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Article

Assessment of the Tissue Resident Memory Cells in Lesional Skin of Patients with Psoriasis and in Healthy Skin of Healthy Volunteers

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Abstract: Background: In the course of plaque psoriasis, tissue resident memory cells (TRM) are responsible for the phenomenon of "immune memory" of lesions, i.e., the appearance of recurrences of lesions in the same location, as well as Koebner phenomenon. We present results determining the location and amount of TRM in psoriatic lesions in patients suffering from plaque psoriasis, as well as an analysis of the relationship between TRM markers expression and the duration and severity of the disease. Methods: TRM markers (CD4, CD8, CD103, CD69, CD49, CXCR6) and tissue expression of cytokines (IL-17, IL-22) in the lesional psoriatic skin of 32 patients compared with 10 healthy skin samples were evaluated by immunohistochemistry. Results: The presence of TRM markers in both the epidermis and skin with psoriatic eruptions was demonstrated in much higher amounts compared with the skin of healthy volunteers. A significant positive relationship was demonstrated between the expression of TRM markers in patients with plaque psoriasis and the duration of skin lesions. There was no relationship between the amount of TRM and the severity of plaque psoriasis. Conclusions: A thorough understanding of the mechanisms responsible for the development and relapse of plaque psoriasis may contribute to the implementation of more effective therapies.

Keywords: psoriasis; tissue resident memory cells; TRM; immune memory

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1. Introduction

In healthy skin, the number of T cells occupying the skin is almost twice as high as in peripheral blood, and most of them are effector memory cells (TEM), formed after antigen exposure from naïve T cells [1]. Effector memory cells have an immune defense function. Most of these cells live briefly and die after an immune response, but some morph into memory T cells. Central memory T cells (TCM) move through lymphoid tissues while TEM circulate in peripheral tissues [2,3]. TRM are subset of memory T cells that persist in non-lymphatic peripheral tissues for a long time without recirculation in the blood, thus providing the first line of adaptive cellular defense [2].

The role of TRM in the body's immune defense is not yet fully understood, but it is likely that these cells can activate both the innate and adaptive immune systems [4]. In addition to protective functions, more and more studies indicate that inappropriate TRM activation may be involved in pathological conditions such as vitiligo, alopecia areata, psoriasis, skin T-cell lymphoma, or melanoma [1,2,5].

Psoriasis is currently regarded as a Th1/Th17/Th22-mediated systemic inflammatory disease characterized by several comorbidities encompassing respiratory [6,7], cardiologic [8,9], and gastrointestinal systems [10].

In the course of plaque psoriasis, TRM are responsible for the phenomenon of "immune memory" of lesions, i.e., the appearance of recurrences of lesions in the same location [11]. Moreover, after the psoriatic plaques have disappeared, signs of inflammation

in the form of TRM cells can still be found in healthy skin. They appear to be able to initiate an inflammatory cascade, causing the recurrence of psoriatic plaques [4]. There are two main types of TRM: CD8 +, which is abundant in the psoriatic epidermis, and TRM CD4 +, which are localized near the vessels in the dermis and have a high proliferative potential [12]. CD8 + epidermal TRM mainly express the antigens CD103, CD69, and CD49a. Other molecules that distinguish TRM cells from other circulating memory T cell types have also been described, including CXCR3, CXCR6, and CD101 [1]. The particular pathogenicity of TRM CD8 + in psoriasis is evidenced by the fact that they express the receptor for IL-23 and can produce pro-inflammatory IL-17 and IL-22 in the skin, even many months after the lesion has resolved [4,13].

An interesting issue in the context of TRM is also the Koebner phenomenon. It turns out that physical stimuli can lead to the accumulation of memory T cells and even their reactivation [12]. The mechanism of this phenomenon has not been fully elucidated; however, numerous studies have shown that intact skin in inflammatory dermatosis differs markedly from normal skin in terms of increased expression of genes related to the immune system and certain T cell-related cytokines and adhesion molecules [12,14,15]. The relationship between the accumulation of TRM cells in nonlesional skin after the initiating stimuli and the Koebner effect is highly probable.

In this article, we present results describing the location and amount of TRM in psoriatic lesions in patients suffering from plaque psoriasis, as well as an analysis of the relationship between TRM markers expression and the duration and severity of the disease.

2. Methods

2.1. Study Group

The study group included 32 patients (26 men and 6 women) with plaque psoriasis, without psoriatic arthritis, who were untreated for at least 4 weeks and who were treated at the outpatient clinic at the Department of Dermatology, Sexually Transmitted Diseases, and Clinical Immunology in Olsztyn. The patients followed a normal diet (omnivores), and they were not addicted to alcohol. Patients with chronic and acute inflammatory diseases and dermatoses other than psoriasis, neoplastic diseases, previous cardiovascular complications, heart, kidney and liver failure were excluded. The average duration of the disease in women was 15 years, in men 14.4 years. In the study group, we also assessed the severity of the disease using the following scales: PASI, BSA, DLQI. The control group consisted of healthy volunteers (n = 10) with no personal or family history of psoriasis and with no concomitant autoimmune and inflammatory diseases. Skin samples from psoriasis patients were collected from psoriatic lesions that recurred at the same sites after the end of previous treatment. Healthy volunteer skin samples were obtained from surgical wastes obtained after removal of pigmentary lesions located on the trunk or limbs.

2.2. Clinical Samples

Assessment of TRM in biopsy specimens was performed by immunofluorescence method. We obtained one 4 mm punch biopsy per patient from the center of the psoriatic plaque and one from healthy volunteers (healthy skin), using local anesthesia (1% lignocaine). Tissue samples (lesional skin from psoriatic patients and non-lesional skin from healthy individuals) were cut into 5 µm thick sections in the CM3050 cryostat system (Leica, Buffalo Grove, IL, USA) and mounted onto glass slides coated with poly-L-lysine (Menzel-Glaser, Braunschweig, Germany). Frozen sections of the examined tissues were thawed to room temperature and fixed in acetone. After rinsing in 0.01 M PBS, they were incubated with 2.5% normal horse serum for 30 min at room temperature (Vector Laboratories, Burlingame, CA, USA) to decrease nonspecific binding. Then, they were incubated at 4 °C overnight with mouse anti-CD8 and CD4 or rabbit anti-CD103, CD69, CD49, CXCR6, IL-17, and Il22 polyclonal antibody (1:50; Merck Millipore, Billerica, Massachusetts). On the following day, the sections were washed 3 times in PBS and incubated for 30 min with secondary horse anti-mouse/rabbit antibodies (commercially diluted; ImmPRESS

Universal reagent Anti-Mouse/Rabbit Ig; Vector Laboratories, Burlingame, CA, USA). In negative controls, 0.01 M PBS was applied instead of primary and/or secondary antibodies (three different controls). To present histology of the tissue section, hematoxylin was used (Figure 1). The immunohistochemical specimens were viewed under a fluorescent microscope (CH30/CH40; Olympus, Tokyo, Japan).

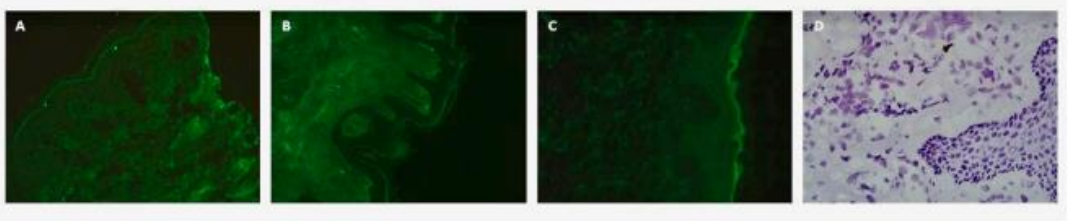


Figure 1. The representative photos of negative controls: without the primary (**A**), secondary (**B**), both types of antibodies (**C**), and staining with hematoxylin (**D**). Magnification: 500×.

The results were processed statistically by non-parametric Mann–Whitney U test. The results are expressed as means ± SEM. Correlation between proteins was analyzed by Spearman's test ($p < 0.05$). All calculations were performed using the Statistica program, release 13 (Statsoft, Inc., Tulsa, OK, USA). Differences were regarded as statistically significant at $p < 0.05$.

The study was approved by the Bioethical Committee of the Warmia and Mazury University in Olsztyn on 29.04.2020 (Resolution 24/2020). Informed consent was obtained from each patient enrolled in the study.

3. Results

3.1. Immunoreactive Area and Skin Localization of TRM Markers in Lesional Skin in Comparison with Healthy Control

The immunoreactive area (%) of CD8, CD4, CD103, CD69, CD49, CXCR6, IL-17, and IL22 was assessed in the affected skin ($n = 32$, dermis and epidermis) compared with the healthy control ($n = 10$, dermis and epidermis) (Table 1; Figure 2). The relationship between the expression of TRM markers and the duration and severity of the disease was also analyzed.

Table 1. Immunoreactive area (%) TRM markers (X ± SEM) in lesional skin ($n = 30$, dermis and epidermis) in comparison with healthy control ($n = 10$, dermis and epidermis). Asterisks show the statistically significant differences between analyzed groups.

	Healthy Control (*n*: 10)		**Lesional Skin (*n*: 32)**	
	Epidermis (%)	**Dermis (%)**	**Epidermis (%)**	**Dermis (%)**
CD8	8.83 ± 2.68	2.04 ± 0.33	15.49 ± 1.53 *	4.27 ± 0.73
CD4	2.77 ± 0.49	7.71 ± 1.80	6.45 ± 0.77 *	15.21 ± 1.11 *
CD103	13.88 ± 1.28	3.37 ± 0.42	22.32 ± 2.10 *	7.86 ± 1.02 *
CD49	10.66 ± 3.23	1.55 ± 0.34	16.17 ± 1.48 *	3.39 ± 0.42 *
CD69	13.89 ± 2.51	6.39 ± 0.69	25.24 ± 1.42 *	7.94 ± 1.13
CXCR6	2.24 ± 0.41	2.05 ± 0.33	12.23 ± 1.04 *	7.34 ± 0.78
IL-17	12.45 ± 0.94	9.85 ± 2.14	23.63 ± 1.82 *	17.19 ± 0.64 *
IL-22	10.54 ± 1.44	8.45 ± 1.03	21.12 ± 1.87 *	17.10 ± 1.01 *

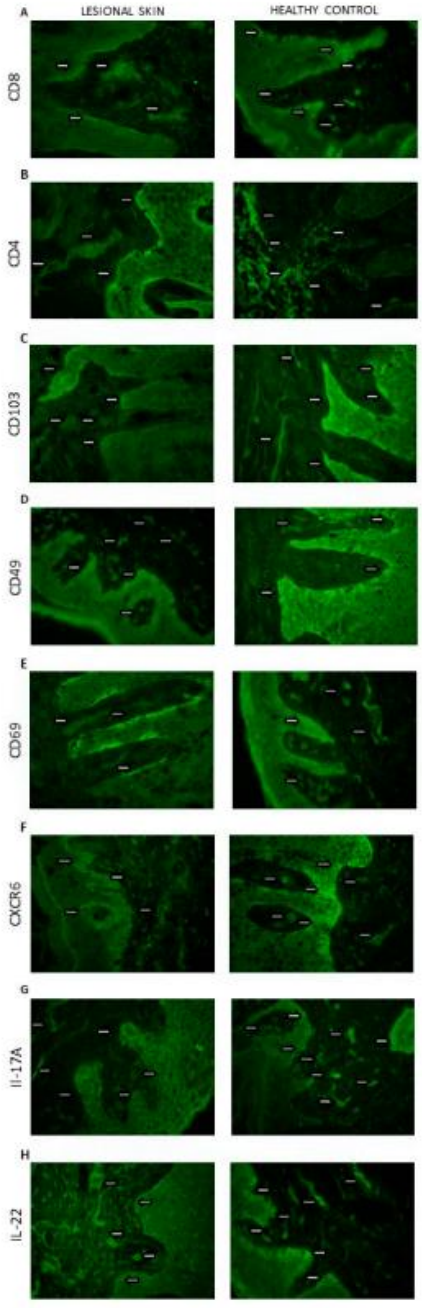


Figure 2. Skin localization of (**A**) CD8; (**B**) CD4; (**C**) CD103; (**D**) CD49; (**E**) CD69; (**F**) CXCR6; (**G**) IL-17A; (**H**) IL-22 proteins (the representative sections): lesional skin (n = 32, dermis and epidermis) and healthy control (n = 10, dermis and epidermis). The proteins are marked in green (fluorescein). Magnification: 500×.

In the case of CD8 (Figure 3A) and CD69 (Figure 3E), a statistically significant difference in their amounts was demonstrated between the epidermis and the dermis, both in the control group and in patients with psoriasis ($p < 0.05$). In both cases, a much larger immunoreactive surface was located in the epidermis. Moreover, in psoriatic patients, an increase in the immunoreactive area of CD8 and CD69 in the epidermis was found, while it did not change significantly in the dermis.

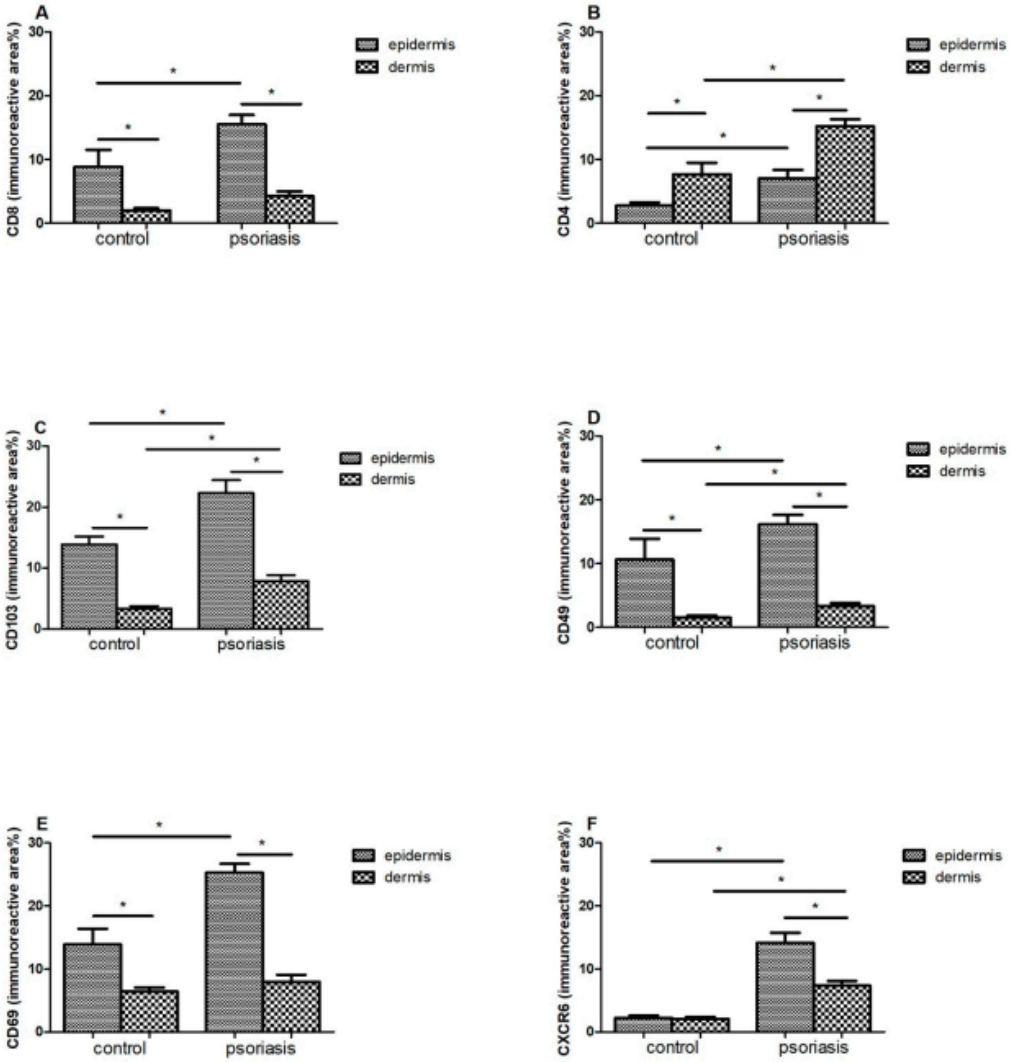


Figure 3. *Cont.*

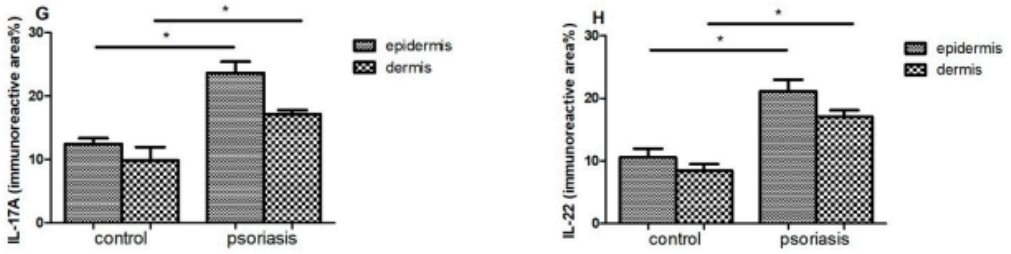


Figure 3. Immunoreactive area (means ± SEM) of (**A**) CD8; (**B**) CD4; (**C**) CD103; (**D**) CD49; (**E**) CD69; (**F**) CXCR6; (**G**) IL-17A; (**H**) IL-22 in lesional skin (n = 32, dermis and epidermis) in comparison with healthy control (n = 10, dermis and epidermis). Bars with asterisks are significantly different ($p < 0.05$, Mann–Whitney U test).

There was a significant difference between the amount of CD4 + in the epidermis and the dermis in the control group ($p < 0.05$) and in people with psoriasis ($p < 0.05$)—the amount was higher in the dermis. Additionally, in the skin of psoriatic patients, an increase in the immunoreactive area of CD4 was found in both the epidermis and skin.

CD103 (Figure 3C) and CD49 (Figure 3D) also showed differences between the epidermis and dermis in the control group ($p < 0.05$) and in people with psoriasis ($p < 0.05$)—there was more in the epidermis. Additionally, in psoriasis patients, a significant increase in the immunoreactive area of CD103 was demonstrated in both the epidermis and the dermis.

In the case of CXCR6 in the control group, there was no difference between the epidermis and the dermis, but there was a clear increase in the area of CXCR6 in the group of patients in two layers of the skin, and in addition, the level was higher in the epidermis compared with the dermis.

In IL17 (Figure 3G) and IL22 (Figure 3H), the levels of the epidermis and dermis in both groups did not differ, but there was a marked increase in area in both the epidermis and dermis of the psoriasis group.

Correlation analysis in the control group showed only two relationships. A relationship was observed between changes in CD8 and CD103 expression (r = 0.62) and between CD8 and CD49 (r = 0.69).

There were many more of these correlations in the diseased tissue. Increases in the expression of most of the markers—CD8, CD4, CD103, CD49, CD69, CXCR6—were correlated with each other (the correlation coefficient "r" is presented in Table 2, $p < 0.05$). Interestingly, negative correlations were observed between CD4 and all other factors, which were related to the different nature of changes in these markers between the individual layers of the skin sections: epidermis and dermis (marked increase in CD4 in the skin). In contrast, IL17 was only correlated with IL22 (r = 0.45, $p < 0.05$).

Table 2. Correlations (r, $p < 0.05$) between individual markers in the study group of patients with psoriasis.

Marker (r)	cd8	cd4	cd103	cd49	cd69	cxcr6
CD8	-	−0.37	0.55	0.65	0.64	0.45
CD4	−0.37	-	−0.29	−0.47	−0.46	−0.26
CD103	0.55	−0.29	-	0.64	0.6	0.51
CD49	0.65	−0.47	0.64	-	0.69	0.54
CD69	0.64	−0.46	0.6	0.69	-	0.52
CXCR6	0.45	−0.26	0.51	0.54	0.52	-

3.2. Correlations between TRM Markers and the Duration of Psoriatic Lesions

In our study, we also analyzed the relationship between marker expression and disease duration, showing a clear relationship between these variables (Table 3). The longer the course of the disease, the higher the expression of TRM markers was observed (time vs. marker, $p < 0.05$).

Table 3. Correlations (r, $p < 0.05$) between individual markers and the duration of psoriatic lesions.

Marker (%)	Duration of the Psoriasis (years)
CD8	0.45
CD4	0.55
CD103	0.44
CD49	0.57
CD69	0.38
CXCR6	0.59
IL-17A	0.37
IL-22	0.51

3.3. Correlations between the Analyzed Variables: Time, Intensity, and Expression of TRM Markers

The evaluation of the PASI, BSA, and DLQI scales allowed for the analysis of the correlation between the obtained values, disease duration, and the expression of the analyzed markers. There was no correlation between the severity of lesions and the time of their occurrence (PASI, BSA, DLQI vs. time). The expression of the analyzed substances was not dependent on the intensity of skin lesions (marker vs. PASI, BSA, DLQI). Only a statistically significant relationship was observed between the scales themselves (the "r" values are presented in Table 4).

Table 4. Correlation coefficient (r) between the analyzed variables: time, intensity, and expression. Statistically significant correlations are marked in italics and bold.

	PASI	BSA	DLQI
time (years)	0.21	0.15	0.29
Cd8 (%)	0.22	0.17	0.33
Cd4 (%)	0.20	0.16	0.27
Cd103 (%)	0.21	0.16	0.31
Cd49 (%)	0.23	0.18	0.28
Cd69 (%)	0.25	0.20	0.29
Cxcr6 (%)	0.18	0.13	0.28
IL-17A (%)	0.20	0.15	0.30
IL-22 (%)	0.24	0.15	0.31
PASI	-	***0.95***	***0.56***
BSA	***0.95***	-	***0.59***
DLQI	***0.56***	***0.59***	-

4. Discussion

CD8 + TRM T cells are plentifully present in the psoriatic epidermis, and their number correlates with the thickness of the epidermis [1,16], while CD4 + TRM preferentially inhabit the dermis [4]. The key skin TRM surface markers are CD69, integrin αE (CD103), integrin α1 (CD49a), and CXCR6 [17]. In our group of 32 psoriasis patients, there was statistically significant increase in the immunoreactive area of CD8 in the epidermis (Figure 3A), CD4 in the dermis and, to a lesser extent, in the epidermis (Figure 3B) compared to the control

group. It seems that the accumulation of epidermal CD8 + cells induces keratinocyte hyperproliferation as well as papillomatosis (increase in CD8 + concurrent to the intensity of Ki67 staining in keratinocytes) [4,13]. In psoriatic patients, epidermal CD8 + TRM cells express the CLA antigen, CCR6, CD103, and IL-23R and produce IL-17A upon ex vivo stimulation, which proves their pathogenicity. The epidermis can also be occupied by CD4 + CD103 + TRM producing IL-22 upon stimulation [1,4]. Both IL-17 and IL-22 are known to play an important role in the pathogenesis of psoriasis. Our studies also confirm a significant increase in IL-17 (Figure 3G) and IL-22 (Figure 3H) levels in both the epidermis and dermis of psoriatic patients. The amounts of these interleukins turned out to be significantly higher than in the skin and epidermis of healthy patients (Table 1). High amounts of pro-inflammatory IL-17 and IL-22 persist for months after the lesion has cleared, and their amount is correlated with the duration of the disease (Table 3).

CD103 is a ligand for E-cadherin, an adhesion molecule expressed by epithelial cells in barrier tissues [18]. Its expression is most pronounced on epidermal CD4 + and CD8 + TRM cells because it allows TRM to bind to E-cadherin, widely expressed by epithelial cells [19]. This determines the adhesion and also promotes local retention of TRM. In addition to adhesion, CD103 + TRM are involved in cytotoxicity through exocytosis of cytolytic granules. Research shows that the lack of CD103 results in a reduction in but not a removal of the TRM population. The above data prove that the presence of TRM in tissues is not only determined by binding to epidermal cells [20]. In the control and study groups, we showed a significant difference between the amount of CD103 in the epidermis and dermis—a much larger immunoreactive area in the epidermis (Figure 2C). In one study assessing phenotypic features of TRM in psoriatic patients without lesions and healthy skin, an immunofluorescence study revealed that TRM CD103 + CD8 was dominant in the epidermis compared with the dermis of the healthy control group [21]. We obtained similar results among our psoriasis patients—CD103 and CD49 were found in significantly greater amounts, mainly in the epidermis, but also in the dermis when compared with healthy subjects (Table 1).

CD49a is the $\alpha 1\beta 1$ integrin receptor α subunit, expressed only on the epidermal CD8 T cells and has been confirmed to be a marker of TRM in the epidermis. CD49a binds to type IV collagen, which is located in the basement membrane zone and also has significant effect on cell migration along the collagen. It is intended to confine CD49a-expressing TRM cells to the epidermis. It is worth noting that the expression of CD49a determines the cytokine production profile of TRM cells [20]. CD8 + CD49a + TRM cells located in the epidermis produce perforin and IFN-γ—crucial in fighting viral infections. In our control group, it turned out that a significantly greater amount of CD49 was found in the epidermis compared with the dermis $10.66 \pm 3.23\%$ vs. $1.55 \pm 0.34\%$ (Figure 2D). The marker CD49a was confirmed to identify two distinct populations of CD8 + CD103 + TRM cells. CD8 + CD49a cells in psoriasis mediate disease by producing interleukin-17 (IL-17) [22,23]. However, blockade of IL-17 may require long-term administration because it blocks a molecule made by TRM and does not target TRM itself. In a clinical study of psoriatic patients (n = 10) treated with secukinumab for 24 weeks, Fujiyama et al. showed that although there was a significant decrease in the number of CD8 + CD103 + cells in the affected skin, there was only a slight decrease in the number of CD8 + CD103 + CD49- cells, suggesting that TRM cells are still preserved [23,24].

Another key TRM marker is CD69-a glycoprotein, which is involved in distinguishing T lymphocytes in tissues from those in the circulation and is responsible for their colonization in tissues, inhibiting their recirculation [4]. CD69 contributes to the downregulation of the sphingosine 1 phosphate receptor (S1P1) by inhibiting its expression. It allows CD69 + TRM to be distinguished from TEM (effector memory), which are CD69-negative. S1P1-expressing T cells are directed from the tissue to the lymph nodes and subsequently to the blood, depending on S1P gradients, the expression levels of which vary with localization (low in tissues, average in lymph nodes, high in blood) [25]. TRM expressing CD69 do not express S1P1, and therefore, they do not migrate from peripheral tissues. Accord-

ingly, CD69 blocks tissue exit mediated by the sphingosine-1-phosphate-1 receptor (S1PR1). However, the lack of CD69 only results in a reduction in, not a complete disappearance of, the TRM population, as is the case with CD103 [20]. In the case of the tested control group, we found a significant difference in the amount of CD69 between the epidermis and the dermis in the control group, in favor of the epidermis (13.89 ± 2.51% vs. 6.39 ± 0.69%) (Figure 3E). Moreover, in an immunohistochemical study of skin lesions in patients with plaque psoriasis, we found a significant increase in the immunoreactive area of CD69 in the epidermis (25.24 ± 1.42%), but these values did not change in the dermis (7.94 ± 1.13%).

CXCR6 is expressed on human skin TRM cells, and the chemokine CXCL16, the ligand CXCR6, is expressed on epidermal keratinocytes and can be released as a chemoattractant. CXCR6-deficient T cells have a low ability to form TRM cells in the skin [1]. In the control group, we found no differences between the immunoreactive area (%) in the epidermis and the dermis—they remained at a very low level in both locations (2.24 ± 0.41% and 2.05 ± 0.33%, respectively) (Figure 3F). However, we observed a marked increase in CXCR6 in the group of psoriatic patients in both layers—higher in the epidermis (12.23 ± 1.04% vs. 7.34 ± 0.78%). These studies clearly indicate that CXCL16–CXCR6 interactions mediate the colonization of T lymphocytes in human skin, and thus contribute to the pathogenesis of psoriasis.

Keratinocytes in lesion-free skin that have never experienced disease in psoriatic patients are also involved in the accumulation of TRM. Undamaged keratinocytes tend to upregulate CCL20 expression upon cytokine stimulation, leading to the migration of IL-17A-producing T cells expressing CCR6 [20]. These data suggest that a CD8 TRM population with an IL-17A-producing profile is constructed in the skin of psoriasis patients before disease onset in response to recruiting signals such as CCL20 and ICAM-1 and cytokines such as IL-23 and IL-15 and contributes to the appearance of psoriasis lesions in the future [20].

Complete TRM suppression appears to be required for complete disease remission. Unfortunately, TRM cells are long-lived and resistant to destructive factors and apoptosis, and their elimination seems impossible [26]. Interestingly, after the psoriatic lesions have disappeared, TRMs can still produce IL-17A, and proper therapy only inhibits their activity [4].

Our analysis of the relationship between the expression of TRM markers and the duration of the disease showed a clear relationship between these variables. Marker expression in the study group was significantly dependent on the time of skin lesions (time vs. marker, $p < 0.05$). Longevity of TRM and their unquestionable ability to accumulate in the skin of psoriatic patients may explain the rapid recurrence of psoriatic lesions at the same site after the causative agent has been triggered.

We found no relationship between the severity of lesions and the time of their occurrence (PASI, BSA, DLQI vs. time). It is interesting that the expression of the analyzed markers was also not dependent on the intensity of skin lesions (marker vs. PASI, BSA, DLQI).

So far, there have been few publications assessing the effect of topical or systemic treatment on the amount of TRM in the skin of patients suffering from plaque psoriasis [27]. Cheuk et al. investigated the effect of nb-UVB, iTNF-α, or IL-12/23 therapy on TRMs. They found the presence of epidermal TRM cells at the site of resolved psoriatic papules and that these cells are capable of producing cytokines that play a key role in the pathogenesis of psoriasis [28]. However, ultraviolet irradiation seems to diminish the number of IL-17A-producing T cells in skin [29], and this T cell fraction includes TRM. Khalil et al. showed that CD8 + cells expressing CD69 remain in healed skin even several months after successful treatment with methotrexate, and CD4 + cells producing IL-22 and CD8 + producing IL-17 also remain in the epidermis of healed lesions [2]. Mehta et al. using flow cytometry, examined the profile of T lymphocytes in the same patients' lesions before and during treatment with guselkumab (IL-23 blocker) or secukinumab (IL-17A blocker). Inflammatory dendritic cells and CD4 + CD49a-CD103- T cells were reduced with both treatments. In contrast, guselkumab decreased memory T cells while maintaining

regulatory T cells, contrary to secukinumab. Neither drug modified the frequency of IL-17A + IL 17F +/− CD4 + or CD8 + T cells [30]. In the case of topical treatment, vitamin D analogues and corticosteroids reduce the lesional IL-17A-producing TRM, possibly also reducing pathogenic TRM [31–33].

Taken together, these results indicate that although we achieve clinical resolution of psoriatic lesions, essential inflammation, as defined by the expression of key cytokines and chemokines, is not completely resolved in all psoriatic lesions and can be stimulated to initiate relapse. Undoubtedly, the issue of the influence of therapy on the amount of TRM requires further research.

5. Conclusions

We have demonstrated an increased number of TRM cells and their markers in the epidermis and dermis of psoriatic eruptions, compared with the skin of healthy volunteers. An interesting and important conclusion is that there is a clear positive relationship between the expression of TRM markers in patients with plaque psoriasis and the duration of skin lesions and the disease. This proves the unquestionable ability of TRM to accumulate in the skin of patients suffering from psoriasis. However, the amount of TRM is not affected by the intensity of skin lesions (assessed according to PASI, BSA scales).

Targeting TRM cells appears to be a new potential therapeutic strategy for reducing psoriasis flare-ups. However, due to the still low number of reports on the effect of local and general treatment on the amount of TRM in the epidermis and dermis of patients suffering from psoriasis, further research is required in relation to this issue. A thorough understanding of the mechanisms responsible for the development and relapse of plaque psoriasis may contribute to the implementation of more effective therapies.

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Original paper

The effect of therapy on TRM in psoriatic lesions

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Abstract

Introduction: The course of psoriasis is associated with recurrence of the lesions at the same location despite effective treatment. It is due to the presence of TRM (tissue-resident memory cells) in the seemingly healthy skin, which may initiate an inflammatory cascade.
Aim: The assessment of TRM in psoriatic lesions prior to and after 12 weeks of systemic therapy with methotrexate (MTX) or secukinumab (SEC) or ixekizumab (IXE) or adalimumab (ADA).
Material and methods: TRM markers (CD4, CD8, CD103, CD69, CD49, CXCR6) and the tissue expression of cytokines (IL-17, IL-22) in the psoriatic lesions obtained from 13 patients compared to 10 healthy skin samples were evaluated with immunohistochemistry. Biopsy specimens were collected three times from the same psoriatic plaque before and after 4 and 12 weeks of therapy.
Results: The expression of TRM markers in the lesions decreased at three time points (W0, W4, W12), revealing the diminished intensity of fluorescence over time with each therapy. The most rapid response was observed with anti-IL-17 therapy at W4 of treatment, while with MTX and ADA at W12.
Conclusions: The decreased expression of TRM markers occurring predominantly in the lesional dermis and not in the epidermis over 12 weeks of observation may be due to the poorer penetration of systemic drugs to the epidermis, or the process of psoriatic lesion regression in the epidermis is secondary to the reduction of inflammation in the skin, or TRM in the epidermis may be more resistant to therapy.

Key words: psoriasis, tissue-resident memory cells, methotrexate, biologics.

Introduction

The course of psoriasis is associated with the persistence of lesions at specific locations and their recurrence at the same location (up to 90% of eruptions) despite effective treatment. According to the literature, after the regression of psoriatic plaques, a trace of inflammation remains in the seemingly healthy skin in the form of tissue-resident memory (TRM) cells. The cells constitute a trace of "immunological memory". They may rapidly induce inflammation triggering the recurrence of the disease [1, 2].

TRM cells are unable to recirculate. In psoriasis they are represented by two main types: TRM CD8+CD69+CD103+ abundant in the lesional epidermis, and TRM CD4+ CD69+CD103+ located in the dermis [1, 3]. Epidermal TRM CD8+ express CD103, a receptor of adhesion for E-cadherin, which determines their location in the epithelial tissue [3–7]. The CD69 antigen plays a key role in differentiating T cells in tissues from those in circulation. It is responsible for their settling, preventing their recirculation via the suppression of the sphingosine 1-phosphate receptor (S1P1). It enables the differentiation between TRM CD69+ and TEM (effector memory) cells which are negative (CD69–) [8]. TRM cells also express CD49a molecule which binds collagen IV present in the stratum basale [6, 9–11]. CD103+ TRM cells produce IFN-γ, IL-17A and IL-22 [3]. As regards *ex vivo*-multiplied T lymphocytes some populations of CD8+CD103+ T lymphocytes produce IFN-γ, IL-17A or IL-22, while CD4+CD103+ T lymphocytes rarely produce the cytokines. As regards CD8+ T lymphocytes, IL-17A is more commonly produced by TRM CD103+ cells than by CD103– T lymphocytes. Therefore, CD8+CD103+ TRM cells are effective in producing IL-17A. Other molecules, discerning TRM cells from other circulating types of T memory cells, includ-

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ing CXCR3, CXCR6 and CD101 were also described in the literature [10–12]. It was determined that CD49a, CD103, CXCR6, CX3CR1 and PD-1 were the essential TRM surface markers which differentiated CD69+ and CD69– human memory cells in tissues [8, 11]. However, some researchers consider CXCR6 as a TRM-specific marker present in the mucous membrane and lymphatic tissue [11].

The particular pathogenic properties of TRM CD8+ in psoriasis are due to the fact that they express the IL-23 receptor and are capable of producing proinflammatory IL-17 and IL-22 in the skin, even many months after the resolution of lesions, and their quantity is correlated with the duration of the disease [1, 2, 4, 6, 9, 13–15]. It also explains the rapid recurrence of lesions at specific locations after a trigger factor is activated.

The implementation of treatment, both systemic and topical, aims at the reduction of inflammation, suppression of the production of TRM and the limitation of the number of TRM cells. It may be of key importance in the explanation of rapid recurrences after topical glucocorticoid (GCS) use and longer remission rates after the administration of systemic preparations [16, 17]. The duration of remission varied even in case of treatment with monoclonal antibodies. The use of IL-23 inhibitors proved to result in longer psoriasis remission after treatment discontinuation compared to TNF inhibitors [3, 18]. TRM quantity is not only influenced by the type of the drug and its mechanism of action, but also by the duration of therapy necessary for the quantity to be reduced. The issue has not been fully elucidated and requires further research.

Aim

The present study aimed at the assessment of the occurrence of TRM in psoriatic eruptions before and after therapy in patients treated systemically with methotrexate (MTX) or secukinumab (SEC) or ixekizumab (IXE) or adalimumab (ADA).

Material and methods

The study group included patients with plaque psoriasis (n = 13), aged 18 to 60 with no concomitant psoriatic arthritis. The patients were treated in our centre. The exclusion criteria were as follows: chronic and acute inflammatory conditions other than psoriasis, neoplastic disease, exacerbation of a cardiovascular disease, cardiac, renal or liver failure. The inclusion criteria were as follows: patients with the severity of psoriatic lesions PASI > 10, no systemic treatment of psoriasis for at least 4 weeks or topical treatment for 1 week prior to enrolment in the study.

The control group (n = 10) included healthy volunteers with no previous/family history of psoriasis, no concomitant autoimmune or inflammatory diseases.

Psoriatic patients were treated with MTX at a dose of 15 mg/week for 12 weeks. They also received folic acid 5 mg on the second day after MTX administration (n = 4). Anti-IL-17 drugs (n = 5): secukinumab and ixekizumab, and anti-TNF drugs (n = 4) were administered according to recommended therapeutic regimens.

Biopsy specimens were collected three times from the same psoriatic plaque before introducing the therapy, after 4 and 12 weeks of therapy initiation (with the use of a 4-mm biopsy punch).

Tissue samples (lesional skin obtained from psoriatic patients and non-lesional skin from healthy individuals) were cut into 5-µm-thick sections with the use of the cryostat system (SLEE MEV, Germany) and mounted onto glass slides coated with poly-L-lysine (Menzel-Glaser, Braunschweig, Germany). Frozen sections of the examined tissues were thawed to room temperature and fixed in acetone. After being rinsed in 0.01 M PBS, they were incubated with 2.5% normal horse serum for 30 min at room temperature (Vector Laboratories, USA) to decrease nonspecific binding. Subsequently, they were incubated at 4°C overnight with mouse anti-CD8 and CD4 or rabbit anti-CD103, CD69, CD49, CXCR6, IL-17 and IL-22 polyclonal antibodies (1 : 50; Biorbyt, UK). On the following day, the sections were washed 3 times in PBS and incubated for 30 min with secondary horse anti-mouse/rabbit antibodies (commercially diluted; ImmPRESS Universal reagent Anti-Mouse/Rabbit Ig; Vector Laboratories). During the analysis of CD4+CD103+ and CD8+CD103+, after being blocked with 2.5% normal goat serum for 30 min, the frozen sections were incubated with the primary mouse anti-CD8 and -CD4 and rabbit anti-CD103 polyclonal antibody (1 : 50; Biorbyt, UK) and the secondary goat anti-mouse/rabbit antibodies (VectaFluor Duet Kit, X-Rabbit IgG/D488 X-Mouse IgG, Vector Laboratories, USA). The immunohistochemical specimens were viewed under a fluorescent microscope (CH30/CH40; Olympus, Tokyo, Japan).

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Bioethical Committee of the Warmia and Mazury University (24/2020).

Statistical analysis

After the assumptions of the normality and homogeneity of variances were checked, the results were processed statistically with non-parametric tests (the system was not in accordance with normal distribution). ANOVA Kruskal-Wallis test was used to compare the studied groups with the controls and Friedman ANOVA (Kendall's W Value) was used to compare repeatable measurements at three time points during different types of therapy in the studied groups. Correlation between proteins was analysed with the Spearman's test. All statistical analyses were performed with Statistica software (release 13, StatSoft Inc., USA). Differences were regarded as statistically significant at $p < 0.05$. The results were expressed as means ± SEM.

Results

The immunoreactive area of CD8 was always larger in the epidermis than in the dermis ($p < 0.05$) of patients undergoing therapy with anti-interleukin 17 (SEC or IXE) (Figure 1). During the 4th week of observation, the decrease in CD8 immunoreactive area was observed in both skin layers ($p < 0.05$) with the levels being comparable to those in healthy individuals. Similarly, the decrease in CD4 immunoreactive area was noted both in the epidermis and in the dermis during the second measurement ($p < 0.05$), but the immunoreactive areas were larger in the dermis during the therapy ($p < 0.05$). The areas of CD103 and CD49 remained unchanged during the therapy, but a higher level was observed in the epidermis at each time point ($p < 0.05$). In case of CD69, the area diminished in the epidermis during the 4th week of the therapy and then it remained unchanged until the 12th week ($p < 0.05$). No changes were observed in the dermis between measurements. Contrary to CD69, the area of CXCR6 did not change in the epidermis, whereas in the dermis it was smaller during the 4th and 12th week than before the therapy ($p < 0.05$). The immunoreactive area of IL-17A and IL-22 was stable in the epidermis during the therapy. However, a significant decrease was noted in the dermis during the 4th week ($p < 0.05$), which was equal to the outcomes of healthy controls. The level of IL-22A in the epidermis correlated with CD103 ($r = 0.6257$, $p < 0.05$) and CD69 ($r = 0.6250$, $p < 0.05$) during the therapy with anti-IL-17.

No changes in the immunoreactive areas of CD8, CD103 and CD49 were reported in the second analysed therapy with anti-tumour necrosis factor α (ADA) (Figure 2). The areas were larger in the epidermis in comparison with the dermis ($p < 0.05$). Interestingly, in case of CD4, the decrease in area size was observed during the third measurement (the 12th week) both in the epidermis as well as in the dermis ($p < 0.05$). The immunoreactive area of CD69 and CXCR6 ($p < 0.05$) in the epidermis was decreased during the 12th week of observation. As regards the dermis, the area diminished only in case of CD69. In contrast, the area of IL-17A and IL-22 remained unchanged in the epidermis, but it was smaller in the dermis during the 4th week of observation than before the therapy ($p < 0.05$).

Similarly to the therapy with anti-IL-17, the immunoreactive area of CD8 was decreased in the epidermis during therapy with MTX. However, the response was delayed (the 12th week of therapy, $p < 0.05$) (Figure 3). The change during the 12th week was also noted in case of CD4 area, but it was observable in the dermis ($p < 0.05$). As regards the third measurement, the decrease in CD103 and CD69 was reported in the epidermis ($p < 0.05$), whereas the area in the dermis was unchanged. The area of CD49 and CXCR6 was constant during therapy, but differences between layers were observed ($p < 0.05$). In contrast, the area of IL-17A remained unchanged in the epidermis. However, a significant decrease was observed in the dermis during the 4th week of the therapy ($p < 0.05$). Similarly, in case of IL-22, the area in the dermis was reduced ($p < 0.05$) during the 4th week (equating to the outcome in healthy controls). Additionally, the decrease in IL-22 was also noted in the epidermis during the 12th week ($p < 0.05$). The changes of the area of CD8 were negatively correlated with IL-17A ($r = -0.8461$, $p < 0.05$) in the epidermis.

The location of typical TRM markers in the lesional epidermis and dermis was assessed at three time points for all the therapies. It was demonstrated that the intensity of marker fluorescence diminished throughout the therapies (Figure 4, Table 1).

The analysis of CD4+CD103+ and CD8+CD103+ during different systemic therapies showed changes in areas only in case of treatment with anti-IL-17. The decrease in the size of areas was reported only in the epidermis during the 4th week – CD4+CD103+ ($p < 0.05$) and during the 12th week – CD8+CD103+ ($p < 0.05$), in case of the absence of changes in the dermis (Figures 5 and 6).

Discussion

Similarly to other authors, we demonstrated the increased quantity of cells expressing CD8+CD103+ in the lesional epidermis and CD4+103+ in the lesional dermis compared to the skin of healthy volunteers (Figure 5) [9, 13, 19, 20]. Moreover, Kurihara *et al.* reported that the number of CD8+CD103+ TRM cells in psoriatic epidermis correlated with the thickness of the psoriatic plaque, i.e. the intensity of keratinocyte proliferation. They were capable of producing IFN-γ, IL-17A or IL-22 [4]. Additionally, the production of IL-17A was higher in patients with the longer duration of the disease [20]. The accumulation of epidermal CD8+ T cells may induce both keratinocyte hyperproliferation and papillomatosis (the parallel increase in CD8+ count and the intensity of Ki67 staining in keratinocytes) [13]. CD8+ cells infiltrating the epidermis are CD103+ and those are considered to be the proper TRM, while CD4+ in the dermis express CD103 to a low extent [3, 9]. The present study also showed the expression of CD103 to be considerably lower (Figure 2).

There is a paucity of research assessing the dynamics of the changes of TRM count under the influence of implemented psoriasis treatment. The present study was performed to assess the location of typical TRM markers in the lesional epidermis and dermis at three time points for three different systemic therapies (MTX, anti-IL-17 and anti-TNF). It revealed that the intensity of the fluorescence of some antigens diminished throughout the therapies. The most rapid reduction in the expression of TRM-specific antigens was observed in case of anti-IL-17 therapy at week 4 of treatment, while with MTX and anti-TNF, the re-

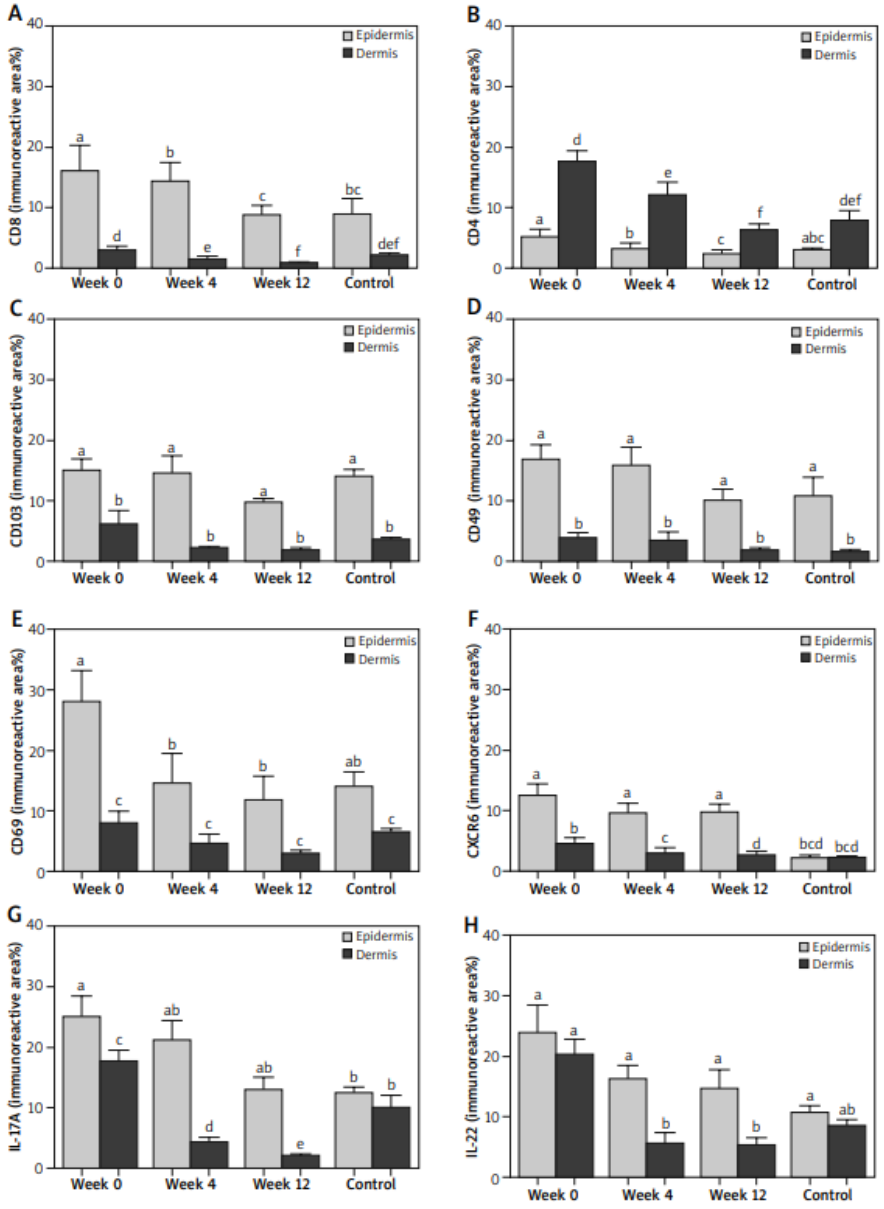


Figure 1. The immunoreactive area (%) of CD8 (x ± SEM) **(A)**, CD4 (x ± SEM) **(B)**, CD103 (x ± SEM) **(C)**, CD49 (x ± SEM) **(D)**, CD69 (x ± SEM) **(E)**, CXCR6 (x ± SEM) **(F)**, IL-17A (x ± SEM) **(G)** and IL-22 (x ± SEM) **(H)** in the lesional skin of patients (n = 5, dermis and epidermis) before (week 0) and during treatment (week 4 and week 12) with anti-interleukin-17 (anti-IL-17) in comparison with healthy controls (n = 10, dermis and epidermis). Bars with different letters are significantly different ($p < 0.05$)

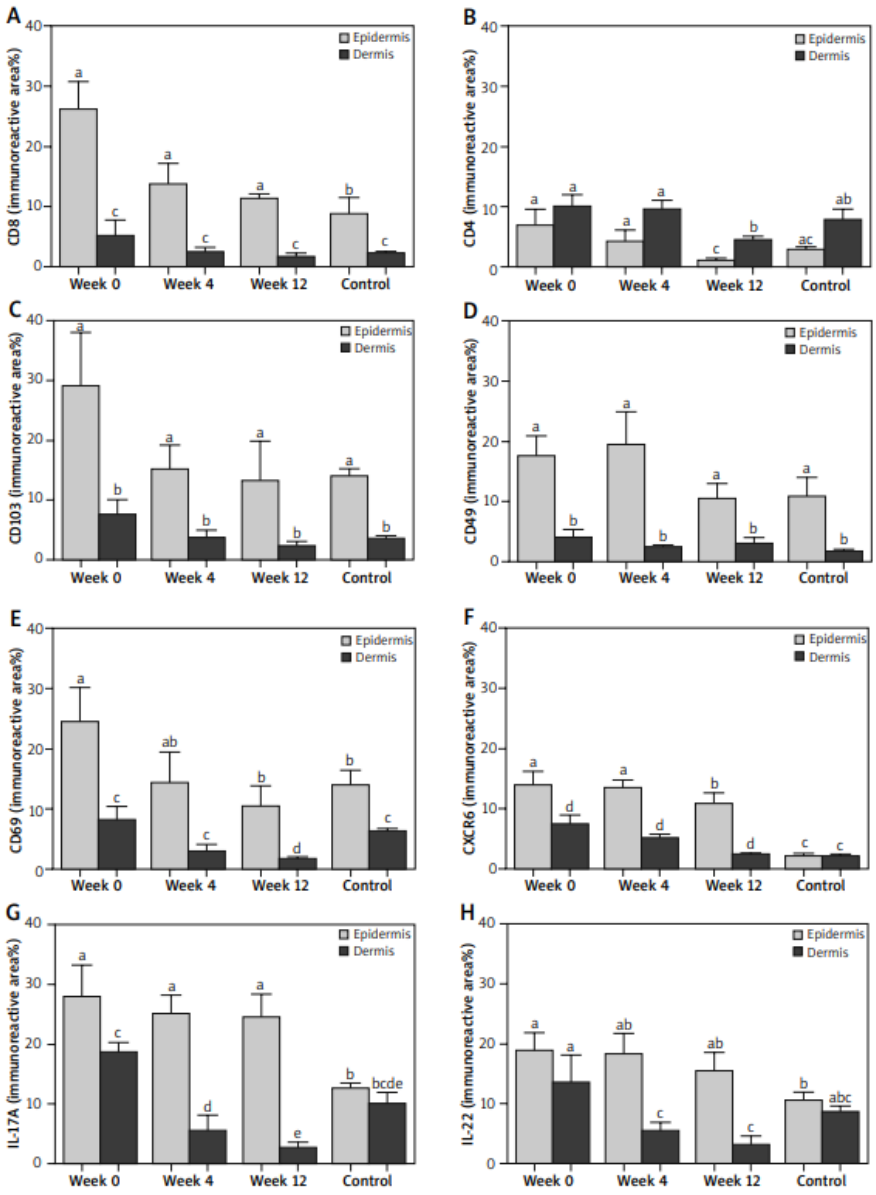


Figure 2. The immunoreactive area (%) of CD8 ($x \pm$ SEM) **(A)**, CD4 ($x \pm$ SEM) **(B)**, CD103 ($x \pm$ SEM) **(C)**, CD49 ($x \pm$ SEM) **(D)**, CD69 ($x \pm$ SEM) **(E)**, CXCR6 ($x \pm$ SEM) **(F)**, IL-17A ($x \pm$ SEM) **(G)** and IL-22 ($x \pm$ SEM) **(H)** in the lesional skin of patients ($n = 4$), dermis and epidermis) before (week 0) and during treatment (week 4 and week 12) with anti-tumour necrosis factor α (TNF-α) in comparison with healthy controls ($n = 10$, dermis and epidermis). Bars with different letters are significantly different ($p < 0.05$)

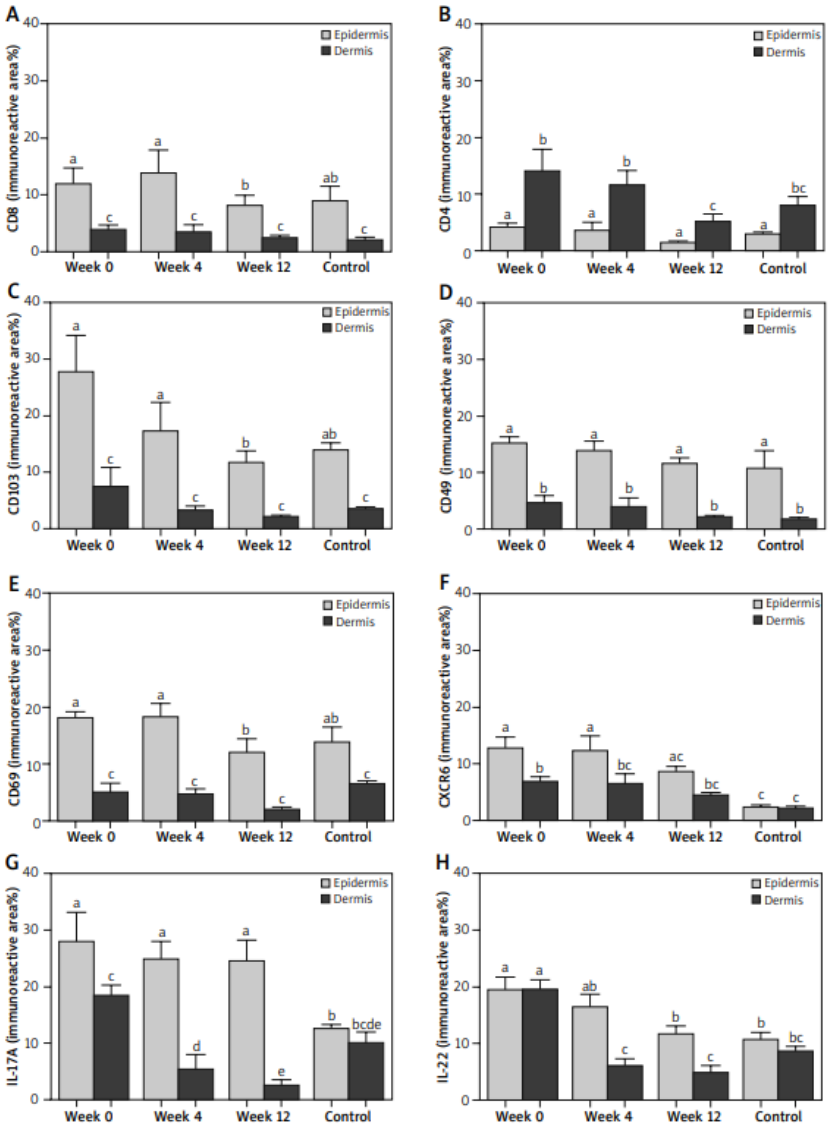


Figure 3. The immunoreactive area (%) of CD8 ($x \pm SEM$) **(A)**, CD4 ($x \pm SEM$) **(B)**, CD103 ($x \pm SEM$) **(C)**, CD49 ($x \pm SEM$) **(D)**, CD69 ($x \pm SEM$) **(E)**, CXCR6 ($x \pm SEM$) **(F)**, IL-17A ($x \pm SEM$) **(G)** and IL-22 ($x \pm SEM$) **(H)** in the lesional skin of patients ($n = 4$, dermis and epidermis) before (week 0) and during treatment (week 4 and week 12) with methotrexate (MTX) in comparison with healthy controls ($n = 10$, dermis and epidermis). Bars with different letters are significantly different ($p < 0.05$)

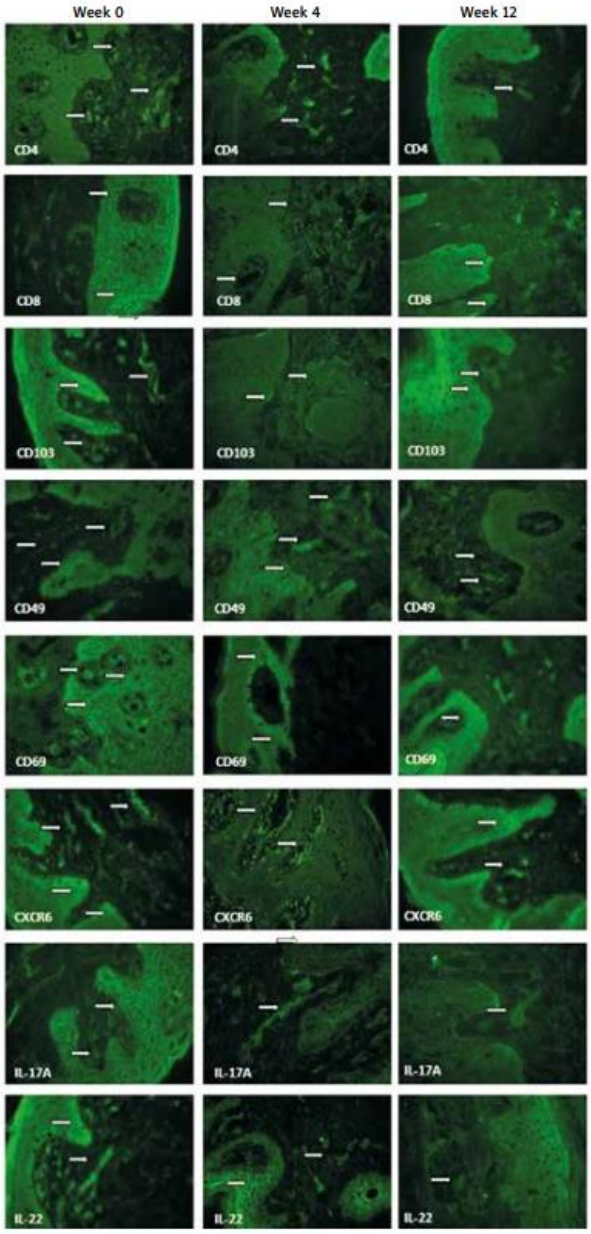


Figure 4. The skin location of CD8, CD4, CD103, CD49, CD69, CXCR6, IL-17A and IL-22 proteins (the representative sections) in the lesional skin (dermis and epidermis) for all the therapies. The proteins are marked in green (fluorescein). Magnification: 500×

Table 1. Changes in the immunoreactive area (%) of CD8, CD4, CD103, CD49, CD69, CXCR6, IL-17A and IL-22 proteins in patients during therapy with: anti-interleukin-17 (anti-IL-17), anti-tumour necrosis factor α (anti-TNF-α), methotrexate (MTX) (week 0, 4 and 12). Bars with an asterisk show statistically significant differences (*$p < 0.05$)

Variable	Week 0 (area %)		Week 4		Week 12	
	Epidermis	Dermis	Epidermis	Dermis	Epidermis	Dermis
Anti-IL-17:						
CD8	16.03	2.91	–12%	–48%	–44%*	–60%*
CD4	5.06	17.37	–37%	–31%	–56%	–64%*
CD103	15.02	5.96	–3%	–61%	–36%	–69%
CD49	16.94	3.71	–6%	–9%	–41%	–49%
CD69	28.00	7.72	–48%	–44%	–58%*	–63%
CXCR6	12.51	4.33	–24%	–35%	–22%	–43%*
IL-17A	25.03	17.46	–15%	–76%	–47%	–89%*
IL-22	23.83	20.18	–32%	–72%	–38%	–74%*
Anti-TNF-α:						
CD8	21.15	5.06	–46%	–54%	–54%	–70%
CD4	6.80	14.95	–39%	–37%	–85%*	–70%*
CD103	29.01	7.25	–48%	–52%	–54%	–72%
CD49	17.55	3.85	–10%	–42%	–41%	–27%
CD69	24.36	7.99	–41%	–66%	–57%*	–80%*
CXCR6	13.77	7.18	–2%	–30%	–22%	–67%
IL-17A	23.17	12.08	–5%	–70%	–19%	–74%*
IL-22	18.83	13.42	–2%	–60%	–18%	–77%*
MTX:						
CD8	9.73	3.86	16%	–12%	–28%	–39%
CD4	4.03	13.82	–14%	–17%	–69%	–63%*
CD103	27.64	7.25	–37%	–57%	–73%*	–58%
CD49	14.96	4.38	–8%	–13%	–24%	–56%
CD69	18.05	4.94	1%	–6%	–66%*	–40%
CXCR6	12.68	6.62	–4%	–4%	–33%	–34%
IL-17A	27.88	18.47	–10%	–71%	–12%	–86%*
IL-22	19.41	19.52	–15%	–69%	–61%*	–75%*

sponse was observable no earlier than in week 12. It is unknown how long the treatment should last so as to eliminate TRM completely and prevent rapid relapses. Seemingly, it may vary depending on the therapy.

Cheuk *et al.* demonstrated the presence of T cells producing both IL-22 and IL-17 in the epidermis and dermis of patients undergoing nb-UVB therapy or patients treated with biological drugs [9]. The cells were significantly reduced in the biopsies collected after the effective treatment with nb-UVB (at least 25 irradiation sessions), after biological drug treatment (infliximab – the median duration of treatment I was 4.5 years and with ustekinumab the median of treatment duration was 1.4 years). However, despite the normalization of the total CD8+ T lymphocyte count, the number of cells expressing CD49a increased 5-fold in samples subjected to nb-UVB radiation and 16-fold in samples collected from patients undergoing biological therapy [9].

Interestingly, *ex vivo* stimulation showed that the levels of CD4+ T lymphocytes producing IL-22 in the resolved lesions were similar or higher compared to active psoriasis and did not correlate with the number of years of treatment, indicating the maintenance of the effector function in the resolved lesions even after 6 years of treatment [9]. It may be explained by TRM resistance to destructive factors and apoptosis. The heterodimeric molecule of CD8+ (αEβ7) marker contains CD103 and β7 subunits and plays a significant role in the stability of CD8+ TRM cells via increasing the expression of Bcl-2 which is characterized by its antiapoptotic properties [1, 4, 5]. Furthermore, active ef-

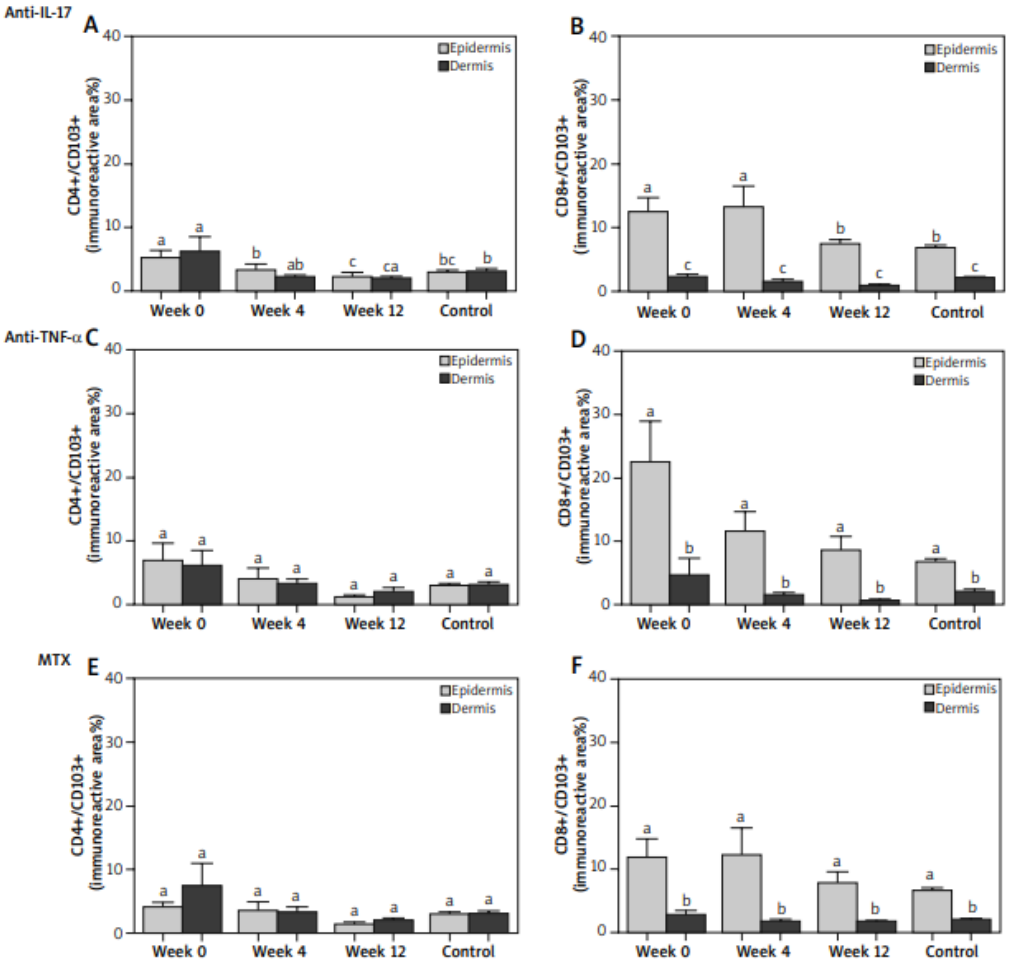


Figure 5. The immunoreactive area (%) of CD8+CD103+ (x ± SEM) and CD4+CD103+ (x ± SEM) in the lesional skin of patients (n = 5, dermis and epidermis) before (week 0) and during treatment (week 4 and week 12) with anti-interleukin-17 (anti-IL-17), anti-tumour necrosis factor α (anti-TNF-α), methotrexate (MTX) in comparison with healthy controls (n = 10, dermis and epidermis). Bars with different letters are significantly different ($p < 0.05$)

fector T cells participating in an inflammatory process may be converted into long-lasting TRM cells in the epidermis throughout disease resolution [1, 4, 5, 21].

Kurihara *et al.* investigated 10 patients who were treated systemically with cyclosporine, a phosphodiesterase-4 inhibitor (PDE4) or biological drugs for a year. The study showed that CD8+CD103+IL-17A+ TRM cells occurred more frequently in those patients. Interestingly, the investigators observed more CD8+ TRM than CD4+ TRM cells, which may be suggestive of their correlation with the duration of disease and not with the intensity of dermal lesions [3, 4]. Our study also confirmed the resistance in the reduction of CD8+ TRM count in the epidermis during the treatment.

The latest study assessing the profile of lymphocytes in psoriatic eruptions after treatment with secukinumab (anti-IL-17) and guselkumab (anti-IL-23) demonstrated that both therapies reduced the number of inflammatory dendritic cells and CD4+CD49a-CD103- T cells. Interestingly, guselkumab reduced TRM count promoting regulatory

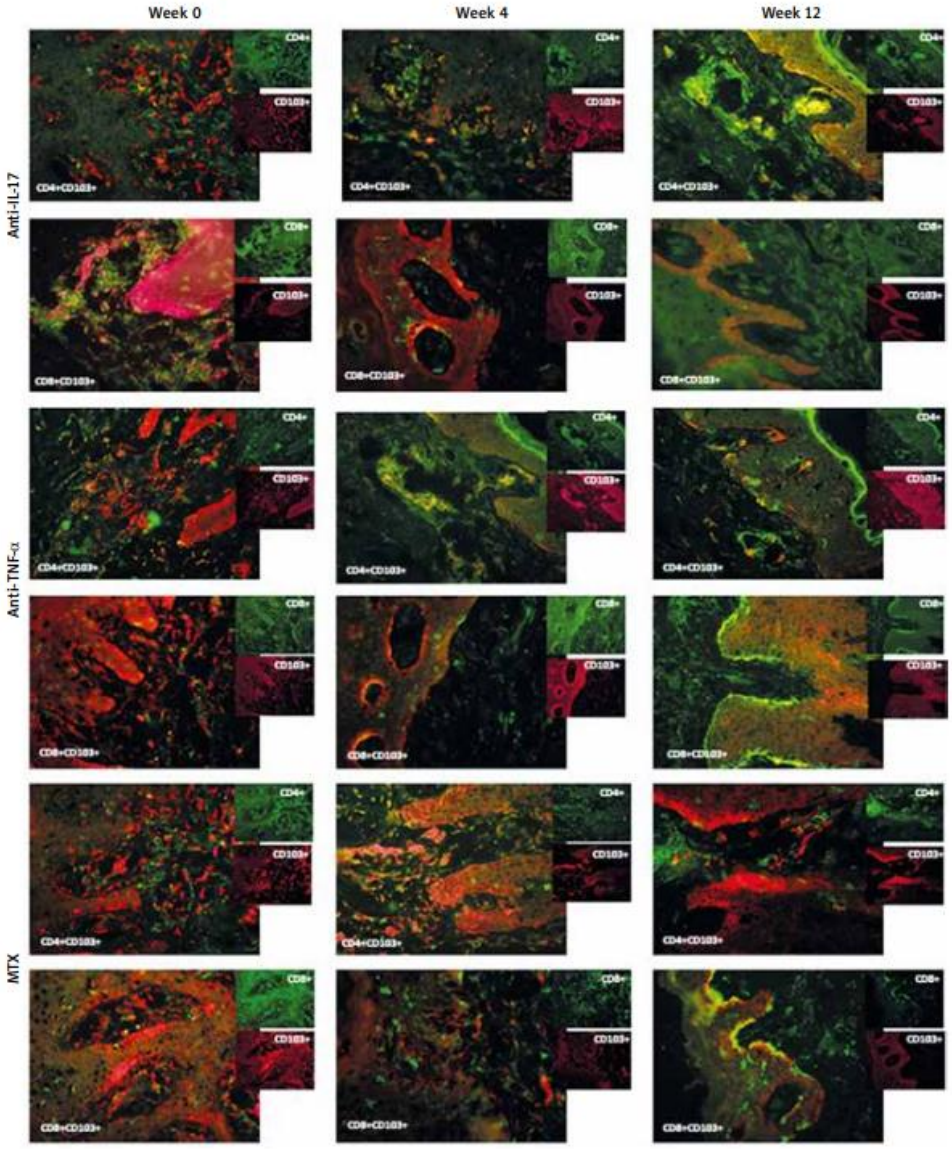


Figure 6. The skin location of CD8 (green – Alexa 488), CD4 (green – Alexa 488) and CD103 (red – Alexa 594) proteins (the representative sections) in the lesional skin of patients (n = 5 or n = 4, dermis and epidermis) before (week 0) and during treatment (week 4 and week 12) with: anti-interleukin-17 (anti-IL-17), anti-tumour necrosis factor α (anti-TNF-α), methotrexate (MTX). Magnification: 500×

T cells at the same time, while in case of secukinumab the situation was reversed. Furthermore, none of the drugs changed the quantity of IL-17A+IL-17F+/– CD4+ or CD8+ T cells [22].

It is a very important conclusion from the study, as longer remissions may be triggered after suppressing only IL-23, and not IL-12, depending on the therapy. Interleukin-12 suppresses the production of γδT (γδT17) cells involved in IL-17 production. Moreover, according to the literature, IL-12 receptor signalling in keratinocytes initiates a protective, anti-inflammatory transcriptional program [23]. It explains more rapid relapses of the disease after blocking IL-17 compared to IL-23 inhibition [17, 22, 24].

Gallais Sérézal *et al.* reported an increased expression of IL-17, which was still produced by CD8+ CD103+ TRM cells in the epidermis of successfully treated psoriasis patients. Trigger factors are responsible for the rapid activation of TRM in the epidermis, IL-17 production by TRM, which stimulates keratinocytes to release chemokines and recruit circulating leukocytes [23, 25, 26]. Our results were similar, i.e. the treatment resulted in a slight decrease in IL-17 expression in the epidermis.

It was noted in the present study that the reduction of the expression of TRM-specific antigens was observable predominantly in the lesional dermis, and not in the lesional epidermis. It may be due to several factors. There is a paucity of data concerning the penetration of systemic drugs into the epidermis – the absence of vasculature may contribute to poorer penetration of systemic drugs translating into poorer treatment outcomes. It is possible that the process of psoriatic lesion resolution in the epidermis and its gradual normalization are secondary to the reduction of inflammation in the skin, or epidermal TRM cells are more resistant to therapy. Therefore, a question arises: "How may topical drugs influence TRM quantity in psoriatic eruptions?" The above question will underlie our subsequent analysis.

We do realize that the small sample size constitutes a limitation of this study. Regrettably, the study was arduous and it required triple biopsy sampling at the same location of an eruption, which was difficult for the patient. The research is still ongoing and a longer observation time would be advisable (a repeat biopsy after 24 weeks).

Another weakness of the study is related to technical limitations. It would be indicated to use a higher number of markers in immunofluorescence in order to increase the specificity of the method.

Conclusions

The understanding of the mechanisms of psoriatic inflammation and the role of TRM may explain the key problems associated with the disease:

– the resistance of lesions to treatment and the reactivation of lesions at the same location,
– the isomorphic Koebner phenomenon,
– the appropriate duration of treatment, longer than the remission of eruptions, in order to suppress and reduce the quantity of TRM [1, 26, 27].

The detailed elucidation of mechanisms responsible for the development/relapses of the disease may contribute to the implementation of more effective therapeutic protocols.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

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Article

The Effect of the Long-Term Calcipotriol/Betamethasone Dipropionate Local Therapy on Tissue Resident Memory Cells Markers in Psoriatic Eruptions

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Abstract: Background: The natural course of psoriasis is characterized by the long-term persistence of lesions and a predilection for relapse in the same area. It is caused by the inherence of TRM (tissue resident memory T cells) in apparently healthy skin. These cells are able to initiate an inflammatory cascade and induce relapse of the disease. These cells are characterized by high resistance to damaging factors and apoptosis, which determines their longevity. Aim: The aim of our study was to evaluate the presence of TRM in psoriatic plaques before, during and after 12 weeks of therapy in patients treated with topical calcipotriol and betamethasone dipropionate (Cal/BD) foam. Methods: TRM markers (CD4, CD8, CD103, CD69, CD49, CXCR6) and tissue expression of cytokines (IL-17A, IL-22) in the lesional psoriatic skin from 10 patients compared to 10 healthy skin samples were estimated by immunohistochemistry. Biopsy samples from the area of the same psoriatic plaque were collected three times: before the initiation of therapy, 4 and 12 weeks after its initiation. Results: The presence of TRM markers in the epidermis and dermis of psoriatic lesions was significantly higher when compared to the skin of control group patients. A reduction in the expression of the characteristic TRM markers (CD8, CD4, CD103, CD69, CXCR6, IL-17A and IL-22) was observed in the epidermis on week 12 of therapy, while a depletion in the expression of TRM in the dermis was demonstrated only in CD4 and IL-22. Conclusions: Topical treatment with Cal/BD foam significantly decreased the expression of TRM markers mainly in the epidermis, and to a lesser extent in the dermis, during the 12-week observation period. It probably results from a worse penetration of the drug into the dermis and the effect of the preparation mainly on the epidermis. The persistence of a high expression of TRM markers in the dermis may result in the rapid recurrence of lesions after discontinuation of topical treatment.

Keywords: psoriasis; tissue resident memory cells; TRM; immune memory; calcipotriol; betamethasone dipropionate

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1. Introduction

There were 4,622,594 (95% uncertainty interval) incident cases of psoriasis worldwide in 2019 [1]. Psoriasis is a chronic and recurrent inflammatory skin disease mediated by T lymphocytes. It is characterized by the appearance of skin lesions in certain places and relapses of the disease in the same location [2]. The recurrent nature of psoriasis is related to the immune memory in the skin and likely depends on tissue resident long-lived memory T cells (TRM) [3]. These cells are able to initiate the inflammatory cascade and thus contribute to disease relapse in apparently healthy skin [4]. Both CD4+ and CD8+ TRM have been described not only in the skin but also in other tissues, including the mucosa, brain, gastrointestinal tract, lungs and pancreas [3]. Resident memory T cells remain in peripheral tissues for extended periods of time, are non-recirculating and assure the first line of adaptive immune responses in resident tissues [5,6]. TRM differ physiologically

from circulating T cells in their expression of tissue residence markers such as CD69 and CD103 and in their distinguishing profile of transcription factors [7]. There are two main types of TRM: CD8+, which predominates in the psoriatic epidermis and express mainly the CD103, CD69, CD49a, and TRM CD4+, which are located close to the blood vessels in the dermis and have a high proliferative capacity [8]. The remaining molecules that differentiate TRM from other types of circulating memory T cells are CD101, CXCR3 and CXCR6 [4,8]. CD8+ TRM in the skin of patients suffering from psoriasis express the IL23 receptor, and, therefore, are able to produce pro-inflammatory interleukin (IL)-17 and IL-22 in the psoriatic skin, even for many months after remission [4,9]. In psoriasis, CD8+ TRM-producing IL-17A are considered to be one of the pathogenic skin populations involved in the pathogenesis of the disease [10].

Although TRM and their role in autoinflammatory diseases have been the subject of research for several years, we still do not have many reports on the effect of specific therapies on the presence of TRM in the skin of patients who are treated general and topical for plaque psoriasis. It is known that these cells are highly resistant to damaging factors and apoptosis, which probably determines their longevity. A significant positive dependence has been demonstrated between the expression of TRM markers in patients with psoriasis vulgaris and the persistence of skin lesions [8,11]. The rapid relapse of psoriatic lesions in the same localization after the action of the trigger factor may be explained by the longevity and accumulation of TRM in the skin of psoriatic patients [4].

2. Methods

2.1. Study Group

The study group included 10 patients of the Outpatient Clinic at the Department of Dermatology, Sexually Transmitted Diseases, and Clinical Immunology in Olsztyn with plaque psoriasis, without psoriatic arthritis, untreated for at least 4 weeks. The inclusion criterion for the study group was a Psoriasis Area Severity Index (PASI) lower than 10 and a body surface area (BSA) lower than 10%. Patients suffering from chronic and acute inflammatory diseases and dermatoses (other than psoriasis), neoplastic diseases, heart, kidney and liver failure, and nicotine or alcohol addiction were excluded. The control group included healthy volunteers (n = 10) with no personal or family history of psoriasis and no concomitant autoimmune and inflammatory disorders. Skin samples from psoriasis patients were obtained from psoriatic plaques which were relapsing at the same sites after the end of previous treatment three times—before the initiation of therapy (week 0), after 4 and 12 weeks of treatment. Patients used the topical drug containing Cal/BD once a day for the 4 subsequent weeks, then as a maintenance (proactively) twice a week. Healthy volunteer skin samples were collected from surgical wastes left after removal of pigmentary lesions located on the trunk or limbs. In the study group, we also assessed the severity of the disease using the following scales: PASI, BSA, DLQI.

2.2. Clinical Samples

Assessment of TRM in biopsy specimens was performed by the immunofluorescence method according to those described previously [8], with modifications. We obtained three 4 mm punch biopsies per patient from lesional skin and one from healthy volunteers (non-lesional skin), using local anesthesia (1% lignocaine). Tissue samples were cut into 5 µm thick sections in the cryostat (SLEE MEV, Nieder-Olm, Germany) and mounted onto glass slides coated with poly-L-lysine (Menzel-Glaser, Braunschweig, Germany). Sections were fixed in acetone for 10 min and rinsed in 0.01 M PBS. Next, they were incubated with 2.5% normal horse serum for 30 min at room temperature (Vector Laboratories, Burlingame, CA, USA) to decrease nonspecific binding. Sections were incubated at 4 °C overnight with mouse anti-CD8 and CD4 or rabbit anti-CD103, CD69, CD49, CXCR6, IL-17A and IL22 polyclonal antibody (1:50; Merck Millipore, Billerica, MA, USA). On the following day, the sections were washed in PBS and incubated for 30 min with secondary horse anti-mouse/rabbit antibodies (commercially diluted; ImmPRESS Universal reagent Anti-

Mouse/Rabbit Ig; Vector Laboratories, Burlingame, CA, USA). In negative controls, primary and/or secondary antibodies were replaced with 0.01 M PBS. The immunohistochemical specimens were evaluated under a fluorescent microscope (CH30/CH40; Olympus, Tokyo, Japan).

The used analysis of images was based on those previously described by the Leska et al. method [12]. TRM staining was performed in triplicates (three slides for each visit of every patient). Next, images of five different areas for epidermis and dermis were taken (under 500 magnification), resulting in 15 images per one visit (separately for epidermis and dermis). The same procedure was performed in for the healthy control. The images were analyzed semiquantitatively. According to the previous publications as the assessed parameter, the immunoreactive area (the ratio of the area occupied by the immunopositive cells to the total area occupied by epidermis/dermis) was chosen. The level of each TRM immunoreactivity was measured using ImageJ using a threshold function to select a range of grey values that were optically identified as positive staining. In the statistical analysis, percentage data were transformed arcsin.

The results were assessed statistically by a non-parametric Mann–Whitney U test. The results are expressed as means ± SEM. Correlation between proteins was analyzed by Spearman's test ($p < 0.05$). All calculations were performed using the Statistica software, release 13 (Statsoft, Inc., Tulsa, OK, USA). Values $p < 0.05$ were considered as statistically significant.

The study was approved by the Bioethical Committee of the Warmia and Mazury University in Olsztyn (Resolution 24/2020). Informed consent was obtained from each patient enrolled in the study.

3. Results

3.1. Immunoreactive Area and Skin Localization of TRM Markers in Lesional Skin before Treatment in Comparison with Healthy Control (Week 0)

In patients with psoriasis, an increase in the CD8 and CD69, IL-17A and IL-22 immunoreactive area in the epidermis was found, but it did not change significantly in the dermis compared to the control group. In the case of CD103, CXCR6, an increase in the immunoreactive area in both the epidermis and the dermis was found compared to the control group. This was similar in the case of the CD4 immunoreactive surface, where the amount of the marker was dominant in the dermis and not in the epidermis, as in the case of CXCR6 (Figure 1).

3.2. Immunoreactive Area and Skin Localization of TRM Markers in Lesional Skin after 4 Weeks of Treatment (Week 4)

After 4 weeks of therapy, a statistically significant decrease in the immunoreactive area was observed only in the case of CD4 markers in the epidermis. Other markers were also reduced, but no statistically significant changes were achieved in the epidermis and dermis (Figure 1).

3.3. Immunoreactive Area and Skin Localization of TRM Markers in Lesional Skin after 12 Weeks of Treatment (Week 12) in Comparison with Healthy Control

Statistically significant decreases in fluorescence area compared to week 0 were observed for the markers: CD8 (8.51 ± 1.2% vs. 15.43 ± 2.1%); CD4 (1.78 ± 0.4% vs. 5.46 ± 1.1%); CD103 (11.72 ± 0.9% vs. 23.26 ± 3.8%); CD49 (16.79 ± 3.7% vs. 11.23 ± 2.1%); CD69 (16.31 ± 1.8% vs. 26.98 ± 1.1%); CXCR6 (8.10 ± 1.1% vs. 11.21 ± 2.5%), IL-17A (15.71 ± 2.1% vs. 25.27 ± 3.8%) and IL-22 (12.16 ± 2.0% vs. 22.29 ± 3.8%) within the epidermis.

It was completely different in the case of the dermis. We observed a decrease in the immunofluorescent surface only in the case of: CD4; IL-22 (Table 1).

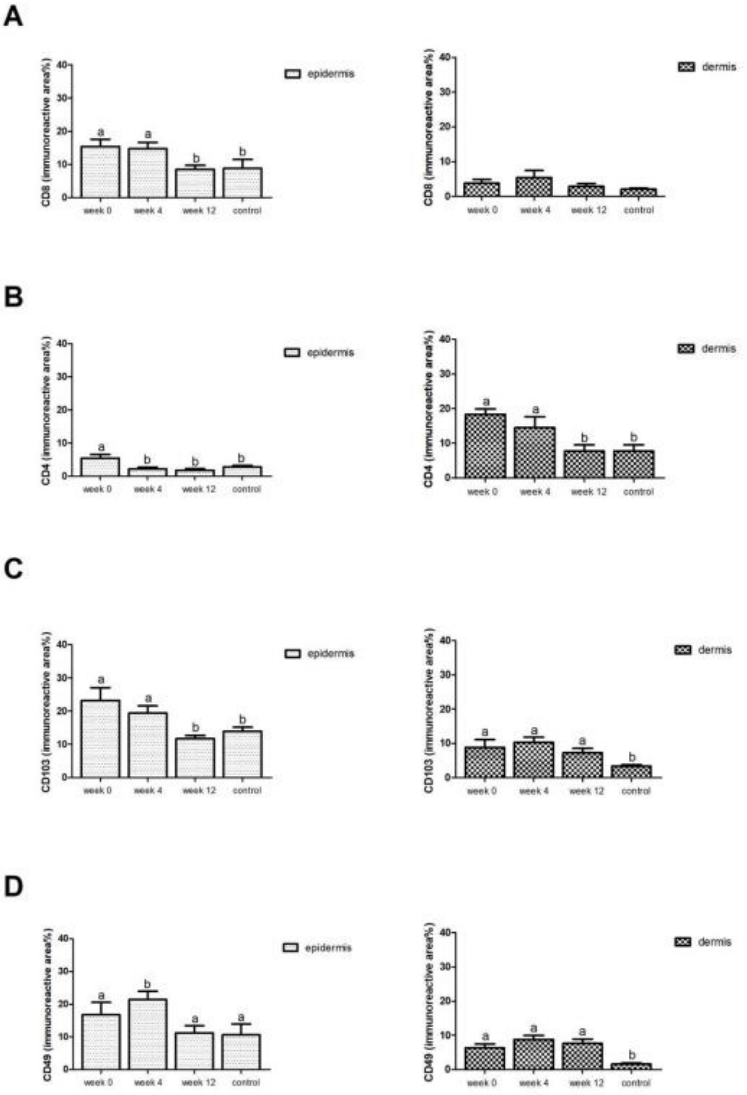


Figure 1. *Cont.*

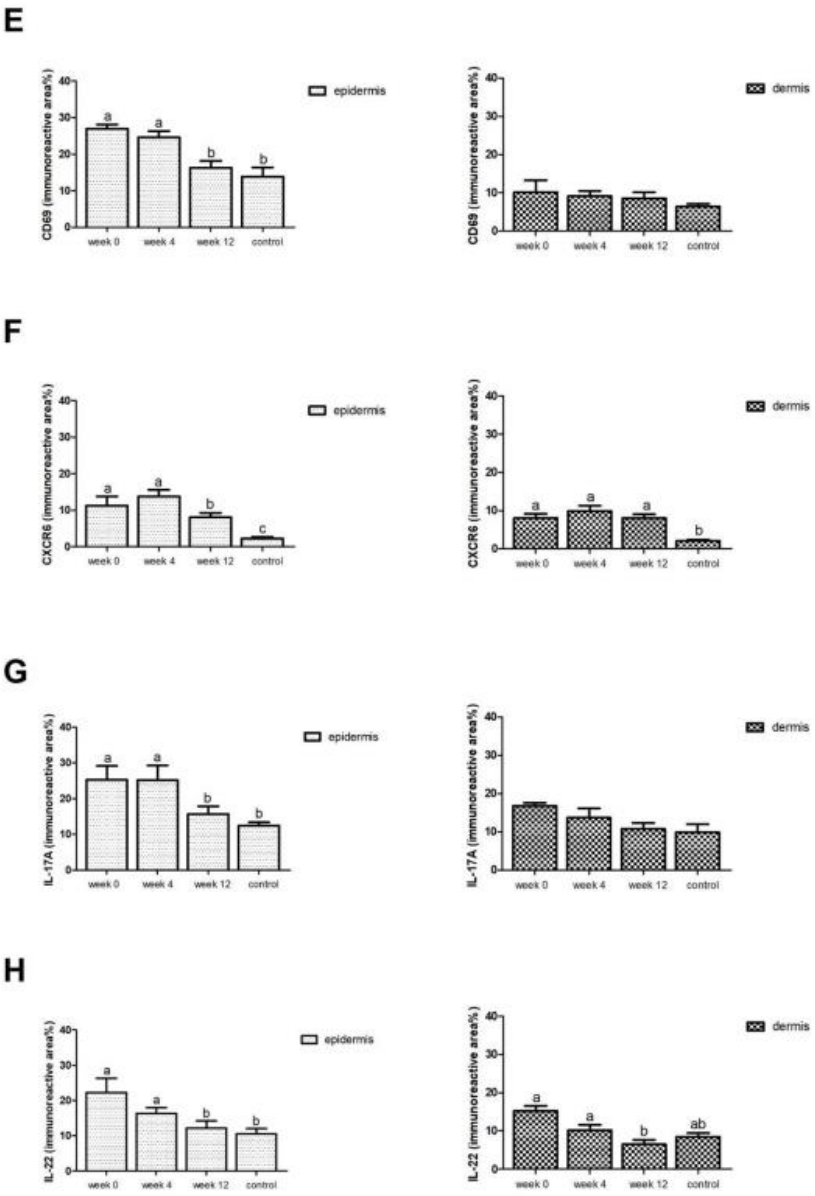


Figure 1. Immunoreactive area (means ± SEM) of TRM (in epidermis and dermis separately) of (**A**) CD8, (**B**) CD4, (**C**) CD103, (**D**) CD49, (**E**) CD69, (**F**) CXCR6, (**G**) IL-17A, (**H**) IL-22 in lesional skin during week 0, 4 and 12 ($n = 10$) in comparison with healthy control ($n = 10$, dermis and epidermis). Different letters indicate significant differences ($p < 0.05$) between the various measurement points, whereas the same letters indicate a lack of differences ($p < 0.05$).

Table 1. Immunoreactive area (%) of TRM markers (X ± SEM) in lesional skin (n = 10, dermis and epidermis) at week 0, week 4 and week 12 in comparison with healthy control (n = 10, dermis and epidermis). Asterisks show the statistically significant differences between analyzed groups (week 0 and 12). ↓—means decrease, ↑—means increase.

	Week 0		Week 4		Week 12		Changes from Week 0 to 12 (%)		Healthy Control	
	Epidermis	Dermis	Epidermis	Dermis	Epidermis	Dermis	Epidermis	Dermis	Epidermis	Dermis
CD8	15.43 ± 2.1	3.83 ± 1.0	14.74 ± 1.9	5.46 ± 2.1	8.51 ± 1.2	2.84 ± 0.9	↓45 *	↓15	8.83 ± 2.68	2.04 ± 0.33
CD4	5.46 ± 1.1	18.34 ± 1.5	2.17 ± 0.5	14.5 ± 3.0	1.78 ± 0.4	7.71 ± 1.8	↓67 *	↓57 *	2.77 ± 0.49	7.71 ± 1.80
CD103	23.26 ± 3.8	8.81 ± 2.2	19.38 ± 2.1	10.25 ± 1.5	11.72 ± 0.9	7.23 ± 1.3	↓49 *	↓17	13.88 ± 1.28	3.37 ± 0.42
CD49	16.79 ± 3.7	6.31 ± 1.1	21.40 ± 2.5	8.78 ± 1.1	11.23 ± 2.1	7.6 ± 1.2	↓33 *	↑20	10.66 ± 3.23	1.55 ± 0.34
CD69	26.98 ± 1.1	10.16 ± 3.0	24.57 ± 1.7	9.1 ± 1.3	16.31 ± 1.8	8.58 ± 1.5	↓39 *	↓15	13.89 ± 2.51	6.39 ± 0.69
CXCR6	11.21 ± 2.5	8.02 ± 1.1	13.7 ± 1.8	9.92 ± 1.3	8.10 ± 1.1	8.03 ± 0.9	↓27 *	0	2.24 ± 0.41	2.05 ± 0.33
IL17	25.27 ± 3.8	16.76 ± 0.8	25.17 ± 4.1	13.69 ± 2.4	15.71 ± 2.1	10.76 ± 1.5	↓37 *	↓35	12.45 ± 0.94	9.85 ± 2.14
IL22	22.29 ± 3.8	15.26 ± 1.3	16.29 ± 1.6	10.22 ± 1.3	12.16 ± 2.0	6.52 ± 1.1	↓45 *	↓57 *	10.54 ± 1.44	8.45 ± 1.03

After 12 weeks, the immunofluorescent surface of TRM markers in patients' epidermis and dermis similar to the control group was observed in the case of: CD8; CD4; CD69; IL17; IL-22 (Figures 1 and 2).

After 12 weeks of treatment in all patients in the study group, we also found a statistically significant clinical improvement in the severity of the disease—as measured by the PASI and BSA scales, and an improvement in quality of life measured by the DLQI (Table 2).

Table 2. Disease activity measured by PASI, BSA and DLQI scales in patients (n = 10) treated with calcipotriol/betamethasone dipropionate foam during week 0, 4 and 12. Asterisks show the statistically significant differences between week 0 and week 12. ↓—means decrease, ↑—means increase.

	Week 0	Week 4	Week 12	Changes from Week 0 to 12 (%)	
PASI	6.91 ± 0.8	3.32 ± 0.59	2.1 ± 0.34	↓69 *	p = 0.0008
BSA	8.13 ± 1.09	5.62 ± 0.98	3.12 ± 0.35	↓61 *	p = 0.002
DLQI	11.18 ± 0.86	8 ± 1.11	6 ± 1.08	↓46 *	p = 0.02

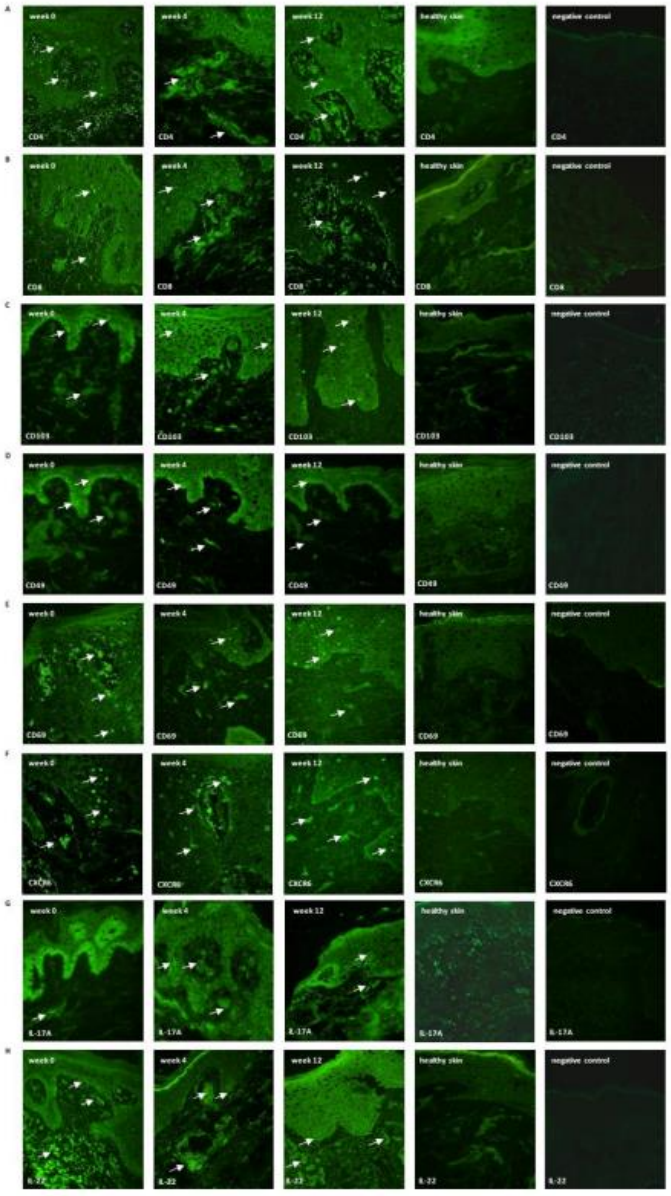


Figure 2. Skin localization of (**A**) CD8, (**B**) CD4, (**C**) CD103, (**D**) CD49, (**E**) CD69, (**F**) CXCR6, (**G**) IL-17A, (**H**) IL-22 (the representative sections): lesional skin during week 0, 4, and 12 (n = 10); healthy skin (n = 10) and negative control (without primary antibody). The proteins are marked in green (white arrows, fluorescein). Magnification: 500×.

4. Discussion

To date, no registered drugs are available that directly and specifically inhibit activity of skin TRM; however, current treatments for psoriasis are known to affect TRM of skin with psoriasis [10,13]. There are still few studies available in the literature describing the effect of systemic therapy—biological drugs, systemic drugs and phototherapy—on TRM in the skin of patients. Even less is known about the effects of various topical treatments on TRM in patients' skin. While therapies targeting tumor necrosis factor (TNF)-α, IL-23 and IL-17 have been proven effective in psoriasis, topical treatment remains the method of choice in most cases. This is due to the severity of skin lesions and the convenience, safety and economic effectiveness of local therapy. Among the topical medications, the most commonly used are glucocorticosteroid preparations, calcineurin inhibitors and vitamin D3 derivatives (vitamin D3 analogues) [14]. The popular drug of the first choice is a combined preparation containing betamethasone dipropionate at a dose of 0.5 mg/g and calcipotriol at a dose of 50 μg/g. Therefore, we decided to assess the effect of topical therapy with a combined foam preparation containing betamethasone dipropionate and calcipotriol on the number of TRM markers in the area of skin lesions subjected to 12-week therapy.

In psoriasis, the balance between the Th1 and Th2 immune response pathways is disturbed, with a predominance towards Th1 [15]. Activated Th1 cells secrete large amounts of TNF-α and interferon γ (IFN-γ), activating dendritic cells (DCs) and stimulating keratinocytes. On the other hand, activation of Th17 and Tc17 lymphocytes leads to an increased production of cytokines such as IL-17A and IL-22, which enhance excessive proliferation and abnormal differentiation of the epidermis [16–18]. In plaque psoriasis, vitamin D and its analogs work mainly by inhibiting proliferation and inducing keratinocyte differentiation by inducing inhibition of transforming growth factor β and cyclin-dependent kinase inhibitors. In addition, vitamin D has an immunomodulatory effect by inhibiting the activation and differentiation of Th17/Th1 cells and inducing a Th2/Threg response [19]. Betamethasone dipropionate belongs to the group of synthetic, fluorinated glucocorticosteroids with a strong effect (class III according to the WHO classification). Glucocorticosteroids in psoriasis suppress the immune system, especially the pro-inflammatory cytokines and chemokines, and thus inhibit the activation of T cells [18]. Betamethasone dipropionate and calcipotriol in the combined preparation complement each other in terms of immunosuppressive, immunomodulating and antiproliferative effects [18]. The secretion of TNF-α by Tc1 and Th1 lymphocytes is inhibited, which prevents the subsequent activation of DCs and keratinocytes, and these substances have an additive effect on reducing IL-17 production by Th and Tc cells [20,21]. The effectiveness of the combination of these drugs is supported by the fact that GCS inhibit both Th1 and Th2 immune mechanisms, while vitamin D3 derivatives have an opposite effect in the Th2 response, which in total results in a slight induction of the Th2 pathway. Balancing the Th1/Th2 ratio may prevent the rebound phenomenon after the end of therapy [18,22].

Dyring et al. investigated the impact of the vitamin D analogue—calcipotriol on the incidence of CD4+ and CD8+ T cells and innate lymphoid cells (ILC) and their production of IL-17A, IFN-γ and IL-22 in psoriatic plaques in patients suffering from psoriasis vulgaris. The authors observed after 14 days of treatment with calcipotriol, a relevant clinical and histological effect; however, they found no significant differences in the incidence of CD4+ and CD8+ T cells or ILC between calcipotriol-treated skin and vehicle. Interestingly, the authors revealed that calcipotriol reduces the incidence of CD8+ IL-17+ T cells in psoriatic plaques, accompanied by clinical and histological improvement. No changes in the frequency of IL-22+ or IFN-γ+ cells were observed [23]. In various studies on the effectiveness of calcipotriol in monotherapy, divergent results were observed for CD4+ and CD8+—some authors reported a significant decrease in these cells after 4 weeks of therapy [24,25], while others found no significant changes in the amount of CD4+ and CD8+ [23,26,27].

Much research has also been done on the combined efficacy of topical corticosteroids and vitamin D3 analogues. Most reports found combination therapy to be more effective

than a single agent in terms of therapeutic efficacy or anti-inflammatory response [21]. During short-term therapy Cal/BD, Dyring et al. did not observe any significant changes in the amount of IL-22 [23]. On the other hand, Ikeda et al. showed a reduction in the level of IL-22 [28].

Fujiyama et al. assessed the effect of topical treatment in monotherapy with calcipotriol or betamethasone, and with two-component combination therapy on psoriatic plaques during 14 days of therapy. In all samples, clinical and histological improvement was achieved and a reduction of IL-17A+ cell infiltration occurred, but the best effectiveness was shown by two-component therapy. There was observed clinical, histologic and IL-17A+ cell-infiltrate improvement [21]. Additionally, evaluation of the number of ex vivo expanded T cells showed the greatest decrease by topical application of combination therapy. The number and frequency of Th17 cells were significantly reduced by Cal/BD and Cal, suggesting that Cal has the ability to selectively suppress Th17 cells.

Vissers et al. determined the effect of combining calcipotriol ointment once daily and betamethasone dipropionate ointment once daily compared to the effect of using each drug twice daily alone over a four-week treatment period. Markers of epidermal proliferation (Ki-67) and epidermal differentiation (keratin-10) were assessed, and a quantitative analysis of the image and subsets of T lymphocytes in the epidermis and dermis (CD4, CD8, CD25, CD45RO, CD45RA, CD94, CD161 and CD2) was performed. Calcipotriol was shown to have a large effect on the Ki-67 proliferation marker and the keratin-10 epidermal differentiation marker, while the effect on T cell subsets was more selective with significant reductions in CD45RO+ and CD8+ T cells. In contrast, the effect of betamethasone dipropionate on the epidermis was limited to normalization of differentiation with a highly significant increase in the surface of the epidermis positive for keratin-10 with no significant effect on the Ki-67 positive nuclei, and the effect on the T cell subsets was limited to the depletion of NK T receptors labeled as CD94 and CD161 in the epidermis. The coupling of the two therapies had no effect on the proliferation marker Ki-67 and the keratin-10 marker, over and above the effect of calcipotriol monotherapy. Moreover, the combination of the two approaches reduced virtually all T-cell subsets, and the efficacy was significantly higher than that of monotherapy [24].

When assessing the presence of TRM markers in psoriatic skin lesions before, during and after 12-week therapy in patients treated with topical Cal/BD, we showed that this therapy reduces the expression of TRM markers mainly in the epidermis, but does not significantly influence the expression of these markers in the dermis. Reduced expression was observed with CD8, CD103, CD69, CD4, CXCR6, IL-17A and IL-22 within the epidermis, and in the dermis, only CD103, CD4 and IL-22 decreased in the immunofluorescent surface area (Figures 1 and 2). This is probably due to the lower penetration of topical drugs into the dermis and acting mainly at the epidermis level. Interestingly, in another study we conducted [29] comparing the effect of various systemic therapies (methotrexate, adalimumab, anti-IL-17) on the amount of TRM in different layers of the skin, a decrease in fluorescence was found for each treatment. The fastest response, already in the 4th week of treatment, was observed in the case of anti-IL17, while in the case of methotrexate and adalimumab, a statistically significant decrease was observed in the 12th week of therapy. An interesting conclusion from the study was the fact that in the case of all the above-described therapies, we observed a decrease in immunofluorescence in the dermis, and not in the epidermis, after 12-week follow-up [29]. Similar conclusions were reached by Kurihara et al., observing the resistance of CD8+ TRM in the epidermis to general treatment (CsA, PDE4 and biological) [13]. Taking into account the literature reports and the results we have published, it seems that combining general therapy with local therapy may have an impact on achieving a more effective reduction of TRM in both the dermis and epidermis. Perhaps this would have a potential impact on the duration of remission?

It is not known how long treatment should take to completely eliminate TRM and prevent rapid relapses, possibly depending on the therapy used. Relapses are common, especially with reactive treatments, since TRM remain capable of initiating an inflammatory

cascade and inducing relapses of psoriatic plaques despite clinical resolution of the skin lesions. These cells are characterized by a high resistance to damaging factors and apoptosis, and also have the ability to transform into long-lived TRM. A long-term, proactive approach to treatment, including supportive care after successful initial treatment, can help to prolong disease remission and improve clinical outcomes, and may positively impact patients' quality of life [30]. The results of the Phase III PSO-LONG trial showed that long-term proactive management was superior to reactive management in reducing the number of relapses and extending the days of remission in adult patients with plaque psoriasis [31]. In our study, a statistically significant effect of local treatment Cal/BD measured by a decrease in the immunofluorescent surface area of specific TRM markers was observed only in the 12th week of therapy (CD8, CD103, CXCR6, IL-17A, IL22), and in the 4th week of treatment, only CD69 and CD4 decreased. Perhaps longer periods of remission and a reduction in the number of relapses during proactive therapy are associated with a significant reduction in TRM in the skin of patients. In the case of most topical medications, according to the characteristics of medicinal products, treatment can be continued for max. 3–4 weeks, which, based on our research, seems to be insufficient time to achieve TRM reduction. So far, only Cal/BD can be used in the treatment of psoriasis in proactive therapy (4 weeks of treatment once a day, then maintenance twice a week).

5. Conclusions

Cal/BD local therapy reduces the expression of TRM markers mainly in the epidermis but does not significantly affect their expression in the dermis.

The decrease in markers was observed in the 12th week of therapy, which confirms the validity of proactive therapy in the local treatment of plaque psoriasis. Four weeks of continuous therapy did not significantly reduce the amount of TRM in the skin of patients with psoriasis vulgaris.

6. Limitations

The small sample size is a limitation of this study. The study required a triple biopsy at the same site, which was difficult for the patient to accept. The studies are still in progress; a longer follow-up period is recommended (repeated biopsy after 24 and 52 weeks), which would enable further evaluation of changes in TRM markers and would also be enable their correlation with the duration of disease remission. Another weakness of the study is related to technical limitations. It would be advisable to use more markers in immunofluorescence and to perform co-staining of the sections to increase the specificity of the method.

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Institutional Review Board Statement: The study was approved by the Bioethical Committee of the Warmia and Mazury University in Olsztyn on 29 April 2020 (Resolution 24/2020). Informed consent was obtained from each patient enrolled in the study.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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Review paper

Does molecular scarring in psoriasis exist? A review of the literature

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Abstract

Plaque psoriasis is a chronic inflammatory dermatosis characterized by a tendency to recur in the same locations after discontinuation of treatment. The implementation of therapy with drugs targeting cytokines like interleukin (IL) 17A (IL-17A) and IL-23 has revolutionized the treatment of psoriasis and enabled the achievement of skin without lesions. However, despite the clinical resolution of psoriatic eruptions, cells that maintain the local memory of the disease remain in the dermis and epidermis, constituting a kind of molecular scar. The cells responsible for maintaining memory in the skin of patients and influencing the rapid relapse of the disease after the triggering factor are primarily tissue resident memory T cells (TRM), but it seems that regulatory T lymphocytes (Treg), dendritic cells (DC), and Langerhans cells (LC) may also play an important role in this process. We reviewed the literature to explain the concept of molecular scarring in psoriasis, and to assess the effect of various therapies on immune memory.

Key words: tissue resident memory T cells, molecular scar, regulatory T lymphocytes, dendritic cells, Langerhans cells.

Introduction

The global prevalence of psoriasis is estimated at 1% to 3%, of which 85–90% is plaque psoriasis, chronic, inflammatory, immune-mediated dermatosis characterized by well demarcated erythematous-papular lesions covered with scales [1]. Histologically, there is hyperproliferation and incomplete differentiation of keratinocytes, acanthosis, retention of cell nuclei within stratum corneum, and loss of the granular layer. The epidermis has a dense infiltrate of T lymphocytes and dendritic cells (DC) [2]. In psoriasis, there is an immunologically induced hyperproliferation and functional stimulation of keratinocytes, which is mediated by numerous cytokines released by T lymphocytes, including interleukin (IL) 1 (IL-1), IL-6, IL-8, IL- 12, IL-17, IL-18, IL-22, IL-23, tumour necrosis factor (TNF), interferon (IFN), and transforming growth factor-β (TGF-β). Proinflammatory cytokines are also secreted by epidermal and dermal cells, including keratinocytes, DC, endothelial cells and fibroblasts [3]. A characteristic feature is the appearance of psoriatic lesions in specific places, as well as recurrences of the disease in the same location after discontinuation of treatment. Despite the clinical resolution of the lesions, within the dermis and epidermis, differences in the populations of cells of the immune system and at the molecular level, constituting local memory, are still observed [4]. Cells responsible for local memory include primarily tissue resident memory T cells (TRM), but regulatory T lymphocytes (Treg), DC, and Langerhans cells (LC) also seem to play an important role in this process. The types of cells, their interactions, and mechanisms that may be involved in molecular scar formation and maintaining the local memory of disease, as well as the effect of various therapies on their levels, are discussed below.

Types of cells forming local memory in the skin

Tissue resident memory T cells

Tissue resident memory T cells are a subset of non-circulating memory T cells. They arise as a result of the activation of naive T cells by antigen-presenting cells. Precursor cells evolve into few subsets of memory T cells, including central memory T cells (TCM), effector memory

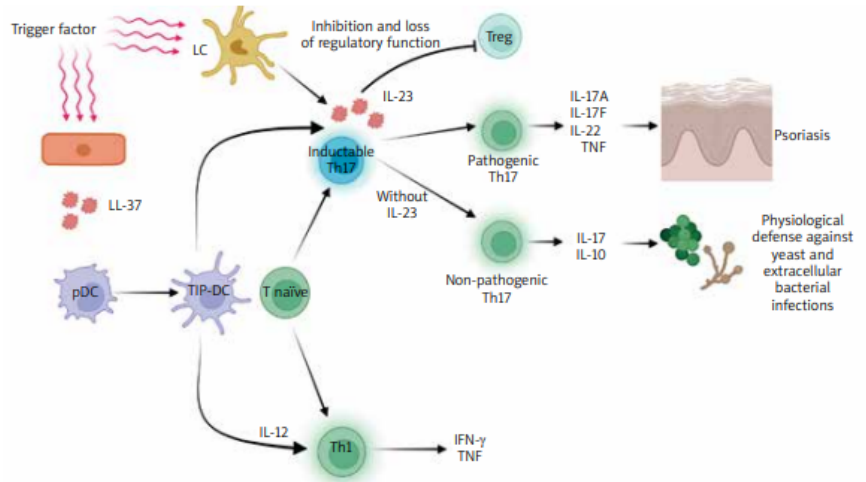
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T cells (TEM), and tissue resident memory T cells (TRM). TEM predominate in peripheral non-lymphoid tissues, while TCM are localized in secondary lymphoid organs [5]. TRM cells are phenotypically distinct because they express molecules that prevent tissue egress, in particular the CD69 antigen responsible for TRM deposition by prevention of their recirculation through suppression of the sphingosine-1-phosphate receptor (S1P1) or the CD103-E-cadherin adhesion receptor, determining the localization of TRM in the epithelial tissue. TRM also express the CD49a molecule, which links collagen IV found in the basal layer [6]. In addition, TRM have receptors for chemokines such as CCR4, CCR8, CCR10, CXCR3, and CXCR6, which are considered as salient molecules for directing or retaining TRM in the skin [7]. The most important task of TRM is the protective function against infections; however, they are also involved in the pathogenesis of many dermatological diseases, including vitiligo, lymphomas, drug eruptions, and psoriasis.

T lymphocytes infiltrating psoriatic lesions play a crucial effector role, stimulating the development and maintenance of the disease [8]. Gilhar *et al.* transplanted psoriatic skin into the severe combined immunodeficient (SCID) mice, which were then injected with autologous T cells. Maintenance of skin pathology within a transplanted psoriatic skin lesion was not only dependent on T-cell, but skin-originated T cells were more effective in maintaining pathology compared to blood-originated T cells from psoriatic patients [9]. Another study revealed that immunodeficient mice can evolve psoriatic plaques after transplantation of healthy-looking skin from mice with psoriasis [10].

In the skin of patients suffering from psoriasis, TRM consists of CD8 and CD4 fractions, which increase the expression of IL-17A, IL-22, and IFN-γ – inflammatory cytokines, which are of key importance in the pathogenesis of plaque psoriasis [7]. TRM can be activated upon recognition of various antigens or cytokines/chemokines, leading to a characteristic relapse after treatment. Autoantigens include but are not limited to neolipid antigens, antimicrobial peptide LL37, and disintegrin and metalloproteinase with thrombospondin motif-like protein 5 (ADAMTSL5) produced by melanocytes [2, 6], PLAG4D from mast cells and keratinocytes, and keratin 17 from keratinocytes [7]. The antimicrobial peptide LL-37, also derived from damaged keratinocytes, binds to its own DNA/RNA thus activating plasmacytoid dendritic cells (pDC) and TNF and nitric oxide releasing dendritic cells (TIP-DC), leading to the formation of key inflammatory cytokines IL-23 and TNF-α. In psoriasis, effector T cells producing IL-17A (Th17 and Tc17) are dependent on IL-23 for survival [11]. IL-23 also inhibits the function of Treg, leading to loss of their regulatory function. Activated DC produce IL-12, which stimulates Th1/Tc1 differentiation; these cells produce IFN-γ and TNF-α (Figure 1). There is also the activation of myeloid dendritic cells (mDC) mi-



IL – interleukin, IFN-γ – interferon γ, Th – T helper cells, LC – Langerhans cells, LL-37 – antimicrobial peptide LL37, pDC – plasmacytoid dendritic cells, TIP-DC – tumour necrosis factor and nitric oxide releasing dendritic cells, TNF – tumour necrosis factor, Treg – T regulatory cells.

Figure 1. Cellular and molecular components of local memory in psoriasis

grating to the lymph nodes where they are taking part in T-cell differentiation and activation. Activated T cells migrate to affected skin through adhesion molecules on vascular endothelial cells, releasing effector molecules such as IL-17, IL-22, and IFN-γ, thereby affecting keratinocytes [2]. Keratinocytes can produce cytokines and chemokines to attract more cells such as neutrophils and macrophages, as well as produce vascular endothelial growth factor, which promotes endothelial cell proliferation and angiogenesis, thus creating highly vascularized psoriatic plaques [2].

In psoriasis, $CD8^+CD49a^+CD103^+$TRM occupying the epidermis produce IFN-g and activate their cytotoxic properties after exposure to IL-15, while $CD8^+CD49a^-CD103^+$TRM more frequently produce IL-17A [12]. $CD4^+CD69^+CD103^+$TRM preferentially inhabit the dermis, being close to blood vessels. IL-22-producing $CD4^+$ and IL-17A-producing $CD8^+$ cells also remain in the epidermis of the healed lesion, possibly determining recurrence of the disease; furthermore, it appears that the percentage of $CD8^+CD103^+$ cells capable of generating IL-17A in previously unaffected skin of a patient with psoriasis increases with the duration of the disease [13, 14]. An important and interesting issue requiring further research is whether early intensive therapy can alter or modulate pathogenic TRM and affect the "molecular scar" remaining in patients' healed skin.

Regulatory T lymphocytes

Regulatory T lymphocytes (Treg) are a subpopulation of lymphocytes capable of influencing immune cells by producing inhibitory cytokines (IL-10, IL-35, galectin-1, TGF-β) or by direct cytotoxic effect (perforin and granzyme B). Treg are involved in the immune homeostasis, as well as in maintaining tolerance and preventing autoimmune diseases [15]. During immunological homeostasis, $CD4^+CD25^+$high Forkhead box $P3^+$ ($Foxp3^+$) Treg maintain self-tolerance by controlling the activation of effector T cells [16]. Foxp3 is a more specific marker of Treg than CD4 and CD25 and is crucial for their evolution and activity [15]. Moreover, Treg inhibit the pro-inflammatory activity of macrophages, which are the main effector immune cells in TNF-dependent psoriasis. In the course of psoriasis, the suppressive function of Treg cells is impaired, which leads to a change in the Th17/Treg balance. It seems that it is the dysregulation of the functioning of Treg that may be important in the pathogenesis of plaque psoriasis (Figure 1) [15].

There are some discrepancies in the results of studies regarding the infiltration of Treg into the affected skin. Zhang *et al.* showed an increase in the number of $Foxp3^+$Treg both in the peripheral circulation and in psoriatic skin, which positively correlated with the severity of the disease [17]. Keijsers *et al.*, comparing samples of asymptomatic skin, lesion area, lesion edge, and centre of the psoriatic plaque, observed a relevant increase in the number of $CD3^+$, $CD4^+$, and $Foxp3^+$ Treg cells from the asymptomatic skin to the centre of the psoriatic lesion [18]. While Kotb *et al.* observed a lower percentage of Treg cells in patients with plaque psoriasis compared to the level in healthy people [19]. Conversely, Yun *et al.* noticed a decrease in the number of $Foxp3^+$Treg in lesioned skin compared to normal skin in patients with exacerbation of psoriasis, but an increase in those with chronic disease, regardless of the severity of the disease [20]. Fujimura *et al.* revealed more $CD3^+$, $CD4^+$, and $CD25^+Foxp3^+$Treg in the epidermis with psoriasis than in the dermis [21]. On the other hand, Bovenschen *et al.*, found a more abundant $CD4^+CD25^+Foxp3^+$Treg infiltration in the dermis than in the epidermis in psoriatic patients [22]. The discrepancies in the studies cited above indicate that the pathogenesis of plaque psoriasis may be influenced not by the number but by the dysfunction of Treg cells. There are several studies describing changes in Treg cell density in the skin of patients after successful treatment of psoriasis. Kotb *et al.* showed an increase in Treg density during UVB treatment or topical vitamin D and betamethasone [19], contrary to Keijsers *et al.*, who reported a reducing the number of Treg cells in psoriatic patients treated with topical vitamin D and betamethasone [23]. In the case of monoclonal antibody therapy (anti-IL17A, anti-IL-23) in a mouse model, an increase in the number of $Foxp3^+$Treg cells in previously changed skin was observed [24].

The role of Treg cells and the effect of therapy on their levels remains unclear and requires further research; however, it seems that long-term remission in psoriasis may be related to the normalization of Treg cell function, enabling the achievement of a balance between pathogenic and effector memory cells [15].

Dendritic cells, Langerhans cells

The skin contains numerous dendritic cells which include i.a. dermal dendritic cells (dDC), plasmacytoid dendritic cells (pDC), and Langerhans cells (LC). Dendritic cells DC and LC are innate immune cells, which are specialized antigen-presenting cells, inducing a T-cell immune response and maintaining tissue tolerance by promoting T-cell development, differentiation, and function. DC play a significant role in establishing and affecting the function of memory T cells in the skin, and abnormal activation of T cells due to DC dysregulation has implications for plaque psoriasis (Figure 1) [4]. Different types of DC are involved in the pathogenesis of psoriasis, and their total number is significantly increased compared to the skin of healthy individuals [4]. Injured keratinocytes release nucleic acids, antimicrobial peptides that bind through the toll-like receptor (TLR) in pDC, secreting large amounts of INF-α, promoting dDC maturation and monocyte differentiation into inflammatory DC. In active lesions, dDC have increased expression of IL-12/IL-23p40 [25] and produce many pro-inflammatory cytokines (IL-1β, IL-12, IL-23, TNF-α) [26], which activate

differentiation naive T cells in Th1, Th17, and Th22. dDC have an increased ability to recruit neutrophils, keratinocytes, and T lymphocytes. Interestingly, DC, as well as being involved in the initiation of inflammation, are also important in sustaining psoriatic inflammation by maintaining the ability to produce IL-23 and TNF-α [26, 27] despite clinical resolution of skin lesions and effective therapy. Together with TRM, these cells seem to remain in the skin, causing relapses of the disease.

LC have the ability to produce IL-23 after stimulation of various types of TLR, which, combined with reduced motility in the affected skin, indicates that LC can stimulate epidermal T lymphocytes [4]. One study showed that epidermal LC present in clinically healed skin share a similar location with epidermal T cells and preserve abnormal profiles of gene expression [28]. The authors observed an increase in the expression of IL-23 and IL-15 by LC after anti- TNF treatment, which play a role in the proliferation of T lymphocytes and the generation of IL-17-producing T lymphocytes [28]. Despite achieving clinically clear skin, antigen- presenting cells appear to be on standby to express pro-inflammatory genes. The ability to cross-communicate with T cells and keratinocytes suggests that LCs play a role in T-cell reactivation and relapse. The concept of innate immune memory exists, but whether DC and LC can be considered as innate memory cells remains to be clarified [4].

The effect of treatment on the molecular scar in plaque psoriasis

Phototherapy

In the treatment of plaque psoriasis, phototherapy is most often used with narrow band UVB (nbUVB) and UVA 320-400 nm radiation. Yones *et al.* conducted a study comparing the effectiveness of 2 well-known therapies: PUVA and nbUVB, showing that PUVA therapy is significantly more effective and gives longer remissions than nbUVB [29]. It may result from the normalization of the expression of keratin 16, filaggrin and involucrin, as well as the suppression and reduction of $CD4^+$ and $CD8^+$ T lymphocytes infiltrating the epidermis and dermis [30]. Phototherapy induces apoptosis of keratinocytes and has an immunosuppressive effect. During phototherapy, the activity of anti-inflammatory cytokines (IL-4, IL-10) increases and the activity of pro-inflammatory cytokines (IL-8, IL-17, IL-22, IL-23, IFN-γ, TNF-α) decreases. Moreover, the induction of Treg and the reduction of LC levels are also observed [2]. A study assessing TRM in psoriatic plaques before nbUVB phototherapy and in healed skin after treatment, revealed that part of the TRM was found to be Th17 dominant and some were Th1 dominant. Faster disease relapses after discontinuation of therapy were found in patients with IL-17A expression (Th17 dominant) compared to TRM Th1-dominant patients [31]. In another study, the ratio of $CD8^+CD103^+$TRM producing IL-17A to those producing IFN-γ increased with the duration of the disease in non-lesional psoriatic skin [13]. The above results suggest that early implementation of treatment may be associated with a reduced risk of disease relapse. Johnson-Huang *et al.* showed in their study that nbUVB leads to inhibition of the IL-17/IL-23 axis. After 6 weeks of therapy, a reduction in the number of mDC and suppression of the INOS, IL-20, IL-23p19, and IL-12/IL-23p40 genes were observed. The expression of IFNG, IL-17, IL-22, and BD4 genes was also reduced, and the production of IFN-γ by 85%, and IL-17 (45%) and IL-22 (89%) was inhibited [32]. This proves that UVB-phototherapy affects the same transmission paths as modern anti-psoriasis therapies.

Biologic treatment

The accelerated TNF-α/IL-23/IL-17 axis is the main pathomechanism of plaque psoriasis, which explains the high effectiveness of biological therapies [33].

Anti-TNF-a

TNF-α inhibitors have been used in the treatment of psoriasis for over 20 years, but despite their high effectiveness, skin lesions recur quickly after treatment. Cheuk *et al.* assessed the TRM in the skin after resolution of psoriatic lesions under the influence of nbUVB and long-lasting biological treatment (anti-TNF-α or anti-IL-12/23). Although both nbUVB and inhibition of TNF-α or IL-12/23 greatly decreased the quantity of T cells in epidermis, the observed increase in $CD8^+$T cells expressing the CD49a marker associated with TRM cells (5-fold for nbUVB and 16-fold for anti-TNF-α or anti-IL-12/23 compared to normal skin) implied expansion or selective retention of pathogenic TRM in resolved lesions. RNA expression profiles implied a less active TRM phenotype in skin treated with biologics. T cells expressing IL-17 and IL-22 were present in both active lesions and skin treated with nbUVB and biologics. The stable TRM population rapidly produces IL-17 or IL-22 upon restimulation, even after 6 years of biologic treatment, indicating that these cells are highly pathogenic and some of T cells located in the epidermis turn into long-living TRM [12]. In another study, the authors observed stabilization of keratinocyte proliferation and decreased expression of Th-17-related genes, i.e. IL-17, CCL20, and DEFB4, after just 1 week of anti-TNF-α treatment. A reduction in the expression of DC inflammatory products (INOS, TNF, IL-20, IL23p40) was also found. With the resolution of psoriatic plaques, a decrease in the number of macrophages [34] and a complete depletion of dendritic cells and $CD3^+$ T lymphocytes was observed [35]. Of interest was the lack of complete depletion of $CD8^+$T cells (64% reduction). This shows that the inflammation is not completely suppressed after the anti-TNF-α treatment, despite macroscopic resolution of the lesions. In the skin

of healed patients, a molecular scar is observed, defined as the "genomic profile of residual disease" containing 248 gene products that do not normalize after treatment [35]. Matos *et al.* in patients treated with etanercept or UVB phototherapy showed a higher quantity of T cells in "cured" skin compared to healthy controls. Before treatment, various T-cell clones were observed in the psoriatic plaques, but only 7% of the T-cell clones remained in the cured skin lesions. After the triggering factor, these cells begin to overproduce IL-17A [2, 36].

Anti-IL-17

Inhibition of the Th-17/IL-17 pathway seems to be of key importance in the treatment of plaque psoriasis [7]. In their study, Krueger *et al.* found that during treatment with ixekizumab there is a reduction in keratinocyte hyperproliferation and infiltration from $CD3^+$T lymphocytes, DC, and AMP (LL-37, S100A7, S100A8, BD2) [37]. They also observed a decrease in gene expression, e.g. IL-17A, IL-17F, IL-19, IL-22, IL-23p19, LCN, KRT16, CXCL8, and CXCL1. Interestingly, after 2 weeks of ixekizumab therapy, it was found that as many as 1200 psoriasis genes change expression, of which 600 were normalized, while etanercept therapy normalized the expression of only 100 genes [37]. Rapid improvement with ixekizumab is associated with suppression of the influence of IL-17 on keratinocytes but does not appear to affect T-cell infiltrates in the skin. In a study of patients treated for 12 weeks with secukinumab, Reich *et al.* observed that after 2 weeks of therapy there was a decrease in the number of neutrophils, a reduction in the expression of IL-17A and IL-17F mRNA, but no effect on TNF mRNA. In the case of the number of DC and T lymphocytes, a much slower decrease was observed. There was no decrease in mast cell counts over 12 weeks of treatment. Surprisingly, patients treated with low doses of secukinumab showed a renewed increase in the number of neutrophils after the 12^{th} week of treatment, and a higher number of neutrophils was associated with a shorter period of clinical remission after treatment discontinuation [38]. It is the effect on neutrophils that is responsible for the rapid therapeutic effect, while the long-term effect of therapy as well as the length of remission are associated with a decrease in T lymphocytes and DC. No DC are found in the cured skin, but LC capable of producing IL-23 are still present [28].

Anti-IL-12/IL-23

Both IL-12 and IL-23 have a relevant impact on the differentiation of T lymphocytes. Brodmerkel *et al.*, in their study, observed a decrease in the expression of genes related to the transmission pathway of IL-1, IL-17, IL-22, IFN-γ, and TNF-α during 12 weeks of treatment with ustekinumab. Interestingly, the expression of 18% of genes after treatment did not return to the baseline level, compared to 23% in the case of etanercept [39]. It seems that this "smaller" molecular scar is responsible for the greater likelihood of a longer clinical remission after ustekinumab therapy (lower expression of IL-17).

Anti-IL-23

IL-23 antagonists appear to reduce the T-cell fraction more effectively than IL-17 antagonists. This is reflected in the length of remission of the disease after biological treatment with specific methods. Although drugs directed against IL-17 give a faster clinical response to treatment, IL-23 antagonists give the longest remissions of the disease. Long-term remission in the case of the use of IL-23p19 antagonists in the psoriasis treatment was demonstrated for many months after discontinuation of therapy, even after the drug was completely washed away [40]. Appropriately early inclusion of anti-IL-23 treatment resulted in a higher rate of sustained remission compared to patients with long-term disease [41]. Flora *et al.*, due to the many pathophysiological similarities between guttate psoriasis and plaque psoriasis, reviewed cases of patients with guttate psoriasis treated with risankizumab. In the case of guttate psoriasis, usually about 25–39% of patients develop chronic plaque psoriasis; however, after 3 doses of risankizumab and discontinuation of treatment, the authors did not document a recurrence of the disease in up to 24 months of observation in any of the patients [42]. IL-23 plays an important role in the survival of TRM in the skin of patients with psoriasis, and early IL-23 antagonism may diminish the formation and survival of TRM, which later form a "molecular scar". Similar results were observed with ustekinumab, secukinumab, ixekizumab, and guselkumab therapies [43, 44]. In a study comparing risankizumab with ustekinumab, it was found that both drugs reduced the thickness of the epidermis, reduced the expression of proteins: CD3, CD11c, K16, Ki67, S100A7, and β-defensin-2 after 4-week treatment. In the case of both drugs, the expression of genes related to the IL-17/IL-23 transmission pathway was reduced, including IL-17A, IL-17C, IL-17F, IL-22, IL-23A, S100A8, and S100A9. However, risankizumab turned out to be more potent than ustekinumab in reducing the expression of disease-related genes [45]. Sofen *et al.* proved that during a 12-week therapy with guselkumab there is a significant thinning of the epidermis, and a decrease in the expression of keratin 16 and the number of $CD3^+$ and $CD11c^+$ cells. Lower expression of KRT16, S100A7, LCN2, CXCL1, CXCL8, and IL-17A genes was also observed. Moreover, normalization to baseline values of more than 70% of genes associated with psoriasis pathogenesis has been demonstrated [46]. Mehta *et al.* compared the efficacy of guselkumab and secukinumab therapy, finding that both treatments decreased the incidence of inflammatory T cells, monocytes, inflammatory DC, and $CD4^+CD49a^-CD103^-$T cells. Guselkumab reduced the number of memory T cells while preserving Treg, and contrary to secukinumab, none

of the drugs modified the incidence of IL-17A$^+$IL-17F$^{+/-}$ CD4$^+$ or CD8$^+$ T cells [47].

Conventional therapies

There is still lack of reports of the "molecular scar" after conventional systemic and topical therapies. Owczarczyk-Saczonek *et al.* evaluated the localization and amount of TRM markers in skin lesions for 3 therapies (methotrexate, anti-IL-17, and anti-TNF). The fastest decrease in the expression of TRM markers was noticed during anti-IL-17 therapy in the fourth week of treatment, while in the case of methotrexate (MTX) and anti-TNF, the response was significant at the 12th week of therapy. The number of markers decreased mainly in the dermis but not in the epidermis [48]. Another study showed that psoriatic TRM persisted in resolved plaques even for months after successful treatment with methotrexate. After an 8-week MTX therapy, reduction in the inflammatory infiltration was observed, but CD8$^+$ T cells with CD69 expression were still present in the healed skin lesions [49]. Kurihara *et al.* studied 2 groups of patients, the first treated only with topical therapy and the second treated with cyclosporine, a phosphodiesterase 4 inhibitor, or biologics. The frequency of CD8$^+$CD103$^+$IL-17A$^+$ and CD4$^+$CD103$^+$IL-17A$^+$ TRM was higher in the systemic therapy group, while CD8$^+$TRM revealed a higher frequency than CD4$^+$TRM. There was no relationship between CD8$^+$CD103$^+$IL-17A$^+$ or CD4$^+$CD103$^+$IL-17A$^+$TRM and the severity of primary psoriatic lesions [50]. In one of our studies, we analysed the correlation between the expression of TRM markers and the duration of the disease, showing a clear dependence between these variables. The longer the course of the disease, the greater the expression of TRM markers [14]. Similarly, to Kurihara, we found no correlation between the expression of the analysed markers and the intensity of skin lesions [14, 50].

The question that arises after analysing the above studies is whether systemic drugs and monoclonal antibodies penetrate the epidermis, where TRM are still present in the healed skin. Clark *et al.* assessed the effect of anti-CD52 treatment on T lymphocytes circulating in the blood and skin. Low doses of the drug were found to be effective in treating patients with refractory L-CTCL but not MF. This was due to the fact that the drug removed T lymphocytes circulating in the blood but did not eliminate T lymphocytes residing in the skin [51]. Similarly, in the case of TRM in psoriasis, systemic drugs can be highly effective in eradicating T lymphocytes in the circulation and in the dermis, leaving TRM in the epidermis [48].

Topical treatment

Kurihara *et al.* studied the result of topical treatments on TRM in the skin of psoriatic patients. Betamethasone dipropionate, calcipotriol, and calcipotriol/betamethasone dipropionate (Cal/BD) ointments were all shown to reduce overall CD8$^+$CD103$^+$TRM counts, but it was the combined preparation that reduced TRM the most. Interestingly CD8+CD103+TRM persisted near the basal membrane zone of the epidermis, being resistant to all tested topical therapies [52]. Another study assessed TRM markers in psoriatic lesions before and after 12 weeks of therapy with Cal/BD. Most markers showed a statistically significant reduction in immunofluorescence at week 12 (CD4, CD8, CD69, CD103, CXCR6, IL-17A, and IL-22) in the epidermal layer. Changes in the expression of TRM markers in the dermis were observed in the case of CD4 and IL-22, which proves that topical therapy does not substantially affect TRM in the dermal layer [53]. Perhaps it is the combination of systemic therapy with topical eradication of TRM at the epidermal level that may be an encouraging therapeutic strategy to avoid psoriasis flare-ups in previously affected skin areas.

Conclusions

Molecular scarring remains in the skin of patients despite the most modern methods of psoriasis treatment. TRM appear to provide a biological elucidation for the clinical observation that psoriatic plaques occur repeatedly in the same anatomical locations throughout the course of the disease. What is more, in "cured" psoriatic lesions there are over 250 genes whose expression does not return to the state of healthy skin [2]. Particular attention and further research are also required for LC, DC, and Treg affecting the activation of dormant TRM and involved in the formation of the "molecular scar". The impact of treatment on TRM remains a difficult and not fully explored issue in dermatology; however, based on current scientific reports, it seems that several factors influence the proper control of the disease and reduction of TRM, including early initiation of treatment, the type of therapy used, as well as its duration. It seems that the use of combined therapies – general and topical treatment – may work more effectively on the number of TRM in the skin of psoriatic patients, giving longer and more durable remissions of the disease.

Conflict of interest

The authors declare no conflict of interest.

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STRESZCZENIE

Naturalny przebieg łuszczycy charakteryzuje długotrwałe utrzymywanie się zmian skórnych i skłonność do nawrotów w tej samej okolicy, pomimo skutecznego leczenia. Wynika to z obecności w pozornie zdrowej skórze rezydujących w tkankach komórek T pamięci (TRM – *tissue-resident memory cells*), będących w stanie zainicjować kaskadę zapalną i wywołać nawrót choroby. Komórki te charakteryzują się dużą odpornością na czynniki uszkadzające i apoptozę, co warunkuje ich długowieczność.

Celem pracy było określenie lokalizacji i ilości markerów TRM w blaszkach łuszczycowych u 32 pacjentów z łuszczycą plackowatą w porównaniu z 10 zdrowymi ochotnikami oraz analiza związku między ekspresją markerów TRM a czasem trwania i nasileniem choroby. Ocenie poddano również ilość markerów TRM w zmianach łuszczycowych przed terapią, w trakcie i po 12 tygodniach terapii ogólnej metotreksatem (MTX) lub sekukinumabem lub iksekizumabem (anty-IL-17) lub adalimumabem (ADA), a także terapii miejscowej preparatem zawierającym kalcypotriol i dipropionian betametazonu (Cal/BD) – łącznie u 23 pacjentów. W tym celu za pomocą badania immunohistochemicznego oceniono ilość i lokalizację markerów TRM (CD4, CD8, CD103, CD69, CD49, CXCR6) oraz tkankową ekspresję cytokin (interleukiny [IL]-17A, IL-22) w zmienionej łuszczycowo skórze. Próbki biopsyjne pobierano z tej samej okolicy przed rozpoczęciem leczenia oraz po 4 i 12 tygodniach terapii. Uzyskane wyniki poddano analizie statystycznej, co umożliwiło ustalenie poniższych wniosków:

1. u pacjentów z rozpoznaną łuszczycą plackowatą w porównaniu do grupy kontrolnej obserwuje się zwiększoną ilość markerów TRM w naskórku i skórze właściwej,
2. istnieje wyraźna dodatnia zależność między ekspresją markerów TRM u pacjentów z łuszczycą plackowatą a czasem trwania choroby,
3. nie stwierdzono zależności między ilością markerów TRM a intensywnością zmian skórnych, ocenianą według skal Psoriasis Area and Severity Index (PASI), Body Surface Area (BSA) i Dermatology Life Quality Index (DLQI),
4. wykazano zmniejszenie ekspresji markerów TRM w zmianach łuszczycowych w czasie z każdą terapią ogólną (anty-IL-17, ADA, MTX), a najszybsza redukcja ekspresji markerów TRM dotyczyła terapii anty-IL-17 już w 4. tygodniu leczenia,

5. w ciągu 12-tygodniowej obserwacji terapia ogólna (anty-IL-17, ADA, MTX) zmniejsza ekspresję markerów TRM głównie w skórze właściwej zmian chorobowych, a nie w naskórku,
6. w ciągu 12-tygodniowej obserwacji terapia miejscowa Cal/BD zmniejsza ekspresję markerów TRM głównie w naskórku, ale nie wpływa znacząco na ich ekspresję w skórze właściwej,
7. spadek ekspresji markerów TRM zaobserwowano w dopiero w 12. tygodniu terapii miejscowej preparatem Cal/BD.

SUMMARY

The natural course of psoriasis is characterized by long-term persistence of skin lesions and predilection for relapse in the same area despite effective treatment. It is caused by the inherence of tissue-resident memory cells (TRM) in apparently healthy skin, which are able to initiate an inflammatory cascade and induce relapse of the disease. These cells are characterized by high resistance to damaging factors and apoptosis, which determines their longevity.

The aim of the study was to determine the location and amount of TRM markers in psoriatic plaques in 32 patients compared to 10 healthy volunteers, and to analyze the relationship between the expression of TRM markers and the duration and severity of the disease. The number of TRM markers in psoriatic lesions was also assessed before, during and after 12 weeks of systemic therapy with methotrexate (MTX) or secukinumab or ixekizumab (anti-IL-17) or adalimumab (ADA), as well as topical therapy with calcipotriol and betamethasone dipropionate (Cal/BD) – a total of 23 patients. For this purpose, the amount and localization of TRM markers (CD4, CD8, CD103, CD69, CD49, CXCR6) and the tissue expression of cytokines (interleukin [IL]-17A, IL-22) in the lesional psoriatic skin were assessed using immunohistochemistry. Biopsy samples were taken from the same area before treatment and after 4 and 12 weeks of treatment. The obtained results were subjected to statistical analysis, which allowed the following conclusions to be drawn:

1. patients diagnosed with plaque psoriasis have an increased amount of TRM markers in the epidermis and dermis compared to the control group,
2. there is a clear positive relationship between the expression of TRM markers in patients with plaque psoriasis and the duration of the disease,
3. no relationship was found between the number of TRM markers and the intensity of skin lesions - assessed according to the Psoriasis Area and Severity Index (PASI), Body Surface Area (BSA) and Dermatology Life Quality Index (DLQI) scales,
4. a decrease in the expression of TRM markers in psoriatic lesions was demonstrated over time with each systemic therapy (anti-IL-17, ADA, MTX), and the fastest reduction in the expression of TRM markers concerned anti-IL-17 therapy in the 4th week of treatment,

5. during the 12-week observation, general therapy (anti-IL-17, ADA, MTX) reduces the expression of TRM markers mainly in the dermis of the lesions, not in the epidermis,
6. during the 12-week observation, topical Cal/BD therapy reduces the expression of TRM markers mainly in the epidermis, but does not significantly affect their expression in the dermis,
7. decrease in TRM markers was observed only in the 12th week of topical therapy with Cal/BD.

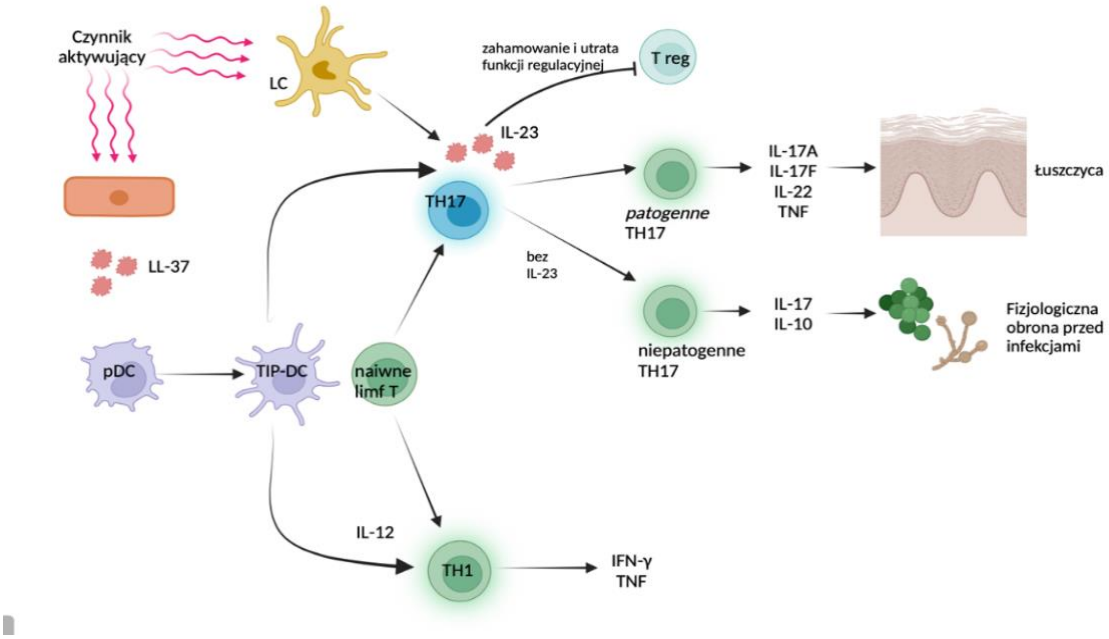
WPROWADZENIE

Komórki T pamięci rezydujące w tkankach (TRM) są nowo opisanym podzbiorem niekrążących komórek T pamięci. Powstają one na skutek aktywacji naiwnych limfocytów T przez komórki prezentujące antygen. Komórki prekursorowe rozwijają się w kilka podzbiorów komórek T pamięci, w tym centralne komórki T pamięci (TCM), efektorowe komórki T pamięci (TEM) i komórki T pamięci rezydujące w tkankach (TRM). TEM dominują w obwodowych tkankach nielimfoidalnych, TCM lokalizują się we wtórnych narządach limfatycznych [1]. Komórki TRM różnią się fenotypowo od krążących TEM i TCM, ponieważ eksprymują cząsteczki, które zapobiegają ich wydostawaniu się z tkanek. Wyróżniamy m.in. antygen CD69 odpowiadający za osadzanie TRM zapobiegając ich recyrkulacji poprzez supresję receptora sfingozyno-1-fosforanowego (S1P1), czy też CD103 – receptor adhezji E-kadheryny, determinujący lokalizację TRM w tkance nabłonkowej. Komórki TRM wyrażają także cząsteczkę CD49a, która wiąże kolagen IV obecny w warstwie podstawnej [2]. Poza tym TRM posiadają receptory dla chemokin takich jak: CCR4, CCR8, CCR10, CXCR3 i CXCR6, które są uważane za ważne cząsteczki naprowadzające lub zatrzymujące TRM w skórze [3]. Komórki T pamięci rezydujące w tkankach pełnią głównie funkcję ochronną przed infekcjami, ale okazuje się, że ich niewłaściwa aktywacja bierze udział w patogenezie wielu chorób dermatologicznych, takich jak bielactwo nabyte, łuszczyca plackowata, chłoniak skóry z komórek T (CTCL), czy też czerniak [1, 4].

W łuszczycy plackowatej TRM odpowiadają za zjawisko „pamięci immunologicznej", warunkującej nawroty zmian chorobowych w tej samej lokalizacji. Pomimo skutecznego leczenia i ustąpienia blaszek łuszczycowych, w skórze pacjentów nadal są obecne oznaki stanu zapalnego w postaci patogennych komórek T pamięci. W przypadku zadziałania czynnika spustowego ulegają one aktywacji i mogą indukować kaskadę zapalną prowadząc do nawrotu choroby. TRM składają się z frakcji CD8 zlokalizowanej w naskórku i CD4 w skórze właściwej, które zwiększają ekspresję cytokin zapalnych takich jak interleukina (IL)-17A, IL-22 i interferon gamma (IFN-γ), mających kluczowe znaczenie w patogenezie łuszczycy plackowatej [5]. Wiadomo, że komórki te są wysoce odporne na czynniki uszkadzające i apoptozę, co prawdopodobnie decyduje o ich długowieczności [2].

Po zadziałaniu czynnika zewnętrznego jak uraz egzogenny, infekcja i/lub utrata tolerancji immunologicznej, dochodzi do aktywacji TRM przez autoantygeny lub

cytokiny/chemokiny. Autoantygeny obejmują: peptyd przeciwdrobnoustrojowy (AMP) LL37, ADAMTSL5 wytwarzany przez melanocyty [2, 6], PLAG4D z komórek tucznych i keratynocytów oraz keratynę 17 z keratynocytów [3]. Mają one zdolność aktywacji plazmocytoidalnych komórek dendrytycznych (pDC) i komórek dendrytycznych wytwarzających czynnik martwicy nowotworów alfa i indukowalną syntetazę tlenku azotu (TIP-DC), a także komórek Langerhansa (LC), prowadząc do produkcji kluczowych cytokin zapalnych IL-23 i czynnika martwicy nowotworów alfa (TNF-α). IL-23 i TNF-α wydzielane przez aktywowane komórki dendrytyczne kierują polaryzacją i ekspansją klonalną komórek T CD4+ i CD8+. Podczas nawrotu choroby CD8+ TRM wytwarzają głównie IL-17, natomiast CD4+ TRM wydzielają IL-22, która zwiększa zdolność antyapoptotyczną keratynocytów i prowadzi do ich nadmiernej proliferacji i nieprawidłowego różnicowania [2]. Co więcej IL-23 hamuje działanie regulatorowych komórek T, prowadząc do utraty ich funkcji regulatorowej [7]. Po zadziałaniu czynnika wyzwalającego dochodzi także do aktywacji mieloidalnych komórek dendrytycznych (mDC), wytwarzających znaczne ilości IL-23 i promujących różnicowanie i aktywację komórek Th17, podtrzymując w ten sposób szlak sygnalizacyjny IL-23/IL-17 w zmianach łuszczycowych [6] (ryc. 1.).



Rycina 1. Komórkowe i molekularne składniki pamięci lokalnej w łuszczycy

Skróty: IL – interleukina, IFN-γ – interferon gamma; TH – limfocyty T pomocnicze, LC – komórki Langerhansa, LL-37 – peptyd przeciwdrobnoustrojowy LL37; pDC – plazmocytoidalne komórki dendrytyczne; TIP-DC – komórki dendrytyczne wytwarzające TNF-α i indukowalną syntetazę tlenku azotu; TNF – czynnik martwicy nowotworów; Treg – limfocyty T regulatorowe.

Proliferujące keratynocyty uwalniają AMPs, cytokiny i chemokiny celem przyciągania innych komórek odpornościowych takich jak neutrofile i makrofagi, a także wytwarzają czynnik wzrostu śródbłonka naczyniowego (VEGF), sprzyjający proliferacji komórek śródbłonka, promując angiogenezę i tworząc wysoce unaczynione blaszki łuszczycowe [6].

Celowanie w komórki TRM wydaje się być nową potencjalną strategią terapeutyczną w leczeniu i ograniczaniu nawrotów łuszczycy. Jednak ze względu na wciąż niewielką liczbę doniesień o wpływie leczenia miejscowego i ogólnego na ilość TRM w naskórku i skórze właściwej pacjentów z łuszczycą, potrzebne są dalsze badania w tym zakresie. Dogłębne poznanie mechanizmów odpowiedzialnych za rozwój i nawrót łuszczycy plackowatej, może przyczynić się do wdrożenia skuteczniejszych terapii.

CELE BADAWCZE

Celem prac badawczych było:

1. Określenie lokalizacji i ilości markerów TRM w zmianach łuszczycowych u pacjentów z łuszczycą plackowatą oraz analiza związku między ekspresją markerów TRM a czasem trwania i nasileniem choroby.
2. Ocena ilości markerów TRM w zmianach łuszczycowych przed terapią, w trakcie i po 12 tygodniach terapii ogólnej metotreksatem lub sekukinumabem lub iksekizumabem lub adalimumabem.
3. Ocena ilości markerów TRM w zmianach łuszczycowych przed terapią, w trakcie i po 12 tygodniach terapii u pacjentów leczonych miejscowo preparatem zawierającym kalcypotriol i dipropionian betametazonu.

Celem przeglądu literatury było usystematyzowanie aktualnie dostępnych danych dotyczących koncepcji bliznowacenia molekularnego ze szczególnym uwzględnieniem TRM w łuszczycy oraz ocena wpływu różnych terapii na pamięć immunologiczną.

ZGODA KOMISJI BIOETYCZNEJ I ŚWIADOMA ZGODA

Badanie zostało zatwierdzone przez Komisję Bioetyczną przy Collegium Medicum Uniwersytetu Warmińsko-Mazurskiego w Olsztynie (Uchwała 24/2020). Każdy pacjent włączony do badania podpisał świadomą zgodę na udział w nim.

OMÓWIENIE WYNIKÓW I DYSKUSJA

Łuszczyca plackowata charakteryzuje się długotrwałym utrzymywaniem się zmian skórnych i skłonnością do nawrotów w tej samej okolicy, pomimo wcześniejszego skutecznego leczenia [9]. Wynika to z obecności w pozornie zdrowej skórze śladu stanu zapalnego w postaci rezydujących w tkankach komórek T pamięci (TRM) [10], mających zdolność inicjowania kaskady zapalnej i wywołujących nawrót choroby po zadziałaniu czynnika spustowego [3, 8].

Pierwsza z prac badawczych miała na celu porównanie ilości markerów TRM w skórze u 32 pacjentów chorych na łuszczycę ze zdrową grupą kontrolną (ocena obszaru immunoreaktywnego (%) CD8, CD4, CD103, CD69, CD49, CXCR6, IL-17A i IL22), a także przeanalizowanie związku między ekspresją markerów TRM a czasem trwania i nasileniem choroby - mierzonych skalami Psoriasis Area and Severity Index (PASI), Body Surface Area (BSA), Dermatology Life Quality Index (DLQI). Badanie wykazało zwiększoną liczbę markerów TRM w naskórku i skórze właściwej wykwitów łuszczycowych w porównaniu ze skórą zdrowych ochotników (tab. 1).

Tabela 1. Obszar immunoreaktywny (%) markerów TRM (X±SEM) w skórze zmienionej chorobowo (n = 32, skóra właściwa i naskórek) w porównaniu ze zdrową grupą kontrolną (n = 10, skóra właściwa i naskórek). Gwiazdki pokazują statystycznie istotne różnice między analizowanymi grupami (* $p < 0,05$)

Marker TRM	**Zdrowa grupa kontrolna (*n*: 10)**		**Skóra zmieniona chorobowo (*n*: 32)**	
	Naskórek (%)	Skóra właściwa (%)	Naskórek (%)	Skóra właściwa (%)
CD8	8.83 ± 2.68	2.04 ± 0.33	15.49 ± 1.53 *	4.27 ± 0.73
CD4	2.77 ± 0.49	7.71 ± 1.80	6.45 ± 0.77 *	15.21 ± 1.11 *
CD103	13.88 ± 1.28	3.37 ± 0.42	22.32 ± 2.10 *	7.86 ± 1.02 *
CD49	10.66 ± 3.23	1.55 ± 0.34	16.17 ± 1.48 *	3.39 ± 0.42 *
CD69	13.89 ± 2.51	6.39 ± 0.69	25.24 ± 1.42 *	7.94 ± 1.13
CXCR6	2.24 ± 0.41	2.05 ± 0.33	12.23 ± 1.04 *	7.34 ± 0.78
IL-17A	12.45 ± 0.94	9.85 ± 2.14	23.63 ± 1.82 *	17.19 ± 0.64 *
IL-22	10.54 ± 1.44	8.45 ± 1.03	21.12 ± 1.87 *	17.10 ± 1.01 *

W badanej grupie chorych na łuszczycę stwierdzono istotny statystycznie wzrost powierzchni immunoreaktywnej CD8 w naskórku, CD4 w skórze właściwej i w mniejszym stopniu w naskórku w porównaniu z grupą kontrolną (tab. 1.). U pacjentów z łuszczycą naskórkowe komórki $CD8^+$TRM wykazują ekspresję antygenu CLA, CCR6, CD103 i IL-23R oraz wytwarzają IL-17A po stymulacji *ex vivo*, co świadczy o ich patogenności. Naskórek może być również zajęty przez $CD4^+CD103^+$TRM wytwarzające IL-22 [11]. Nasze badania potwierdzają również istotny statystycznie wzrost poziomu IL-17A i IL-22 zarówno w naskórku, jak i w skórze właściwej pacjentów z łuszczycą w porównaniu do zdrowych ochotników. Duże ilości prozapalnych IL-17A i IL-22 utrzymują się w skórze pacjentów przez wiele miesięcy po ustąpieniu zmian skórnych, a ich ilość okazuje się być skorelowana z czasem trwania choroby (tab. 2.).

Tabela 2. Korelacje (r, $p < 0,05$) między poszczególnymi markerami TRM a czasem trwania zmian łuszczycowych

Marker TRM	**Czas trwania choroby (lata)**
CD8	0.45
CD4	0.55
CD103	0.44
CD49	0.57
CD69	0.38
CXCR6	0.59
IL-17A	0.37
IL-22	0.51

Zaobserwowano także istotne różnice w odniesieniu do markerów TRM odpowiadających za zasiedlanie i utrzymanie komórek pamięci w skórze. CD103 jest ligandem dla E-kadheryny, cząsteczki adhezyjnej wyrażanej przez komórki nabłonkowe w tkankach barierowych takich jak skóra, płuca czy jelita. Warunkuje adhezję, a także promuje lokalną retencję TRM. CD49a jest podjednostką α receptora integryny α1β1, ulegającą ekspresji tylko na naskórkowych komórkach T CD8 [12]. CD49a wiąże się z kolagenem typu IV, który znajduje się w strefie błony podstawnej, a także ma znaczący wpływ na migrację komórek pamięci wzdłuż kolagenu [4]. W grupie kontrolnej i badanej

wykazano znaczącą różnicę między ilością CD103 i CD49 w naskórku i skórze właściwej – stwierdzając znacznie większy obszar immunoreaktywny w naskórku. CD103 i CD49 stwierdzono w istotnie większych ilościach w grupie chorych na łuszczycę, głównie w naskórku, ale także w skórze właściwej w porównaniu z osobami zdrowymi. W przypadku CD69 – glikoproteiny, biorącej udział w kolonizacji i zapobieganiu recyrkulacji TRM [2] stwierdzono istotną statystycznie różnicę w ilości CD69 między naskórkiem a skórą właściwą w grupie kontrolnej na korzyść naskórka. Ponadto w badaniu immunohistochemicznym zmian skórnych u pacjentów z łuszczycą plackowatą zaobserwowano istotny wzrost powierzchni immunoreaktywnej CD69 w naskórku, natomiast w skórze właściwej wartości te nie uległy zmianie w porównaniu do grupy kontrolnej. W przypadku CXCR6 w grupie kontrolnej nie stwierdzono różnic między obszarem immunoreaktywnym w naskórku i skórze właściwej – pozostawały one na bardzo niskim poziomie w obu lokalizacjach. Zaobserwowano natomiast wyraźny wzrost CXCR6 w grupie chorych na łuszczycę w obu warstwach – wyższy w naskórku (tab. 1.).

W badaniu przeanalizowano również związek między ekspresją markerów TRM a czasem trwania choroby, wykazując wyraźny związek między tymi zmiennymi. Ekspresja markerów w grupie badanej była istotnie zależna od czasu wystąpienia zmian skórnych. Im dłuższy był przebieg choroby, tym wyższa była ekspresja markerów TRM (czas vs. marker, $p < 0,05$) (tab. 2.). Długowieczność TRM i ich niekwestionowana zdolność do kumulowania się w skórze chorych na łuszczycę mogą wyjaśniać szybki nawrót zmian łuszczycowych w tym samym miejscu po zadziałaniu czynnika sprawczego. Nie stwierdzono zależności między ekspresją analizowanych markerów TRM a nasileniem zmian skórnych (marker vs. PASI, BSA, DLQI).

Kolejne prace badawcze przeprowadzono w celu oceny ilości i lokalizacji typowych markerów TRM w zmienionym naskórku i skórze właściwej w trzech punktach czasowych dla trzech różnych terapii ogólnoustrojowych (MTX, anty-IL-17, anty-TNF-α), a także terapii miejscowej preparatem zawierającym kalcypotriol i dipropionian betametazonu (Cal/BD).

Podczas oceny immunohistochemicznej wycinków pobranych ze zmian łuszczycowych u 13 pacjentów leczonych ogólnie metotreksatem (MTX), sekukinumabem (SEC), iksekizumabem (IXE) lub adalimumabem (ADA) oceniono markery TRM (CD4, CD8, CD103, CD69, CD49, CXCR6) oraz tkankową ekspresję cytokin (IL-17A, IL-22) w porównaniu z próbkami pobranymi od 10 zdrowych

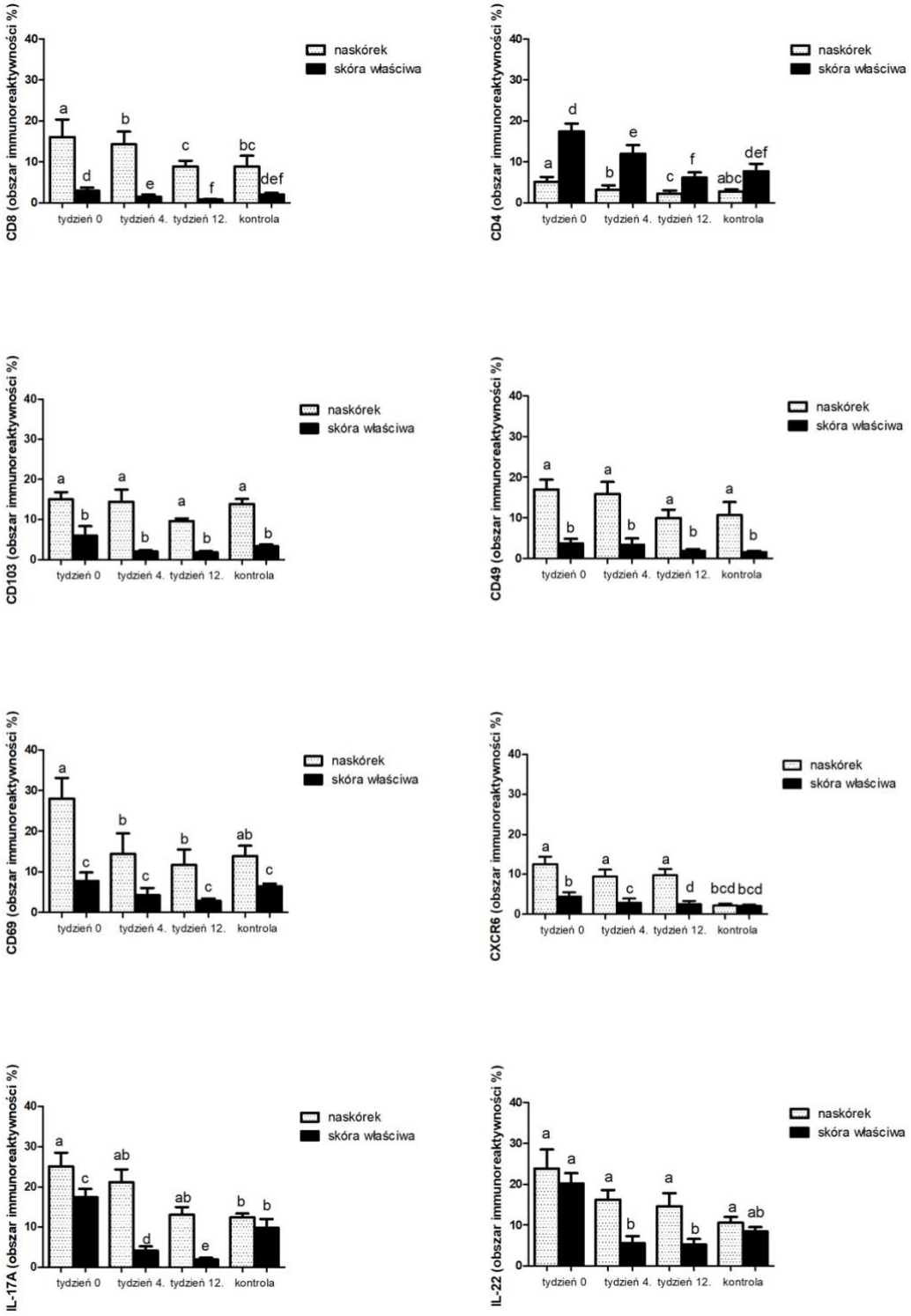
pacjentów. Próbki biopsyjne pobierano trzykrotnie z tej samej blaszki łuszczycowej przed i po 4. oraz 12. tygodniach terapii.

W przypadku chorych poddawanych terapii anty-IL-17 (SEC lub IXE) obszar immunoreaktywny CD8 był zawsze większy w naskórku niż w skórze właściwej ($p < 0,05$). W 4. tygodniu obserwacji wykazano zmniejszenie powierzchni immunoreaktywnej CD8 w obu warstwach skóry ($p < 0,05$) na poziomie porównywalnym z osobami zdrowymi. Podobnie spadek powierzchni immunoreaktywnej CD4 odnotowano zarówno w naskórku, jak i w skórze właściwej podczas drugiego pomiaru ($p < 0,05$), ale obszary immunoreaktywne były zdecydowanie większe w skórze właściwej ($p < 0,05$). Obszary CD103 i CD49 pozostały niezmienione podczas terapii, ale w każdym punkcie czasowym obserwowano wyższy poziom w naskórku ($p < 0,05$). W przypadku CD69 obszar immunoreaktywny w naskórku zmniejszał się w 4. tygodniu terapii, a następnie utrzymywał się na niezmienionym poziomie do 12. tygodnia ($p < 0,05$). Nie zaobserwowano zmian istotnych statystycznie w skórze właściwej. W przeciwieństwie do CD69, powierzchnia CXCR6 nie zmieniła się w naskórku, natomiast w skórze właściwej była mniejsza w 4. i 12. tygodniu niż przed terapią ($p < 0,05$). Obszar immunoreaktywny IL-17A i IL-22 w naskórku był stabilny podczas terapii, jednak w 4. tygodniu odnotowano istotny spadek w obrębie skóry właściwej ($p < 0,05$), uzyskując wartości zbliżone do wyników zdrowych osób z grupy kontrolnej (ryc. 2.).

Nie odnotowano istotnych statycznie zmian w obszarach immunoreaktywnych CD8, CD103 i CD49 w grupie leczonej anty-TNF-α (ADA). Obszary te były większe w naskórku w porównaniu ze skórą właściwą ($p < 0,05$). Co ciekawe, w przypadku CD4 zmniejszenie powierzchni obserwowano podczas trzeciego pomiaru (12. tydzień) zarówno w naskórku, jak i w skórze właściwej ($p < 0,05$). Powierzchnia immunoreaktywna CD69 i CXCR6 ($p < 0,05$) w naskórku uległa zmniejszeniu w 12. tygodniu obserwacji. W skórze właściwej obszar ten zmniejszył się tylko w przypadku CD69. Natomiast powierzchnia IL-17A i IL-22 pozostała niezmieniona w naskórku, zaś w skórze właściwej była mniejsza w 4. tygodniu obserwacji niż przed terapią ($p < 0,05$) (ryc. 3).

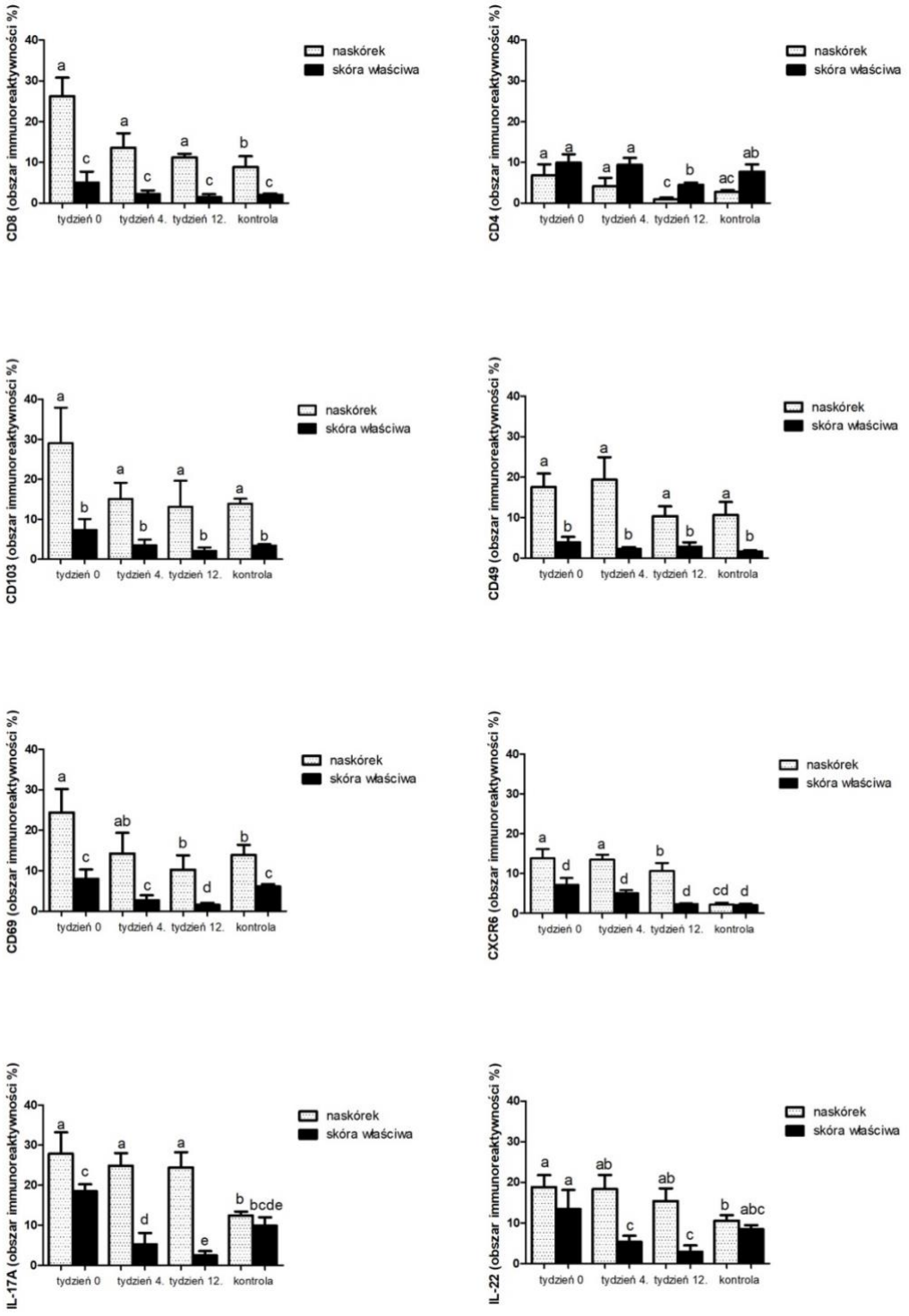
Podobnie jak w przypadku terapii anty-IL-17, podczas leczenia MTX doszło do zmniejszenia powierzchni immunoreaktywnej CD8 w naskórku, odpowiedź była jednak opóźniona (12. tydzień terapii, $p<0,05$). W przypadku CD4 odnotowano zmniejszenie obszaru immunofluorescencji jedynie w skórze właściwej w 12. tygodniu

leczenia ($p < 0,05$). W trzecim pomiarze odnotowano także spadek powierzchni immunoreaktywnej CD103 i CD69 w naskórku ($p < 0,05$), natomiast w skórze właściwej nie uległa ona zmianie. Powierzchnia CD49 i CXCR6 utrzymywała się na stałym poziomie podczas terapii. Obszar powierzchni immunoreaktywnej IL-17A pozostał niezmieniony w naskórku, jednakże w 4. tygodniu terapii zaobserwowano istotny spadek w skórze właściwej ($p < 0,05$). Podobnie w przypadku IL-22 powierzchnia immunofluorescencyjna w skórze właściwej uległa zmniejszeniu ($p < 0,05$) w 4. tygodniu. Dodatkowo spadek IL-22 odnotowano również w 12. tygodniu w obrębie naskórka ($p < 0,05$) (ryc. 4).



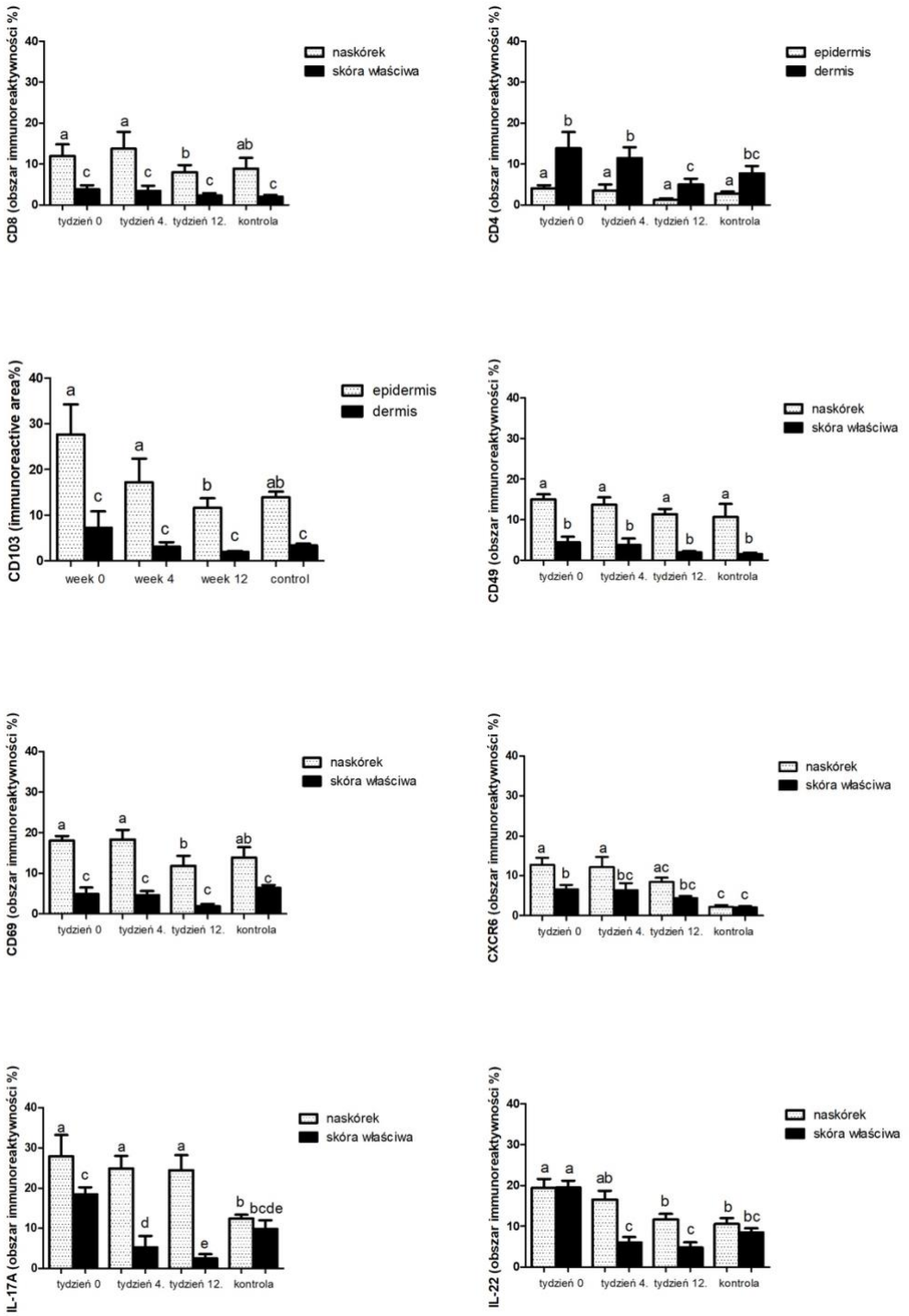
Rycina 2. Obszar immunoreaktywny (%) (x ± SEM) CD8; CD4; CD103; CD49; CD69; CXCR6; IL-17A; IL-22 w zmienionej chorobowo skórze pacjentów (n = 5, skóra właściwa i naskórek) przed (tydz. 0) i w trakcie terapii (tydz. 4. i 12.) anty-interleukiną-17 (anty-IL-17) w porównaniu ze zdrową grupą kontrolną (n = 10, skóra właściwa i naskórek).

Różne litery oznaczają istotne statystycznie różnice pomiędzy różnymi punktami pomiarowymi i warstwami skóry, natomiast te same litery oznaczają brak różnic. ($p < 0,05$, test U Manna–Whitneya).



Rycina 3. Obszar immunoreaktywny (%) (x ± SEM) CD8; CD4; CD103; CD49; CD69; CXCR6; IL-17A; IL-22 w zmienionej chorobowo skórze pacjentów (n = 4, skóra właściwa i naskórek) przed (tydz. 0) i w trakcie terapii (tydz. 4. i 12.) anty-tumour necrosis factor alfa (anty-TNF-α) w porównaniu ze zdrową grupą kontrolną (n = 10, skóra właściwa i naskórek).

Różne litery oznaczają istotne statystycznie różnice pomiędzy różnymi punktami pomiarowymi i warstwami skóry, natomiast te same litery oznaczają brak różnic ($p < 0,05$, test U Manna-Whitneya).



Rycina 4. Obszar immunoreaktywny (%) (x ± SEM) CD8; CD4; CD103; CD49; CD69; CXCR6; IL-17A; IL-22 w zmienionej chorobowo skórze pacjentów (n = 4, skóra właściwa i naskórek) przed (tydz. 0) i w trakcie terapii (tydz. 4. i 12.) metotreksatem (MTX) w porównaniu ze zdrową grupą kontrolną (n = 10, skóra właściwa i naskórek)

Różne litery oznaczają istotne statystycznie różnice pomiędzy różnymi punktami pomiarowymi i warstwami skóry, natomiast te same litery oznaczają brak różnic. ($p < 0,05$, test U Manna–Whitneya).

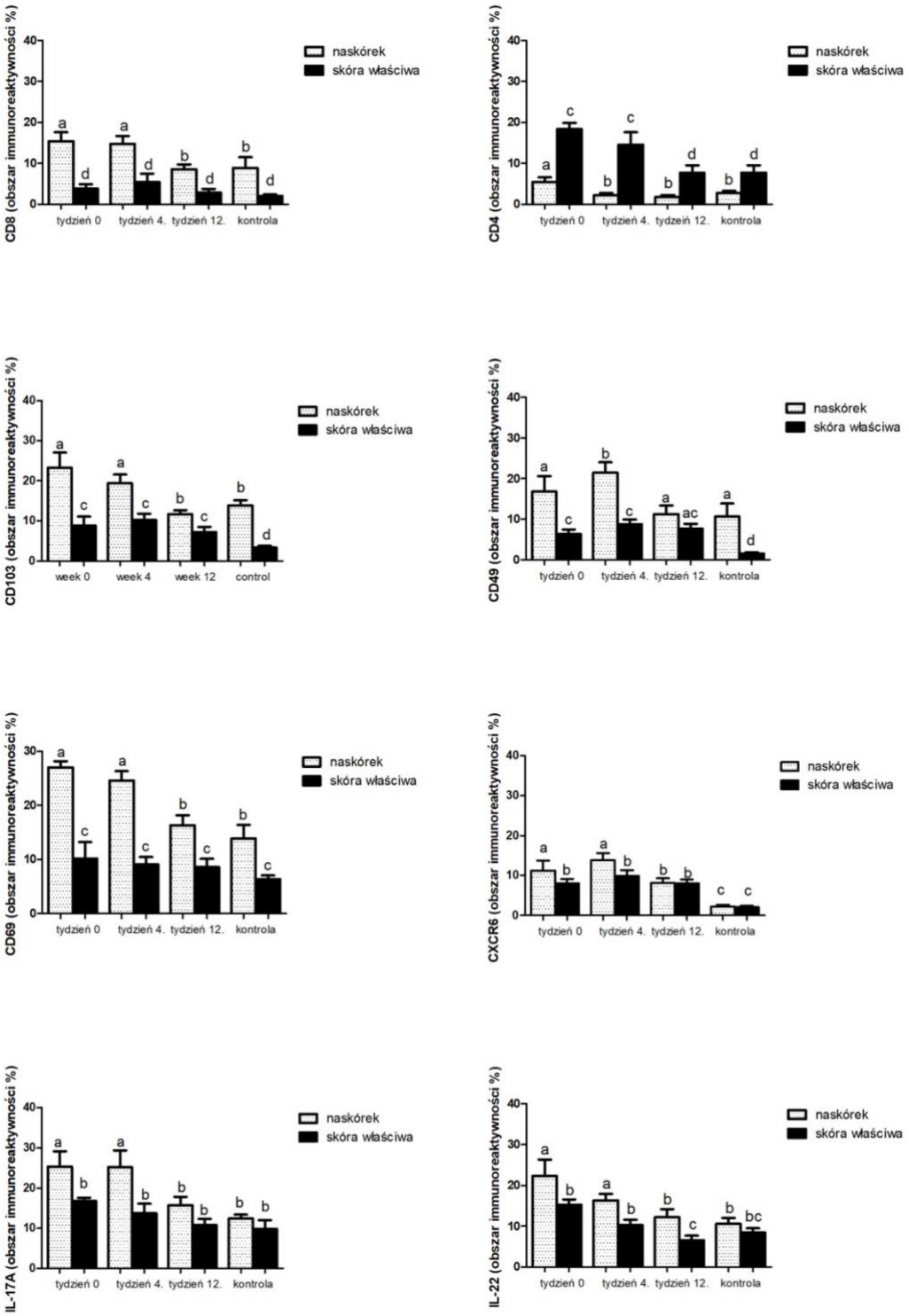
W przypadku leków stosowanych ogólnie najszybszą odpowiedź, już w 4. tygodniu leczenia, zaobserwowano w przypadku terapii anty-IL17, natomiast w przypadku metotreksatu i adalimumabu statystycznie istotny spadek większości markerów TRM zaobserwowano dopiero w 12. tygodniu leczenia. W niniejszym badaniu zauważono również, że zmniejszenie ekspresji antygenów specyficznych dla TRM obserwowano głównie w zmienionej chorobowo skórze właściwej, a w mniejszym stopniu w uszkodzonym naskórku. Może to wynikać z kilku czynników. Istnieje niewiele danych dotyczących przenikania leków ogólnoustrojowych do naskórka – brak unaczynienia może przyczyniać się do gorszej penetracji leków ogólnoustrojowych, co przekłada się na gorsze wyniki leczenia. Możliwe, że proces ustępowania zmiany łuszczycowej w naskórku i jej stopniowa normalizacja są wtórne do redukcji stanu zapalnego w skórze lub komórki TRM naskórka są bardziej oporne na terapię. W związku z tym powstaje pytanie: „Jak leki miejscowe mogą wpływać na ilość TRM w wykwitach łuszczycowych?".

W grupie 10 pacjentów chorujących na łuszczycę plackowatą o nasileniu lekkim wdrożono terapię miejscową preparatem zawierającym Cal/BD w pianie. Lek aplikowany był na zmiany łuszczycowe raz dziennie przez 4. tygodnie, następnie dwa razy w tygodniu. Po 4 tygodniach terapii statystycznie istotny spadek pola immunoreaktywnego zaobserwowano tylko w przypadku CD4 w naskórku. W 12. tygodniu terapii statystycznie istotne spadki powierzchni fluorescencji w porównaniu z tygodniem 0 zaobserwowano dla markerów: CD8, CD4, CD103, CD69, CXCR6, IL-17A oraz IL-22 w obrębie naskórka ($p < 0,05$). Zupełnie inaczej było w przypadku skóry właściwej. Po 12. tygodniach terapii zaobserwowano spadek powierzchni immunofluorescencyjnej jedynie w przypadku: CD4, IL-22 ($p < 0,05$). Co więcej po 12. tygodniach terapii powierzchnia immunoreaktywna markerów TRM w naskórku i skórze właściwej u pacjentów z grupy badanej była podobna do grupy kontrolnej w przypadku: CD8; CD4; CD69; IL17; IL-22 (ryc. 5). Powyższe wyniki świadczą o tym, że leczenie miejscowe preparatem zawierającym Cal/BD istotnie obniża ekspresję markerów TRM głównie w naskórku i w mniejszym stopniu w skórze właściwej w 12-tygodniowym okresie obserwacji. Wynika to prawdopodobnie ze słabszego przenikania leku do skóry właściwej oraz działania preparatu głównie na naskórek. Utrzymywanie się wysokiej ekspresji markerów TRM w skórze właściwej w przypadku stosowania jedynie terapii miejscowej może skutkować szybkim nawrotem zmian po zaprzestaniu leczenia.

Warto także zwrócić uwagę na czas niezbędny do uzyskania istotnych statystycznie zmian w ilości markerów TRM. W badaniu statystycznie istotny efekt leczenia miejscowego Cal/BD mierzony zmniejszeniem powierzchni immunofluorescencyjnej swoistych markerów TRM zaobserwowano dopiero w 12. tygodniu terapii (CD8, CD103, CXCR6, IL-17A, IL22), a w 4. tygodniu spadek dotyczył tylko CD4. Powyższe wyniki potwierdzają zasadność i konieczność stosowania terapii proaktywnej. W przypadku większości leków miejscowych, zgodnie z charakterystyką produktów leczniczych, terapia może być kontynuowana przez maksymalnie 3–4 tygodnie, co okazuje się być niewystarczającym czasem na osiągnięcie redukcji TRM. Do tej pory w leczeniu łuszczycy w terapii proaktywnej można stosować tylko Cal/BD (1 raz dziennie na zmiany łuszczycowe przez 4 tygodnie, następnie podtrzymująco dwa razy w tygodniu) [13]. Nie wiadomo, jak długo powinno trwać leczenie, aby całkowicie wyeliminować TRM i uzyskać długotrwałą remisję. Nawroty choroby są częste, zwłaszcza w przypadku leczenia reaktywnego, ponieważ utrzymujące się w skórze TRM pozostają zdolne do inicjowania kaskady zapalnej i promują tworzenie nowych blaszek łuszczycowych pomimo klinicznego ustąpienia zmian skórnych. Długofalowe, proaktywne podejście do leczenia, w tym terapia podtrzymująca po skutecznym leczeniu wstępnym, może pomóc w osiągnięciu dłuższych remisji choroby, poprawie wyników klinicznych oraz pozytywnie wpłynąć na jakość życia pacjentów [14].

Biorąc pod uwagę uzyskane wyniki badań, wydaje się, że połączenie terapii ogólnej z terapią miejscową może mieć wpływ na uzyskanie skuteczniejszej redukcji TRM zarówno w skórze właściwej, jak i naskórku.

Mały rozmiar prób stanowi ograniczenie wyżej opisanych badań. Niestety były one żmudne i wymagały pobrania potrójnej biopsji z tej samej okolicy, co było trudne dla pacjentów. Zalecany jest także dłuższy okres obserwacji (powtórna biopsja po 24 i 52 tyg.), co umożliwiłoby dalszą ocenę markerów TRM oraz ich korelację z czasem trwania remisji choroby.



Rycina 5. Obszar immunoreaktywny (%) (x ± SEM) CD8; CD4; CD103; CD49; CD69; CXCR6; IL-17A; IL-22 w zmienionej chorobowo skórze pacjentów (n = 10, skóra właściwa i naskórek) przed (tydz. 0) i w trakcie terapii (tydz. 4. i 12.) preparatem zawierającym kalcypotriol i dipropionian betametazonu (Cal/BD) w porównaniu ze zdrową grupą kontrolną (n = 10, skóra właściwa i naskórek).

Różne litery oznaczają istotne statystycznie różnice ($p < 0,05$) pomiędzy różnymi punktami pomiarowymi i warstwami skóry, natomiast te same litery oznaczają brak różnic. ($p < 0,05$, test U Manna–Whitneya).

WNIOSKI

1. Wykazano zwiększoną ilość markerów TRM w naskórku i skórze właściwej wykwitów łuszczycowych w porównaniu ze skórą zdrowych ochotników.
2. Istnieje wyraźna dodatnia zależność między ekspresją markerów TRM u pacjentów z łuszczycą plackowatą a czasem trwania choroby.
3. Na ilość markerów TRM nie ma wpływu intensywność zmian skórnych (oceniana według skal PASI, BSA, DLQI).
4. Wykazano zmniejszenie ekspresji markerów TRM w zmianach łuszczycowych, ujawniając zmniejszoną intensywność fluorescencji w czasie z każdą terapią ogólną (anty-IL-17, ADA, MTX).
5. Najszybsza redukcja ekspresji markerów TRM dotyczyła terapii anty-IL-17 w 4. tygodniu leczenia, natomiast w przypadku MTX i ADA w 12. tygodniu leczenia.
6. W ciągu 12-tygodniowej obserwacji terapia ogólna (anty-IL-17, ADA, MTX) zmniejsza ekspresję markerów TRM głównie w skórze właściwej zmian chorobowych, a w zdecydowanie mniejszym stopniu w naskórku.
7. W ciągu 12-tygodniowej obserwacji terapia miejscowa Cal/BD zmniejsza ekspresję markerów TRM głównie w naskórku, ale nie wpływa znacząco na ich ekspresję w skórze właściwej.
8. Spadek markerów TRM zaobserwowano dopiero w 12. tygodniu terapii miejscowej preparatem Cal/BD, co potwierdza zasadność terapii proaktywnej w miejscowym leczeniu łuszczycy plackowatej. Cztery tygodnie ciągłej terapii nie zmniejszyły istotnie ilości TRM w skórze pacjentów z łuszczycą zwykłą.

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ANKIETA PRACY ZAWODOWEJ I NAUKOWEJ

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4. Analiza bibliometryczna dorobku:

Mój całkowity dorobek naukowy wynosi 23 artykuły. Sumaryczny Impact Factor (IF) moich publikacji wynosi 26.965, przy punktacji MNiSW/KBN 1336.

5. Nagrody i wyróżnienia:

Stypendystka Warmińsko-Mazurskiej Izby Lekarskiej w Olsztynie w związku z uzyskaniem najwyższego wyniku w Lekarskim Egzaminie Końcowym wśród lekarzy stażystów Warmińsko-Mazurskiej Izby Lekarskiej w roku 2017.

Nagroda Rektora Uniwersytetu Warmińsko-Mazurskiego w Olsztynie za wyróżniającą się publikację naukową wydaną w 2020 roku.

Nagroda Rektora Uniwersytetu Warmińsko-Mazurskiego w Olsztynie za wyróżniającą się publikację naukową wydaną w 2021 roku.

6. Członkostwo w towarzystwach naukowych:

Polskie Towarzystwo Dermatologiczne od 2017 roku (od 2022 roku Sekretarz Oddziału Warmińsko-Mazurskiego PTD).

Członek EADV od 2022 roku.

7. Działalność dydaktyczna:

Prowadzenie zajęć ze studentami polsko- i angielskojęzycznymi na Uniwersytecie Warmińsko-Mazurskim w Olsztynie od 2020 r. (Klinika Dermatologii, Chorób Przenoszonych Drogą Płciową i Immunologii Klinicznej, Uniwersytet Warmińsko-Mazurski w Olsztynie).

OŚWIADCZENIA WSPÓŁAUTORÓW PUBLIKACJI

Olsztyn, 31.08.2023 r.

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1. Kasprowicz-Furmańczyk, M., Czerwińska, J., Placek, W., & Owczarczyk-Saczonek, A. (2021). Assessment of the tissue resident memory cells in lesional skin of patients with psoriasis and in healthy skin of healthy volunteers. *International Journal of Environmental Research and Public Health*, *18*(21), 11251. doi.org/10.3390/ijerph182111251

2. Owczarczyk-Saczonek, A., Kasprowicz-Furmańczyk, M., Czerwińska, J., Krajewska-Włodarczyk, M., & Placek, W. (2022). The effect of therapy on TRM in psoriatic lesions. *Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii*, *38*(1). doi.org/10.5114/ada.2021.113125

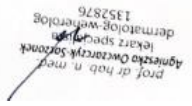
3. Kasprowicz-Furmańczyk, M., Czerwińska, J., Placek, W., & Owczarczyk-Saczonek, A. (2022). The Effect of the Long-Term Calcipotriol/Betamethasone Dipropionate Local Therapy on Tissue Resident Memory Cells Markers in Psoriatic Eruptions. *International Journal of Environmental Research and Public Health*, *19*(14), 8345. doi.org/10.3390/ijerph19148345

4. Kasprowicz-Furmańczyk, M., Narbutt, J., Borzęcki, A., & Owczarczyk-Saczonek, A. (2023). Does molecular scarring in psoriasis exist? A review of the literature. *Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii*, *40*(1). doi.org/10.5114/ada.2023.129322

Mój udział polegał na współtworzeniu koncepcji badań, redakcji wstępnej wersji manuskryptów oraz na nadzorze naukowym.

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Olsztyn, 14.03.2023 r.

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2. Owczarczyk-Saczonek, A., Kasprowicz-Furmańczyk, M., Czerwińska, J., Krajewska-Włodarczyk, M., & Placek, W. (2022). The effect of therapy on TRM in psoriatic lesions. *Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii*, *38*(1). doi.org/10.5114/ada.2021.113125

3. Kasprowicz-Furmańczyk, M., Czerwińska, J., Placek, W., & Owczarczyk-Saczonek, A. (2022). The Effect of the Long-Term Calcipotriol/Betamethasone Dipropionate Local Therapy on Tissue Resident Memory Cells Markers in Psoriatic Eruptions. *International Journal of Environmental Research and Public Health*, *19*(14), 8345. doi.org/10.3390/ijerph19148345

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3. Kasprowicz-Furmańczyk, M., Czerwińska, J., Placek, W., & Owczarczyk-Saczonek, A. (2022). The Effect of the Long-Term Calcipotriol/Betamethasone Dipropionate Local Therapy on Tissue Resident Memory Cells Markers in Psoriatic Eruptions. *International Journal of Environmental Research and Public Health*, *19*(14), 8345. doi.org/10.3390/ijerph19148345

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Mój udział polegał na zbieraniu/opracowywaniu piśmiennictwa oraz napisaniu wstępnej wersji manuskryptu.

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Oświadczam, że samodzielna i możliwa do wyodrębnienia część w/w pracy wykazuje indywidualny wkład lek. Marty Kasprowicz-Furmańczyk przy opracowaniu koncepcji tej pracy oraz nad opracowaniem manuskryptu.

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Mój udział polegał na zbieraniu/opracowywaniu piśmiennictwa oraz napisaniu wstępnej wersji manuskryptu.

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Oświadczam, że samodzielna i możliwa do wyodrębnienia część w/w pracy wykazuje indywidualny wkład lek. Marty Kasprowicz-Furmańczyk przy opracowaniu koncepcji tej pracy oraz nad opracowaniem manuskryptu.

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KOPIA POŚWIADCZENIA DOROBKU NAUKOWEGO

BIBLIOTEKA UNIWERSYTECKA
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Olsztyn, 08.08.2023 r.

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Wydział Lekarski
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Analiza bibliometryczna dorobku naukowego lek. Marty Kasprowicz-Furmańczyk
(na podstawie bazy Bibliografia Publikacji Pracowników Uniwersytetu Warmińsko-Mazurskiego w Olsztynie)

I. Analiza cytowań

Lp.	Rok	Tytuł publikacji	Ilość cytowań w bazie Web of Science Core Collection	Ilość cytowań w bazie Scopus
1	2022	*Changes in Tumor Necrosis Factor α (TNFα) and Peptidyl Arginine Deiminase 4 (PAD-4) Levels in Serum of General Treated Psoriatic Patients* **International Journal of Environmental Research and Public Health**	2	2
2	2022	*Surgical Treatment of Vitiligo* **International Journal of Environmental Research and Public Health**	5	3
3	2022	*The effect of therapy on TRM in psoriatic lesions* **Advances in Dermatology and Allergology**	1	1

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4	2022	*Effect of diet and weight loss on the severity of psoriasis* **Postępy Higieny i Medycyny Doświadczalnej**	1	1
5	2021	*Assessment of the Tissue Resident Memory Cells* in Lesional Skin of Patients with Psoriasis and in Healthy Skin of Healthy Volunteers **International Journal of Environmental Research and Public Health**	2	2
6	2021	*The Role of Chemokines in Psoriasis - An Overview* **Medicina-Lithuania**	12	12
7	2021	*D-chiro-inositol as a treatment in plaque psoriasis: a randomized placebo-controlled clinical trial* **Dermatologic Therapy**	5	6
8	2020	*The Correction of Facial Morphea Lesions by Hyaluronic Acid: A Case Series and Literature Review* **Dermatology and Therapy**	5	5
9	2020	*Immunological memory of psoriatic lesions* **International Journal of Molecular Sciences**	17	19
		Suma cytowań:	50	51

Podsumowanie cytowań w bazie Web of Science Core Collection/Basic Search w dniu 08.08.2023 r.:

liczba prac cytowanych = 9	**liczba cytowań = 50**	**Index Hirscha (Index h) = 5**

Podsumowanie cytowań w bazie Scopus w dniu 08.08.2023 r.:

liczba prac cytowanych = 9	**liczba cytowań = 51**	**Index Hirscha (Index h) = 4**

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Artykuł w czasopiśmie zagranicznym	9	6	9	23.208	950
Rozdział (fragment) w książce	2	0	0	0	0
Rozdział (fragment) w monografii naukowej	1	0	1	0	20
Razem:	**23**	**11**	**21**	**26.965**	**1366**

III. Wykaz publikacji

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Czasopismo: Advances in Dermatology and Allergology
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IDT: 0000078687.
Rok: 2022
Autorzy: Owczarczyk-Saczonek Agnieszka, Kasprowicz-Furmańczyk Marta, Czerwińska Joanna, Krajewska-Włodarczyk Magdalena, Placek Waldemar.
Tytuł oryginału: The effect of therapy on TRM in psoriatic lesions
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Impact Factor: 1.400
Punktacja MNiSW: 70.000
Dyscypliny czasopisma na podstawie wykazu MNiSW z 2021.12.21: nauki farmaceutyczne;nauki medyczne;nauki o zdrowiu;nauki chemiczne
Tytuł czasopisma w wykazie MNiSW z 2021.12.21: Postępy Dermatologii i Alergologii

Afiliacja: Owczarczyk-Saczonek Agnieszka [*] 0000-0002-8660-4889 3938531;335;23, Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;335;23, Czerwińska Joanna [*] 0000-0001-6371-8130 3961456;335;23, Krajewska-Włodarczyk Magdalena [*] 0000-0002-7857-347X;479;23, Placek Waldemar [*] 0000-0001-8310-9199 1273980;335;23

3.
IDT: 0000079879.
Rok: 2022
Autorzy: Płaszczyńska Anna, Klimek Paulina, Kasprowicz-Furmańczyk Marta, Owczarczyk-Saczonek Agnieszka.
Tytuł oryginału: Zastosowanie dipropionianu betametazonu w połączeniu z kalcypotriolem w terapii łuszczycy
Czasopismo: Aesthetica
Opis fizyczny: 2022, 51 (3), s. 46-52 ; bibliogr. 14 poz.
Adres url: https://aesthetica.com.pl/clients/25/files/files/046-052.pdf
p-ISSN: 2353-3412
Język publikacji: POL
Data ukazania się publikacji: 2022.05.31
Charakt. formalna: artykuł w czasopiśmie polskim
Charakt. merytoryczna: praca oryginalna
Punktacja MNiSW: 5.000
Uwaga dot. automatycznej identyfikacji: Niezidentyfikowano na wykazie dyscyplin
Afiliacja: Płaszczyńska Anna [*];355;23, Klimek Paulina [*] 0009-0006-5719-0177;355;23, Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;335;23, Owczarczyk-Saczonek Agnieszka [*] 0000-0002-8660-4889 3938531;335;23

4.
IDT: 0000082886.
Rok: 2022
Autorzy: Czerwińska Joanna, Kasprowicz-Furmańczyk Marta, Placek Waldemar, Owczarczyk-Saczonek Agnieszka.
Tytuł oryginału: Changes in Tumor Necrosis Factor α (TNFα) and Peptidyl Arginine Deiminase 4 (PAD-4) Levels in Serum of General Treated Psoriatic Patients
Czasopismo: International Journal of Environmental Research and Public Health
Opis fizyczny: 2022, 19 (14), s. 1-14 ; bibliogr. ; nr art. 8723
Adres url: https://www.mdpi.com/1660-4601/19/14/8723
p-ISSN: 1660-4601
Język publikacji: ENG
DOI: 10.3390/ijerph19148723
open-access-text-version: FINAL_PUBLISHED
open-access-licence: CC-BY

open-access-release-time: AT_PUBLICATION
open-access-article-mode: OPEN_JOURNAL
Data ukazania się publikacji: 2023.01.09
Charakt. formalna: artykuł w czasopiśmie zagranicznym
Charakt. merytoryczna: praca oryginalna
Inne bazy podające opis: Web of Science Core Collection ; Scopus
Punktacja MNiSW: 140.000
Dyscypliny czasopisma na podstawie wykazu MNiSW z 2021.12.21: architektura i urbanistyka;inżynieria lądowa i transport;inżynieria środowiska, górnictwo i energetyka;nauki farmaceutyczne;nauki medyczne;nauki o kulturze fizycznej;nauki o zdrowiu;nauki leśne;rolnictwo i ogrodnictwo;technologia żywności i żywienia;weterynaria;geografia społeczno-ekonomiczna i gospodarka przestrzenna;nauki o zarządzaniu i jakości;nauki prawne;nauki biologiczne;nauki chemiczne
Tytuł czasopisma w wykazie MNiSW z 2021.12.21: International Journal of Environmental Research and Public Health
Afiliacja: Czerwińska Joanna [*] 0000-0001-6371-8130 3961456;335;23, Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;335;23, Placek Waldemar [*] 0000-0001-8310-9199 1273980;335;23, Owczarczyk-Saczonek Agnieszka [*] 0000-0002-8660-4889 3938531;335;23

5.
IDT: 0000079488.
Rok: 2022
Autorzy: Frączek Alicja, Kasprowicz-Furmańczyk Marta, Placek Waldemar, Owczarczyk-Saczonek Agnieszka.
Tytuł oryginału: Surgical Treatment of Vitiligo
Czasopismo: International Journal of Environmental Research and Public Health
Opis fizyczny: 2022, 19 (8), s. 1-21 ; bibliogr. 84 poz. ; nr art. 4812
Adres url: https://www.mdpi.com/1660-4601/19/8/4812
p-ISSN: 1660-4601
Język publikacji: ENG
DOI: 10.3390/ijerph19084812
open-access-text-version: FINAL_PUBLISHED
open-access-licence: CC-BY
open-access-release-time: AT_PUBLICATION
open-access-article-mode: OPEN_JOURNAL
Data ukazania się publikacji: 2022.04.15
Charakt. formalna: artykuł w czasopiśmie zagranicznym
Charakt. merytoryczna: praca przeglądowa
Inne bazy podające opis: Web of Science Core Collection
Punktacja MNiSW: 140.000

Dyscypliny czasopisma na podstawie wykazu MNiSW z 2021.12.21: architektura i urbanistyka;inżynieria lądowa i transport;inżynieria środowiska, górnictwo i energetyka;nauki farmaceutyczne;nauki medyczne;nauki o kulturze fizycznej;nauki o zdrowiu;nauki leśne;rolnictwo i ogrodnictwo;technologia żywności i żywienia;weterynaria;geografia społeczno-ekonomiczna i gospodarka przestrzenna;nauki o zarządzaniu i jakości;nauki prawne;nauki biologiczne;nauki chemiczne
Tytuł czasopisma w wykazie MNiSW z 2021.12.21: International Journal of Environmental Research and Public Health
Afiliacja: Frączek Alicja [*];355;23, Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;335;23, Placek Waldemar [*] 0000-0001-8310-9199 1273980;335;23, Owczarczyk-Saczonek Agnieszka [*] 0000-0002-8660-4889 3938531;335;23

6.
IDT: 0000082896.
Rok: 2022
Autorzy: Kasprowicz-Furmańczyk Marta, Czerwińska Joanna, Placek Waldemar, Owczarczyk-Saczonek Agnieszka.
Tytuł oryginału: The Effect of the Long-Term Calcipotriol/Betamethasone Dipropionate Local Therapy on Tissue Resident Memory Cells Markers in Psoriatic Eruptions
Czasopismo: International Journal of Environmental Research and Public Health
Opis fizyczny: 2022, 19 (14), s. 1-12 ; bibliogr. ; nr art. 8845
Adres url: https://www.mdpi.com/1660-4601/19/14/8345
p-ISSN: 1660-4601
Język publikacji: ENG
DOI: 10.3390/ijerph19148345
open-access-text-version: FINAL_PUBLISHED
open-access-licence: CC-BY
open-access-release-time: AT_PUBLICATION
open-access-article-mode: OPEN_JOURNAL
Data ukazania się publikacji: 2023.01.10
Charakt. formalna: artykuł w czasopiśmie zagranicznym
Charakt. merytoryczna: praca oryginalna
Inne bazy podające opis: Scopus ; Web of Science Core Collection
Punktacja MNiSW: 140.000
Dyscypliny czasopisma na podstawie wykazu MNiSW z 2021.12.21: architektura i urbanistyka;inżynieria lądowa i transport;inżynieria środowiska, górnictwo i energetyka;nauki farmaceutyczne;nauki medyczne;nauki o kulturze fizycznej;nauki o zdrowiu;nauki leśne;rolnictwo i ogrodnictwo;technologia żywności i żywienia;weterynaria;geografia społeczno-ekonomiczna i gospodarka przestrzenna;nauki o zarządzaniu i jakości;nauki prawne;nauki biologiczne;nauki chemiczne
Tytuł czasopisma w wykazie MNiSW z 2021.12.21: International Journal of Environmental

Research and Public Health
Afiliacja: Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;335;23, Czerwińska Joanna [*] 0000-0001-6371-8130 3961456;335;23, Placek Waldemar [*] 0000-0001-8310-9199 1273980;335;23, Owczarczyk-Saczonek Agnieszka [*] 0000-0002-8660-4889 3938531;335;23

7.
IDT: 0000081326.
Rok: 2022
Autorzy: Owczarczyk-Saczonek Agnieszka, Kasprowicz-Furmańczyk Marta, Kuna Jakub, Klimek Paulina, Krajewska-Włodarczyk Magdalena.
Tytuł oryginału: Aseptic Abscess Syndrome in Rheumatoid Arthritis Patient
Czasopismo: Medicina-Lithuania
Opis fizyczny: 2022, 58 (10), s. 1-7 ; bibliogr. 15 poz. ; nr art. 1354
Adres url: https://www.mdpi.com/1648-9144/58/10/1354
p-ISSN: 1010-660X
e-ISSN: 1648-9144
Język publikacji: ENG
DOI: 10.3390/medicina58101354
open-access-text-version: FINAL_PUBLISHED
open-access-licence: CC-BY
open-access-release-time: AT_PUBLICATION
open-access-article-mode: OPEN_JOURNAL
Data ukazania się publikacji: 2022.10.13
Charakt. formalna: artykuł w czasopiśmie zagranicznym
Charakt. merytoryczna: praca oryginalna
Inne bazy podające opis: Web of Science Core Collection ; Scopus
Impact Factor: 2.600
Punktacja MNiSW: 40.000
Dyscypliny czasopisma na podstawie wykazu MNiSW z 2021.12.21: inżynieria biomedyczna;nauki farmaceutyczne;nauki medyczne;nauki o zdrowiu;weterynaria
Tytuł czasopisma w wykazie MNiSW z 2021.12.21: Medicina-Lithuania
Afiliacja: Owczarczyk-Saczonek Agnieszka [*] 0000-0002-8660-4889 3938531;335;23, Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;335;23, Kuna Jakub [*] 0000-0002-4216-9731;355;23, Klimek Paulina [*] 0009-0006-5719-0177;355;23, Krajewska-Włodarczyk Magdalena [*] 0000-0002-7857-347X;355;23

8.
IDT: 0000082424.
Rok: 2022
Autorzy: Owczarczyk-Saczonek Agnieszka, Kasprowicz-Furmańczyk Marta, Krajewska-

Włodarczyk Magdalena, Szepietowski Jacek.
Tytuł oryginału: Effect of diet and weight loss on the severity of psoriasis
Czasopismo: Postępy Higieny i Medycyny Doświadczalnej
Opis fizyczny: 2022, 76 (1), s. 450 - 460 ; bibliogr. 109 poz.
p-ISSN: 0032-5449
e-ISSN: 1732-2693
Język publikacji: ENG
DOI: 10.2478/ahem-2022-0039
open-access-text-version: FINAL_PUBLISHED
open-access-licence: CC-BY-NC-ND
open-access-release-time: AT_PUBLICATION
open-access-article-mode: OPEN_JOURNAL
Data ukazania się publikacji: 2022.12.15
Charakt. formalna: artykuł w czasopiśmie polskim
Charakt. merytoryczna: praca przeglądowa
Inne bazy podające opis: Scopus ; Web of Science Core Collection
Impact Factor: 0.300
Punktacja MNiSW: 40.000
Dyscypliny czasopisma na podstawie wykazu MNiSW z 2021.12.21: inżynieria biomedyczna;nauki farmaceutyczne;nauki medyczne;nauki o kulturze fizycznej;nauki o zdrowiu;rolnictwo i ogrodnictwo;technologia żywności i żywienia;weterynaria;zootechnika i rybactwo;nauki chemiczne
Tytuł czasopisma w wykazie MNiSW z 2021.12.21: Postępy Higieny I Medycyny Doświadczalnej
Afiliacja: Owczarczyk-Saczonek Agnieszka [*] 0000-0002-8660-4889 3938531;335;23, Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;335;23, Krajewska-Włodarczyk Magdalena [*] 0000-0002-7857-347X;355;23, Szepietowski Jacek [ ];000;000

9.
IDT: 0000080379.
Rok: 2022
Autorzy: Owczarczyk-Saczonek Agnieszka, Kasprowicz-Furmańczyk Marta, Krajewska-Włodarczyk Magdalena, Placek Waldemar.
Tytuł oryginału: The impact of external factors on psoriasis
Czasopismo: Postępy Higieny i Medycyny Doświadczalnej
Opis fizyczny: 2022, 76 (1), s. 177-187 ; bibliogr. 80 poz.
p-ISSN: 0032-5449
e-ISSN: 1732-2693
Język publikacji: ENG
DOI: 10.2478/ahem-2022-0017
open-access-text-version: FINAL_PUBLISHED

open-access-licence: CC-BY-NC-ND
open-access-release-time: AFTER_PUBLICATION
open-access-months: 1
open-access-article-mode: OPEN_JOURNAL
Data ukazania się publikacji: 2022.01.15
Charakt. formalna: artykuł w czasopiśmie polskim
Charakt. merytoryczna: praca przeglądowa
Inne bazy podające opis: Web of Science Core Collection ; Scopus
Impact Factor: 0.300
Punktacja MNiSW: 40.000
Dyscypliny czasopisma na podstawie wykazu MNiSW z 2021.12.21: inżynieria biomedyczna;nauki farmaceutyczne;nauki medyczne;nauki o kulturze fizycznej;nauki o zdrowiu;rolnictwo i ogrodnictwo;technologia żywności i żywienia;weterynaria;zootechnika i rybactwo;nauki chemiczne
Tytuł czasopisma w wykazie MNiSW z 2021.12.21: Postępy Higieny I Medycyny Doświadczalnej
Afiliacja: Owczarczyk-Saczonek Agnieszka [*] 0000-0002-8660-4889 3938531;335;23, Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;335;23, Krajewska-Włodarczyk Magdalena [*] 0000-0002-7857-347X;334;23, Placek Waldemar [*] 0000-0001-8310-9199 1273980;335;23

10.
IDT: 0000076065.
Rok: 2021
Autorzy: Kasprowicz-Furmańczyk Marta.
Tytuł oryginału: Choroby grudkowo-rumieniowe wymagające pilnej interwencji
Tytuł całości: Dermatologia interwencyjna / pod red. Agnieszki Owczarczyk-Saczonek
Adres wydawniczy, liczba stron: Poznań : Termedia Wydawnictwa Medyczne, 2021, s. 253-273 ; bibliogr.
Adres url: https://www.termedia.pl/Ksiazka/-298
p-ISBN: 978-83-7988-290-8
Język publikacji: POL
Liczba arkuszy: 1.05
Data ukazania się publikacji: 2021.09.02
Charakt. formalna: rozdział (fragment) w książce
Charakt. merytoryczna: praca oryginalna
Afiliacja: Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;335;23

11.
IDT: 0000070846.
Rok: 2021

Autorzy: Owczarczyk-Saczonek Agnieszka, Czerwińska Joanna, Wygonowska Ewa, Kasprowicz-Furmańczyk Marta, Placek Waldemar.
Tytuł oryginału: D-chiro-inositol as a treatment in plaque psoriasis: a randomized placebo-controlled clinical trial
Czasopismo: Dermatologic Therapy
Opis fizyczny: 2021, 34 (1), s. 1-17 ; bibliogr. 28 poz. ; nr art. e14538
p-ISSN: 1396-0296
Język publikacji: ENG
DOI: 10.1111/dth.14538
Data ukazania się publikacji: 2020.12.03
Charakt. formalna: artykuł w czasopiśmie zagranicznym
Charakt. merytoryczna: praca oryginalna
Inne bazy podające opis: Web of Science Core Collection ; Scopus
Impact Factor: 3.858
Punktacja MNiSW: 70.000
Dyscypliny czasopisma na podstawie wykazu MNiSW z 2021.12.21: inżynieria biomedyczna;nauki farmaceutyczne;nauki medyczne;nauki o zdrowiu;weterynaria
Tytuł czasopisma w wykazie MNiSW z 2021.12.21: Dermatologic Therapy
Afiliacja: Owczarczyk-Saczonek Agnieszka [*] 0000-0002-8660-4889 3938531;335;23, Czerwińska Joanna [*] 0000-0001-6371-8130 3961456;335;23, Wygonowska Ewa [*] 0000-0001-8701-1922 3928736;335;23, Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;335;23, Placek Waldemar [*] 0000-0001-8310-9199 1273980;335;23

12.
IDT: 0000076980.
Rok: 2021
Autorzy: Kasprowicz-Furmańczyk Marta, Czerwińska Joanna, Placek Waldemar, Owczarczyk-Saczonek Agnieszka.
Tytuł oryginału: Assessment of the tissue resident memory cells in lesional skin of patients with psoriasis and in healthy skin of healthy volunteers
Czasopismo: International Journal of Environmental Research and Public Health
Opis fizyczny: 2021, 18 (21), s. 1-12 ; bibliogr. 33 poz. ; nr art.11251
p-ISSN: 1660-4601
Język publikacji: ENG
DOI: 10.3390/ijerph182111251
open-access-text-version: FINAL_PUBLISHED
open-access-licence: CC-BY
open-access-release-time: AFTER_PUBLICATION
open-access-months: 1
open-access-article-mode: OPEN_JOURNAL
Data ukazania się publikacji: 2021.10.29

Charakt. formalna: artykuł w czasopiśmie zagranicznym
Charakt. merytoryczna: praca oryginalna
Inne bazy podające opis: Web of Science Core Collection ; Scopus
Impact Factor: 4.614
Punktacja MNiSW: 140.000
Dyscypliny czasopisma na podstawie wykazu MNiSW z 2021.12.21: architektura i urbanistyka;inżynieria lądowa i transport;inżynieria środowiska, górnictwo i energetyka;nauki farmaceutyczne;nauki medyczne;nauki o kulturze fizycznej;nauki o zdrowiu;nauki leśne;rolnictwo i ogrodnictwo;technologia żywności i żywienia;weterynaria;geografia społeczno-ekonomiczna i gospodarka przestrzenna;nauki o zarządzaniu i jakości;nauki prawne;nauki biologiczne;nauki chemiczne
Tytuł czasopisma w wykazie MNiSW z 2021.12.21: International Journal of Environmental Research and Public Health
Afiliacja: Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;335;23, Czerwińska Joanna [*] 0000-0001-6371-8130 3961456;355;23, Placek Waldemar [*] 0000-0001-8310-9199 1273980;335;23, Owczarczyk-Saczonek Agnieszka [*] 0000-0002-8660-4889 3938531;335;23

13.
IDT: 0000075724.
Rok: 2021
Autorzy: Zdanowska Natalia, Kasprowicz-Furmańczyk Marta, Placek Waldemar, Owczarczyk-Saczonek Agnieszka.
Tytuł oryginału: The role of chemokines in psoriasis - an overview
Czasopismo: Medicina-Lithuania
Opis fizyczny: 2021, 57 (8), s. 1-7 ; bibliogr. 51 poz. ; nr art. 754
p-ISSN: 1648-9144
Język publikacji: ENG
DOI: 10.3390/medicina57080754
open-access-text-version: FINAL_PUBLISHED
open-access-licence: CC-BY
open-access-release-time: AT_PUBLICATION
open-access-article-mode: OPEN_JOURNAL
Data ukazania się publikacji: 2021.07.27
Charakt. formalna: artykuł w czasopiśmie zagranicznym
Charakt. merytoryczna: praca oryginalna
Inne bazy podające opis: Web of Science Core Collection ; Scopus
Impact Factor: 2.948
Punktacja MNiSW: 40.000
Dyscypliny czasopisma na podstawie wykazu MNiSW z 2021.12.21: inżynieria biomedyczna;nauki farmaceutyczne;nauki medyczne;nauki o zdrowiu;weterynaria

Tytuł czasopisma w wykazie MNiSW z 2021.12.21: Medicina-Lithuania
Afiliacja: Zdanowska Natalia [*] 0000-0001-8939-4680;335;23, Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;335;23, Placek Waldemar [*] 0000-0001-8310-9199 1273980;335;23, Owczarczyk-Saczonek Agnieszka [*] 0000-0002-8660-4889 3938531;335;23

14.
IDT: 0000075594.
Rok: 2021
Autorzy: Placek Waldemar, Bechtold Agata, Kasprowicz-Furmańczyk Marta, Przekwas Jana, Budzyńska Anna, Gospodarek-Komkowska Eugenia.
Tytuł oryginału: Zakażenia skóry i jej przydatków - aspekty kliniczne
Tytuł całości: Mikrobiologia w dermatologii, wenerologii oraz w medycynie estetycznej i kosmetologii / redakcja naukowa Eugenia Gospodarek-Komkowska, Agnieszka Mikucka
Adres wydawniczy, liczba stron: Warszawa : Wydawnictwo Lekarskie PZWL, 2021, s. 135-156 ; bibliogr.
Uwagi: Praca rec.
Adres url:
http://katalog.nukat.edu.pl/lib/item?id=chamo:5076393&fromLocationLink=false&theme=nukat
p-ISBN: 978-83-200-6211-3
Język publikacji: POL
Liczba arkuszy: 2.10
Data ukazania się publikacji: 2021.03.01
Charakt. formalna: rozdział (fragment) w monografii naukowej
Charakt. merytoryczna: praca oryginalna
Punktacja MNiSW: 20.000
Afiliacja: Placek Waldemar [*] 0000-0001-8310-9199 1273980;335;23, Bechtold Agata [*];355;23, Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;335;23, Przekwas Jana [ ];000;000, Budzyńska Anna [ ];000;000, Gospodarek-Komkowska Eugenia [ ];000;000

15.
IDT: 0000078622.
Rok: 2021
Autorzy: Owczarczyk-Saczonek Agnieszka, Kasprowicz-Furmańczyk Marta, Krajewska-Włodarczyk Magdalena, Griffiths Christopher E..
Tytuł oryginału: Does the lifestyle of patients with psoriasis affect their illness?
Czasopismo: Postępy Higieny i Medycyny Doświadczalnej
Opis fizyczny: 2021, 75 (1), s. 643-654 ; bibliogr. 107 poz.
Adres url: https://sciendo.com/article/10.2478/ahem-2021-0033
p-ISSN: 0032-5449

e-ISSN: 1732-2693
Język publikacji: POL
DOI: 10.2478/ahem-2021-0033
open-access-text-version: FINAL_PUBLISHED
open-access-licence: CC-BY-NC-ND
open-access-release-time: AFTER_PUBLICATION
open-access-months: 1
open-access-article-mode: OPEN_JOURNAL
Data ukazania się publikacji: 2022.02.10
Charakt. formalna: artykuł w czasopiśmie polskim
Charakt. merytoryczna: praca przeglądowa
Inne bazy podające opis: Web of Science Core Collection ; Scopus
Impact Factor: 0.357
Punktacja MNiSW: 40.000
Dyscypliny czasopisma na podstawie wykazu MNiSW z 2021.12.21: inżynieria biomedyczna;nauki farmaceutyczne;nauki medyczne;nauki o kulturze fizycznej;nauki o zdrowiu;rolnictwo i ogrodnictwo;technologia żywności i żywienia;weterynaria;zootechnika i rybactwo;nauki chemiczne
Tytuł czasopisma w wykazie MNiSW z 2021.12.21: Postępy Higieny I Medycyny Doświadczalnej
Afiliacja: Owczarczyk-Saczonek Agnieszka [*] 0000-0002-8660-4889 3938531;335;23, Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;335;23, Krajewska-Włodarczyk Magdalena [*] 0000-0002-7857-347X;355;23, Griffiths Christopher E. [ ];000;000

16.
IDT: 0000077238.
Rok: 2021
Autorzy: Aleksandrowicz Hubert, Kasprowicz-Furmańczyk Marta, Owczarczyk-Saczonek Agnieszka, Placek Waldemar.
Tytuł oryginału: Cutaneous manifestations in a patient with adult-onset Stills disease
Tytuł równoległy: Objawy dermatologiczne u dorosłego pacjenta z chorobą Stilla
Czasopismo: Przegląd Dermatologiczny
Opis fizyczny: 2021, 108 (2), s. 184-190 ; bibliogr. 15 poz.
p-ISSN: 0033-2526
Język publikacji: ENG, POL
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Charakt. merytoryczna: praca kazuistyczna
Inne bazy podające opis: Web of Science Core Collection ; Scopus
Punktacja MNiSW: 20.000
Dyscypliny czasopisma na podstawie wykazu MNiSW z 2021.12.21: nauki farmaceutyczne;nauki medyczne;nauki o zdrowiu;weterynaria
Tytuł czasopisma w wykazie MNiSW z 2021.12.21: Przegląd Dermatologiczny
Afiliacja: Aleksandrowicz Hubert [*];355;23, Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;335;23, Owczarczyk-Saczonek Agnieszka [*] 0000-0002-8660-4889 3938531;335;23, Placek Waldemar [*] 0000-0001-8310-9199 1273980;335;23

17.
IDT: 0000078620.
Rok: 2021
Autorzy: Kasprowicz-Furmańczyk Marta, Owczarczyk-Saczonek Agnieszka.
Tytuł oryginału: The significance of secukinumab in the treatment of plaque psoriasis
Tytuł równoległy: Znaczenie sekukinumabu w leczeniu łuszczycy plackowatej
Czasopismo: Przegląd Dermatologiczny
Opis fizyczny: 2021, 108 (5), s. 372-384 ; bibliogr. 37 poz.
Adres url: https://www.termedia.pl/The-significance-of-secukinumab-in-the-treatment-of-plaque-psoriasis,56,46319,0,1.html
p-ISSN: 0033-2526
Język publikacji: ENG, POL
DOI: 10.5114/dr.2021.113155
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Data ukazania się publikacji: 2022.02.10
Charakt. formalna: artykuł w czasopiśmie polskim
Charakt. merytoryczna: praca przeglądowa
Inne bazy podające opis: Scopus ; Web of Science Core Collection
Punktacja MNiSW: 20.000
Dyscypliny czasopisma na podstawie wykazu MNiSW z 2021.12.21: nauki farmaceutyczne;nauki medyczne;nauki o zdrowiu;weterynaria
Tytuł czasopisma w wykazie MNiSW z 2021.12.21: Przegląd Dermatologiczny
Afiliacja: Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;335;23, Owczarczyk-Saczonek Agnieszka [*] 0000-0002-8660-4889 3938531;335;23

18.
IDT: 0000070871.
Rok: 2020
Autorzy: Owczarczyk-Saczonek Agnieszka, Kasprowicz-Furmańczyk Marta, Kruszewska Anna, Krajewska-Włodarczyk Magdalena, Bechtold Agata, Klimek Paulina, Placek Waldemar.
Tytuł oryginału: The Correction of Facial Morphea Lesions by Hyaluronic Acid: A Case Series and Literature Review
Czasopismo: Dermatology and Therapy
Opis fizyczny: 2020, 10 (6), s. 1423-1434 ; bibliogr. 45 poz.
p-ISSN: 2193-8210
Język publikacji: ENG
DOI: 10.1007/s13555-020-00438-z
open-access-text-version: FINAL_PUBLISHED
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Data ukazania się publikacji: 2020.09.02
Charakt. formalna: artykuł w czasopiśmie zagranicznym
Charakt. merytoryczna: praca kazuistyczna
Inne bazy podające opis: Web of Science Core Collection ; Scopus
Impact Factor: 3.264
Punktacja MNiSW: 100.000
Dyscypliny czasopisma na podstawie wykazu MNiSW z 2021.12.21: inżynieria biomedyczna;nauki farmaceutyczne;nauki medyczne;nauki o zdrowiu
Tytuł czasopisma w wykazie MNiSW z 2021.12.21: Dermatology and Therapy
Afiliacja: Owczarczyk-Saczonek Agnieszka [*] 0000-0002-8660-4889 3938531;335;23, Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;335;23, Kruszewska Anna [*];355;23, Krajewska-Włodarczyk Magdalena [*] 0000-0002-7857-347X;334;23, Bechtold Agata [*];355;23, Klimek Paulina [*] 0009-0006-5719-0177;355;23, Placek Waldemar [*] 0000-0001-8310-9199 1273980;335;23

19.
IDT: 0000065337.
Rok: 2020
Autorzy: Owczarczyk-Saczonek Agnieszka, Krajewska-Włodarczyk Magdalena, Kasprowicz-Furmańczyk Marta, Placek Waldemar.
Tytuł oryginału: Immunological memory of psoriatic lesions
Czasopismo: International Journal of Molecular Sciences
Opis fizyczny: 2020, 21 (2), s. 1-11 ; bibliogr. 52 poz. ; nr art. 625

p-ISSN: 1422-0067
Język publikacji: ENG
DOI: 10.3390/ijms21020625
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Data ukazania się publikacji: 2020.01.17
Charakt. formalna: artykuł w czasopiśmie zagranicznym
Charakt. merytoryczna: praca przeglądowa
Inne bazy podające opis: Web of Science Core Collection ; Scopus
Impact Factor: 5.924
Punktacja MNiSW: 140.000
Dyscypliny czasopisma na podstawie wykazu MNiSW z 2021.12.21: inżynieria biomedyczna;inżynieria chemiczna;inżynieria materiałowa;nauki farmaceutyczne;nauki medyczne;nauki o kulturze fizycznej;nauki o zdrowiu;nauki leśne;rolnictwo i ogrodnictwo;technologia żywności i żywienia;weterynaria;zootechnika i rybactwo;nauki biologiczne;nauki chemiczne;nauki fizyczne
Tytuł czasopisma w wykazie MNiSW z 2021.12.21: INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES
Afiliacja: Owczarczyk-Saczonek Agnieszka [*] 0000-0002-8660-4889 3938531;335;23, Krajewska-Włodarczyk Magdalena [*] 0000-0002-7857-347X;334;23, Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;335;23, Placek Waldemar [*] 0000-0001-8310-9199 1273980;335;23

20.
IDT: 0000070708.
Rok: 2020
Autorzy: Kasprowicz-Furmańczyk Marta, Owczarczyk-Saczonek Agnieszka, Kwaśny Mirosław, Placek Waldemar.
Tytuł oryginału: Lichen sclerosus: A review of the literature
Czasopismo: Polish Annals of Medicine
Opis fizyczny: 2020, 27 (2), s. 230-231 ; bibliogr. 54 poz.
p-ISSN: 1230-8013
Język publikacji: ENG
DOI: 10.29089/2020.20.00131
open-access-text-version: FINAL_PUBLISHED
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Data ukazania się publikacji: 2020.11.30
Charakt. formalna: artykuł w czasopiśmie polskim
Charakt. merytoryczna: praca kazuistyczna
Inne bazy podające opis: Scopus
Punktacja MNiSW: 40.000
Dyscypliny czasopisma na podstawie wykazu MNiSW z 2021.12.21: inżynieria biomedyczna;nauki farmaceutyczne;nauki medyczne;nauki o zdrowiu
Afiliacja: Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;335;23, Owczarczyk-Saczonek Agnieszka [*] 0000-0002-8660-4889 3938531;335;23, Kwaśny Mirosław [ ];000;000, Placek Waldemar [*] 0000-0001-8310-9199 1273980;335;23

21.
IDT: 0000070873.
Rok: 2020
Autorzy: Kasprowicz-Furmańczyk Marta, Owczarczyk-Saczonek Agnieszka, Placek Waldemar.
Tytuł oryginału: Cutaneous reactions after contact with santos palisander (Machaerium scleroxylon)
Tytuł równoległy: Reakcje niepożądane po kontakcie z palisandrem boliwijskim (Machaerium scleroxylon)
Czasopismo: Przegląd Dermatologiczny
Opis fizyczny: 2020, 107 (2), s. 184-190 ; bibliogr. 14 poz.
p-ISSN: 0033-2526
Język publikacji: ENG, POL
DOI: 10.5114/dr.2020.96362
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Data ukazania się publikacji: 2020.03.15
Charakt. formalna: artykuł w czasopiśmie polskim
Charakt. merytoryczna: praca kazuistyczna
Inne bazy podające opis: Web of Science Core Collection ; Scopus
Punktacja MNiSW: 20.000
Dyscypliny czasopisma na podstawie wykazu MNiSW z 2021.12.21: nauki farmaceutyczne;nauki medyczne;nauki o zdrowiu;weterynaria
Tytuł czasopisma w wykazie MNiSW z 2021.12.21: Przegląd Dermatologiczny
Afiliacja: Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;335;23, Owczarczyk-Saczonek Agnieszka [*] 0000-0002-8660-4889 3938531;335;23, Placek Waldemar [*] 0000-

0001-8310-9199 1273980;335;23

22.
IDT: 0000069905.
Rok: 2019
Autorzy: Kasprowicz-Furmańczyk Marta.
Tytuł oryginału: Choroby grudkowo-rumieniowe wymagające pilnej interwencji
Tytuł całości: Dermatologia interwencyjna / pod red. Agnieszki Owczarczyk-Saczonek
Adres wydawniczy, liczba stron: Poznań : Termedia Wydawnictwa Medyczne, 2019, s. 243-263 ; bibliogr.
Adres url: https://www.termedia.pl/Ksiazka/-276
p-ISBN: 978-83-7988-290-8
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Afiliacja: Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;355;23

23.
IDT: 0000059554.
Rok: 2018
Autorzy: Kasprowicz-Furmańczyk Marta, Kruszewska Anna, Owczarczyk-Saczonek Agnieszka, Placek Waldemar.
Tytuł oryginału: Neutrofilowe zapalenie grzbietów rąk
Tytuł równoległy: Neutrophilic dermatosis of the hand
Czasopismo: Forum Dermatologicum
Opis fizyczny: 2018, 4 (4), s. 148-152 ; bibliogr. 13 poz.
Adres url: https://journals.viamedica.pl/forum_dermatologicum/article/view/60327
p-ISSN: 2451-1501
Język publikacji: POL
Data ukazania się publikacji: 2018.04.30
Charakt. formalna: artykuł w czasopiśmie polskim
Charakt. merytoryczna: praca kazuistyczna
Punktacja MNiSW: 1.000
Dyscypliny czasopisma na podstawie wykazu MNiSW z 2021.12.21: nauki medyczne
Tytuł czasopisma w wykazie MNiSW z 2021.12.21: Forum Dermatologicum
Afiliacja: Kasprowicz-Furmańczyk Marta [*] 0000-0002-4177-8404;355;23, Kruszewska Anna [*];335;23, Owczarczyk-Saczonek Agnieszka [*] 0000-0002-8660-4889 3938531;335;23, Placek Waldemar [*] 0000-0001-8310-9199 1273980;335;23