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From Cell Biology to Tissue Engineering

Microvascular changes in relation to inflammation and epidermal hyperplasia in chronic cutaneous lesions of psoriasis vulgaris

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Summary. Epidermal proliferation, inflammatory changes and microvascular augmentation are prominent features of chronic cutaneous psoriatic lesions. The objective of this study was the investigation of blood and lymphatic microvascular changes in relation to epidermal changes and inflammatory infiltration in dermis.

Immunohistochemical analysis with antibodies to CD34, podoplanin, vascular endothelial growth factors-A and C (VEGF-A and C) and morphometric software were used for quantification of the following parameters: blood and lymphatic vessel area (BVA and LVA), VEGF-A and VEGF-C positive area, inflammatory cell infiltration in dermis (CIA) and epidermal area (EA).

In comparison to healthy skin psoriatic lesions showed remarkable elevation of all measured parameters with the following average increase: BVA (2.8-times increased), LVA (2.6-times increased), VEGF-A and VEGF-C area (in epidermis 29-times and 19-times increased, in dermis 25-times and 15-times increased, respectively), and EA (3-times increased).

Statistical analysis revealed significant positive correlation between CIA and EA in psoriatic samples. Blood vessels area and VEGF-A expression in epidermis showed mild positive correlation with epidermal hyperplasia and weak positive correlation with dermal inflammatory infiltration. VEGF-A expression in epidermis also significantly correlated with blood vessels area. As for the lymphatic microcirculation we found a statistically significant positive correlation between lymphatic vessels area and the cellular infiltration in dermis but only weak correlation with epidermal hyperplasia.

We hypothesize that angiogenesis in psoriasis is to a greater extent responding to epidermal hyperplasia and in a lesser way to inflammatory infiltration in dermis. However, lymphangiogenesis is significantly related to dermal inflammatory infiltration.

Key words: Psoriasis, Cutaneous blood and lymphatic microcirculation, Inflammation, Epidermal hyperpasia

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Introduction

Psoriasis vulgaris is a chronic inflammatory and relapsing skin disorder, whose prevalence in different populations varies from 1-11% (Gudjonsson and Elder, 2007). The etiology of psoriasis is still elusive, although, some genetic predisposing factors, and endogenous or exogenous provocation factors, especially trauma, light and infections, are known (Braun-Falco et al., 2001).

Originally, psoriasis was considered to be a primary keratinization disorder of the skin, because of the prominent epidermal hyperplasia with hyperkeratotic scales (Bos et al., 2005; Griffiths and Barker, 2007). Nowadays, psoriasis is classified as T-cell-mediated autoimmune disease (Griffiths and Barker, 2007). Abnormal regulation of T-cells coupled with the interaction between keratinocytes and complex cytokine network is supposed to be predominant in the pathogenesis of psoriasis (Das et al., 2009). Recent insights have revealed that aquired as well as innate

immunity is crucial in the initiation and maintaince of psoriatic lesions (Griffiths and Barker, 2007). In plaque type psoriasis, acquired immunity response is more apparent, whereas in pustular forms innate immune responses are more aggravated (Bos et al., 2005).

Clinical manifestation of psoriatic lesion develops from the initial small red macules to mature erythematous sharply bordered psoriatic plaques which are covered with loosely adherent white or silver scales (Braun-Falco et al., 2001; Naldi and Gambini, 2007). Psoriatic lesions are preferentially situated at the elbows, knees, scalp, lumbosacral and umbilical areas (Griffiths and Barker, 2007).

Histopathological manifestation depends on the stage of the psoriatic lesion. At early onset the psoriatic macule shows moderate perivascular lymphocytic infiltrate and more obvious microvessels. Incipient epidermal hyperplasia with mounds of parakeratosis, infiltration of neutrophils and formation of Munro microabsceses are characteristic for the scaly psoriatic papule. Histopathological features of fully developed scaly silver plaque are: epidermal hyperplasia, defined as regular elongation of the rete ridges with preservation of the rete ridge-dermal papillae pattern, abnormal differentiation of the keratinocytes, spongiosis, parakeratosis, hypogranulosis and dermal accumulation of neutrophils and T-lymphocytes, and prominent expansion with elongated and tortuous capillary loops of the superficial subpapillary plexus within the dermal papillae. The resolving lesion reveals a reduced number of neutrophils, fibrosis and more compact parakeratosis (Braverman and Sibley, 1982; Braun-Falco et al., 2001; Murphy et al., 2007).

Thus, epidermal proliferation, inflammatory changes and microvascular augmentation are typical features of psoriasis. In histolopathogical manifestation just microvascular changes are the most prominent. Althoug microvascular changes alone are not causal in the pathogenesis of psoriasis, they assist in the inflammatory process and provide indispensable nutritional support for the epidermal hyperproliferation. Althought proliferation of blood microvessels in psoriasis was confirmed by several studies (Braverman, 1972; Braverman and Yen, 1974, 1977a,b; Barton et al., 1992; Creamer et al., 1997, 2002; Heidenreich et al., 2009), lymphatic microvascular changes are far less explored. It prompted us to investigate lymphatic microvascular changes and compare them with blood microvascular changes, as well as evaluate their relations to epidermal hyperplasia and inflammatory infiltration in dermis.

Material and methods

Tissue samples

Lesional psoriatic skin samples were taken from untreated patients with no systemic disorder. Control healthy skin samples were obtained from subjects without any skin and systemic disease in anamnesis. Both lesional and control skin samples (25) were taken from individuals of both genders, aged 35-57 years to eliminate age-dependent variability in skin microcirculation (Výbohová et al., 2012). All specimens were excised from regions of the trunk (umbilical region of abdomen and lumbar part of the back) to eliminate regional variability in skin microcirculation and epidermal thickness (Pasyk et al., 1989; Alper et al., 2004; Vybohova et al., 2015). Specimens were fixed in 10% neutral buffered formalin and embedded into paraffin blocks. Lesions were verified for clinical and histopathological diagnostic criteria by experienced dermatologists and pathologists. The study was approved by the local Ethics Committee at Jessenius Faculty of Medicine in Martin and registered with the Office for Human Research Protection, U.S. Department of Health and Human Services under No. IORG0004721.

Immunohistochemical analysis

Four micrometers thick sections were mounted on silane coated slides to achieve greater adherence (Dako, Denmark). Sections were deparaffinized with xylene, rehydrated in a series of descending ethanol concentrations and washed in phosphate-buffer saline. Endogenous peroxidase was blocked by 0.3% hydrogen peroxide. Heat-induced epitope retrieval was done after the immersion of the slides in the retrieval solution (Target Retrieval Solution High pH, Code No. S3308, DakoCytomation Denmark A/S). Double immunohistochemical staining was performed with CD34 (Monoclonal Mouse Anti-Human CD34 Class II Clone QBEnd-10 M 7165, DakoCytomation Denmark A/S) and D2-40 (Monoclonal Mouse Anti-Human D2-40 Clone D2-40, DakoCytomation Denmark A/S) antibodies to detect blood and lymphatic microvessels. The primary antibodies were revealed by polymer technique-EnVision Doublestain System (Code K 1395; Dako, Denmark) which permitted the simultaneous demonstration of both antigens (CD34 and D2-40) within one specimen by double immunoenzymatic staining with two chromogens: peroxidase chromogen diaminobenzidine (DAB) and alkaline phosphatase chromogen permanent red.

Expression of VEGF-A (vascular endothelial growth factor A) and VEGF-C (vascular endothelial growth factor C) was analysed by using Monoclonal Mouse Anti-Human Vascular Endothelial Growth Factor (Clone VG1 M7273, Dako, Denmark) and Monoclonal Mouse Anti-Human Vascular Endothelial Growth Factor-C (Clone MM0006-2E65, Santa Cruz Biotechnology, USA). These primary antibodies were detected by biotinylated immunoglobulins and horseradish peroxidase streptavidin-biotin complex (LSAB™+/HRP kit, code No. K0679; Dako, Denmark). Staining was completed with DAB as chromogen.

All sections were stained with Mayer's hematoxylin (Dako, Denmark). Negative controls were obtained by

omitting the primary antibodies. All steps of the immunohistochemical staining were conducted according to the manufacturer's instructions.

Computer-assisted morphometric analysis

Sections were screened and digital images were taken at magnifications of x200 and x400 with a camera (Olympus Evolt E-420) installed in a brightfield microscope (Olympus BX41N). Morphometric analysis of the digital images was done using QuickPhoto Micro software version 2.3 (Promicra, Prague, Czech Republic). The blood and lymphatic microcirculatory beds were evaluated by blood vessel area (BVA) and lymphatic vessel area (LVA). Slides from each skin sample were examined field per field by two experienced investigators to identify the areas with highest vascularization-hotspot areas. At least 5 standard fields (hotspot areas) in each slide were evaluated (Weidner, 1995). Fields with the capillary networks around hair follicules and glands were eliminated.

The blood vessel area (BVA) / lymphatic vessels area (LVA) was defined as percentage of CD34 / D2-40-stained area to standard field (0.1 mm^2) of the papillary and upper reticular dermis (Mylona et al. 2007; Deb et al., 2012; Boruah et al., 2014). VEGF-A and VEGF-C expression was evaluated by VEGF-A and VEGF-C positive area which was defined as the average percentage in standard fields of epidermis and dermis. Similarly, cellular infiltration area (CIA) was defined as the average percentage of the cells in the same standard fields of dermis (Mylona et al., 2007; Boruah et al., 2014). Epidermal area (EA) was defined as the ratio between the area of epidermis around the hotspot area of the dermis (in which BVA, LVA and CIA were evaluated) and the epidermal length at the external surface (Jones et al., 1994; Sauerbronn et al., 2010).

Statistical analysis

Morphometric data were expressed as the mean ± standard deviation. Statistical analysis was performed using the analysis of variance, post-hoc tests, and Pearson's correlation test. A p value less than 0.05 was considered the minimum for statistical significance.

Results

The blood vessel area (BVA), lymphatic vessel area (LVA), VEGF-A and VEGF-C positive area, cellular infiltration area (CIA) and epidermal area (EA) were quantified and statistically analysed in psoriatic lesions as well as in control healthy skin. Individual variability of the specimens was rejected on the basis of ANOVA tests.

In both healthy skin samples and psoriatic lesions we confirmed neither gender nor regional statistically significant differences in all studied parameters (BVA, LVA, VEGF-A and VEGF-C area, CIA and EA).

Pathological samples showed all required criteria for histopathological diagnosis of chronic psoriatic plaques (Figs. 1, 2).

In comparison to healthy skin psoriatic lesions showed statistically significant elevation of all measured parameters: BVA, LVA, VEGF-A and VEGF-C area and EA.

Cellular infiltration area (CIA)

Within the healthy human dermis the cellular component comprised fibroblasts, macrophages and mast cells. CIA values in control healthy skin ranged from 0.51 to 0.88%. Cellular infiltration in pathological lesions consisted mainly of inflammatory cells, especially T-lymphocytes, monocytes, macrophages and neutrophils. CIA values in psoriatic lesions were

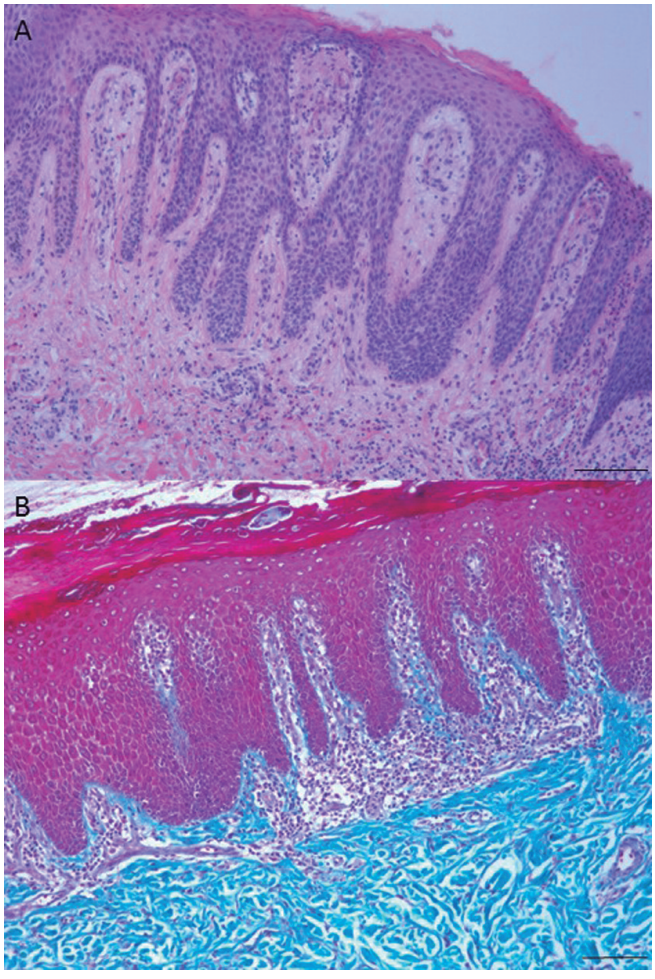

Fig. 1. histopathological manifestation of chronic cutaneous lesions of psoriasis vulgaris: epidermal hyperplasia, elongated rete ridges, spongiosis, parakeratosis and hypogranulosis, an accumulation of inflammatory cells within the dermis. **A.** Hematoxylin-eosin staining. **B.** Masson's trichrome staining. Scale bars: 100 μm.

significantly higher than in control healthy skin (from 2.17% to 11.19%). Lesional samples were subdivided into three groups according to the degree of cellular infiltration (Table 1): weak (CIA range: 2.57-3.67%), mild (CIA range: 4.49-6.28%) and strong (CIA range: 8.23-11.19%).

Epidermal area (EA)

Lesional psoriatic samples showed significant increase in average epidermal area in comparison with healthy skin (Table 1). The average EA increased with the increased cellular infiltration in dermis. We confirmed a statistically significant correlation between CIA and EA (r=0.683, p=0.005).

Blood and lymphatic vessel area (BVA and LVA)

BVA values in psoriatic lesions with weak, mild and strong degree of inflammation did not differ significantly, and correlation between CIA and BVA was only low (r=0.217, p=0.437). However, results showed higher-mild correlation between EA and BVA (r=0.457, p=0.087) (Table 2, Fig. 3).

Statistical analysis confirmed significant increase of LVA values within the samples with strong inflammatory infiltrate in comparison with weak and mild degree (Table 1), moreover, correlation test affirmed statistically

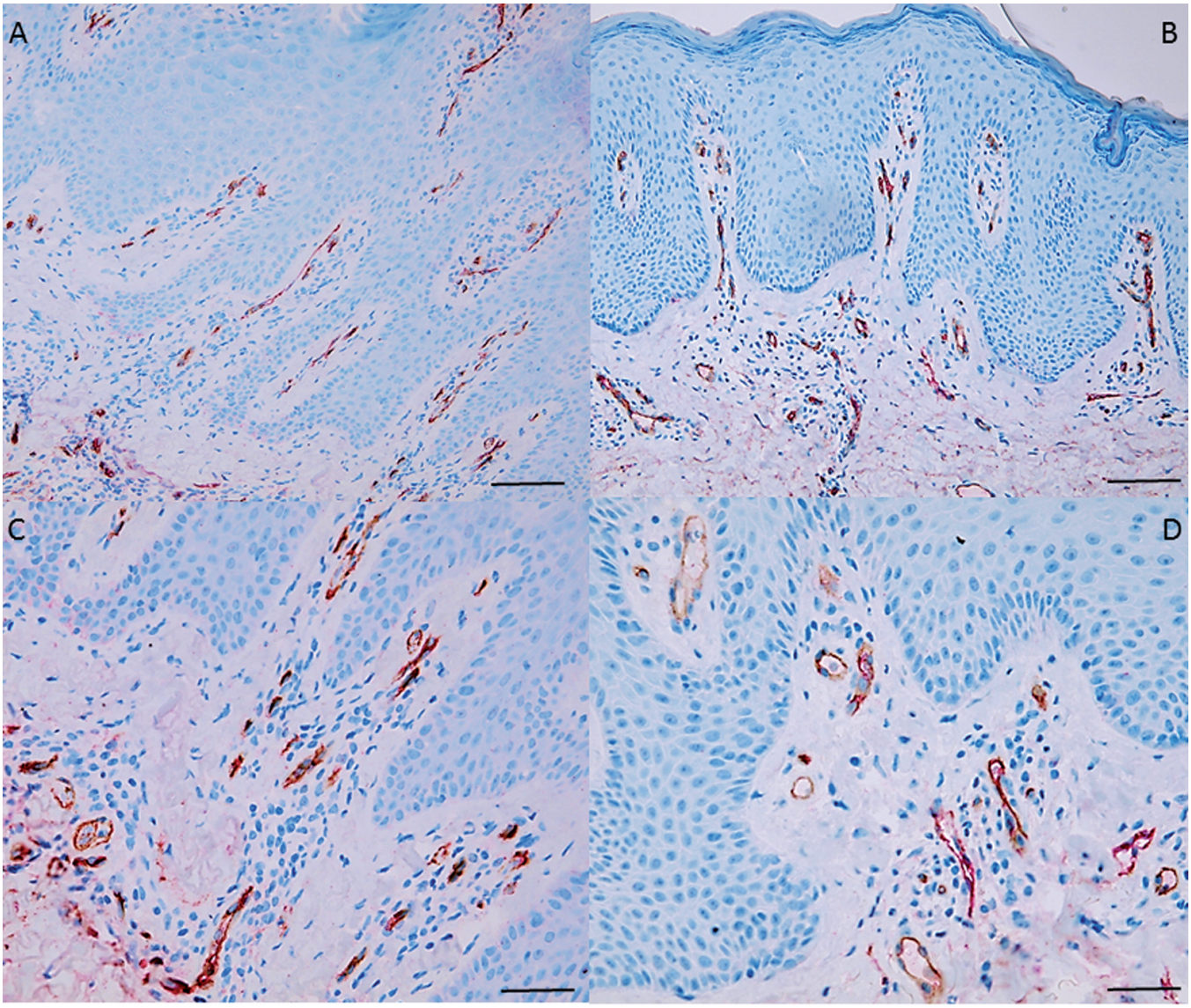

Fig. 2. Blood (brown) and lymphatic (pink-red) microvessels in cutaneous psoriatic lesions. Double immunohistochemical staining with CD34 and D2-40 antibodies. Scale bars: A, B, 100 μm; C, D, 50 μm.

Table 1. Cellular infiltration area (CIA), epidermal area (EA), blood and lymphatic vessels area (BVA and LVA) in healthy skin and in chronic cutaneous psoriatic lesions. Values are expressed as mean ± SD.

	CIA	EA	BVA	LVA
Healthy skin	0.70±0.11	72.64±3.61	0.77±0.11	0.32±0.08
Healthy vs psoriatic skin	<0.0001	<0.0001	<0.0001	<0.0001
Psoriatic lesions				
weak degree of inflammation in dermis	2.99±0.41	185.28±26.85	1.99±0.17	0.74±0.12
mild degree of inflammation in dermis	5.28±0.76	200.02±38.80	2.19±0.24	0.76±0.16
strong degree of inflammation in dermis	10.70±1.21	252.65±33.33	2.17±0.30	1.02±0.24
ANOVA p-value	<0.0001	0.019	0.377	0.054
Student Neuman Keuls test p-value				
weak vs mild	0.001	0.498	0.417	0.837
mild vs strong	<0.0001	0.020	0.251	0.070
weak vs strong	<0.0001	0.028	0.919	0.051

Table 2. Correlation between cellular infiltration area (CIA) and epidermal area (EA) with blood and lymphatic vessels area (BVA and LVA) and VEGF-A and VEGF-C expression.

correlation	Healthy skin		Psoriatic lesions	
	CIA	EPIA	CIA	EPIA
BVA	r=0.395 p=0.259	r=0.342 p=0.333	r=0.217 p=0.437	r=0.457 p=0.087
VEGF-A in epidermis	r=0.044 p=0.988	r=0.001 p=0.998	r=0.199 p=0.477	r=0.388 p=0.153
VEGF-A in dermis	r=-0.417 p=0.122	r=0.558 p=0.094	r=0.191 p=0.496	r=0.351 p=0.199
LVA	r=0.366 p=0.180	r=0.121 p=0.739	r=0.537 p=0.039	r=0.185 p=0.509
VEGF-C in epidermis	r=0.320 p=0.245	r=-0.221 p=0.539	r=0.434 p=0.106	r=0.325 p=0.232
VEGF-C in dermis	r=-0.071 p=0.801	r=0.183 p=0.613	r=0.391 p=0.149	r=0.369 p=0.144

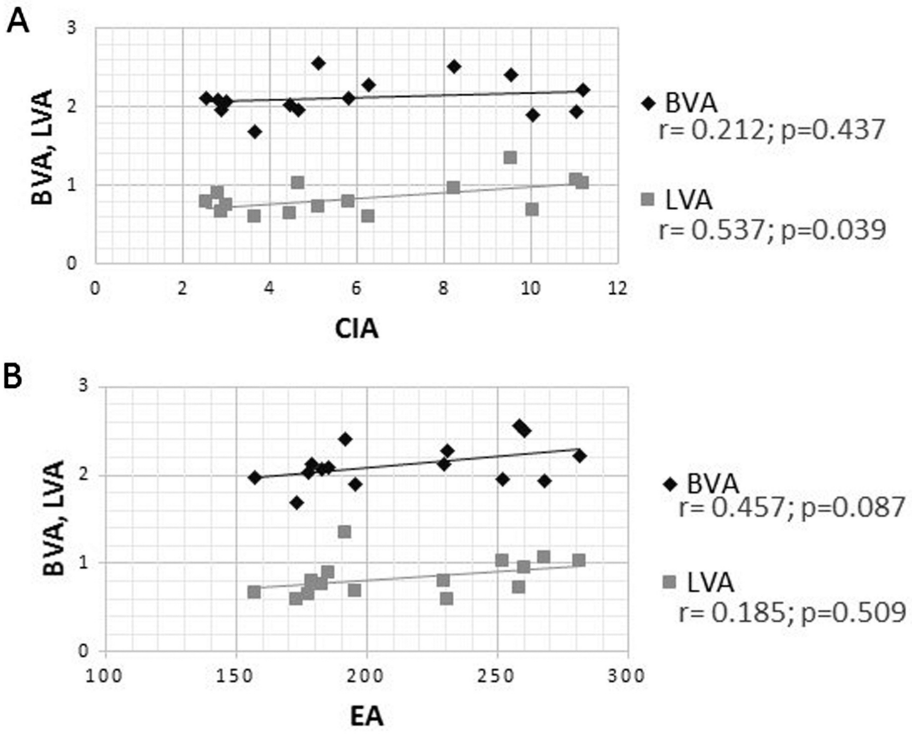

Fig. 3. Graph-Correlation between cellular infiltration area (CIA) and epidermal area (EA) with blood and lymphatic vessels area (BVA and LVA).

significant positive correlation between CIA and LVA (r=0.537, p=0.039) (Table 2, Fig. 3). Interestingly, in comparison with BVA, correlation between epidermal thickness and LVA was only low (r=0.185, p=0.509).

VEGF-A positive area

VEGF-A was immunohistochemically detected mainly in the cytoplasm of keratinocytes in epidermis, to a lesser extent in fibroblasts, and blood endothelial cells of the dermis (Fig. 4A). We confirmed significantly higher VEGF-A expression in psoriatic lesions than in healthy skin in both epidermis and dermis (Table 3). Althought VEGF-A positive area in both epidermis and dermis showed an increase from weak to strong degree of inflammation, the difference was not statistically significant (Table 3). Analysis confirmed weak correlation between CIA and VEGF-A positive area in epidermis and dermis (r=0.199, p=0.477 and r=0.191, p=0.495, respectively). Mild positive correlation was revealed between epidermal thickness and VEGF-A expression in epidermis and dermis (r=0.388, p=0.153 and r=0.351, p=0.199, respectively). Statistical analysis confirmed significant positive correlation between VEGF-A positive area in epidermis and BVA (r=0.578, p=0.024) in lesions.

VEGF-C positive area

VEGF-C was predominantly expressed in the cytoplasm of keratinocytes in the basal and suprabasal layers and rarely in fibroblasts of the dermis (Fig. 4B). Althought values of VEGF-C positive area increased from weak to strong degree of cellular infiltration, differences were not statistically significant (Table 3). Correlation analysis revealed mild correlation between CIA and VEGF-C area in epidermis and dermis (r=0.434, p=0.106 and r=0.391, p=0.149, respectively) as well as between epidermal thicknes and VEGF-C area in epidermis and dermis (r=0.325, p=0.232 and r=0.369, p=0.144, respectively).

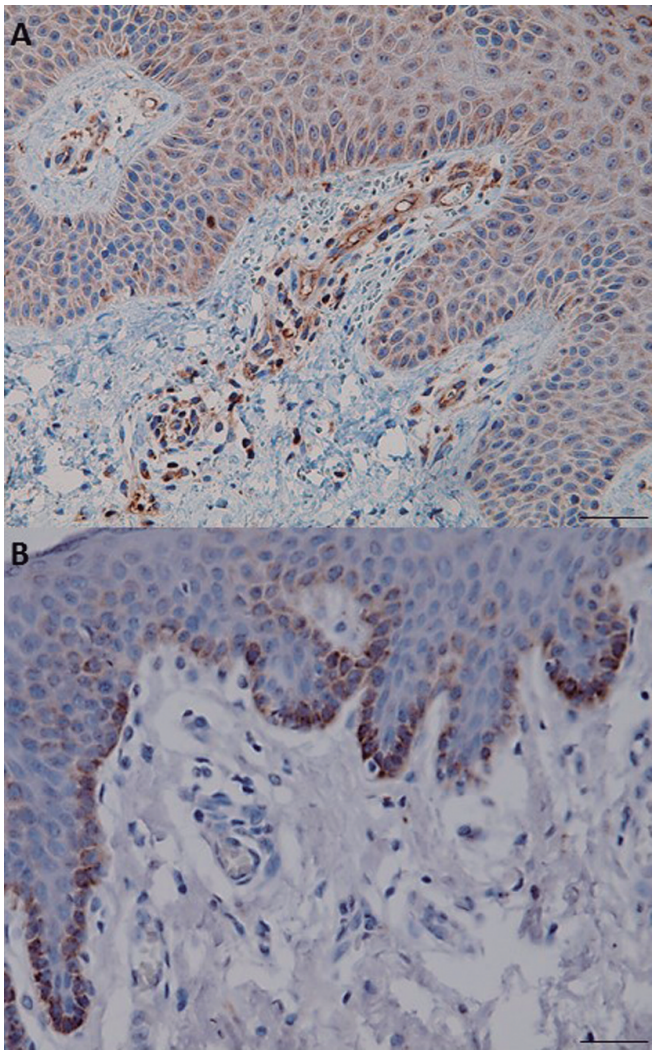

Fig. 4. A. Immunohistochemical positivity of VEGF-A expression in epidermis and dermis within the cutaneous psoriatic lesions. **B.** Immunohistochemical positivity of VEGF-C (B) expression in epidermis and dermis within the cutaneous psoriatic lesions. Scale bars: 50 μm.

Table 3. VEGF-A and VEGF-C positive area in epidermis and dermis in healthy skin and psoriatic lesions. Values are expressed as mean ± SD.

	VEGF-A positive area		VEGF-C positive area	
	in epidermis	in dermis	in epidermis	in dermis
Healthy skin	0.46±0.40	0.20±0.40	0.41±0.32	0.10±0.07
Healthy vs psoriatic skin	<0.0001	<0.0001	<0.0001	<0.0001
Psoriatic lesions				
weak degree of inflammation in dermis	12.62±7.32	4.75±2.70	6.47±3.76	1.13±0.74
mild degree of inflammation in dermis	13.13±7.84	4.62±2.59	7.76±0.78	1.86±0.52
strong degree of inflammation in dermis	15.13±2.61	5.65±0.33	9.02±0.69	1.94±0.37
ANOVA p-value	0.809	0.724	0.242	0.080
Student Neuman Keuls test p-value				
weak vs mild	0.902	0.929	0.382	0.063
mild vs strong	0.811	0.524	0.214	0.098
weak vs strong	0.629	0.741	0.395	0.822

All angiogenesis and lymphangiogenesis parameters BVA, LVA, VEGF-A and VEGF-C positive area showed positive correlation with dermal cellular infiltration and epidermal thickness in psoriatic lesions.

Seventy percent of healthy skin samples showed negative VEGF-A and/or VEGF-C expression in epidermis and/or dermis. It caused a negative correlation between CIA, EA and VEGF-A and VEGF-C expression in healthy skin (see Table 3).

Discussion

Microvascular changes in psoriasis became an objective for researchers in the early 1970s. Braverman and Yen first provided a detailed description of microvascular changes in psoriasis in their electron-microspcopic studies (Braverman, 1972; Braverman and Yen, 1974, 1977a,b). They found out that capillary loops within the psoriatic dermal papillae show morphologic features of the venous capillary with the bridge fenestrations and multilayered basement membrane. According their theory, proliferation of the capillary loop starts from its venous limb. Thus venous limb is enlarged and arterial limb is consequently shortened until the whole capillary loop has a venous configuration, with typical tortuosity at the apical segment. This microvascular expansion is limited only to the superficial vascular plexus (Braverman, 1972; Braverman and Yen, 1974, 1977a, 1977b). Microvascular changes already occur in the early stages of the lesion development (Pinkus and Mehregan, 1966).

Modern immunohistochemical methods allowed deeper insight into the process of angiogenesis in psoriasis. Quantitative immunohistochemical morphometric studies have demonstrated from 2-fold to 4-fold enlargment of the blood microcirculatory bed within the upper dermis (Barton et al., 1992; Creamer et al., 1997, 2002). Our results showed that in psoriatic lesions BVA values were 2.6-3.3 times higher than in healthy skin and the BVA values did not show the tendency to elevate with the higher degree of inflammatory infiltrate in dermis. Data of our previous study revealed that psoriatic lesions taken from 4 various topographical regions (abdomen, back, face and palm of the hand) with comparable strong degree of the dermal inflammation did not show regional differences in blood vessel density (Výbohová et al., 2015).

The evidence that angiogenesis is tightly connected to chronic inflammation was revealed only recently. However, at this moment it is still unclear if these processes are cause or result (Costa et al., 2007). Althought both angiogenesis and inflammation are distinct and separable processes they potentiate each other (Granger et Senchenkova, 2010). Angiogenesis in psoriasis is initiated by vasodilatation, increased vascular permeability, enzymatic degradation of the capillary basement membrane and subsequent activation, proliferation and migration of endothelial cells. Migration of the endothelial cells is directed by a gradient of angiogenic factors (mainly vascular endothelial growth factor-A), and mediated by integrins ($\alpha v\beta 3$), cell-adhesion molecules on the endothelial cells surface (ICAM-1, VCAM-1, E-selectin). Lumen creation is followed by stabilization of the capillary by pericyte recruitment and basement membrane synthesis (Carmeliet, 2000, 2003; Creamer et al., 2002; Karamysheva, 2008).

Growing tissue mass and abundance of inflammatory cells in chronic inflammation site leads to increased metabolic demands and hypoxia which is consecutively a stimulus for angiogenesis and another immune cell accumulation (Jackson et al., 1997; Costa et al., 2007). Recruitment and attraction of T-cells is activated by dendritic cells and cytokine-mediated upregulation of adhesion molecules on endothelial cells. Successively T-cells activate macrophages, endothelial cells and fibroblasts to produce pro-inflammatory cytokines, chemokines and matrix metalloproteinases. Moreover, T-cells activated by cytokines like tumour necrosis factor α, interleukin-6 or interleukin-2 can stimulate fibroblasts and macrophages to produce VEGF-A, one of the most important angiogenesis stimulators (Monaco et al., 2004; Costa et al., 2007). Newly formed vessels provide both nutrition and transport of inflammatory cells to the site of inflammation, and in this way they maintain the chronic inflammation process (Jackson et al., 1997).

The chronic inflammatory process is also regulated by lymphatic microcirculation which assumes responsibility for transport of fluid, extravasated leukocytes, and antigen-presenting cells from the inflamed tissue to the lymph nodes and to other secondary lymphoid organs (Zgraggen et al., 2013). However, it has been supposed that some inflammatory mediators, especially prostaglandins and histamin, can diminish lymphatic vessels contractility resulting in lymphatic insuficiency (Alitalo et al., 2005; Tamella and Alitalo, 2010). This leads to new lymphatic vessel growth-compensatory lymphangiogenesis. Key lymphangiogenesis stimulating factors (VEGF-C and VEGF-D) are produced by transformed macrophages in dermis (Schoppmann et al., 2002) and keratinocytes in epidermis (Huggenberger, 2010). Experimental studies have revealed that lymphangiogenesis can be also induced by VEGF-A, although, VEGF-A induced lymphatics are structurally abnormal and functionally insufficient (Nagy et al., 2002). On the contrary, stimulation of lymphangiogenesis by VEGF-C significantly inhibits chronic skin inflammation (Huggenberger et al., 2010). It can be stated that newly formed lymphatic vessels in chronic inflammation allow a reduction of the tissue edema and enhance immune responses by promoting macrophages and dendritic cell recruitment (Baluk et al., 2005; Kataru et al., 2009). In our previous study we revealed that psoriatic lesions taken from various topographical regions of the body with comparable strong degree of dermal inflammation did not show significant differences in lymphatic vessel

density (Výbohová et al., 2015). In the present study we confirmed 1.8-3.1 times increase of LVA values in comparison with healthy skin. Furthermore, LVA values showed a significant positive correlation with the level of inflammatory infiltrate in dermis. Henno et al. (2010) found that the expansion of lymphatic vessels occurs after the blood vascular development when they compared acute psoriatic pinpoints and chronic psoriatic plaques.

Chronic inflammation within the dermis is closely related to epidermal hyperplasia in psoriasis. Cytokines released by T-lymphocytes, macrophages, dendritic cells or neutrophils (IL-23, IL-22, IL-17, IL-1, IL-6 and IFN-γ) can trigger or directly induce keratinocyte hyperproliferation. Moreover, it is suggested that T-lymphocytes, which infiltrate the epidermis, disrupt epidermal integrity (by breaking the basement membrane and disrupting the desmosome connection between keratinocytes) and it can be interpreted as an injury. Subsequently it leads to wound repair response resulting in "regenerative" epidermal growth (Krueger, 2002; Tonel and Conrad, 2009; Flatz and Conrad, 2013). The latest evidence from mouse models and translational studies indicate that epidermal hyperplasia in psoriasis is caused not only by cytokines released by T-cells and other inflammatory cells, but also by the primary defects in keratinocytes (underexpression of keratinocyte differentiation markers, overexpression of keratinocyte proliferation markers) (Krueger, 2002; Griffiths and Barker, 2007; Tonel and Conrad, 2009; Flatz and Conrad, 2013). Some studies showed a resistence of psoriatic keratinocytes to apoptotic stimuli (Wrone-Smith et al., 1997; Oztas et al., 2006). Braun-Falco et al. (2001) state that epidermis in psoriatic lesion has a volume that is four to six times larger than normal skin from the same region, there are more keratinocytes and individual cells are larger. In the present study we found that in comparison with healthy skin the epidermal area in psoriatic lesions was 2.2-3.9 times larger and it significantly correlated with the inflammatory cell infiltrate in dermis. Some other morphometric studies revealed that epidermal area or epidermal thickness was 1.5-4-times larger in psoriatic lesions than in healthy skin (Rowe, 1966; Alper et al., 2004; Boruah et al., 2013).

Hyperproliferation of keratinocytes increases metabolic demands of epidermis resulting in hypoxia which leads to excessive VEGF expression by proliferating keratinocytes (Detmar et al., 1997). Our data also revealed a remarkable increase of VEGF-A expression in psoriatic epidermis. In lesional skin VEGF-A is produced predominantly in the epidermis by keratinocytes and to a lesser extent by fibroblasts of the dermis (Detmar et al., 1995). Similarly, in our study, in comparison with healthy skin we confirmed 29-times higher VEGF-A expression in the epidermis and 25-times higher VEGF-A expression in dermis of psoriatic plaques.

Psoriatic epidermis is not only the source of angiogenesis stimulating factors but also the source of inflammatory mediators. Recent studies revealed that keratinocytes in psoriatic lesion can be activated by infiltrating T-cells to produce various inflammatory cytokines and growth factors which are able to modify the microenvironment within the psoriatic lesion. In this way the intrinsic epidermal components play a role in sustaining of the inflammatory process and they contribute to the chronicity of the disease (Tschachler, 2007).

It is supposed that chronic inflammation, angiogenesis, lymphangiogenesis and epidermal hyperplasia in psoriatic lesions influence each other and they work in a tightly regulated manner. The results of the present study confirmed a significant correlation between dermal inflammatory infiltration and epidermal hyperplasia. Blood vessels area and VEGF-A expression in epidermis showed mild positive correlation with epidermal hyperplasia and weak positive correlation with dermal inflammatory infiltration. VEGF-A expression in epidermis also significantly correlated with blood vessels area. Boruah et al. (2014) in their morphometric study disclosed that blood vessel density showed weak positive correlation with dermal inflammatory infiltration and mild positive correlation with epidermal parameters. As for the lymphatic microcirculation we found a statistically significant positive correlation between lymphatic vessels area and the cellular infiltration in dermis but only weak correlation with epidermal hyperplasia.

On the basis of our results we hypothesize that angiogenesis in psoriasis is to a greater extent responding to epidermal hyperplasia and to a lesser extent to the inflammatory infiltration in dermis. Lymphangiogenesis is significantly related to dermal inflammatory infiltration.

Although a lot of studies support the relation between inflammation and angio/lymphangiogenesis and epidermal hyperplasia, detailed pathophysiological consequences and molecular mechanisms underlying this association are not fully understood and they need more investigation. The interplay between these processes could be a potential benefit for therapeutic approaches because inhibition of angiogenesis and, surprisingly, activation of lymphangiogenesis might serve as a novel strategy for treating chronic inflammation. In reverse, targeting inflammation can decrease production of angiogenesis and lymphangiogenesis stimulators. Hopeful results were gained from transgenic mouse models of psoriasis where systemic anti-VEGF treatment significantly reduced skin inflammation (Jackson et al., 1997; Costa et al., 2007; Schonthaler et al., 2009).

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