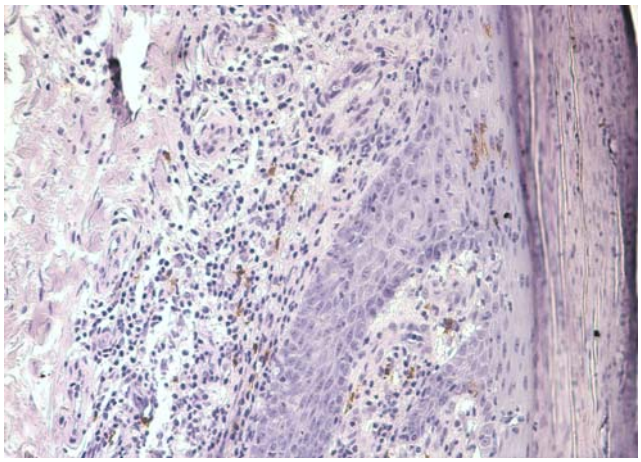

Figure 5 – ***Seborrheic dermatitis: (+) immunomarking for CD20 in rare lymphoid cells of the inflammatory infiltrate situated in the papillary derma***

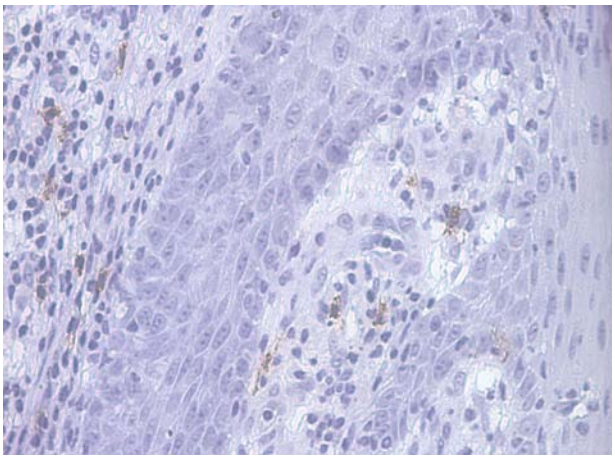

Figure 6 – ***Seborrheic dermatitis: (+) immunomarking of membrane for CD20, ×20***

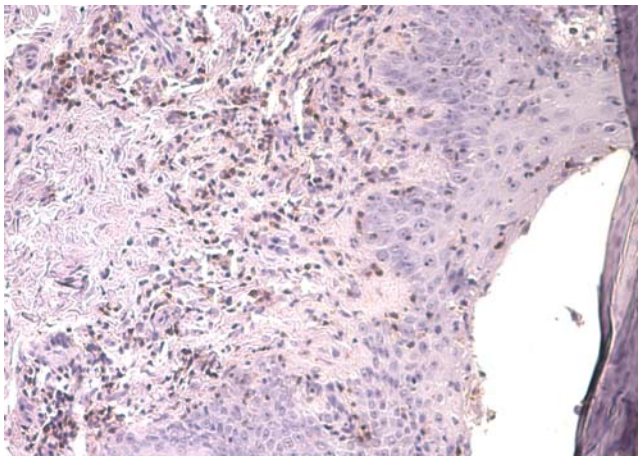

Figure 7 – ***Seborrheic dermatitis: (+) immunomarking for CD45.Ro in many lymphoid cells of the inflammatory infiltrate situated in the papillary derma, ×10***

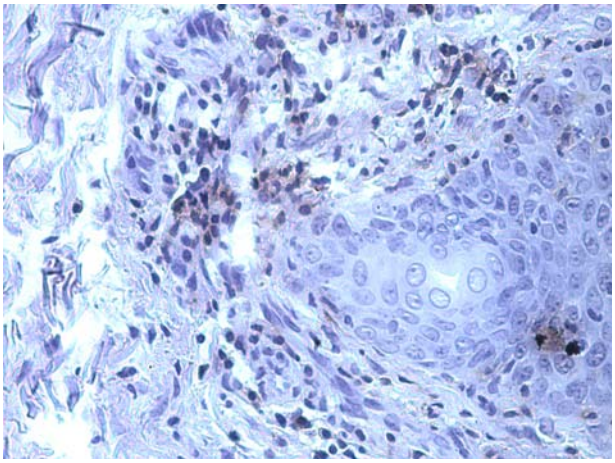

Figure 8 – ***Seborrheic dermatitis: (+) immunomarking for CD45.Ro in many lymphoid cells of the inflammatory infiltrate situated in the papillary derma predominantly disposed perivascularly. Rare T-lymphocytes at the intraepidermic level, ×20***

CD20 intensely expresses in about half of the leukemias and lymphoblastic lymphomas in almost all the lymphomas with B mature cells; in Reed–Sternberg cells from about a quarter of the classic Hodgkin cases and it does not express in lymphomas with T-cells.

The common leukocytary antigen (CD45.Ro) is a glycoprotein of membrane with a great molecular weight (also known as t200) expressed almost exclusively by the cells of haematopoietic origin. It is present in most of the benign or malignant lymphocytes and also in the precursors of the erythrocytes and plasmocytes but not in their mature homologues [17].

Anti CD45.Ro antibody recognizes a variant of CLA, limited to the T cell. It marks T cells, granulocytes and some histiocytes. We must consider as positive just the membranary marking though a cytoplasmatic mark can be often seen in different tissues. Most of the lymphomas with T-cells and very rarely lymphomas with B cells are marked.

Conclusions

The absence of the cells marked with CD45.Ro in the proximity of the pilosebaceous follicle interested in acne excludes the direct participation of B- and T-lymphocytes in the perifollicular inflammatory process, though the T-lymphocytes can be revealed in a small number at a distance from the affected follicle.

The inflammatory infiltrate from the seborrheic dermatitis proved to be rich in positive CD45.Ro cells and poorer in positive CD20 cells.

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Received: April 16th, 2007

Accepted: July 15th, 2007