Dear Editors,

Psoriasis vulgaris is a chronic, immune-mediated, inflammatory skin disorder with high worldwide prevalence (2–3 %). It has a great impact on quality of life and is associated with several comorbidities. Even though psoriasis is the focus of many studies, its pathogenesis is still not completely understood and is the result of complex interactions between keratinocytes, dendritic cells, T cells, neutrophils and mast cells. Interleukins such as IL-23 and IL-17, TGF-β1 and IL-10 also play important roles in its pathogenesis [1].

Dysregulation of T cell activity and regulatory counterparts lead to chronic T cell-mediated inflammatory conditions [1, 2]. Many research groups focus on the contribution of keratinocytes, lymphocytes and recently immunosuppressive regulatory T cells (Treg) to the pathogenesis of psoriasis [1]. Additionally, CD15-/CD14+/CD33high/HLA-DRlow monocytic myeloid-derived suppressor cells (M-MDSC) suppress T cell proliferation [3, 4]. In patients with psoriasis, peripheral blood-derived Treg are elevated and correlate with disease severity [5, 6]. Interestingly, Treg in psoriasis patients are poorly activated and exhibit little suppressive capacity [7]. Recent publications indicate that CD15-/CD14+/CD33high/HLA-DRlow M-MDSC suffer the same fate as Treg [2, 8]. However, little is known about the role of the rather immature, LIN- (CD3-/CD15-/CD14-/CD19-)/HLA-DRlow/CD11b+/CD33+ early-stage MDSC (E-MDSC) and CD14+/CD124+ M-MDSC, or the effect of topical versus systemic treatment regimens on the regulatory cell compartment.

We aimed to investigate the frequency and function of immune regulatory peripheral blood-derived MDSC and Treg in psoriasis, correlated with disease severity in patients undergoing topical versus systemic therapy. The monocentric study (ethical approval obtained from the Landesärztekammer Rheinland-Pfalz, #837.065.11), was performed between 5/2012 and 5/2018, and included 104 patients with different forms of psoriasis and different treatment regimens, as well as 23 healthy donors (for patient characteristics see Table 1). Patients were recruited from our outpatient clinic and written consent was obtained prior to their participation. Psoriatic patients with various disease presentations/therapies were included, with ages ≥ 18 years. Patients received systemic treatment for a minimum of three months. Patients with malignant disease or pregnancy were excluded. Our study design was aimed at determining differences in immune regulatory cell subsets in patients with psoriasis and their correlation with systemic therapy. Baseline levels of these cell types were defined in patients receiving topical therapy only, assuming that this would not modulate systemic immune regulatory phenomena. We chose healthy individuals with no history of skin disease as controls.

The frequency of all investigated subtypes of MDSC in psoriatic patients who received only topical therapy tended

| Table 1 Patient characteristics. |
|---------------------------------|
| **Psoriasis patients**          |
| **Number of patients, n**       |
| **All**                         |
| **Topical therapy**             |
| **Systemic therapy**            |
| **Healthy donors**              |
| **Age in years, median (min-max)** | 44 (19–82) | 49 (18–82) | 32 (24–51)* |
| **Male/Female**                 | 21/9 | 52/22 | 5/10* |
| **Number of patients**          | 104 | 30 | 74 | 23 |
| **Psoriasis form**              |
| Psoriasis vulgaris (PASI range 0–45.8) | 85 | 26 | 59 |
| PASI < 10                       | 7 | 69 |
| PASI 10–20                      | 12 | 2 |
| PASI > 20                       | 10 | 1 |
| Psoriasis arthritis (PASI range 0–30.2) | 16 | 3 | 13 |
| Nail psoriasis (PASI n.a.)      | 3 | 1 | 2 |

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to be greater than in healthy individuals (Figure 1). MDSC have already been studied in cancer patients due to their increased numbers in peripheral blood [3]. In line with our findings, recent studies also revealed an impact of MDSC in inflammatory diseases including asthma, inflammatory bowel disease and arthritis. Especially E-MDSC and M-MDSC appear to be suppressive and lead to reduced effector T cell proliferation [9].

While the percentage of CD14+/CD124+ M-MDSC in psoriatic patients with systemic therapy was comparable to that of controls (and less than that of patients with topical therapy), the mean percentage of CD15+/CD14+/CD33low/HLA-DRlow M-MDSC and LIN−(CD3−/CD15−/CD14−/CD19−)/HLA-DRlow/CD11b+/CD33 E-MDSC tended to be higher in patients with systemic therapy than in patients with topical treatment. This also correlated with increasing PASI scores. With regard to MDSC playing a role in the immunosuppression of inflammatory immune responses, our observation that frequencies of E- and CD15+/CD14+/CD33low/HLA-DRlow M-MDSC were increased under systemic therapy may be relevant to the therapeutic efficacy of systemic approaches.

We next analyzed the effect of systemic therapy on MDSC in more detail, in particular the effect of various approved biologicals in comparison to conventional anti-psoriatic drugs and targeted intracellularly active small molecules (Figure 1). Systemic therapy with secukinumab and apremilast clearly reduced the frequency of M-MDSC. In addition, secukinumab treatment was associated with a significantly decreased frequency of CD15+/CD14+/CD33low/HLA-DRlow M-MDSC as compared to treatment with fumaric acid. The other biologicals and immunosuppressive agents mentioned had no consistent effect on CD15+/CD14+/CD33low/HLA-DRlow M-MDSC, CD14+/CD124+ M-MDSC or LIN−(CD3−/CD15−/CD14−/CD19−)/HLA-DRlow/CD11b+/CD33 E-MDSC.

In the next step, we assessed the correlation of Treg frequencies with treatment modality. The percentage of CD4+CD25+CD127lowFoxp3+ Treg in CD4+ cells was not different in psoriatic patients with topical treatment than in controls (Figure 1). However, psoriasis patients with topical therapy had lower frequencies of functional HLA-DR+ Treg than controls, and systemic therapy significantly increased the percentage of highly suppressive HLA-DR+ Treg. In addition, in 19 out of a group of 74 patients with systemic therapy, we found a higher frequency of activated GARP+ Treg than in patients with only topical therapy, even though the difference did not reach statistical significance. The frequency of activated GARP+ Treg increased with increasing PASI scores in both therapy groups. Thus, systemic treatment led to increased frequencies of highly functional, activated Treg comparable to the frequencies in healthy controls, implying that frequencies of activated Treg could serve as markers for treatment success. In addition, our study showed that systemic treatment may act by affecting the activation status of regulatory cells, rather than by altering their frequencies.

We also assessed the effect of different types of systemic treatment on Treg frequencies (Figure 1). Most drugs appeared to increase the frequency of HLA-DR+ Treg. Enhanced expression of GARP+ Treg was induced by anti-TNF and anti-IL-12/23 biologicals (Figure 1), but not by anti-IL-17A, suggesting a stronger immunomodulation toward suppression.

Finally, we investigated the levels of immunosuppressive interleukin-10 (IL-10), which is known to attenuate pathogenic T cell development in peripheral blood. Systemic therapy was associated with significantly increased IL-10 secretion in psoriatic patients compared to controls (Figure 2). While IL-10 levels in controls and patients with topical therapy were below the lower limit of detection, systemic therapy increased IL-10 secretion significantly. Further investigation showed that apremilast, etanercept and ustekinumab strongly increased IL-10 secretion. This is consistent with data of other groups showing higher levels of circulating IL-10 after treatment with e.g. etanercept [10]. In the present study, the cellular source of IL-10 was not assessed. Preliminary data indicated that B cells, Treg and MDSC were responsible for producing IL-10; frequencies of pDC and monocytes were not different between the groups studied (data not shown).

Many studies have indicated that the frequencies of immunocompetent cells differ between circulating and skin-infiltrating cells. It is therefore difficult to draw conclusions from a study investigating the effect of PBMCs in the peripheral blood on the complex immunoregulatory skin network in general, and the fact that each patient was examined at a different time point since commencement of therapy may also affect the study results. However, our goal was not to perform a longitudinal observational study under therapy, but to generally assess the immune status of patients under local and systemic therapy. Circulating regulatory cells and cytokines in the peripheral blood of patients may serve as peripheral biomarkers that are more accessible for measuring therapeutic efficacy. The number of patients in the individual systemic treatment arm was low, so conclusions from treatment arm comparisons should be regarded with caution. In addition, we cannot exclude the possibility that systemic treatment did not indirectly affect immune parameters by influencing systemic inflammation, or that the drugs had a direct effect. We hypothesize that due to the efficacy of the drugs in controlling skin inflammation (indicated by strongly reduced PASI scores), immune parameters of the periphery are modulated as well.

Taken together, we found that immunosuppressive CD15+/CD14+/CD33low/HLA-DRlow M-MDSC and E-MDSC
Figure 1 Modulation of the frequency of various subtypes of MDSC and Treg function in psoriatic patients depends on treatment. Peripheral blood mononuclear cells (PBMCs) were isolated, frozen and stored at –180°C until analysis. For flow cytometry, thawed cells were stained with fluorochrome-conjugated antibodies against CD3 (clone UCHT1), CD4 (clone M-T466), CD11b (clone ICRF44), CD14 (clone 18D11), CD15 (clone VIMCD6), CD19 (clone HIB19), CD25 (clone M-A251), CD33 (clone WM53), CD124/IL-4Ra (Clone G07F6), CD127 (clone A019D5), GARP (clone REA166), HLA-DR (clone L243), and Foxp3 (clone 259D). Foxp3 was stained intranuclearly. Flow cytometry analysis was performed on a BD LSR II. Flow cytometric data were analyzed on the free cloud-based platform Cytobank (Santa Clara, CA, USA). After gating on live cells, myeloid cell gating differentiated between two M-MDSC subpopulations: the CD14+/CD124+ M-MDSC and CD15−/CD14+/CD33high/HLA-DR low M-MDSC, and lineage negative, LIN− (CD3−/CD15−/CD14−/CD19−)/HLA-DR low/CD11b+/CD33+ E-MDSC. The mean fluorescence intensity of HLA-DR and GARP indicated the level of suppressive activity/activation of CD4+CD25+CD127−Foxp3+ Treg. Values represent percentages in total number of MDSCs and Treg in the peripheral blood of 104 patients (topical therapy: n = 30; systemic therapy: n = 74) and 23 healthy controls. Means ± SEM are shown (Dunn’s Multiple Comparison Test: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001).

Abbr.: PASI, Psoriasis Area and Severity Index; Ctr., healthy controls

frequencies were greater in psoriasis patients under systemic therapy than in patients with topical therapy and healthy donors. The overall Treg frequency in the total CD4+ cells did not differ in general between psoriasis patients and controls. Nevertheless, systemic treatment appeared to “normalize” the frequencies of activated, highly suppressive Treg to the le-
vel of healthy donor controls, implying that the frequency of HLA-DR+ Treg could serve as a marker for treatment success. This inhibitory signature may be relevant to the therapeutic efficacy of systemic approaches. The present study was intended to define the immune status in topically versus systemically treated patients at any time point during therapy, and it will be of great interest to compare the pretherapeutic immune status and its development under different therapies including more detailed clinical correlation in further prospective studies.

Our results indicate that elevated levels of MDSC, activated Treg and immunosuppressive IL-10 in the peripheral blood of psoriatic patients differ and correlate with treatment regimens, suggesting the induction of an immunosuppressive phenotype in systemically treated patients. More functional studies are needed in order to understand the underlying regulatory mechanisms in detail.

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Conflict of interest

None.

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