Original paper

Expression of CD1a, CD207, CD11b, CD11c, CD103, and HLA-DR receptors on the surface of dendritic cells in the skin of patients with atopic dermatitis

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Abstract

Introduction: Atopic dermatitis (AD) is a chronic skin disorder of unknown etiopathogenesis. Its development is based on the influence of environmental factors, genetic and immunologic disorders. Undoubtedly, an important role is played by changes in quantitative and qualitative information on dendritic cells.
Aim: Assessment of CD1a, CD207, CD11b, CD11c, CD103, and HLA-DR receptors on the surface of dendritic cells in the skin of patients with atopic dermatitis.
Material and methods: The study group consisted of 45 patients with clinically diagnosed AD from whom biopsies were taken from the lesions. The control group was the material of 20 healthy people. To carry out the study the method of indirect immunofluorescence double staining reaction was used.
Results: Studied receptors gave positive reactions in both groups. The number of cells in healthy individuals was significantly lower than in patients. They also differed in appearance and location of the skin.
Conclusions: The CD1a/CD207 and CD1a/CD11c, CD1a/HLA-DR cell density was higher in AD patients compared to controls. There were differences in the location and appearance of the cells of AD patients compared to controls. All cells in the epidermis identified with antibodies CD1a, CD11c and CD207 were dendritic cells.

Key words: atopic dermatitis, dendritic cells, Langerhans cells.

Introduction

Atopic dermatitis (AD) is a common, chronic and recurrent skin disorder. Changes begin in infancy or early childhood. It is characterized by intense itching, dry skin, and lichenification with typical location [1–6].

Despite numerous studies the etiology is not fully explained, but two of the relevant factors are ectodermal defect and dysregulation of the immune system [1, 4, 7, 8]. Undoubtedly, an important role is played by dendritic cells (DC). This is the main population of antigen-presenting cells (APC) [8–10].

There are immature DC and mature forms. Immature cells occur in tissues that are in contact with the external environment. These include primarily the skin and the lining of respiratory and digestive systems. The immature form of cells possesses the ability the fagocytosis and the absorption of antigen, the form mature the introduction of antigen. Dendritic cells process and present antigen to T cells or other cells to initiate an immune response [9, 11–14]. The absorption of the antigen can be expressed on respective receptors and adhesion molecules on the cell surface.

During maturation, DC change the function of the absorbing cells and antigen-processing function to the antigen presentation function.

Mature DC already have the ability to present antigen to T cells and are characterized by numerous, long and branched dendritic protrusions. Mature DC are able to induce a primary immune response involving T lymphocytes [9, 10].

Dendritic cells morphological appearance differs depending on the location in the body. They are found in the skin

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population of Langerhans cells. Cells these after contact from antigen transform in forme matur. They gather in the vicinity of inflammatory and necrotic foci. Their most important function is to capture and absorb the antigen and to stimulate inflammation by antigen presentation to lymphocytes. Dendritic cells can alone without prior contact with the antigen present it to the naive T cell, and affect all populations of T cells [12–14].

Dendritic cells are a heterogeneous cell population. Three subpopulations of DC have been isolated according to the expression of cell surface antigens on: myeloid, follicular and plasmacytoid cells. They differ in function and immunophenotype. Myeloid DC are characterized by strong expression of the CD11c receptor and HLA-DR, including blood DC antigen (BDCA), mainly BDCA-1 and BDCA-2 receptors, also have toll-like receptors, so they can recognize foreign pathogens and other receptors. The primary function of myeloid DC is to recognize foreign antigens, their processing and presentation. They are necessary for T-cell activation [8, 10, 14]. Lymphoid (plasmacytoid) dendritic cells (pDC) express CD123 receptor, BDCA-2 and BDCA-4. Also they express CD4, CD10, and CD45RA, while expression of MHC class II is much lower than in the case of myeloid DC.

This population of DC has a weak function of antigen processing and presentation; however, it is the largest producer of interferon I, the level of which increases rapidly following a viral infection. It is part of the innate immunity [8, 10, 12].

Follicular DC present in lymphoid follicles of the spleen and lymph nodes are not capable of migration; cells are sessile. They probably derive from connective tissue cells: fibroblast precursors or stromal cells. They play a very important role in the formation of B memory cells. In places their propagation leads to their interaction with B cells [12, 14].

Aim

1) Evaluation of density and morphological characteristics of DC in patients with AD.
2) To determine whether there is a difference in the population of DC depending on their location.
3) Evaluation of the correlation between the presence of DC and their receptors located in the skin.
4) Comparison of cell population, and morphological receptors correlation between the control group and the study group.

Material and methods

The study group consisted of 45 patients with clinically diagnosed AD, 33 patients with acute phase of the disease and 12 with chronic. According to SCORAD the acute phase is atopic dermatitis with moderate and severe disease and the chronic phase is SCORAD with the mild course of disease. The control group was material derived from 20 healthy patients who underwent surgery in the Department of Plastic Surgery. All patients underwent biopsy of the affected area punched with a diameter of 5 mm. In the study indirect immunofluorescence double staining was used. In the first stage of the study the primary antibody anti-human CD1a was used, followed by fluorescent conjugated secondary antibody (TRITC). In the second reaction step, first CD207, CD11c, CD11b, CD103, HLA-DR were applied and then the secondary antibody FITC. At the end the cells were counted.

Results

All kinds of cells were found in patients. CD1a cells have on their surface a receptor for antibodies CD207 CD11c, CD11b, CD103, and HLA-DR.

CD1a/CD207 and CD1a/CD11c cells occurred only in the epidermis, not in the dermis. They were unevenly distributed in clusters and long dendritic protrusions (Figure 1).

CD1a/CD103 (Figure 2) and CD1a/CD11b cells were found both in the epidermis and within the dermis. The morphological appearance of all cells in the study group was similar, in the form of clusters, unevenly distributed, in contact with each other with long, branched tabs. In all patients there were observed from 3 to 4× more cells than in the control group. Distribution differed as well. In the patient group very numerous cells were presented throughout the epidermis, whereas in the control group they occurred separately.

Between patients with the moderate and acute course of the disease according to the SCORAD scale and the patients with the mild course of the atopic dermatitis there were not found any significant differences in the amount and morphological appearance of the CD1a, CD207, CD1a/CD11c, CD1a/CD11b, CD1a/CD103, CD1a/HLA-DR cells (Figure 3). Among the people with mild atopic dermatitis the amount of the cells varied between 411 and 582 cells/mm^2 of the epidermis cells (Table 1); however, among patients with the moderate and acute phase of the disease it ranged from 423 to 674 cells/mm^2 of the epidermis (Table 2).

In the control gorup found only in the spinous layer of epidermal cells CD1a/CD207, CD1a/CD11c and CD1a/HLA-DR. The distribution was uniform along the skin, having short and dendritic extensions in contact with each other. All cells were stained with TRITC-CD1a and contained on their surface a receptor for CD207, CD11c and HLA-DR. Compared with test cells there was 4 times less (Figure 4).

Statistical analysis

The results of the Shapiro-Wilk test confirmed the normality of the parameters on the designated cell markers CD1a/CD207, CD1a/CD11c, CD1a/HLA-DR

Statistical power of the used test indicates that for the studied population size we obtained the power of

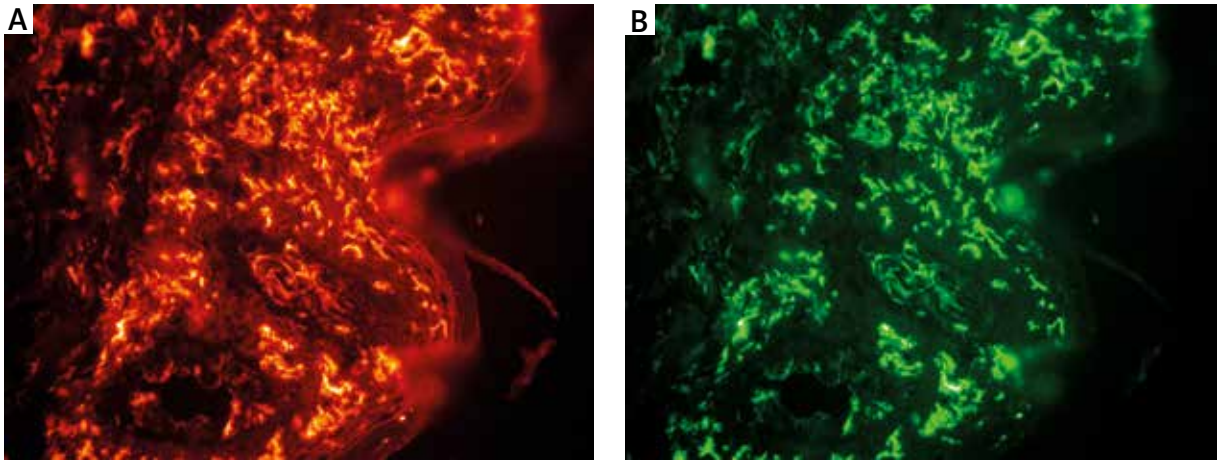

Figure 1. Indirect immunofluorescence double staining, using anti-human anti-CD1a/TRITC (**A**) and anti-human CD207/FITC (**B**); magnification 400×

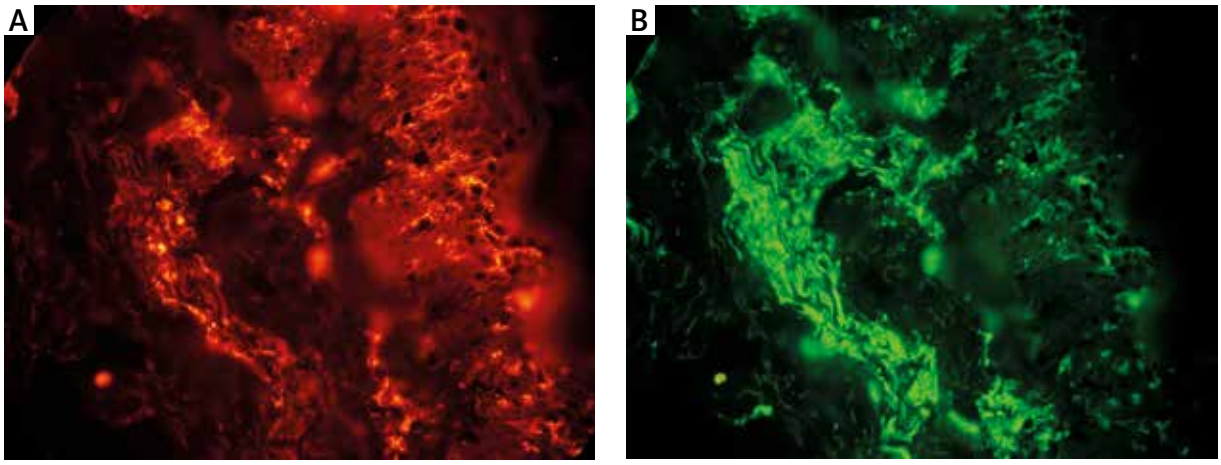

Figure 2. Indirect immunofluorescence double staining, using anti-human anti-CD1a/TRITC (**A**) and anti-human CD103/FITC (**B**); magnification 400×

100% for each of the analyzed markers compared between the groups.

Among the patients with the moderate and acute course of the disease, according to the SCORAD scale, higher density of the marked cells was found on the surface of the skin (four times more than in the control group). The comparison of the results of the patients with the mild course of the disease shows that the number of analyzed cells was three times higher than in the tested group.

Comparison of the results with chronic phase of the disease shows that the cells analyzed were more than three times more numerous than in the study group.

Statistical analysis performed using ANOVA confirms posed hypotheses about the occurrence of the differences for the analyzed factors between the compared groups, at a level below the threshold of statistical significance α.

Additional statistical analysis performed in the post-hoc algorithm (Fisher's LSD) confirms the presence of statistically significant differences between the groups for each variable (Figure 4).

Analysis of markers CD1a/CD11b, CD1a/CD103

The results of the test confirmed the normality of the parameters on cell markers CD1a/CD11b, CD1a/CD103.

Statistical analysis performed with Student's *t* test confirmed the hypothesis of the occurrence of differences for the analyzed factors.

Discussion

Atopic dermatitis is a chronic inflammatory dermatosis of the skin, which begins in early childhood. Despite several studies, the pathogenesis is still not fully explained. It is the result of dysregulation of both the

Table 1. Number of cells in patients with atopic dermatitis with mild course per mm² of the epidermis

Patient no.	CD1a/CD207	CD1a/CD11c	CD1a/CD11b	CD1a/CD103	CD1a/HLA-DR
1	409	455	410	425	445
2	347	359	360	355	345
3	480	494	475	485	490
4	480	492	477	490	485
5	400	397	415	407	410
6	402	446	447	428	439
7	428	440	444	438	439
8	479	510	500	499	515
9	563	570	559	568	572
10	478	421	420	456	468
11	455	550	545	558	560
12	411	403	400	418	408

mechanisms of humoral and cellular defect ectodermal and microbiome disorders. For the proper functioning of the immune system it is necessary to have correct structure of the epidermis, particularly the stratum corneum, which in AD patients is defective [1, 3, 4]. One of the many molecules that is present on the surface of both CD and LC is CD1a. It is able to present antigens derived from mycobacteria, microorganisms and their own cells, to T cells. It is a membrane protein with a weight of 43–49 kDa; its structure is similar to MHC class I. In our study, the presence of CD1a+ cells was observed only in the epidermis, both in the control group and study group. Lesiak *et al.* observed the presence of CD1a+ in their control group in the basal layer of the epidermis [15].

Langerin (CD207) receptor protein of a C-lectin character was discovered in Birbeck granules in Langerhans cells; it is believed to have a vital function in their creation. In our study we revealed the presence of cells CD1a/CD207 in patients, whereas in the control group, there were statistically fewer. Similar test results were obtained by Henri *et al.*, who identified CD207+ cells in people with AD in the epidermis and dermis. According to the authors the CD207+/CD103+ cells are able to cross-present antigen whose expression is limited to keratinocytes. They also demonstrated that it is not connected to the increased expression of CD80, CD86 and MHC class II [7].

Kwiek *et al.* in their study qualify the number of dendritic cells CD1a and langerin. Patients in the acute phase of atopic dermatitis have a greater level of CD1a+ cells

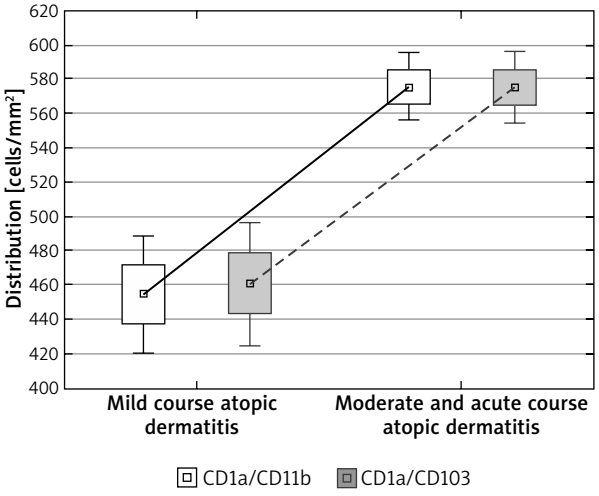

Figure 3. Distribution of cell surface markers CD1a/CD11b, and CD1a/CD103 in patients with moderate and acute course of atopic dermatitis and those with mild course of atopic dermatitis

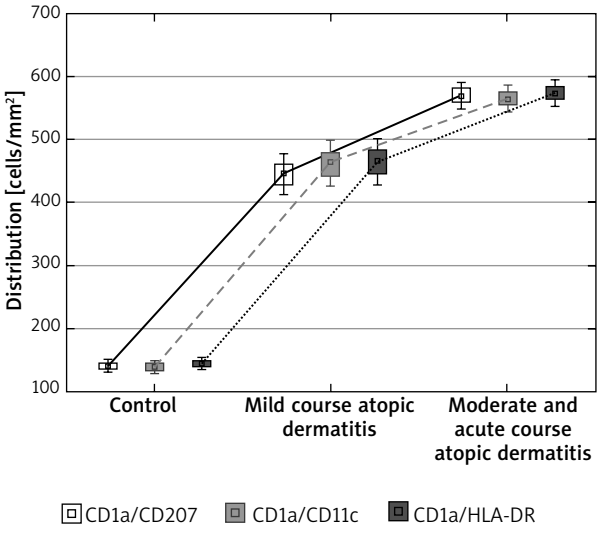

Figure 4. Number of cells per square millimeter of the present marker CD1a/CD207, CD1a/CD11c and CD1a/HLA-DR

Table 2. Number of cells in patients with atopic dermatitis in moderate and acute course per mm^2

Patient no.	CD1a/CD207	CD1a/CD11c	CD1a/CD11b	CD1a/CD103	CD1a/HLA-DR
1	554	582	562	578	580
2	610	589	615	602	611
3	651	666	649	655	659
4	558	525	550	561	534
5	472	423	451	461	455
6	661	665	658	666	670
7	548	492	501	489	482
8	547	545	554	532	560
9	618	605	600	620	620
10	588	603	601	590	600
11	597	540	600	589	567
12	674	631	629	650	649
13	541	512	550	548	550
14	598	625	600	627	611
15	601	589	610	600	608
16	443	463	470	459	469
17	521	501	515	525	515
18	489	500	506	499	501
19	522	521	536	548	533
20	610	600	628	616	614
21	521	550	555	526	539
22	487	495	515	500	490
23	499	550	515	536	529
24	514	520	530	548	536
25	611	663	652	660	655
26	656	603	624	666	648
27	587	543	590	564	580
28	621	664	650	666	651
29	631	611	645	625	633
30	505	524	516	500	508
31	555	554	590	569	588
32	581	603	599	607	597
33	636	589	630	600	597

compared to patients in the chronic phase [16]. In our study there was no difference in the number of CD1a cells depending on the stage of the disease. It is considered that the CD1a+ cells are IDAC cells – inflammatory dendritic epidermal cells. IDAC cells are present in patients with AD; it is an indication of damage to skin [16].

Tanei *et al.* in their research also used CD1a to identify Langerhans cells and found that there is more CD1a+ in the skin of AD patients than in healthy people; they are found in the epidermis and single ones in the dermis [17]. In our study, the presence of CD1a+ cells was found only in the skin in both study and control groups. In Tanei's *et al.* research the number of CD1a+ cells in patients was significantly higher than in the control group [17]. IDAC cells have higher expression of CD80, MHC class II and FcεRI. They also found that increased expression of FcεRI increases the production of IL-8, while there is a sharp decrease in IL-1. Langerhans cells may play a role in the maturation of other cells such as monocytes, CD4+ T cells, and CD8+ T cells, which at the time of skin dam-

age are present in high densities. The authors also found that IgE mediated by allergen in capturing and passing through the LC FcεRI and IDAC cells may enhance allergic resistance [16, 17].

In our study, and those of other authors, the presence of HLA-DR was found in both the control group and the study group, and there was no difference in the number of CD1a and HLA-DR cells. Greater level of expression of HLA-DR in patients with atopic dermatitis is due to increased activity of antigen presentation to T cells. The presence of this marker also provides for ongoing inflammation [15, 18].

Van den Berg *et al.* in their study of healthy skin found no difference between the expression of CD1a and HLA-DR. Also, the number of CD1a and CD207 cells was the same and they were Langerhans cells [19]. On the other hand, Le *et al.* to identify the DC in their study used HLA-DR and CD11c. According to them, therefore, the DC contain high levels of MHC class II molecules; those used to identify DC, and CD11c, are myeloid DC markers.

According to Le *et al.* the antibody anti-HLA-DR is a characteristic marker of DC, but not specific. These authors found that the MHC II cells are capable of antigen presentation to T cells, but it is not clear whether the increased expression of epidermal MHC precedes the disease or is a consequence of it [20]. Also, Li *et al.* using flow cytometry detected high levels of HLA-DR blood DC [21].

Le *et al.* in their studies marked the DC in the skin in healthy people using antibodies CD11c [20]. It is a marker for myeloid cells and to distinguish them from the lymphoid lineage. However, macrophages can also have CD11c expression and some CD11c+ cells are macrophages, but not DC. All HLA-DR cells had on their surface the receptor for CD11c. These cells present the morphology of DC [20]. In our study, we also observed CD11c cells in the skin of healthy individuals. Expression of CD11c is higher on DC than on myeloid origin Langerhans cells [20].

According to individual research, in the epidermis of people (in the tested group) with moderate and severe atopic dermatitis according to the SCORAD scale, CD11c cells were observed. All CD1a cells gave a positive reaction with the antibody anti-human CD11c. They are DC, of myeloid origin. In this regard, the antibody identifies CD1a Langerhans cells, DC and CD11c. It can be concluded that in our study, in the epidermis, all of the DC are Langerhans cells. Compared with the control group quantity of the cells was significantly higher in the epidermis. A large amount of DC in the epidermis is evidence of ongoing inflammation and the ongoing immune response.

Persson *et al.* in their study identified DC by using antibodies CD103 and CD11b. In this connection, CD11b+ cells and CD103+ are present in the epidermis and in the intestine. We believe that they are the main population of migratory DC [22]. In contrast, Santegoets *et al.* also identified myeloid blood DC using anti-CD11c and CD11b. On the basis of these studies, they found that CD14+ CD11b+ cells are the precursors of Langerhans cells. Among all blood DC LC constitute approximately 20–40%. These cells differ significantly from CD11b DC, and all of the precursor DC of both myeloid and follicular plasmacytoid form are CD34+ cells [23].

The researchers Waithman *et al.* believe that CD11b is a marker of myeloid DC. Thanks to that, it is possible to distinguish DC from macrophages [24]. IDAC cells apart from expression of CD1a and CD207 have a high level of CD11b and CD11c molecules. Thanks to these receptors cell adhesion to the endothelium is possible [25].

Milne *et al.* studied blood DC in healthy individuals. To determine DC used antibody CD1c, CD14 and CD16 or monocytes, and for dendritic plasmoidal cells CD103. These cells were isolated from the blood by flow cytometry. CD1c+ cells have low levels of CD11b, like Langerhans cells, while high expression of CD11b was observed on CD11c+ monocytes [26]. Other research results were presented by Henri *et al.* They analyzed cells present in the epidermis and dermis, by using flow cytometry. In the epidermis, they found cells with the expression of CD207 and CD11b. In the dermis there were cells having CD207+ CD11b+, CD207+, CD207+ and CD103+ CD11b expression [10]. Interesting studies conducted by Kim *et al.* checked if blood DC have CD103+ expression. On the basis of these studies, there was noted increase of antibody expression with increase of regulatory T cells. It was found that distinct populations of DC can induce proliferation of T-cells [27].

Atopic dermatitis is a severe disease that affects mainly children. It causes a lot of discomfort for the young patients. Regardless of widely known etiopathogenesis, intensive research around the world is taking place, trying to explore the mechanisms of action in AD much more deeply.

Conclusions

Density of CD1a/CD207 and CD1a/CD11c, CD1a/HLA-DR cells is higher in AD patients compared to the control group. There are differences in the location and appearance of cells in AD patients compared to the control group. All cells identified in the epidermis with antibodies CD1a, CD11c and CD207 are DC.

Conflict of interest

The authors declare no conflict of interest.

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