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## Persistent Expression of CD26/DPPIV After Treatment with Infliximab in Psoriasis Despite Clinical Improvement

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Sir,

Tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) is a proven adequate target for treatment of psoriasis. The chimeric TNF- $\alpha$  antibody infliximab binds to the transmembrane receptors and induces a neutralizing effect on soluble TNF by reversed signalling, ultimately causing a reduction in lesional T cells and, indirectly, increased programmed cell death of keratinocytes (1).

CD26/dipeptidylpeptidase IV (DPPIV) is a multifunctional glycoprotein known to be present and upregulated on the surface of keratinocytes and T cells in psoriatic skin compared with the skin of healthy volunteers (2). The expression of CD26/DPPIV on peripheral blood T-lymphocytes of untreated psoriatic patients, however, was found to be substantially reduced, in particular on the CD8+CD26bright subset (3). Furthermore, TNF- $\alpha$  has been reported to influence CD26/DPPIV expression (4, 5). *In vitro* cell cultures have shown that anti-TNF- $\alpha$  can upregulate the expression of CD26/DPPIV on human-activated lymphocytes (6).

CD26/DPPIV expression may have a crucial role and might function as a marker correlating with disease activity in the pathogenesis of psoriasis. The objective of this study was therefore to determine whether anti-TNF- $\alpha$  therapy with infliximab modulates CD26/DPPIV expression on keratinocytes and T cells.

### PATIENTS AND METHODS

Eight patients with moderate-to-severe plaque-type psoriasis (defined as Psoriasis Area and Severity Index (PASI)  $\geq 12$ ) participated in this 16-week study. At baseline, after 2, 6 and 14 weeks patients received an infusion with infliximab 5 mg/kg. Approval of the medical ethics committee was obtained and all patients provided written informed consent before any study-related procedures were performed. Interfering topical or systemic treatments had been suspended for at least 2 and 4 weeks, respectively, prior to participation, and their use was prohibited throughout the study.

One solitary plaque of at least 3 cm diameter was chosen as a target lesion. In all patients 4-mm punch biopsies were taken at weeks 0 (T=0), 2 (T=2) and 16 (T=16) from representative sites in the designated target lesion, after anaesthesia with 1% xylocaine/adrenaline. The obtained specimens were embedded in Tissue-Tek OCT compound (Sakura, Zoeterwoude, The Netherlands), snap-frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until use.

Peripheral blood for enumeration of lymphocytes was obtained at the same time-points as the biopsies were collected.

At each visit severity scores (PASI) and sum-score (for the severity of the target lesion) were assessed. The sum-score comprises the total score for erythema (0–4), induration (0–4) and scaling (0–4).

Immunohistochemical staining was performed on all obtained biopsies, as described previously (7). The following primary antibodies (mouse anti-human) were used: anti-CD3 1:100 (clone UCTH1), anti-CD8 1:200 (clone C8/144B) both from DAKO (Copenhagen, Denmark) and anti-CD26 1:50 (clone BA-5) from SantaCruz Biotechnology (Santa Cruz, USA). Furthermore, haematoxylin and eosin (H&E) staining was performed.

The immunohistochemical CD26/DPPIV stained sections were analysed as described previously (8). The scoring of the epidermal CD26/DPPIV expression comprised assessing the honeycomb- and column-like patterns of epidermal staining. In addition, the percentage of the total keratinocytes positive for CD26/DPPIV was estimated along a 5-point scale: 0=0%, 1=1–29%, 2=30–59%, 3=60–89% and 4=90–100%.

Quantification of the CD3+ and CD8+ T-cell subsets in the immunohistochemically stained sections was carried out as described previously (7). Quantitative cell counts of these T-cell subsets were expressed as "Positive cells per mm skin length".

The Zenon labelling technique, as described previously (9), was used to combine the aforementioned antibodies. Microscopy and image analysis of the sections and antibodies was performed as described previously (2).

The following monoclonal antibodies were used for flow cytometry: CD3-FITC (Beckman Coulter), CD4-PC7 (Beckman Coulter), CD8-PECy5 (Beckman Coulter) and CD26-PE (Becton Dickinson) (quadruple staining). Aliquots of 100  $\mu\text{l}$  peripheral blood were analyzed as previously described (3).

All assays were run on a FC500 flow cytometer (Beckman Coulter, Cytomics FC500). A regional gate was set on CD3+ (FITC) vs. forward scatter (FSC) to gate for T lymphocytes in the sample being studied. At least 20,000 gated events were acquired for each antibody combination.

Statistical analyses were performed using SPSS 14.0 software. Analysis of variance with repeated measurement (ANOVA) was used to analyse the effect of treatment and time. If significant, Bonferroni's *post-hoc* comparison was performed to assess time-related effects. Statistical significance was set at 0.05.

### RESULTS

Of 8 enrolled patients, 6 completed the study as 2 dropped out at between 2 and 16 weeks of treatment, both due to infusion-related adverse events, and were excluded from further analysis. The participating patients had an age range of 36–55 years and the male:female ratio was 3:3. The PASI had decreased significantly after 2 weeks of therapy, from  $19.27 \pm 3.17$  (mean  $\pm$  SEM) to  $12.35 \pm 2.45$  ( $p < 0.05$ ). This decrease extended after 16 weeks of therapy to a mean PASI of  $2.52 \pm 0.38$ , equivalent to a mean reduction of 86.9% ( $p < 0.001$ ). Equally, the sum-score of the biopsied target lesion decreased significantly from baseline to 16 weeks of treatment ( $p < 0.001$ ). In line with this, the thickness of

the epidermis narrowed from  $266 \pm 48 \mu\text{m}$  at baseline to  $74 \pm 7 \mu\text{m}$  after 16 weeks ( $p < 0.001$ ).

In general, compared with the epidermis, the dermis contained larger amounts of CD3+ and CD8+ T cells per mm skin length. At week 2, treatment with infliximab had induced a significant reduction in the CD3+ T-cell subset in the dermis (mean reduction 48.4% ( $p = 0.009$ ), but not yet in the epidermis (mean reduction 36.2%). Subsequently, at week 16, mean decreases in the number of CD3+ T cells in the dermis and epidermis were 69.6% ( $p = 0.001$ ) and 83.8% ( $p = 0.002$ ), respectively. With respect to the CD8+ T-cell subset, a significant decrease was seen after 16 weeks of treatment in both the dermis ( $p = 0.01$ ) and epidermis ( $p < 0.01$ ). However, this decrease was significant after 2 weeks of therapy only in the dermis, not in the epidermis.

Treatment with infliximab did not provoke significant alterations in the honeycomb-like pattern of CD26/DPPIV staining, nor did it influence the percentage of CD26/DPPIV positively stained keratinocytes during therapy in our patients.

CD8+ T cells in the psoriatic dermis and epidermis expressed CD26+ on their surface, although in small amounts (Fig 1; top panel).

The lower panel in Fig. 1 shows that both the double-labelled CD3CD26 and CD8CD26 T cells in the dermis and epidermis tend to decrease under therapy with infliximab (a non-significant difference between baseline, 2 weeks and 16 weeks of therapy).

No significant changes were observed in the mean percentages of CD3+, CD4+ and CD8+ T-cell subsets. When focusing on the CD26-negative/dim/bright subpopulations (7) of the CD3+, CD4+ and CD8+ T cells in the present study, no significant alterations in mean percentages were found during therapy with infliximab.

## DISCUSSION

In agreement with previous findings, the results of this study show that infliximab induces a remarkable clinical

effect, together with a reducing effect on lesional T cells and thinning of the epidermis. Nevertheless, after effective anti-TNF- $\alpha$  therapy, CD26/DPPIV appeared to remain present in equal amounts on keratinocytes in the epidermis, and on T cells in the peripheral blood. In the dermis and epidermis, however, the expression of CD26/DPPIV on CD3+ T cells tended to decrease. Moreover, the clinical improvement after infliximab therapy paralleled the reduction in epidermal thickness and of CD3+ and CD8+ T cells in the dermis and epidermis. Within the population of double-labelled T cells, the CD3CD26- and CD8CD26-positive cells tended to decrease after treatment. It is attractive to speculate that the hypothesized upregulation of CD26 on T cells in response to anti-TNF- $\alpha$  therapy could be evened out by a downward effect of the clinical response on the T-cell-related expression of CD26/DPPIV.

The fact that CD26/DPPIV remains present on the surface of keratinocytes can be compared to previous findings with respect to CD26/DPPIV enzyme activity in uninvolved skin of psoriatic patients (8). Both the uninvolved skin and the “post-treatment plaque” skin after effective treatment in psoriatic patients encompass and retain their expression of CD26/DPPIV. The uninvolved psoriatic skin can be regarded as a subclinical psoriatic manifestation. Therefore, CD26/DPPIV may play an important role in the initiation or triggering of the local immune system in psoriasis. In contrast, T-cell-related expression of CD26 tended to reduce during infliximab therapy. It is therefore plausible that these T cells possess independent functions and patterns of expression, which are likely to be separately regulated in time and context. This confirms the attributed moonlighting character of the CD26 protein (5, 10).

In a previous investigation, it was found that the CD8CD26bright subpopulation was reduced (<10% of peripheral CD8+ T cells) in the peripheral blood of psoriatic patients compared with healthy volunteers (3). The current study shows that the specific CD26 subsets of the CD3+, CD4+ and CD8+ peripheral blood T-cell

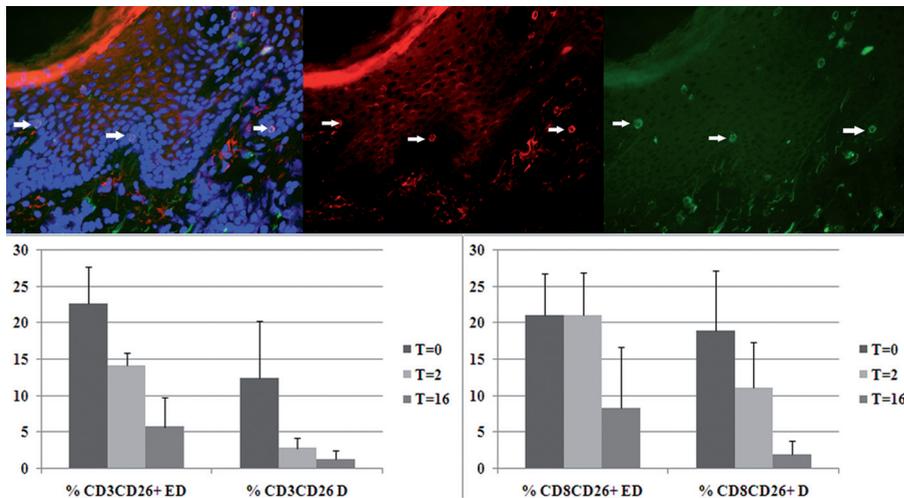


Fig. 1. Phenotyping of CD8+CD26+ T cells in lesional psoriatic epidermis using the Zenon immunofluorescence double-labelling technique. (Top panel) Co-staining of CD8 (green) and CD26 (red) in lesional psoriatic skin revealed co-localization of these two proteins (merge; yellow) in the epidermis (white arrows). (Panel below) Overview of mean changes in, respectively, the percentage of (epi)dermal double-labelled CD3+CD26+ T cells, and the percentage of (epi)dermal double-labelled CD8+CD26+ T cells during treatment with infliximab. T: time-point in weeks; ED: epidermis; D: dermis. Error bars indicate standard error of the mean (SEM).

populations (incorporating the CD26negative, CD26dim and CD26bright T cells) were stable over time during treatment. These data suggest that significant clinical improvement of psoriasis after infliximab therapy does not go hand in hand with significant fluctuations in the various T-cell populations and CD26-related T-cell subsets in peripheral blood. As a result, the CD26bright subpopulation of the CD8+ T cells in the peripheral blood remained consistently reduced (less than 10%) during therapy with infliximab.

Although the present findings add insight into the expression of CD26/DPPIV in psoriasis, the small sample size defines the pilot character of this study, and larger studies are needed to confirm the present observations.

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