BJD
British Journal of Dermatology

Differential effects of phototherapy, adalimumab and betamethasone–calcipotriol on effector and regulatory T cells in psoriasis

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Summary

Background Psoriasis is a chronic T-cell-mediated skin disease with marked social and economic burdens. Current treatments are unsatisfactory, with unpredictable remission times and incompletely understood modes of action. Recent advances in our understanding of the pathogenesis of psoriasis have identified the imbalance between $CD4^+$ T effector cells, particularly the T helper (Th)17 subset, and regulatory T cells (Tregs) as key to the development of psoriatic lesions, and therefore a novel therapeutic target.

Objectives To quantify in patients the effects of three commonly used psoriasis treatment modalities on the Th1, Th2, Th17 and Treg subsets, and to test whether any change correlates with clinical response.

Methods Flow cytometry was used to enumerate Th1, Th2, Th17 and Treg subsets in blood and skin of patients with psoriasis before and after receiving any of the following treatments: narrowband ultraviolet B (NB-UVB), adalimumab and topical betamethasone–calcipotriol combination (Dovobet®)

Results All patients responded clinically to the treatments. NB-UVB significantly increased the numbers of circulating and skin Tregs, while, by contrast, adalimumab reduced Th17 cells in these compartments, and Dovobet had dual effects by both increasing Tregs and reducing Th17 cells.

Conclusions The differential effects reported here for the above-mentioned treatment modalities could be exploited to optimize or design therapeutic strategies to overcome the inflammatory drivers more effectively and restore the Th17–Treg balance in psoriasis.

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Accepted for publication
14 December 2017

Funding sources
The Egyptian Cultural Bureau Office (London) and Grampian NHS Endowments, grant RG12745.

Conflicts of interest
None to declare.

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DOI 10.1111/bjd.16336

What's already known about this topic?

- The imbalance between $CD4^+$ T effector cells, particularly the T helper (Th)17 subset, and regulatory T cells (Tregs) is key to the development of psoriatic lesions, and therefore a novel therapeutic target.

What does this study add?

- This study quantifies in patients the effects of three commonly used psoriasis treatment modalities on the Th1, Th2, Th17 and Treg subsets, and tests whether any changes correlate with clinical response.

What is the translational message?

- The results can be used to optimize or design therapeutic strategies to overcome the inflammatory drivers and restore the Th17–Treg balance in psoriasis.

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Psoriasis is a chronic relapsing skin disease that affects 2–4% of the population in Western countries.[1] Although not contagious, psoriasis greatly impacts on patients' quality of life, and has been linked with social stigmatization, discomfort and psychological distress.[2] Despite the availability of many treatments, responses remain unsatisfactory, and the frequent relapses, together with absence of long-term control, make psoriasis a challenging condition. This therapeutic inadequacy reflects the need for a more complete understanding of the cellular and molecular determinants of psoriasis.

An important advance has been recognition that the disease is mediated by T cells, and that different $CD4^+$ T helper (Th) cell or regulatory T (Treg) subsets can drive or control the pathogenic responses. For many years, psoriasis was classified as a Th1-mediated skin disease.[3] However, the recent description of abundant Th17 cells in psoriasis lesions,[4] and the evidence of impaired Treg function,[5] have led to a new model in which the disease is caused by an imbalance between inflammatory effector T (Teff) cells, represented by Th17 and to a lesser extent Th1 subsets, and Tregs.[5] Although skewing a Th17 bias back towards Tregs is emerging as a rational therapeutic goal in psoriasis, it is not known how effectively conventional and more recent treatments correct this balance, and whether any such restoration correlates with clinical improvement. Nonselective treatments such as ciclosporin[6] inhibit both Teffs and Tregs, leading to rebound of psoriasis on stopping therapy.

Narrowband ultraviolet B (NB-UVB) is a popular and effective tool, yet the molecular mechanisms underlying its efficacy are incompletely understood.[7] More recently, anti-tumour necrosis factor (TNF)-α reagents such as adalimumab have emerged as valuable biological agents with significant anti-inflammatory effects, although with introduction to regular clinical use, they are associated with side-effects, loss of efficacy and paradoxical cases of psoriasis.[8] Dovobet® (LEO Pharma, Princes Risborough, U.K.), containing betamethasone and the synthetic vitamin D derivative calcipotriol, is widely used in clinical practice owing to its complementary antiproliferative and anti-inflammatory mechanisms.[9] Vitamin D3 analogues were also found to have beneficial effects in murine models of autoimmune, allergic and inflammatory diseases.[10] Overall, we suggest that better understanding of the mechanisms of success of these treatments can lead to innovation of new, more targeted therapeutic strategies with fewer side-effects.

In this study, we quantified the effects of NB-UVB, adalimumab and Dovobet on circulating and skin T cells, with particular interest in the critical balance between Th17 and Treg cells, and correlated these changes to clinical response measured by Psoriasis Area and Severity Index (PASI) score. In particular, we wanted to test whether the most effective treatments would skew this balance in favour of Tregs, which are predicted to be important for immune tolerance and prevention of autoimmunity.

Patients and methods

Patients

This study was approved by the North of Scotland Research Ethics Committee and the Medicines and Healthcare Products Regulatory Agency according to the Declaration of Helsinki protocols. All participants signed written informed consent. Thirty-four patients (23 male and 11 female; mean age $50 \pm 14{\cdot}5$) were recruited from Aberdeen Royal Infirmary, Scotland, U.K., with moderate-to-severe psoriasis untreated with topical therapy for 2 weeks or with systemic therapy for 4 weeks. Blood samples and 6-mm punch biopsies were taken from lesional skin at baseline and 6 weeks after treatment with each of NB-UVB ($n = 15$), adalimumab ($n = 11$) and Dovobet® ($n = 8$). Nonlesional 6-mm punches were taken from one group of patients ($n = 8$) at baseline.

The number of patients studied was limited by the restricted inclusion and exclusion criteria, as we were looking for treatment-naive patients to avoid bias in the results. Lesional biopsies were taken from the centre of plaques, and nonlesional samples were taken from the adjacent psoriasis-free skin. Post-treatment samples were collected from cleared psoriatic skin at the same site as pretreatment samples. We chose 6 weeks for post-treatment samples as data from previous similar experiments showed significant immune response to treatment as early as 4–6 weeks.

Patients with psoriasis for whom the decision to treat with NB-UVB was made were exposed to known amounts of NB-UVB three times weekly according to a standard escalating protocol based on minimal erythemal dose. This is defined as the dose that caused just perceptible erythema 24 h after irradiation. Standard dose ranges were between 0·55 and 3·13 J cm^{-2}. Whole-body UVB was given in a Waldmann 7001 cabinet (Waldmann Lichttechnik GmbH, Villingen-Schwenningen, Germany), incorporating 24 100-W Philips TL-01 fluorescent lamps (311–313 nm).

Adalimumab is a preloaded pen device that automatically injects the drug by subcutaneous infusion. Injection was done by the patient at home after initial illustration and training by the doctor or biological nurse. The dose was 80 mg subcutaneously at week 0, then 40 mg at week 1 and then every other week thereafter. Betamethasone–calcipotriol (Dovobet) is prescribed as a twice-daily dose for 6 weeks to the affected areas. The selection of patients to the treatment arm was not part of the study and was dictated solely by the clinical need. Control samples were provided by psoriasis-free patients who attended the dermatology clinic for excision of naevi.

Samples

Skin samples were physically disaggregated *ex vivo* for 1 min using 50-μmol L^{-1} medicons in the Medimachine (BD Biosciences, San Jose, CA, U.S.A.), allowing for collection of viable lymphocytes.[11] The cell suspension was then used for

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culture or flow cytometry. Peripheral blood mononuclear cells were obtained by density gradient centrifugation (Ficoll-Paque; GE Healthcare, Waukesha, WI, U.S.A.).

Flow cytometric analysis

Cell suspensions were analysed using BD LSRII research flowcytometry, after staining with combinations of the following antibodies and their isotype controls: anti-CD4-APC-CY7, anti-CD25-Alexa Fluor 700, anti-CCR4-PE-CY7 (all BD Biosciences), anti-CCR6-Alexa Fluor 647 (BioLegend, San Diego, CA, U.S.A.), anti-CD127-Alexa Fluor 647, anti-forkhead box (Fox)P3-Alexa Fluor 488, anti-GATA-3-Alexa Fluor 647 (all BD Biosciences) and anti-Tbet-PE (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.). For intracellular staining, cells were fixed and permeabilized using the Cytofix/Cytoperm kit (BD Biosciences) as per the manufacturer's protocol. Data were analysed using FlowJo version 7.6 (Tree Star Inc., Ashland, OR, U.S.A.). The lymphocyte population was selected, then identification of the pure $CD4^+$ proportion of T cells was gated on the isotype control. The cell populations were calculated as percentages of $CD4^+$ T cells in the whole lymphocytic count and are presented as the mean ± SD. Identification of the different T-cell subsets and gating is shown in Figures S1–S4 (see Supporting Information).

Statistical analysis

Statistical analyses were carried out using Prism GraphPad 5 for windows, version 5.02, 2008 (GraphPad Software, La Jolla, CA, U.S.A.). A two-tailed paired t-test was used to compare proportions of cells in lesional vs. nonlesional skin, and blood of patients. The Mann–Whitney U-test was used to compare the proportions of cells between blood and skin of patients and healthy controls. The changes in proportions of T-cell subsets with treatment were correlated to changes in PASI score using Spearman correlation. The level of significance was taken as $P \leq 0{\cdot}05$.

Results

Clinical response

To study the clinical and immunological effects of the three different therapies, 34 patients with moderate-to-severe psoriasis were recruited and received 6-week treatment regimens of NB-UVB ($n = 15$), adalimumab ($n = 11$) or Dovobet ($n = 8$). All patients responded clinically to treatment; the mean ± SD PASI scores for each treatment group decreased from 5·7 ± 4·3 to 1·5 ± 1·2, 16·0 ± 6·1 to 5·6 ± 5 and 5·0 ± 5 to 1·7 ± 8, respectively.

The skin and blood of patients with psoriasis contain significantly higher proportions of T helper 17 cells and lower proportions of regulatory T cells compared with healthy controls

The proportions of $CD4^+$ Th cells with Th1, Th2, Th17 and Treg phenotypes were compared in lesional and nonlesional skin, and in blood, of patients with psoriasis at enrolment to the study. In line with reports describing the phenotypes of these subsets, and our own previous studies,[4] we used the characteristic combinations of markers $CD4^+$ $Tbet^+$, $CD4^+$ $GATA\text{-}3^+$, $CD4^+$ $CCR4^+$ $CCR6^+$ interleukin (IL)-$23R^+$ and $CD4^+$ $CD25^+$ $FoxP3^+$ $CD127^{lo}$ to identify Th1, Th2, Th17 and Treg cells, respectively. The choice of Th17 markers was based on literature indicating significant expression of the characteristic combination of $CCR4^+$ and $CCR6^+$ on Th17 cells,[12–14] and CCR6 is critically required for IL-23-induced psoriasis lesions.[15] IL-23 helps in the development of Th17 cells[16,17] and IL-23R is expressed only on activated T cells.[18] RORγT is the transcription factor for Th17 cells,[19] and is also expressed by dermal γδ T cells.[15] We did not rely on IL-17A expression to identify the Th17-cell population, because IL-17A can be produced by other cells, including γδ T cells, $CD8^+$ memory T cells, eosinophils, neutrophils, monocytes and mast cells, contributing to the common pole of IL-17 in psoriasis.[20–23] Due to this complex origin and phenotyping of Th17 cells, we chose to combine the characteristic chemokines ($CCR4^+$ $CCR6^+$), with IL-23R for purification and identification of the Th17-cell population.

Examples of representative flow cytometric data from the blood and skin of patients and healthy controls are illustrated in Figures S5 and S6 (see Supporting Information), and the results are summarized in Figure 1. The most striking findings were that patients' lesional skin and blood contained both significantly higher proportions of Th17 cells than respective samples from healthy controls ($P < 0{\cdot}001$ and $P = 0{\cdot}004$, respectively) and lower proportions of Tregs ($P < 0{\cdot}001$ and $P = 0{\cdot}001$, respectively). Treg proportions were also significantly lower in patients' nonlesional skin than in healthy donors' skin ($P = 0{\cdot}007$). The highest proportions of Th17 cells were found in lesional skin, and they were also significantly higher than in patients' nonlesional skin ($P < 0{\cdot}001$). Although Th1 cell numbers were generally higher in blood and skin samples from patients than from healthy controls, these increases were not significant, and there were also no differences in the proportions of Th2 cells in samples between the groups. Taken together, these findings support the view that an imbalance between Th17 and Treg cells contributes to psoriasis pathogenesis.

Narrowband ultraviolet B therapy significantly increased the percentages of circulating and skin regulatory T cells

The most marked effect of NB-UVB on the $CD4^+$ subsets of patients was shown on Tregs. Examples of flow cytometric analyses are illustrated in Figure S7(a–c) (see Supporting Information). A summary of the effects of NB-UVB on various T-cell subsets is shown in Figure 2. In the 15 tested samples, mean circulating and skin Treg percentages were significantly increased from 2·96 ± 0·6 to 8·92 ± 1·28 ($P < 0{\cdot}001$) and from 1·47 ± 0·8 to 13·6 ± 2·53 ($P < 0{\cdot}001$), respectively, after treatment. Th1 cells were significantly reduced, from 4·3 ± 0·9 to 1·2 ± 0·6 ($P = 0{\cdot}007$) in the blood and from

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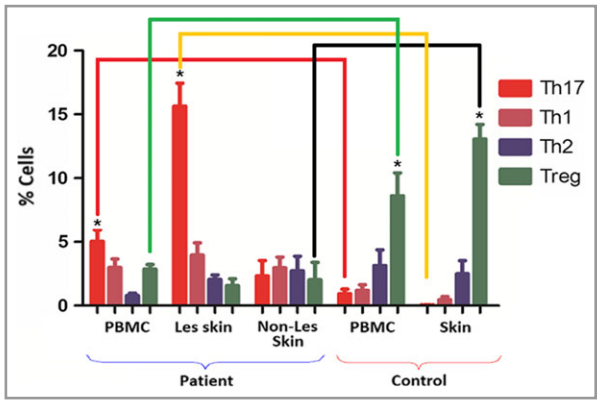

Fig 1. Proportions of $CD4^+$ cell subsets from lesional (Les) and nonlesional skin and blood of patients with psoriasis at baseline (n = 8), and from skin and blood of healthy controls (n = 5). Flow cytometric analyses classified $CD4^+$ T cells as T helper (Th)1 ($CD4^+$ $Tbet^+$), Th2 ($CD4^+$ $GATA\text{-}3^+$), Th17 ($CD4^+$ $CCR4^+$ $CCR6^+$ $IL\text{-}23R^+$ and regulatory T cells (Tregs) ($CD4^+$ $CD25^+$ $FoxP3^+$ $CD127^{lo}$). Note the significantly (*) higher proportions of Tregs in the skin and blood of healthy donors compared with patients ($P < 0{\cdot}001$ and $P = 0{\cdot}001$, respectively). The proportions of Th17 cells were significantly higher in the blood ($P = 0{\cdot}004$) and lesional skin ($P < 0{\cdot}001$) of patients than in the respective samples in the control. Also, skin Treg levels in healthy donors were significantly higher than nonlesional Treg levels of patients ($P = 0{\cdot}007$). PBMC, peripheral blood mononuclear cell.

$6{\cdot}0 \pm 0{\cdot}9$ to $1{\cdot}5 \pm 0{\cdot}5$ ($P = 0{\cdot}02$) in the skin, while Th2 cells were significantly increased from $0{\cdot}6 \pm 0{\cdot}4$ to $3{\cdot}1 \pm 2{\cdot}1$ in the blood ($P = 0{\cdot}04$) and from $2{\cdot}7 \pm 0{\cdot}5$ to $4{\cdot}7 \pm 2{\cdot}7$ in the skin ($P = 0{\cdot}01$). A reduction in Th17 cells was not statistically significant in either the blood or the skin. Strikingly, the change in skin Tregs, but not in the other subsets, was positively correlated to clinical improvement assessed by change in PASI score (Fig. S7d; see Supporting Information).

Adalimumab therapy significantly reduced T helper 17 proportions without significant effect on regulatory T cells

Patients treated with adalimumab for 6 weeks exhibited a different pattern of shifts in the proportions of $CD4^+$ T-cell subsets from those seen after NB-UVB treatment, with the main effects seen on Th17 cells. Examples of flow cytometric analyses are illustrated in Figure S8(a–c) (see Supporting Information) and a summary is displayed in Figure 3. The greatest effect of adalimumab was on the percentages of Th17 cells, which were significantly decreased from $7{\cdot}1 \pm 0{\cdot}4$ to $2{\cdot}7 \pm 0{\cdot}1$ in the blood ($P = 0{\cdot}05$) and from $21{\cdot}7 \pm 11{\cdot}6$ to $9{\cdot}5 \pm 3{\cdot}8$ in the skin ($P = 0{\cdot}005$). The proportions of Th1 and Th2 cells showed small nonsignificant increases, in both the blood and the skin, and there was also no statistically significant effect of adalimumab on Tregs, with opposing trends for small decreases in blood but small increases in skin. The fall in skin Th17 numbers, but none of the other changes, was related to clinical improvement, with change in skin Th17 cells significantly correlated to change in PASI. (Fig. S8d).

Treatment with Dovobet ointment significantly affects T helper 1, 2 and 17 cells and regulatory T cells

Dovobet is a combination of betamethasone dipropionate and active vitamin D (calcipotriol), and has a well-established therapeutic role in psoriasis. We showed highly significant, reciprocal systemic effects of Dovobet on Th17 and Treg cells after treatment for 6 weeks. Examples of flow cytometric analyses are illustrated in Figure S9(a–c) (see Supporting Information) and are summarized in Figure 4. Dovobet significantly increased the percentages of Tregs in the circulation (from $3{\cdot}3 \pm 0{\cdot}6$ to $7{\cdot}3 \pm 0{\cdot}9$, $P = 0{\cdot}005$) and in the skin (from $1{\cdot}9 \pm 1{\cdot}4$ to $8{\cdot}7 \pm 2{\cdot}3$, $P = 0{\cdot}01$), and decreased those of Th17 cells (from $4{\cdot}3 \pm 1{\cdot}1$ to $3{\cdot}4 \pm 0{\cdot}4$, $P = 0{\cdot}003$ and from $8{\cdot}7 \pm 0{\cdot}9$ to $5{\cdot}3 \pm 1{\cdot}8$, $P = 0{\cdot}01$, respectively). The percentages of Th1 cells were also significantly decreased in the skin ($P = 0{\cdot}01$), while Th2 cells were increased in the blood ($P = 0{\cdot}01$). The fall in circulating Th17 numbers, but none of the other changes, was related to clinical improvement, as change in Th17 blood levels correlated significantly to change in PASI (Fig. S9d; see Supporting Information).

Discussion

The present study of immunological changes associated with three effective treatments for psoriasis has, for the first time, revealed differential effects on $CD4^+$ T-cell subsets and identified changes that correlate with clinical improvement. Patterns of change in the proportions of Teff and Treg subsets in the circulation and skin, including those that correlated with clinical improvement, depended on treatment type. The design of the study, comparing patients before and after treatment, allowed significant results to be obtained while applying stringent inclusion and exclusion criteria, and with patients allocated to different treatment arms on the basis of clinical decisions. The relatively short treatment interval focused on initial response rates, which are higher than those expected in longer studies that include relapses. Taken together, the results build on the understanding of psoriasis pathogenesis as an imbalance between $CD4^+$ subsets by demonstrating that a range of current treatments target the imbalance in different ways and point to the possibility of improved therapies in future.

NB-UVB enhanced the number of Tregs, reduced Th1 cells and had no significant effect on Th17 cells, while, by contrast, adalimumab lowered Th17 cells without noticeable effects on either Th1 or Treg cells. However, the topical Dovobet ointment was remarkable for its reciprocal effect in reducing effector (Th1 and Th17) subsets while increasing the proportion of Tregs. Moreover, the increase in UV-induced Tregs, and the Th17 falls caused by adalimumab and Dovobet, were correlated to improvements in PASI score. These results collectively emphasize the importance to therapeutic efficacy of

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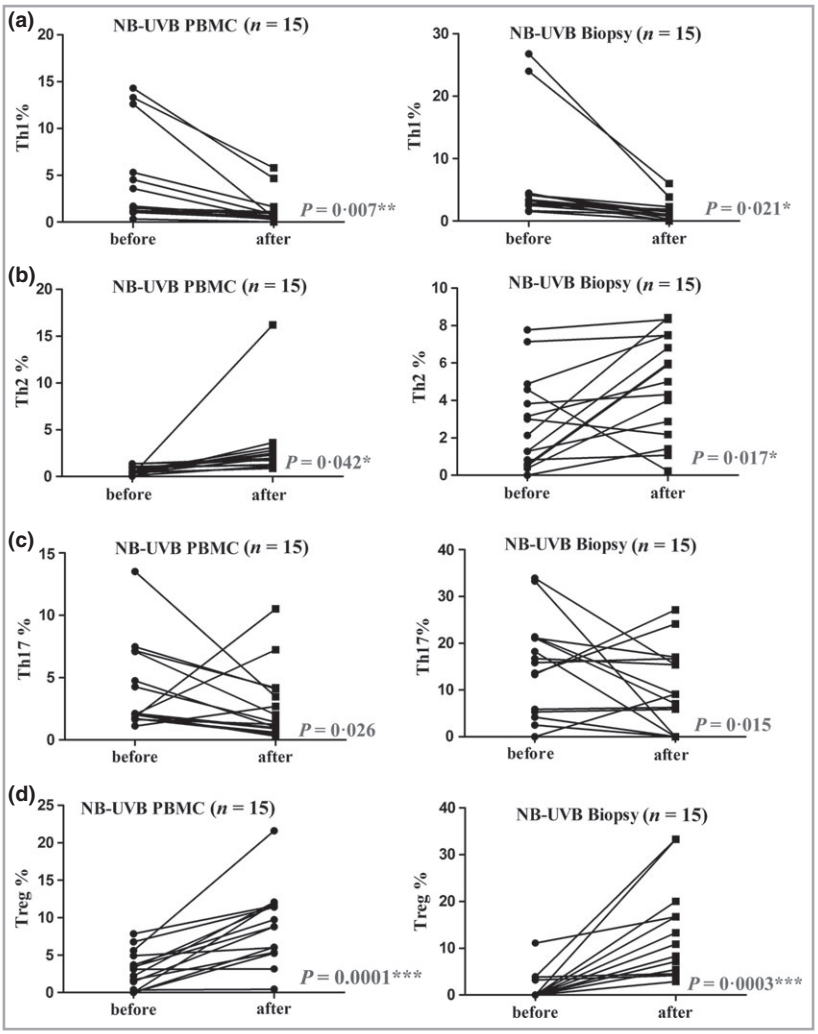

Fig 2. The effects of narrowband ultraviolet B (NB-UVB) treatment of patients with psoriasis on T-cell subsets: T helper (Th)1, Th2, Th17 and regulatory T cells (Tregs). Summary of flow cytometric data showing proportions of T-cell subsets circulating, and in lesional biopsies, in patients with psoriasis before and after treatment. Significant changes are indicated. The proportions of (a) Th1 ($CD4^+$ $Tbet^+$) cells, (b) Th2 ($CD4^+$ $GATA\text{-}3^+$) cells, (c) Th17 ($CD4^+$ $CCR4^+$ $CCR6^+$ $IL\text{-}23R^+$) cells and (d) Tregs ($CD4^+$ $CD25^+$ $FoxP3^+$ $CD127^{lo}$). PBMC, peripheral blood mononuclear cell.

restoring a Th17–Treg balance in psoriasis, and indicate that successful treatment can target either one or both sides of the imbalance.

Previous reports showed that psoriasis lesional Tregs are dysfunctional[5] or numerically deficient.[24] Our data showed significant differences in Treg numbers between patients and healthy controls, supporting previous findings[25,26] and indicating that Tregs are important to maintain skin immune homeostasis in healthy skin.[27] The demonstration of Th17 cells enriched in psoriasis lesions compared with normal skin and healthy donor skin strongly supports the model of a Th17–Treg immune disturbance in psoriasis and reflects the frequent occurrence of skin-homing $CD4^+$ T lymphocytes ($CCR4^+$ $CCR6^+$) in psoriasis plaques, providing more evidence that activation and recruitment of Th17 cells is pivotal for psoriasis pathogenesis.[4]

Our findings suggest that lesional Tregs are numerous but are either dysfunctional or overwhelmed by chronic inflammatory microenvironments due to high numbers of Th17 cells and their cytokines. This in agreement with data shown by Keijsers *et al.*[28] and Rodriguez *et al.*,[29] who reported increased percentages and absolute numbers of lesional FoxP3-expressing T cells, as well as production of IL-17 and interferon-γ from these cells compared with nonlesional skin, supporting the concept of T-cell plasticity.[30] The reciprocal increase in Tregs and fall in Th17 cell numbers in response to Dovobet may be an example of a treatment driving subset plasticity in a beneficial direction.

The striking increase in $CD4^+$ $CD25^+$ $FoxP3^+$ $CD127^{lo}$ Treg percentages, correlated to clinical response to NB-UVB, supports the notion that enhanced Tregs are key to improving psoriasis through restoration of immune tolerance, and the results also raise the possibility that a fall of Th1/Th2 ratio contributes to the beneficial effects of NB-UVB. Our use of $CD127^{lo}$ and $FoxP3^+$ in addition to $CD25^+$ cells to define the Treg population better confirmed earlier suggestions that restoration of dysfunctional $CD4^+$ $CD25^+$ T cells could be achieved via induction of $CD127^{lo}$ $CD4^+$ $CD25^+$ T cells.[31] Although it was not statistically significant, NB-UVB may have reduced Th17 cells, possibly secondarily to Treg stimulation.

Notwithstanding such a secondary effect, NB-UVB emerges as a possible therapeutic tool to enhance Tregs without compromising the immune defending role of Th17 cells. The effect of NB-UVB on resident dermal dendritic cells to boost

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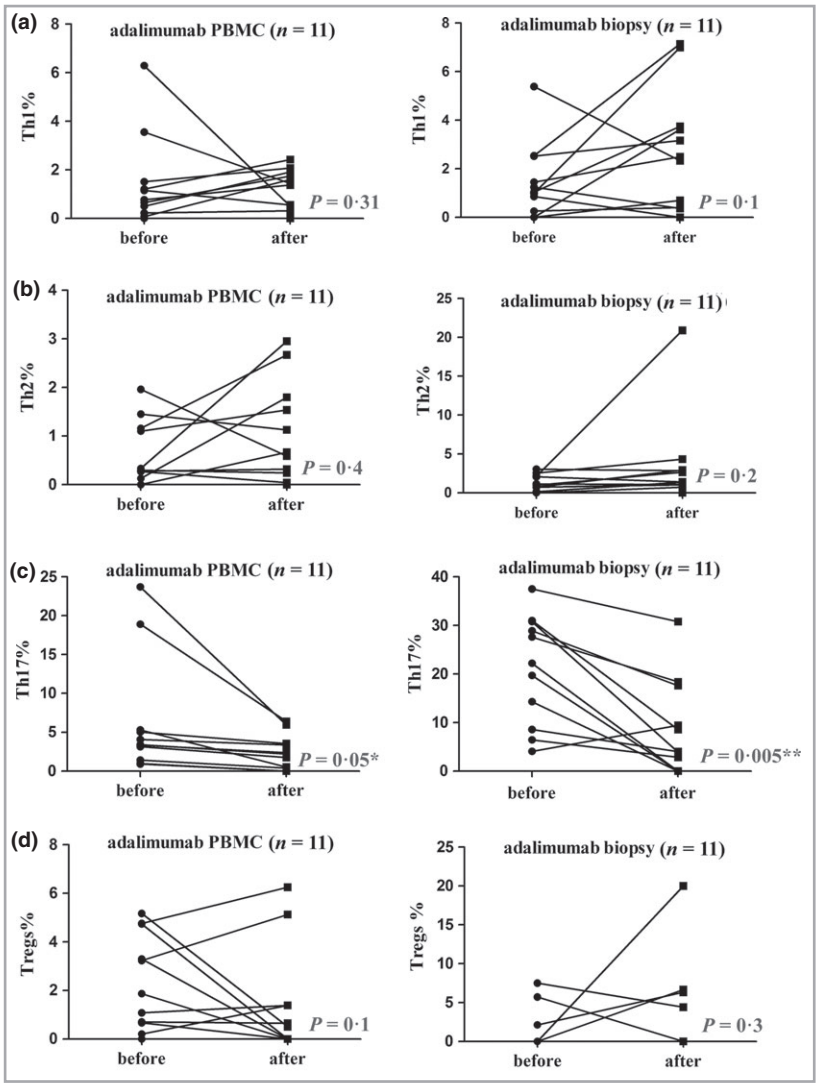

Fig 3. The effects of adalimumab treatment in patients with psoriasis on T-cell subsets: T helper (Th)1, Th2, Th17 and regulatory T cells (Tregs). Summary of flow cytometric data showing proportions of T-cell subsets circulating, and in lesional biopsies, in patients with psoriasis before and after treatment. Significant changes are indicated. The proportions of (a) Th1 ($CD4^+$ $Tbet^+$) cells, (b) Th2 ($CD4^+$ $GATA\text{-}3^+$) cells, (c) Th17 ($CD4^+$ $CCR4^+$ $CCR6^+$ $IL\text{-}23R^+$) cells and (d) Tregs ($CD4^+$ $CD25^+$ $FoxP3^+$ $CD127^{lo}$). PBMC, peripheral blood mononuclear cell.

Tregs has been the focus of a relatively recent study.[32] Furthermore, previous work in our laboratory highlighted a strongly correlated Treg response to serum vitamin D level following 2 weeks of NB-UVB therapy,[33] but other mediators such as nitric oxide may also be responsible for the induction by phototherapy of a $CD4^+$ $CD25^+$ $FoxP3^-$ Treg population from $CD4^+$ $CD25^-$ $FoxP3^-$ cells.[34]

We showed for the first time in psoriasis that 6 weeks of treatment with adalimumab significantly reduced the percentages of lesional and peripheral blood Th17 cells, with a strong correlation to clinical response ($r^2 = -0{\cdot}8$, $P = 0{\cdot}03$). Other studies have revealed that TNF-α antagonists suppressed Th17[35] and Th22 signalling,[36] while others reported enhancement of Th1/Th17 cell activation in peripheral blood and inhibition of T-cell responses in the skin.[37] Although it has also been reported that anti-TNF-α agents can increase $CD4^+$ $CD25^+$ $FoxP3^+$ cells in responding patients,[25,26] we did not see such an effect. This may be because we used combined markers for Treg identification or due to the use of a different anti-TNF-α agent in the other studies.

There has been a growing interest in the role of vitamin D3 and analogues in the prevention of immune-mediated diseases. Some studies suggested that vitamin D deficiency is associated with autoimmune conditions[38] and active vitamin D analogues may prevent such disease by induction of Tregs.[33] Strikingly, Dovobet in our study showed significant systemic effects on Teffs and Tregs. Moreover, the fall in circulating Th17 cells was significantly correlated to clinical response ($r^2 = -0{\cdot}7$, $P = 0{\cdot}04$). This systemic effect of Dovobet may be mediated by the response to vitamin D by plasmacytoid dendritic cells.[39] Previous studies showed that vitamin D arrests the maturation of dendritic cells and increases the level of regulatory cytokines, IL-10 and transforming growth factor (TGF)-β in murine models of allergic asthma.[40] Thus, it is plausible that Dovobet improves psoriasis primarily by inducing Tregs, and that the effect on Th17 cells is secondary to the release of suppressive cytokines such as TGF-β and IL-10. However, the direct suppressive effects of betamethasone on Teff responses are also likely.

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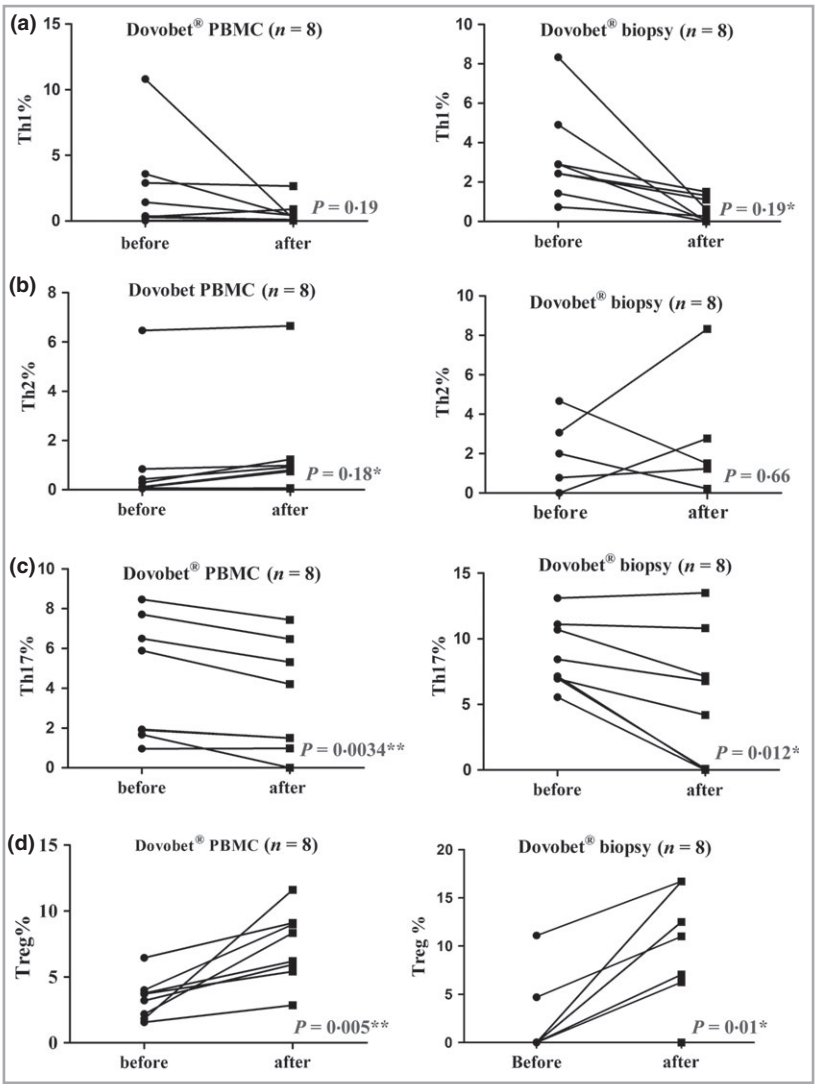

Fig 4. The effects of Dovobet (betamethasone–calcipotriol) treatment of patients with psoriasis on T-cell subsets: T helper (Th)1, Th2, Th17 and regulatory T cells (Tregs). Summary of flow cytometric data showing proportions of T-cell subsets circulating, and in lesional biopsies, in patients with psoriasis before and after treatment. Significant changes are indicated. The proportions of (a) Th1 ($CD4^+$ $Tbet^+$) cells, (b) Th2 ($CD4^+$ $GATA\text{-}3^+$) cells, (c) Th17 ($CD4^+$ $CCR4^+$ $CCR6^+$ $IL\text{-}23R^+$) cells and (d) Tregs ($CD4^+$ $CD25^+$ $FoxP3^+$ $CD127^{lo}$). PBMC, peripheral blood mononuclear cell.

In conclusion, this study identified immunological mechanisms of action of three commonly used psoriasis treatments. The results firstly support the model of psoriasis as an immune-mediated disease, in which there is a shift in the balance between Tregs that maintains self-tolerance towards a predominance of pathogenic Th17 cells. Furthermore, the work also has implications for optimizing existing, or developing new, treatment regimens. This study reveals that enhancing Treg proportions without necessarily compromising Th17 function is an effective treatment approach. The demonstration that existing treatments for psoriasis can exert selective effects on Treg and Teff subsets will also encourage a search for new strategies that restore tolerance without compromising protection against infectious disease.

Acknowledgments

I.S.K. thanks the Egyptian Government for financial support through the Egyptian Cultural Bureau Office. This work was partially supported by a National Health Service endowment grant RG12745 to A.D.O. and I.S.K. We thank Linda Lawson, the biologics nurse, all the staff members at the dermatology department and the participants.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig S1. Diagrams showing first the selection of the lymphocyte population, and then identification of the pure $CD4^+$ proportion of T cells gated from the isotype control.

Fig S2. Diagrams to show the steps involved in subgating of regulatory T cells, firstly on $CD4^+$, then $CD25^+$ cells, to be identified as $CD4^+$ $CD25^+$ $FoxP3^+$ $CD127^{lo}$.

Fig S3. Diagrams to show the steps involved in subgating of T helper 17 cells, firstly on $CD4^+$, then $CCR4^+$ cells, to be identified as $CD4^+$ $CCR4^+$ $CCR6^+$ $IL\text{-}23R^+$.

Fig S4. Diagrams to show identification of T helper (Th)1 cells as $CD4^+$ $Tbet^+$, and Th2 as $CD4^+$ $GATA\text{-}3^+$.

Fig S5. A set of representative flow cytometric data showing proportions of circulating T-cell subsets in patients with psoriasis at baseline (n = 3).

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Fig S6. A set of representative flow cytometric data showing proportions of T-cell subsets in peripheral blood mononuclear cells and skin of healthy controls at baseline ($n = 3$).

Fig S7. (a–c) Representative flow cytometric data showing the effect of narrowband ultraviolet B on T helper (Th)1 ($CD4^+$ $Tbet^+$), Th2 ($CD4^+$ $GATA\text{-}3^+$), Th17 ($CD4^+$ $CCR4^+$ $CCR6^+$ $IL\text{-}23R^+$) and regulatory T cells (Tregs) ($CD4^+$ $CD25^+$ $FoxP3^+$ $CD127^{lo}$) in the blood and skin of patients with psoriasis. (d) Correlation of change in skin Tregs to change in Psoriasis Area and Severity Index.

Fig S8. (a–c) Representative flow cytometric data showing the effect of adalimumab on the proportions of T helper (Th) 1 ($CD4^+$ $Tbet^+$), Th2 ($CD4^+$ $GATA\text{-}3^+$), Th17 ($CD4^+$ $CCR4^+$ $CCR6^+$ $IL\text{-}23R^+$) and regulatory T cells (Tregs) ($CD4^+$ $CD25^+$ $FoxP3^+$ $CD127^{lo}$) in the blood and skin of patients treated with adalimumab for 6 weeks. (d) Correlation change in skin Th17 cells to Psoriasis Area and Severity Index.

Fig S9. (a–c) Representative flow cytometric data showing the effect of Dovobet on T helper (Th)1 ($CD4^+$ $Tbet^+$), Th2 ($CD4^+$ $GATA\text{-}3^+$), Th17 ($CD4^+$ $CCR4^+$ $CCR6^+$ $IL\text{-}23R^+$) and regulatory T cells (Tregs) ($CD4^+$ $CD25^+$ $FoxP3^+$ $CD127^{lo}$). (d) Correlation of change in blood Th17 cells to Psoriasis Area and Severity Index.

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