Treatment-Related Restoration of Langerhans Cell Migration in Psoriasis

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TO THE EDITOR
The mobilization and migration of epidermal Langerhans cells (LCs) to draining lymph nodes is dependent upon receipt of (at least) two independent cytokine signals; one provided by IL-1β and the second by tumor necrosis factor-α (TNF-α) (Cumberbatch et al., 1997). Approximately 20–30% of epidermal LCs are mobilized in response to these signals (Griffiths et al., 2005). Attention has focused recently on the potential importance of LCs in uninvolved skin sites of patients with...
psoriasis (Cumberbatch et al., 2006; Shaw et al., 2010). We have shown previously that in subjects with early-onset psoriasis (onset before 40 years of age), LCs are refractory to all of those stimuli (chemical allergen, IL-1β, and TNF-α) that cause significant migration in healthy controls (Cumberbatch et al., 2006). Further, we have shown that impairment of LC migration in early-onset psoriasis is likely a consequence of the epidermal microenvironment rather than an abnormality of LCs themselves. However, the contribution of impaired LC migration to the pathogenesis of psoriasis has not been defined.

In the present investigation, we have sought to determine whether effective treatment of early-onset psoriasis, using systemic therapies, can restore LC mobilization. Examples of systemic therapies include drugs that predominantly act as T-cell antagonists (ciclosporin and methotrexate; Menter and Griffiths, 2007), and biologics that inhibit cytokine signaling. Treatment of early-onset psoriasis using one-way analysis of variance followed by Dunnett’s multiple comparison post hoc tests using the untreated early-onset psoriasis patients as the comparator. P < 0.05 was chosen as the threshold for statistical significance.

The historical data (Cumberbatch et al., 2003, 2006) demonstrate that although intradermal administration of IL-1β to healthy volunteers induced a significant decrease (n = 10; 19.7 ± 2.0%; P < 0.05; Figure 1a) in LC frequency, treatment of uninvolved skin of early-onset psoriasis patients failed to induce any LC migration (n = 7; 1.1 ± 0.5%; Figure 1b). An identical pattern was recapitulated in the ex vivo epidermal explant model. LCs migrated spontaneously from all explants derived from healthy controls after 24 h in culture (n = 6; 19.8 ± 3.7%; P < 0.05; Figure 1c). The release of factors in response to the trauma of the biopsy procedure is thought to drive the spontaneous migration of LCs in the explant model (Ratzinger et al., 2002). The extent of migration (approximately 20% of LCs) observed was similar to that provoked by in vivo stimuli such as IL-1β (Figure 1a).

Patients with early-onset psoriasis (n = 40; mean age 39.9 ± 1.4 years; 15 female and 25 male) and healthy controls (n = 6; mean age 24.3 ± 2.4 years; 4 female and 2 male) were recruited following provision of written informed consent. The study was approved by the Salford and Trafford Research Ethics Committee (05/Q1404/249) and was conducted according to the Declaration of Helsinki. Individuals with early-onset psoriasis who were either on topical therapy alone (untreated) or were recruited because they had shown physician-determined clinical improvement while receiving one of the aforementioned six systemic therapies. Inclusion criteria for patients on topical therapy alone included: no use of systemic therapies for at least 4 weeks, and for healthy volunteers, no history of any skin disease. Clinical severity of individuals with early-onset psoriasis ranged from psoriasis area severity index (PASI) scores of 0–25.7. For the individual groups, PASI scores were as follows: untreated: 0–25.7; methotrexate: 5–7.8; ciclosporin: 0.5–9.5; etanercept: 6.2–12.2; adalimumab: 2–5.3; ustekinumab: 0–8.5; and FAEs: 2.4–3.6.

Two, 6-mm diameter skin biopsies were taken from sun-protected buttock skin under 1% lidocaine local anesthesia. For psoriasis patients, biopsies were taken from normal-appearing clinically uninvolved skin >5 cm from a plaque of psoriasis. Biopsies were collected into 10% fetal calf serum (FCS)/RPMI containing 2.5 μg ml⁻¹ amphotericin B, 200 μg ml⁻¹ streptomycin and 200 U ml⁻¹ penicillin (all from Life Technologies, Paisley, UK), and epidermal sheets were prepared as described previously (Cumberbatch et al., 2006). In every experiment, one epidermal sheet was processed immediately for LC counting (n = 24 from each sample). The remaining epidermal sheets were washed briefly in phosphate-buffered saline (PBS), processed for staining with a monoclonal antibody specific for CD1a (clone NA1/34; 10 μg ml⁻¹ in 0.1% bovine serum albumin/PBS; Dako Ltd., Stockport, UK) and assessed and counted as described previously (Cumberbatch et al., 2006). For each sample, 50 consecutive fields in the central portion of the biopsy were examined, and the results were expressed as the mean number of cells per mm². Data are expressed either as LC frequency at T = 0 and 24 for each individual donor or with respect to the percentage change in LC frequency at T = 24 compared with baseline (paired T = 0) data:

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\% \text{Migration} = \left( \frac{(T = 0 \text{ LC mm}^{-2}) - (T = 24 \text{ LC mm}^{-2})}{(T = 0 \text{ LC mm}^{-2})} \right) \times 100
\]

LC frequency (T = 0 versus T = 24) data were analyzed by paired t-test, and % migration data were analyzed using one-way analysis of variance followed by Dunnett’s multiple comparison post hoc tests using the untreated early-onset psoriasis patients as the comparator. P < 0.05 was chosen as the threshold for statistical significance.

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To investigate the effect of systemic therapies on LC migration, LC frequencies at baseline (T = 0) and T = 24 from
untreated individuals were compared with healthy controls and patients who had shown a clinical response (reduction in PASI) to systemic therapy. The overall patterns of responses are displayed as percentage migration of LCs following 24 h incubation of epidermal explants compared with baseline T=0 levels (Figure 1e). Given the relatively low numbers for some groups, in order to aid statistical analyses data have been combined for those therapy groups with a common mechanism (the T-cell therapies, methotrexate and ciclosporin, and the TNF-α inhibitors, etanercept and adalimumab). Despite inter-donor variability in all groups, it is nevertheless apparent that, compared with the untreated psoriasis group (n = 16; 4.0 ± 1.4%), there was significant spontaneous migration of LCs in explants taken from healthy donors (19.8 ± 3.7%; raw data previously illustrated in Figure 1c; P < 0.05), and from patients receiving systemic therapy with TNF-α inhibitors (etanercept (n = 6) and adalimumab (n = 2); 14.1 ± 2.9%; P < 0.05), ustekinumab (n = 6; 14.0 ± 3.8%; P < 0.05) or FAEs (n = 4; 22.4 ± 5.7%; P < 0.05). In contrast, there was little or no restoration of LC mobilization observed in patients receiving T-cell-targeted therapies (methotrexate (n = 4) and ciclosporin (n = 3); 4.3 ± 2.0%) despite clinical improvement.

Collectively, these data indicate that systemic treatment of psoriasis patients with non-T-cell-targeted therapies is associated with a significant restoration of epidermal LC migration in uninvolved skin. Adalimumab and etanercept are TNF-α inhibitors, whereas ustekinumab targets the p40 subunit common to IL-12 and IL-23. The mechanism of action of FAEs has yet to be elucidated fully, although one study suggests that it may also target IL-12/IL-23 signaling in psoriasis (Ghoreschi et al., 2011). A previous study reported restoration of epidermal LC frequency in plaques of psoriasis that preceded clinical response to treatment with adalimumab (Gordon et al., 2005). Our findings support the importance of the regulatory role of LCs in psoriasis, although we have not investigated their function in involved plaques. In contrast, we have shown that the predominantly T-cell-targeted therapies failed to restore LC migration despite effective clearance of psoriasis. This observation is consistent with a previous study that showed that successful treatment of patients with ciclosporin was not associated with an increase in the frequency of LCs within plaques compared with pretreatment values (Gupta et al., 1989).

In summary, we have developed an ex vivo epidermal explant model that can be used to interrogate the mechanisms underlying LC migration and the effect of therapy on LC migration in psoriasis. Furthermore, we have shown that LC mobilization is restored in patients on therapies that target key cytokines in psoriasis pathogenesis and hence cell signaling within the epidermal environment. Although the influence of impaired LC mobilization on the pathogenesis of psoriasis is presently uncertain, a speculation is that the loss of LC motility may have an important

Figure 1. Explant model to investigate Langerhans cell (LC) migration in early-onset psoriasis: impact of systemic therapies. Historical data (Cumberbatch et al., 2003; 2006) showing LC frequencies 2 h post in vivo intradermal injection of 50 or 100 U IL-1β or saline control in: (a) healthy individuals and (b) patients with early-onset psoriasis. LC frequencies assessed using the explant model for epidermal sheets from (c) healthy individuals and (d) patients with psoriasis processed immediately (T = 0) and at 24 h (T = 24). (e) Percentage LC migration in the explant model for untreated psoriasis patients, healthy volunteers, and psoriasis patients on various treatments: TNF-α inhibitors (etanercept (△) and adalimumab (■)), T-cell therapies (ciclosporin (▼) and methotrexate (●)), fumaric acid esters (FAEs), or ustekinumab. Each line/data point represents an individual donor (for ustekinumab, one patient made two independent visits). Statistical analyses: paired t-test (a–d) or one-way analysis of variance and Dunnett’s post hoc test (e). *P < 0.05.
TO THE EDITOR

Recessive mutations of the gene encoding the interleukin-36 receptor antagonist (IL36RN) have been associated with generalized pustular psoriasis, palmar-plantar pustulosis, and acrodermatitis continua of Hallopeau (Marrakchi et al., 2011; Onoufiadiis et al., 2011; Setta-Kaffetzi et al., 2013). As patients suffering from these pustular conditions often present with concomitant psoriasis vulgaris (PV), it has been proposed that IL36RN deficiency may also contribute to PV susceptibility (Marrakchi et al., 2011). This hypothesis is supported by the observation that mice lacking il36rn show exacerbated symptoms of imiquimod-induced psoriasiform dermatitis and enhanced infiltration of inflammatory cells in the dermis and the epidermis (Tortola et al., 2012). The elevated expression of IL-36 cytokines in psoriatic skin (Carrier et al., 2011; Johnston et al., 2011) is also consistent with the notion that abnormal IL-36 signaling has an important role in the establishment of cutaneous inflammation (Supplementary Figure 1 online).

On the basis of the above findings, it has recently been suggested that IL-36 blockade could be an innovative approach to the treatment of PV.

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Loss of IL36RN Function Does Not Confer Susceptibility to Psoriasis Vulgaris

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Abbreviations: IL36RN, interleukin-36 receptor antagonist gene; PV, psoriasis vulgaris

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