CD44 expression in human skin: high expression in irritant and allergic contact dermatitis and moderate expression in psoriasis lesions in comparison with healthy subjects

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Abstract

Background. Previous research using animal models demonstrated that CD44 expression may contribute to directing inflammatory cells into skin lesions during inflammation development in allergic contact dermatitis (ACD).

Objectives. To examine CD44 expression in patients with ACD and irritant contact dermatitis (ICD), and to compare it to expression in psoriatic lesions and healthy subjects' (HCs) skin.

Materials/Methods. This study included 200 subjects from 4 groups of 50 subjects each: ACD, ICD, psoriasis vulgaris and HCs. CD44 expression was determined by immunohistochemical analysis using an optical microscope, and the results were visualised semiquantitatively by determining the percentage of immunoreactive cells in the epidermis, dermis, and on lymphocytes.

Results. The highest CD44 expression was found in ICD, followed by ACD, psoriasis vulgaris, and lastly, the HCs ($P<.001$). Epidermal CD44 expression was significantly higher in contact dermatoses (especially in ICD) compared to psoriasis and healthy skin ($P<.001$). Similarly, CD44 expression in the dermis and on lymphocytes was strongest in ICD, although less pronounced than in the epidermis.

Conclusion. Since significantly elevated CD44 expression in ICD might be related to its function in maintaining and preserving the skin barrier in affected patients, further research on disease pathogeneses and new treatment options is needed.

Keywords: allergic contact dermatitis; irritant contact dermatitis; CD44; psoriasis; healthy skin; immunohistochemical analysis; skin inflammation; skin markers

1. Introduction

Recent studies have shown that allergic contact dermatitis (ACD) involves complex immune pathways and inflammatory mediators, genetic factors (predominantly filaggrin mutation), environmental triggers, antimicrobial peptides produced from various skin cells (e.g., keratinocytes, sebocytes), etc.[1-6]. There are still many unknown factors concerning the pathogenesis of ACD, but some animal studies indicate the potential role of CD44 in ACD[7]. The CD44 antigen, found on cell membranes, is a glycoprotein and is encoded by chromosome 11. It is involved in intercellular interactions, cell adhesion and migration, activation of lymphocytes, haematopoiesis, and tumor metastasis[8-10]. This multistructural and multifunctional molecule is also involved in cell proliferation and differentiation, cellular migration, angiogenesis, the expression and binding of cytokines, chemokines and growth factors to corresponding receptors, adhesion of protease to cell membranes, and is a signal for cell survival[11]. Also, CD44 is the major cellular receptor for hyaluronan and may interact with other ligands (collagen, laminin and fibronectin)[9,12,13]. Concerning CD44 structure, there are also post-translational modifications (e.g. phosphorylation and glycosylation) that have the effect of increasing/decreasing the functionality of the whole CD44 or part of it[14].

CD44 is expressed to a certain extent in all healthy human skin, and its forms are CD44s, CD44v3- v4, v5, v6 and v9. The smallest CD44 molecule is called standard CD44 (CD44s), and its broader variables (CD44v6, CD44v9, Cd44v2-10 and Cd44v3-10) are generated by insertion of one or more alternative exons into standard CD44[15,16]. By immunohistochemistry, all forms of CD44 can be observed in the epidermis, with the strongest expression in the *stratum spinosum* and *stratum basale*[17]. According to current findings, skin affected by inflammation or neoplasms increasingly expresses CD44 on keratinocyte membranes and on infiltrated lymphocytes near the inflammation or tumor process[18]. Concerning the pathohistological comparison of ACD and irritant contact dermatitis (ICD), there are no

prominent differences[19,20]. Immunohistochemically, the most important observation for both ICD and ACD is the presence of CD4+ and some CD8+ T lymphocytes. In the acute phase of contact dermatitis, keratinocytes increasingly express IL-2, ICAM-1 and HLA-DR[21-24]. In determining the role of certain factors in the complex immune cascade in dermatoses and other inflammatory dermatoses, immunohistochemistry provides important diagnostic, prognostic and predictive information and serves scientific purposes for a better understanding of biomarker distribution/localization and protein expression in different tissues[25-27]. Until now, research has only been conducted on animal models. Hence, we wanted to examine whether CD44 expression in human skin (the dermis, epidermis and on lymphocytes) is increased in ACD compared to ICD, psoriasis vulgaris and healthy controls (HCs).

2. Methods

2.1 Subjects

This research was conducted at the Department of Dermatovenereology, University Hospital Center Sestre Milosrdnice (April 2016 - June 2018) and included 200 subjects from four groups of 50 subjects each: ACD, ICD, psoriasis vulgaris and HCs. The study was approved by the Ethics Committee of the University Hospital Center Sestre Milosrdnice (No. EP-4433/15-14). After providing informed consent, patients were incorporated into the study that was conducted according to the guidelines of the Helsinki Declaration.

We included patients with acute skin lesions on their hands histopathologically confirmed as contact dermatitis; these lesions were compared with histologically verified psoriasis lesions and with healthy skin samples. Patients with clinical and histological findings of other hand dermatoses, those with mycological infections (confirmed by mycological exam) and patients whose skin samples had unclear or non-specific histopathological findings were excluded. ACD was diagnosed based on clinically relevant sensitization to contact allergens

(improvement of lesions after avoidance of contact allergens positive in patch tests) while irrelevant reactions were not considered. A diagnosis of ICD, on the other hand, was based on the following criteria: negative patch tests, a history of skin contact with irritants and the exclusion of other dermatoses by histological examination. The biopsies of the first 50 patients with acute hand ACD and the first 50 patients with acute hand ICD were included in the study.

Additionally, histologically verfied skin samples of 50 patients with psoriatic lesions on the hands were analyzed. As a control, healthy skin samples (50 biopsies) from resected edges of excised moles (various localizations) were stored and examined at the Department of Pathology "Ljudevit Jurak" at the above-mentioned hospital.

2.2 Patch testing

In patients with a clinical picture of hand contact dermatitis, patch testing was performed following ESCD guidelines, with the baseline series of allergens (Patch Test Strips Curatest, Lohman & Rauscher International, Rangsdorf, Germany) applied to patients' upper backs[28]. Baseline allergens were used supplied by the Institute of Immunology, Zagreb, Croatia[29]: potassium dichromate (0.5% pet.), nickel sulfate (5.0% pet.), cobalt chloride (1.0% pet.), fragrance mix (8.0% pet.), *p*-phenylenediamine (PPD) (0.5% pet.), epoxy resin (1.0% pet.), *N*-Isopropyl-*N*-phenyl-*p*-phenylenediamine (IPPD) (0.1% pet.), mercapto mix (2.0% pet.), carba mix (3.0% pet.), thiuram mix (1.0% pet), paraben mix (15.0% pet.), neomycin sulfate (20.0% pet.), *Myroxylon pereirae* (balsam of Peru; 25.0% pet.), colophonium (20.0% pet.), formaldehyde (1.0% aq.), quaternium-15 (1.0% pet.), thimerosal (0.1% pet.), lanolin alcohol (30.0% pet.), phenylmercuric acetate (0.01% aq.), ammoniated mercury (10.0% pet.), sulfur precipitated (10.0% pet.), and ichtammol (10.0% pet.). Test results were read and recorded on days 2 and 4 (D2 and D4).

2.3 Histopathological and immunohistochemical analysis

Skin samples (punch biopsies 4mm in diameter) from the 50 ACD patients, 50 ICD patients, 50 psoriasis patients, and the 50 samples of healthy skin (in the latter, excised from tissues surrounding moles) were processed and stored in paraffin blocks and analysed at the Clinical Department of Pathology "Ljudevit Jurak" in the aforementioned hospital. Skin samples were processed by the standard histological method of tissue fixation in 10% buffered formaline, embedded in paraffin blocks, cut to 4μm thickness and stained by the standard hematoxylin and eosin staining method.

Immunohistochemical analysis was performed using a primary antibody to CD44 (anti-human CD44 from monoclonal mice; clone DF1485, Dako) in a 1:50 dilution for determination of the standard CD44 expression (CD44s). The LSAB method was used as a visualization system on the automated DAKO TechMate TM (DAKO, Denmark*)*. Human tonsil section was used as a positive control for CD44 antibody, also in a 1:50 dilution. As a negative control, the primary CD44 antibody was omitted**.**

The expression of CD44 was observed by estimation of the percentage of immunoreactive cells in the selected specimens in three areas: the epidermis, the dermis (observation was done on the collagen network, elastic fibers and the glycosaminoglycan matrix) and lymphocytes (clearly seen as separate cells, observed mainly in the dermis, rarely in the epidermis). CD44 expression was determined by light microscopy, and the results were presented semi-quantitatively by determining the percentage of immunoreactive cells (keratinocytes, lymphocytes, dermal cells) in the following manner: no reaction (0); low reaction (<33% positive); moderate reaction (33-66% positive); strong reaction (>66% positive).

2.4 Statistical analysis

Descriptive statistics were calculated for all variables. Regression analysis (parametric and nonparametric, depending on distribution type) was used to determine correlations to individual variables, and the Chi^2 -test or Fischer's exact test were used for comparing qualitative variables between subgroups. Differences in CD44 expression among the groups

were analyzed by Fisher's exact test for 2x2 size tables or by Fisher-Freeman-Halton's exact test in the case of a larger table. Age differences between the examined groups were processed by one-way variance analysis. Absolute correlation coefficient values >0.600 were considered a strong correlation. Values from 0.300 to 0.599 were medium-strong, and values <0.300 were weak, whether positive or negative.

Statistical analysis was carried out using the STATISTICA statistical software package. 6.0 (StatSoft, Tulsa, Oklahoma) and the commercial statistical software IBM SPSS 25.0 (IBM, Armonk, New York). The G*Power for Windows version 3.1.9.2 was used in the power test analysis. The statistical significance was set at $P < .05$, results set in bold.

3. Results

The study groups did not differ significantly by gender, but by age: the HC group was significantly younger (mean 42.9) than the other groups (ACD mean 48.8; ICD mean 52.3; psoriasis mean 51.0) ($P = .011$) (Table 1).

3.1 CD44 expression in the epidermis, dermis and on lymphocytes

The results of CD44 expression in the epidermis, dermis and on lymphocytes by groups are presented in Figs. 1, 2 and 3 and Tables 2-4. Concerning the epidermal CD44 expression in the examined biopsies, a significant difference between the groups ($P < .001$) was found (Fig. 1). The highest epidermal CD44 expression was found in ICD lesions (strong expression in 26% of cells; moderate expression in 46% of cells), followed by ACD lesions (strong expression in 4% of cells and moderate expression in 62% of cells). In psoriatic lesions, there was no biopsy with strong epidermal CD44 reactions; low CD44 reactions were found in 58%, followed by moderate reactions (26%) and the biopsies without CD44 reactions (16%). Among the biopsies of healthy skin, those without epidermal CD44 reactions predominated (54.90%); low reactions were found in 45.10%; there were no biopsies with strong and moderate reactions.

Dermal CD44 expression in skin biopsies in the four groups (Fig. 2) also differed significantly. ICD lesions without dermal CD44 reactions were found in 42% patients, followed by low (40%) and moderate expressions (18%). In ACD lesions, low dermal CD44 reactions predominated (76%), followed by biopsies without CD44 reactions (20%) and moderate expression (4%). Among psoriatic biopsies, those without dermal CD44 predominated (64%), while low expression was found in 36%. In HCs, biopsies without dermal CD44 predominated (78.43%); in others, low reactions were found (21.57%).

CD44 expression on lymphocytes (Fig. 3) was significantly different between the groups, and the strongest expression was again noted in ICD and also psoriasis vulgaris (P <.001). In the majority of ICD biopsies, low CD44 expression on lymphocytes was found (58%), followed by moderate reactions (22%) and those without reactions (20%). In ACD lesions, low CD44 reactions predominated (76%), followed by biopsies without CD44 (22%) and moderate CD44 reactions (2%). In psoriatic lesions, low CD44 reactions on lymphocytes predominated (64%), followed by moderate reactions (20%) and those without CD44 expression (16%). In HCs skin, the samples were predominantly without CD44 reactions (56.86%) or showed low expressions (43.14%).

3.2 CD44 expression in ACD and ICD compared to controls

Results of CD44 expression in contact dermatitis (ACD, ICD) in comparison to the control group are presented in Tables 2 and 3 . The comparison between ACD patients and the HCs as regards CD44 expression for the three parts (in the epidermis, dermis and on lymphocytes) is presented in Table 2. In comparison to HCs, ACD patients had a significantly higher skin CD44 expression in all three parts: in the epidermis (total positive CD44 reactions: ACD 100% versus HCs 45%), dermis (ACD 80% versus HCs 21.6%) and on lymphocytes (ACD 78% versus HCs 43.1%) (P <.001).

The differences in CD44 expression for the three parts of the skin between the ICD and HC groups are presented in Table 3. Compared to healthy skin, ICD skin showed significantly higher CD44 expression in all three parts (P <.001): in the epidermis (total positive reaction:

ICD 98% versus HCs 45.1%) dermis (ICD 58% versus HCs 21.6) and on lymphocytes (total positive ICD 80% versus HCs 43.1%).

A comparison of CD44 expression between ACD patients and ICD patients (in the three parts of the skin) is presented in Table 4. Positive dermal CD44 reactions were significantly more frequent in ACD lesions than in ICD lesions ($P = .030$). Immunohistochemistry and histology pictures for each of the four groups are presented in Figures 4-7, with the comparison of different magnifications and different levels of skin CD44 expressions (low, moderate, strong).

Based on the data from Table 2-4 the main conclusions are: In comparison to HCs, ACD and ICD patients had significantly higher CD44 expression in the epidermis, dermis and on lymphocytes. The results also show statistically significant differences between the groups ($P<.001$), with the highest skin CD44 expression in ICD, followed by ACD, psoriasis vulgaris, and finally, the control group.

4. Discussion

To understand the significance of CD44 for dermatology, it is particularly important to understand how CD44 participates in processes such as lymphocyte guidance and activation, extravasation of leukocytes and matrix adherence, which are important in autoimmune and allergic reactions. In previous studies on animals and healthy human skin, the roles of the particular CD44 forms were not entirely clarified. According to literature data, in skin inflammatory or neoplastic lesions, CD44 expression can be widespread on keratinocyte membranes and on infiltrating lymphocytes in the vicinity of the process[7,9]. Looking at epidermal CD44 expression, we found significant differences between subject groups with the highest CD44 expression predominantly in ICD, followed by ACD. According to previous immunofluorescent skin analysis, the basic (standard) CD44s form is most pronounced in the

epidermis and dermis, while its v6 and v9 forms are more pronounced only in the epidermis[30]. Although we did not specifically look at the different CD44 forms, based on previous experience and research we assume that the CD44v6 and v9 forms were those that were statistically significantly more pronounced in the epidermis of ICD lesions. .

As in the epidermis, higher CD44 expression was also found in the dermis, more often in ICD than ACD, but in the dermis, it was less pronounced than in the epidermis. Our results confirm and emphasize the role of CD44 in contact dermatoses, especially in ICD, likely because of its involvement in inflammatory reactions and skin barriers. According to a recent study with immunohistochemical determination of CD3, CD4, CD8, CD11c, CD34, CD123, S100 and IL-17 expression in ACD, ICD and atopic dermatitis (AD) lesions, there were no significant differences in epidermal and dermal marker expressions between the diseases[31]. Another study, concerning the role of CD44 in AD lesions in animal models (using the IL-4 transgenic mouse models), also stands out, suggesting that CD4+ and CD8+ T lymphocyte-expressing activation markers (CD44 and CD69) and costimulatory molecules increase with AD progression[32]. According to other studies on cutaneous inflammation (animal models), acute disruption of the epidermal permeability barrier function increases epidermal CD44 expression[7,9], which corresponds to our results conducted on CD patients. Generally, most studies have demonstrated the prominent role of CD44 only in acute inflammations, although some authors found increased epidermal CD44 expressions in both acute skin reactions and subacute inflammatory reactions (chronic delayed-type hypersensitivity)[7].According to animal model results (acute ICD and ACD), inflammatory responses did not differ between CD44 knockout animals (with blocked genes for CD44) and wild-type mice, but following repeated hapten challenges in CD44 knockout mice, both inflammatory responses and epidermal hyperplasia increased. Also, according to literature data (in a subacute murine ACD model), permeability barrier disruption and inflammation stimulate epidermal CD44 expression which modulates epidermal proliferation and inflammatory responses[7,9]. This is in accordance with our study results on CD patients.

Furthermore, CD44 is particularly important for cellular skin barrier and epidermal intercellular junctions (intercellular adhesion is crucial for epidermal development, barrier

creation and structural integrity)[33-37], and cell adhesion proteins are important for tissue functions, including cell growth control, differentiation and inflammation[33]. The results of previous research on mice indicate the importance of CD44 in skin tight junctions, which are necessary for barrier functionality and maintenance[35]. Moreover, according to a genetic study on animal models, there is an association between genes for CD44 and keratinocyte differentiation markers. Specifically, in the skin of animals with blocked genes for CD44 (CD44 knockdown or CD44 knockout mice), the expression of keratinocyte differentiation markers (involucrin and filaggrin) are less expressed compared to wild-type mice (without blocked CD44 genes). According to earlier findings, CD44 deficiency was associated with significant changes in keratinocyte barrier function including changed proliferation, differentiation and lipid synthesis[36]. This connection is supported by our results showing particularly strong CD44 expression (high activity) in ICD (predominantly in the epidermis) and in ACD compared to other groups. Our results in humans point to low CD44 expression in healthy skin and high CD44 expression in contact dermatoses, consistent with inflammatory activity, which corresponds to previous research on animals.

Furthermore, CD44 is the principal cell surface receptor for hyaluronan, a common component of extracellular matrices and extracellular fluids. As a cell surface glycoprotein, CD44 participates in important cellular functions which mostly involve hyaluronan (cell adhesion, migration, and cellular receptor signal modulation)[37,38]. For keratinocyte activity and hemidesmosomes, the association between CD44, matrix hyaluronan (MH) and keratinocyte activity is very important. Specifically, it has been proven that MH promotes CD44 signaling that actively supports keratinocyte activity and improves disturbed epidermal functions[36]. It is precisely this CD44/hyaluronic acid (HA) interaction that is crucial in cell differentiation, proliferation and cell migration, and it plays a particularly important role in tumors, especially in their progression[39]. Our results, which confirm increased CD44 expression on lymphocytes in inflammatory dermatoses (ACD, ICD, psoriasis) compared to healthy skin, support the fact that CD44 activates lymphocytes and participates in inflammatory cascades.

The knowledge of CD44 being a leukocyte-directed (leukocyte-homing) receptor may be useful due to its role during skin inflammation but also for its possible future usefulness in dermatoses management since CD44 plays a crucial role in extravasation of T lymphocytes as well as monocytes[37]. According to other results from animal models, targeted CD44 deletion on leukocytes causes their decreased entry into inflamed areas. Blocking effector cell migration could be achieved with the isoform of CD44 specific antibodies, and there are several assumptions and possible explanations regarding these processes. Since CD44 is the major receptor for HA, their binding (CD44-HA) is crucial for leukocyte migration. In chronic inflammation, T lymphocyte rolling is regulated, and the percentage of T lymphocytes that express activated CD44 is increased. In addition, direct contact between CD44 and CD49d results in the strong adhesion of CD44[16,37]. Depending on the activation state, CD44 binds to CD49d which supports firm adhesion, migration of lymphocytes, activation and apoptosis resistance due to transduction molecules by appropriate binding. Therefore, in the CD44-mediated blockade of leukocyte migration, CD44-integrin associations are prominent[37].

Considering the importance of CD44 in leukocyte extravasation in allergic and autoimmune diseases, the possible targets of anti-CD44 therapy are endothelial cells and CD44-expressing leukocytes, whose blockage would reduce leukocyte migration. Thus, anti-pan CD44 and anti-CD49d antibodies primarily inhibit T lymphocyte migration, while anti-CD11b and anti-CD44v10 inhibit macrophage migration. The anti-CD44 antibody, particularly anti-CD44v10, inhibits only the homing of T lymphocytes in the skin. According to previous studies, the most promising method is CD49d/CD44-blockage of effector T lymphocytes with double-specific antibodies, which should not affect the migration and accumulation or effectiveness of effector cells at active sites. Such a selective blockade of CD44 has already proven to be successful in B-lymphocytic leukemia[37,40].

Even though our study is the first to examine CD44 expression in human dermatoses, it has a few limitations. For example, we only looked at CD44 even though other immunohistochemistry markers could and should be explored as well. We did not take into

account a possible difference in lesional duration (chronicity); in our study, CD44 was analyzed only in the acute ACD/ICD lesions in terms of its predominant participation in acute elicitation reactions. In addition, we compared the CD44 expression in the acute ACD/ICD lesions with those of the psoriatic and healthy skin biopsies, which did not involve acute inflammations. Therefore, there is a need for future examination of the CD44 expression in chronic ACD/ICD lesions in comparison to acute lesions of ACD/ICD, especially concerning the previous inconsistent study results. Furthermore, the skin CD44 expression in biopsies of the examined groups could differ in other aspects, e.g. in anatomic localizations, indicating that further studies are necessary to expand the knowledge of other lesional characteristics. We hope that our results will inspire further studies involving more factors (additional markers and cytokines), additional methods and confirmatory techniques (e.g. western blot, flow cytometry) as well as additional clinical patient features.

5. Conclusion

Based on our results of low CD44 expression in healthy skin, moderate expression in psoriasis, and strong CD44 expression in ICD and ACD, the possible application of a CD44 blockade for these diseases and its usefulness in patients with contact dermatoses could be envisaged. There have already been such attempts with monoclonal antibodies against CD44 variants, including bivatuzumab for v6. Since CD44 is a potential therapeutic target for modifying disease pathology and leukocyte recruitment, further studies on CD44 expression would be valuable.

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Table 1. Gender and age of the four subsamples

	Healthy control	**Vulgar psoriasis**	**Irritant contact dermatitis**	**Allergic contact dermatitis**	***P*-value**
Female: n (%)	30 (58.8%)	25 (50.0%)	32 (64.0%)	33 (66.0%)	.37
Age: arithmetic mean ± SD	42.9 ± 15.6	51.0 ± 15.9	52.3 ± 15.1	48.8 ± 14.4	.011

Table 2. A comparison of CD44 expression in the epidermis, dermis and on lymphocytes in skin biopsies of ACD patients and the HC group (Fisher-Freeman-Halton's exact test)

		Control group		Allergic contact dermatitis		*P*-value
		N	%	N	%	
Epidermal CD44	no reaction	28	54.9	0	0.0	**<.001**
	low reaction	23	45.1	17	34.0	
	moderately expressed reaction	0	0.0	31	62.0	
	strongly expressed reaction	0	0.0	2	4.0	
Dermal CD44	no reaction	40	78.4	10	20.0	**<.001**
	low reaction	11	21.6	38	76.0	
	moderately expressed reaction	0	0.0	2	4.0	
	strongly expressed reaction	0	0.0	0	0.0	
CD44 on lymphocytes	no reaction	29	56.9	11	22.0	**<.001**
	low reaction	22	43.1	38	76.0	
	moderately expressed reaction	0	0.0	1	2.0	
	strongly expressed reaction	0	0.0	0	0.0	

low reaction: 1-33% positive cells; moderately expressed reaction: 33-66% positive cells; strongly expressed reaction: >66% positive cells

Table 3 A comparison of CD44 expression in the epidermis, dermis and on lymphocytes in skin biopsies of patients with ICD and the HC group (Fisher-Freeman-Halton's exact test)

		Control group		Irritant contact dermatitis		*P*-value
		N	%	N	%	
Epidermal CD44	No reaction	28	54.9	1	2.0	**<.001**
	low reaction	23	45.1	13	26.0	
	moderately expressed reaction	0	0.0	23	46.0	

	strongly expressed reaction	0	0.0	13	26.0	
Dermal CD44	No reaction	40	78.4	21	42.0	**<.001**
	low reaction	11	21.6	20	40.0	
	moderately expressed reaction	0	0.0	9	18.0	
	strongly expressed reaction	0	0.0	0	0.0	
CD44 on lymphocytes	No reaction	29	56.9	10	20.0	**<.001**
	low expressed reaction	22	43.1	29	58.0	
	moderately expressed reaction	0	0.0	11	22.0	
	strongly expressed reaction	0	0.0	0	0.0	

low reaction: 1-33% positive cells; moderately expressed reaction: 33-66% positive cells; strongly expressed reaction: >66% positive cells

Table 4. . A comparison of CD44 expression in the epidermis, dermis and on lymphocytes in skin biopsies of ACD and ICD patients (Fisher-Freeman-Halton's exact test)

		Irritant contact dermatitis		Allergic CD		*P*-value
		N	%	N	%	
Epidermal CD44	No reaction	1	2.0	0	0,0	**.007**
	low reaction	13	26.0	17	34.0	
	moderately expressed reaction	23	46.0	31	62.0	
	strongly expressed cells	13	26.0	2	4.0	
Dermal CD44	No reaction	21	42.0	10	20.0	**.001**
	low reaction	20	40.0	38	76.0	
	moderately expressed reaction	9	18.0	2	4.0	
	strongly expressed reaction	0	0.0	0	0.0	
CD44 on lymphocytes	No reaction	10	20.0	11	22.0	**.008**
	low reaction	29	58.0	38	76.0	
	moderately expressed reaction	11	22.0	1	2.0	
	strongly expressed reaction	0	0.0	0	0.0	

low reaction: 1-33% positive cells; moderately expressed reaction: 33-66% positive cells; strongly expressed reaction: >66% positive cells

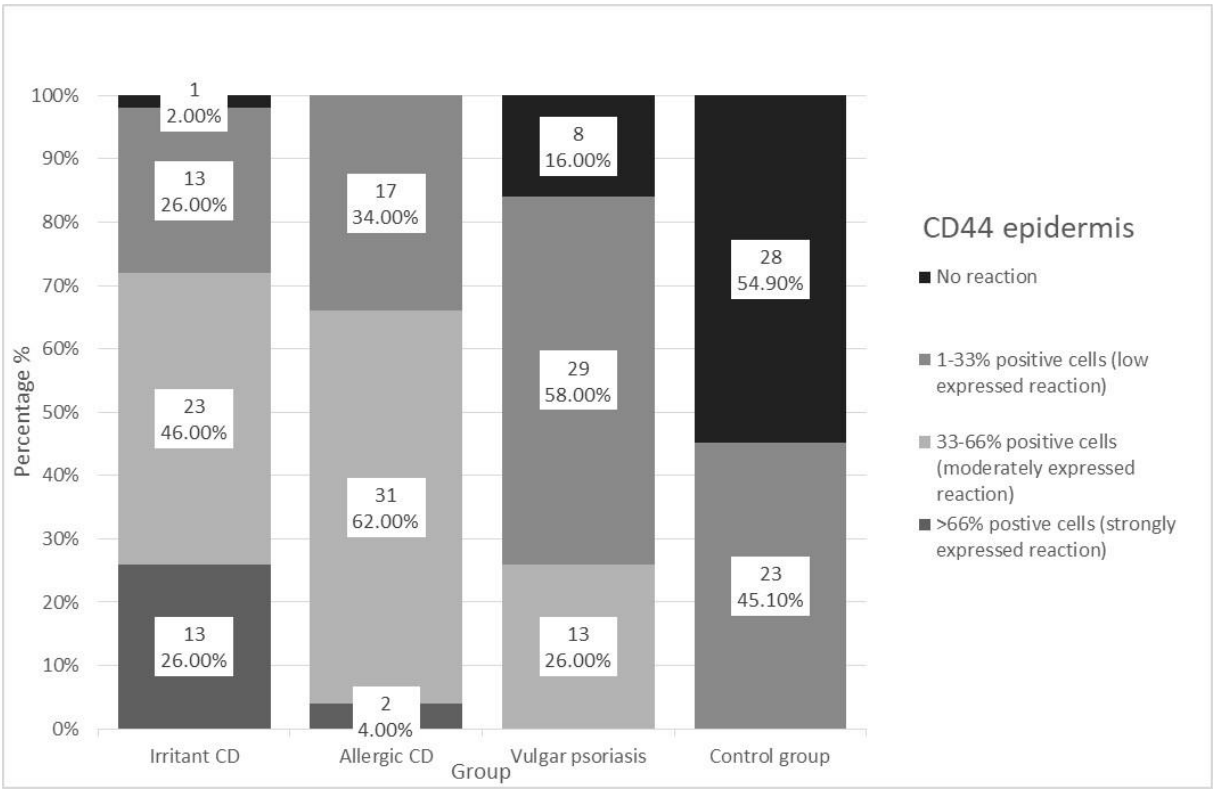

Figure 1. Expression of CD44 in the epidermis in the four groups (ACD, ICD, psoriasis and the healthy control group): P <.001

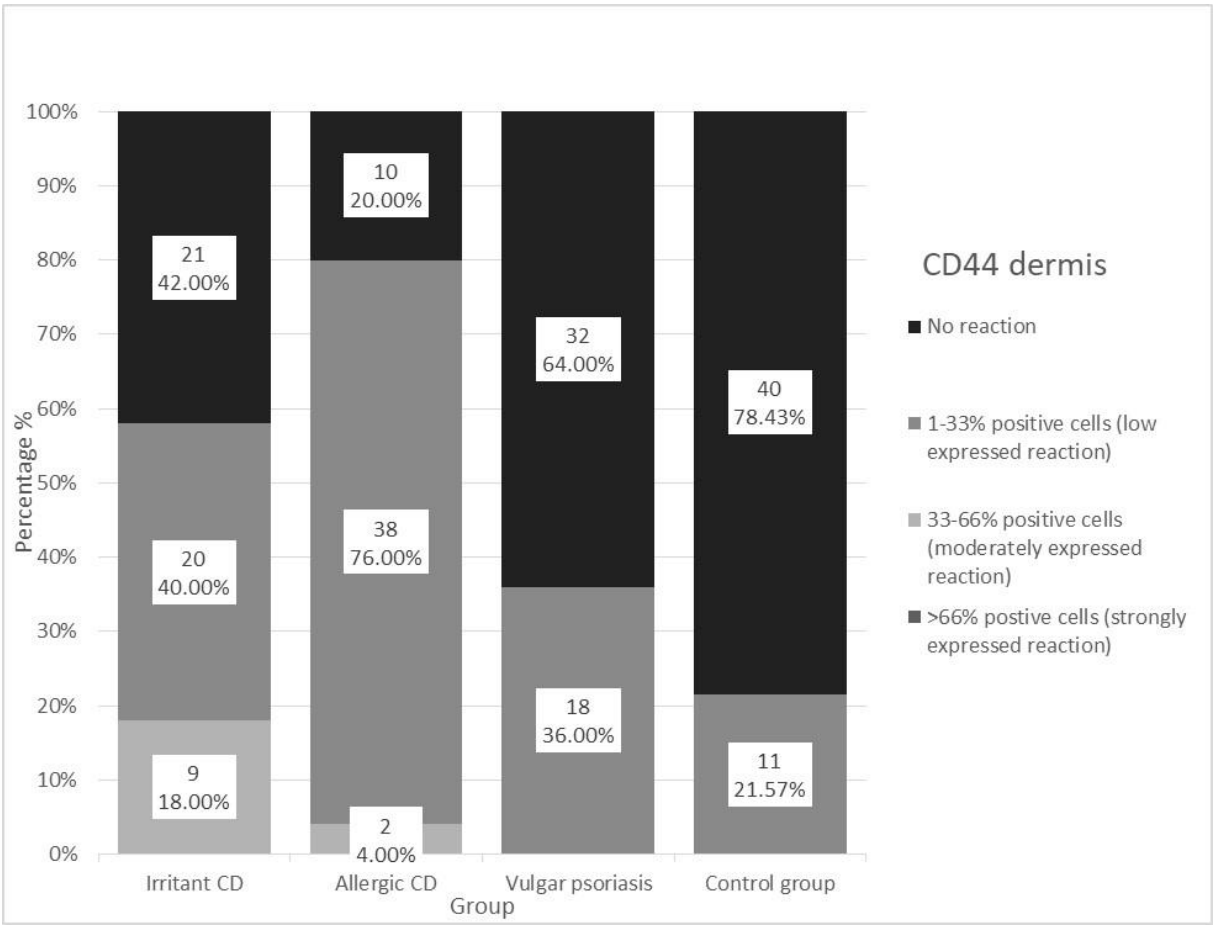

Figure 2. Expression of CD44 in the dermis in skin biopsies in the four groups: *P*<.001

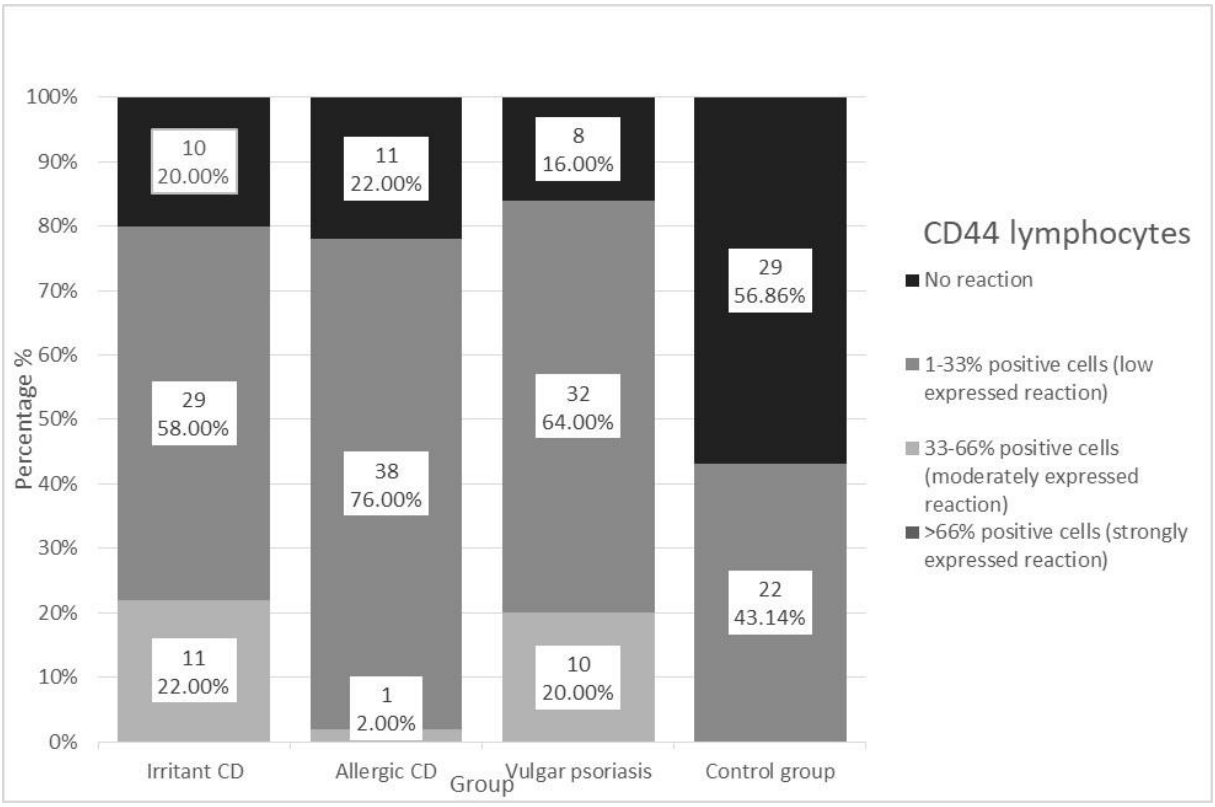

Figure 3. Expression of CD44 on the lymphocytes in skin biopsies in the four groups: *P*<.001

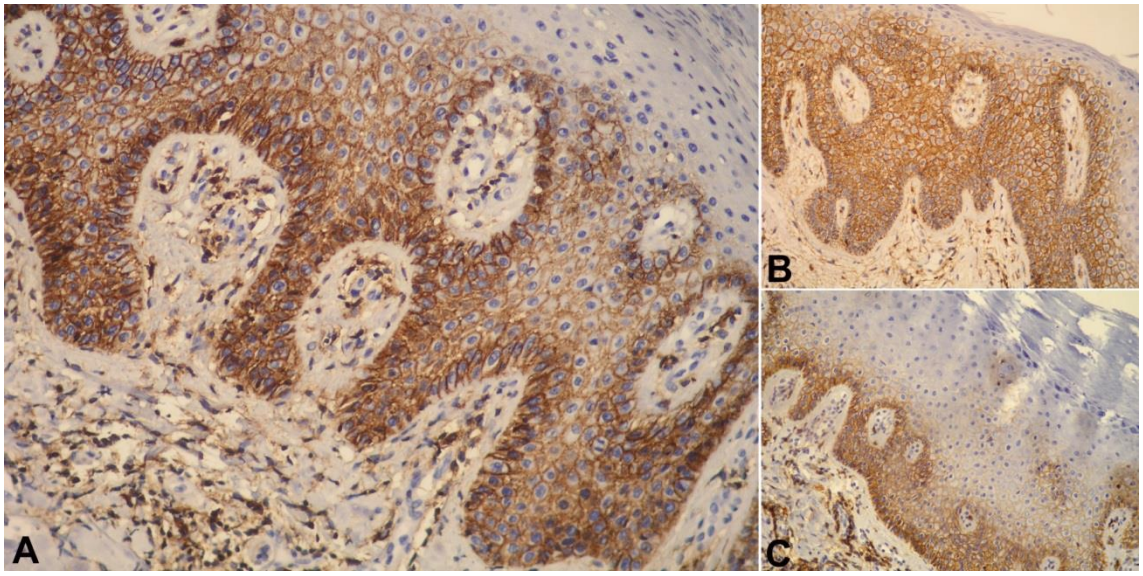

Figure 4 (A) Moderate CD44 expression in epidermal basal and suprabasal layers and on the lymphocytes and dermal stroma in an ICD lesion (X200), (**B)** Strong CD44 expression in the epidermis and on dermal lymphocytes in an ICD lesion (X200) (**C)** Low CD44 expression mainly in the basal and suprabasal epidermal cells and on dermal lymphocytes in an ICD lesion (X200)

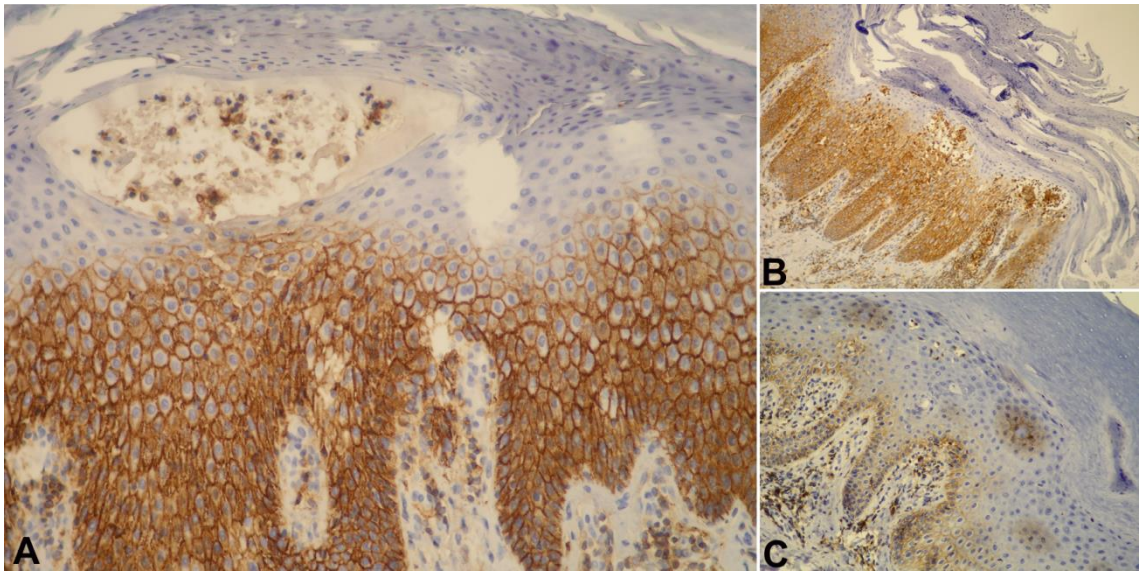

Figure 5 (A) Moderate CD44 expression in the epidermal basal and part of the spinous layer and on dermal lymphocytes in an ACD lesion (X200). (**B)** Strong CD44 expression in the epidermis and on dermal lymphocytes in an ACD lesion. (X200) (**C).** Low CD44 expression mainly in the basal epidermal cells and on dermal lymphocytes in an ACD lesion (X100)

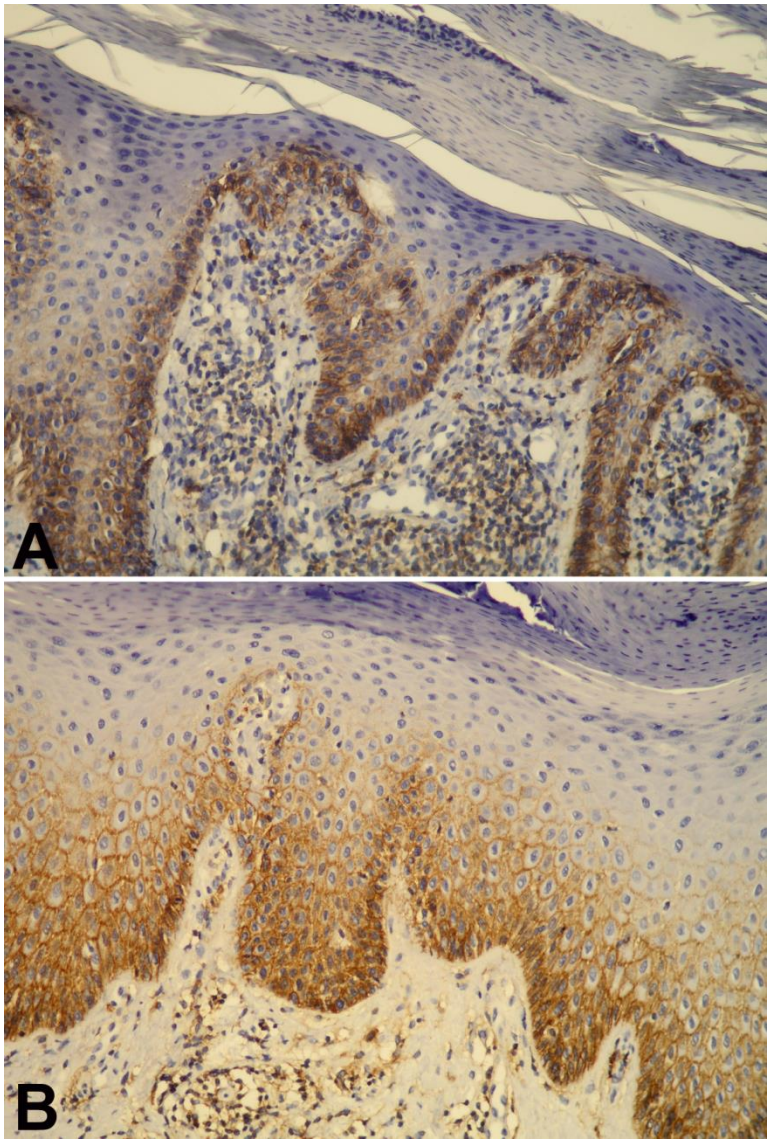

Figure 6 (A) Low CD44 expression in the epidermal basal and suprabasal layers and on dermal lymphocytes in a psoriasis lesion (X200) (**B).** Moderate CD44 expression in the epidermal basal and spinous layers and on dermal lymphocytes in a psoriatic lesion (X200)

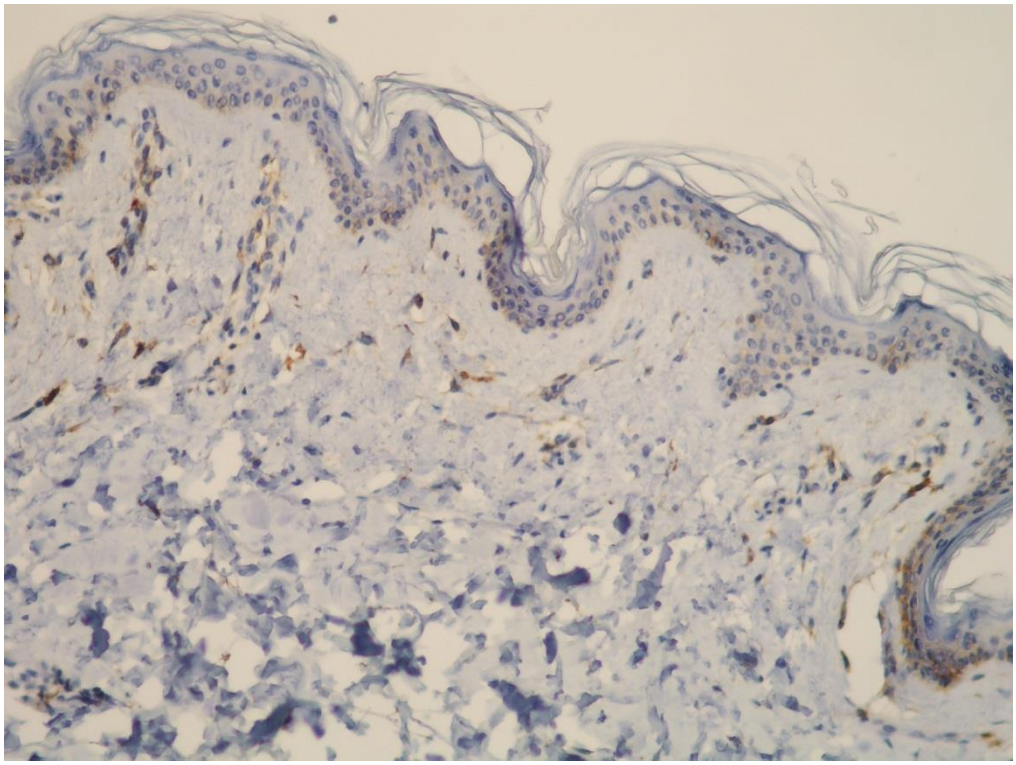

Figure 7. Low CD44 expression in basal epidermal layers with rare dermal lymphocytes in the healthy skin of a control subject (X100)