Regulatory T cells (Treg) are characterized as a functionally highly specialized T cell subset responsible for optimal control of the immune response. Several subsets of Treg cells have already been identified, such as natural CD4+ Treg cells, Th3 cells, Tr1 cells, and CD8+ Treg cells. It is very likely that other distinct subsets will be added to this list in future. Natural Treg cells represent 5–10% of total CD4+ helper inducer T cells. They are characterized by the intermediate to high expression of the cell surface α-subunit of IL-2 receptor (CD25), as well as intracellular expression of transcription factor Foxp3. Phenotype of Tr1 cells is CD25−Foxp3−CD4+. These cells, upon activation via the TcR, produce high amounts of IL-10 and very low levels of IL-2 but they do not produce IL-4 (7). Th3 cells are phenotypically resembling Tr1 cells but their principal modulator is TGFB. CD8+ Treg cells are CD25+FoxP3+CD8+ and they play an important role in a protective immunity (3, 16, 36). Treg cells reveal suppressive function via a mechanism that requires cell to cell contact (natural CD4+ Treg cells) or the secretion of anti-inflammatory cytokines (adaptive Treg cells) (5, 17, 33). Another mechanism of Treg cells action is their cytotoxic activity which is mediated by granzymes and perforin release. Numerous target cells including both CD4+ and CD8+ subpopulations of T cells (11, 13), NK, and NKT cells, monocytes, dendritic cells, and granulocytes are regulated by this cytotoxic activity of Treg cells (26, 32, 34). Treg are able to restrict CD8+ memory effector T cells (20, 26). Thus, it is conceivable that increased number of cutaneous Treg cells could diminish pathogenic inflammatory CD8+ T cell functions in the psoriasis (23).

It is widely accepted that psoriasis is a systemic, predominantly T cell-mediated inflammatory disorder with skin manifestation. This disease is characterized by hyperproliferation and abnormal differentiation of keratinocytes with activated T cells and granulocytes accumulated in involved skin areas. Psoriasis is a multifactorial disease in which both genetic predisposition and many variable inciting factors such as infection, stress, skin trauma, to list only some, are involved (2, 31). Psoriatic Treg cells are functionally deficient in suppressing effector T cell responses. Although this deficiency is not absolute, higher numbers of psoriatic Treg cells are required to provide suppression similar to that of normal regulatory cells (31). These findings suggest that Treg cells are involved in the pathogenesis of psoriasis (23, 29, 31). Defects in the function or count of Treg appear to be common in different autoimmune diseases (10).
Goeckerman therapy (GT) is based on daily skin application of pharmaceutical coal tar with subsequent body exposition to UV light. This therapeutic approach is still preferred for its simple application, good clinical response, and low cost (12, 22). There are very sparse information addressing the influence of GT therapy of psoriasis on Treg cells. Therefore we aimed our research on this topic. Here we present our results which, to the best of our knowledge, for the first time confirmed that the relative count of CD3+/CD4+/CD25+/CD127low/- Treg is increased after Goeckerman therapy of psoriasis.

**Materials and Methods**

**Study group**

The study was approved by the Ethics Committee of the University Hospital in Hradec Králové. Informed written consent was obtained from each patient. 27 adult patients with psoriasis were enrolled to this study. Our study group consisted of 16 females and 11 males (average age: 44.5 ± 22.7 years). Patients with psoriatic arthritis were excluded from the study. 19 otherwise healthy blood donors (6 females, 13 males, average age: 38.9 ± 10.9 years) served as a control group.

**Goeckerman therapy**

Goeckerman therapy was indicated by the consulting dermatologist with patient-to-patient adjustments based on the activity of disease. The average duration of therapy was 15 days. The efficacy of Goeckerman therapy was assessed by clinical evaluation of erythema, desquamation, and skin infiltration using PASI score (Psoriasis Area Severity Index). The PASI score was calculated before and after treatment for each patient. GT is ceased when 30% decrease of PASI is achieved. Coal tar ointment with 5% pharmaceutical grade coal tar was applied daily overnight on affected skin (10–75% of total body surface). Each morning, the excess tar ointment was removed with oil bath. After the tar removal, the patient was irradiated with UV light. The duration of UV irradiation was individual, depending on disease activity (range 1–15 minutes). The light beam density (dose) was controlled by Sola–Scope 2000 spectrometer (Solatell, UK) and was 245.60 μW/cm² for UV-B radiation and 134.4 μW/cm² for UV-A radiation. Previous exposure of patients to UV irradiation and polyaromatic hydrocarbons was assessed by a questionnaire. Patients with this positive personal history were excluded from the study. Samples of heparinized venous blood were obtained by venipuncture of the cubital vein before treatment and again after completion of Goeckerman therapy (at the day of dismissal from the hospital) using BD Vacutainer sampling tubes. Venous blood was also collected from otherwise healthy blood donors who served as control.

![Fig. 1: Gating strategy to identify Treg cells. Peripheral blood obtained from healthy volunteers or psoriatic patients was stained with antibodies against CD3, CD127, CD25 and CD4. Lymphocyte population was gated by using SS (side scatter) and FS (forward scatter) dot plot (gate A). Then we used CD3 and CD4 antibodies to select CD3⁺CD4⁺ population (gate B). By using CD25 antibody we gated CD3⁺CD4⁺CD25high positive cells (gate C). Then we used antibody CD127 and defined final population of CD3⁺CD4⁺CD25highCD127low/- – Treg cells (gate D).](image-url)
Flow cytometry

The method which is now readily available to identify Treg cells is flow cytometry. However it is a difficult task to identify Treg cells by flow cytometry, as the most specific marker FoxP3 which is localized intracellularly is only detectable after cell permeabilization (7, 8, 30). Permeabilization step is laborious and confounding results are sometimes found. Therefore, it has been recently recognized that FoxP3+ cells are expressing CD127 in a significantly lower density. CD127 is a subunit of IL-7R (15). It was proved experimentally that CD3+/CD4+/CD25high/CD127low− cells are FoxP3+. It is now generally accepted that this phenotype can serve as a surrogate marker for Treg cells (24, 35). For routine testing, CD25high/CD127low− phenotype is enough specific and sensitive to identify Treg cells.

The immunophenotypic analysis was performed on erythrocyte lysed peripheral blood using a flow cytometer FC500 Cytomics (Beckman Coulter) with a 4-color antibody panel (Immunotech): CD3-FITC/CD127-PE/CD25-PC5/CD4-PC7. Appropriate isotype-matched negative control was used to avoid the influence of the background fluorescence staining. The obtained data were analysed using software CXP Analysis. Treg were characterized by the expression of CD3+CD4+CD25highCD127low− phenotype (Fig. 1). Treg counts are expressed as percentage of CD4+ T lymphocyte subpopulation in peripheral blood. Cytometric analyses were run by K. K. and D. V. who were blind to patient’s status to eliminate bias.

Statistical analysis

Statistical differences between the groups were evaluated by non-paired and paired t-test (MedCalc software, Belgium) after data normality evaluation. To exclude confounding effect of different age and sex presentation in patients and controls, unpaired t-test and chi-square was performed. The results are given as the mean ± standard deviation. P value less than 0.05 was considered as significant.

Results

Disease activity was significantly positively affected by Goeckerman therapy. Good clinical response was achieved in all patients. Pre-therapy PASI score 17.5 ± 6.5 dropped to 8.4 ± 4.6 after therapy (P < 0.0001).

At first, we compared Treg levels in normal and psoriatic blood. There was no significant difference in the number of Treg cells in the peripheral blood of healthy blood donors (2.9 ± 1.0%) and patients with psoriasis before initiation of GT (3.3 ± 1.2%); P = 0.2668. Compared to controls (2.9 ± 1.0%) the relative number of Treg cells in peripheral blood of patients with psoriasis after GT (4.3 ± 1.6%) was significantly elevated (P = 0.0019). The relative number of Treg was significantly higher in the patients with psoriasis after GT (4.3 ± 1.6%) than at the beginning of the therapy (3.3 ± 1.2%); P = 0.0042 (Fig. 2). Our results are summarized in Fig. 3.

![Fig. 2: Relative number of Treg cells in peripheral blood of one patient with psoriasis before and after Goeckerman therapy](image-url)
tumors and pathogenic microorganisms (25). or decreasing treg number might dampen immunity against exert their local suppressive function on pathogenic t cells cD4+cD25+Foxp3+ treg cells were completely absent in contribute to overall disease pathogenesis (29, 31). genetic predisposition), defects in treg cell function may modulate immune response, thereby maintaining homeostasis and self-tolerance (17, 37). Defect in a distinct treg subset results in enhanced inflammatory reaction directed predominantly to the mucosal surfaces. treg are a good target for ululating immune response, thereby maintaining homeostasis and, or prevention of immunological disorders.

Skin is normally covered with innocuous and weakly immunogenic normal microbial flora, to which vigorous ongoing immune responses would cause immunological chaos and chronic inflammation. Treg limit effector T cell activation to this normal nonpathogenic resident flora, while permitting the necessary T cell activation to acute infection (14, 18). Bovenschen et al. reported that whereas CD4+CD25–FoxP3+ Treg cells were completely absent in normal skin biopsies, these cells were both found in the upper psoriatic dermis and to a minimal extent in the epidermis of psoriatic skin. They indicated that Treg cells are not able to exert their local suppressive function on pathogenic T cells in the dermis and the epidermis of patients with psoriasis (5).

The ability to induce Treg cells and to promote their long-term survival in the periphery could be the proof of efficacy of immunosuppressive therapy. These induced Treg are capable of inhibiting proliferation of effector T cells (1, 21, 39). Up-regulation of Treg cell function or increase in the number of these cells might be beneficial for treatment of autoimmune diseases and allergies, and to prevent allograft rejection. Moreover, inhibiting Treg cell function or decreasing Treg number might dampen immunity against tumors and pathogenic microorganisms (25).

Chen et al. emphasized that the balance between activated effector cells and Treg cells is important for efficient immune response (6). In psoriasis, the imbalance between Treg and effector T cells has been recently described by several groups. Their findings suggest that the number and/or function of Treg cells may be impaired with consequent hyperproliferation of pathogenic T cell in psoriasis (6, 31, 38). In accordance with other reports (6, 29, 31, 38), we found that Treg frequency in peripheral blood of psoriasis patients is comparable to that of healthy controls. Saito et al. reported that the percentage of CD4+CD25–FoxP3+ Treg in peripheral blood mononuclear cells isolated from patients with psoriasis before bath-PUVA therapy was slightly lower than that in cells from healthy volunteer, but the difference was not significant (29). In line with our results, the percentage of Treg cells in the psoriatic patients was significantly higher after bath-PUVA therapy. Chen et al. as well as Zhang et al. reported that there is no significant difference in Treg cells in patients with psoriasis compared with normal controls (6, 38). Sugiyama et al. found that Treg in psoriatic patients displayed impaired suppressor activities that are not associated with a decrease in their number in the peripheral blood. They proposed that this impairment of Treg cells can ultimate to the failure of regulation of autoreactive T cells with their subsequent overproliferation in patients with psoriasis (31). Quaglino et al. demonstrated that therapy with biological drugs (infliximab, etanercept, efalizumab) is able to up-regulate the expression of the CD4+CD25brightFoxP3– Treg cell subset and that this increase is associated with the achievement of a clinical response (27). Furuhashi et al. reported that levels of Treg cells in patients with palmoplantar pustulosis after excimer light therapy (308 nm) were significantly higher than those at baseline (9).

This study in which we showed that the number of Treg cells in peripheral blood of patients with psoriasis is increased after Goeckerman therapy, adds a substantial supplementary piece to the list of GT immunomodulatory effects. Despite the long-time history of GT its immunomodulatory mechanisms are still not known in details. The most important drawback of this therapy is entire lack of knowledge of coal tar composition. Coal tar consists of largely undefined aliphatic and aromatic hydrocarbons which in combination with immunomodulatory effect of UV irradiation are responsible for diminished hyperproliferation of epidermal cells (12, 22). The combination of coal tar and UV light has been reported to be more effective than either therapy alone (19). Coal tar displays anti-inflammatory, antibacterial and antipruritic effects. It reveals also a photodynamic effect that makes the skin more sensitive to UV light (28). UV exposure induces the local and systemic suppression of the inflammatory response. It induces T cells with suppressor activity, inhibits the function of antigen-presenting cells, and stimulates the release of immunosuppressive cytokines (4).

Data addressing the influence of Goeckerman therapy on the number of T regulatory cells in patients with
psoriasis are entirely lacking. Our study is the first attempt to study effects of GT on Treg cells.

**Conclusion**

In conclusion, we found that the number of Treg cells is significantly increased in patients with psoriasis after Goeckerman therapy. With regard to our results we propose that the number and/or function of Treg cells may be involved in the pathogenesis of psoriasis. The elevation of these cells after GT might be functional and may contribute to the achievement of a good clinical response followed by long-term remission. Assessment of Treg cells may be useful marker of beneficial effects of Goeckerman therapy in patients with psoriasis.

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**References**

4. Borska L, Andrys C, Krejsek J et al. Genotoxic and apoptotic effects of Goeckerman therapy. with regard to our results we propose that the anti-inflammatory action of GT could be explained by the anti-inflammatory effect of GT. We hypothesize that Treg cells induced by GT might be functional and may contribute to the achievement of a good clinical response followed by long-term remission. Assessment of Treg cells may be useful marker of beneficial effects of Goeckerman therapy in patients with psoriasis.

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