interpreted as having an ''extremely large effect on patient's life'' (Hongbo *et al.*, 2005). Imagine you want to evaluate the effect of a treatment on the patient's quality of life that is extremely affected by a dermatological disease (DLQI scores between 21 and 30). You define success when the score after the treatment is between 11 and 20, because it is considered clinically relevant. After treatment, scores of one patient decrease from 29 to 22, and scores of another patient decrease from 22 to 18. Thus, the treatment improved the quality of life by seven points in case one, and by four in case two. However, according to the chosen cutoff score, only the latter would be called ''success,'' although the treatment effect was much lower. Dichotomization blurs the sizes of treatment effects, and it is therefore not ''more clinically meaningful'' (Nassar *et al.*, 2013, p 374).

Finally, we recommend not confusing outcome measurements in RCTs with clinical decision-making (Streiner, 2002). RCTs are conducted to demonstrate superiority, equivalence, or noninferiority of treatment effects. As such, they must be designed to maximize their internal validity (Shadish *et al.*, 2002)—that is, to reduce bias and to increase the probability of finding a difference when one exists (i.e., power). This is best achieved when the outcome variable more closely matches the phenomenon being studied. When that phenomenon is itself a continuum, then its measurement should be on a continuum. After—and only after—the study has determined whether there is a relationship between the independent and dependent variables, clinicians can then use this information to make decisions regarding treatment. Techniques, such as receiver operating characteristic analysis, can be used to determine optimal cutoff points (Streiner and Cairney, 2007). As we mentioned previously, these cutoff points can later be changed in the light of new information, but this can be done only if the outcome had been measured on a continuum to begin with, and cannot be done if the outcome had been dichotomized a priori.

We completely agree with Nassar *et al.* (2013) on the importance of primary outcome constructions in dermatology and other fields. The decision on outcome variables should be based on the objective of the RCT, on the validity and reliability of the operationalization, on the clinical relevance, and on available resources. Because parametric variables allow stronger inferences with smaller sample sizes, they have advantages over binary variables. If the outcome in an RCT is measured on a continuum, it should never be dichotomized.

CONFLICT OF INTEREST

The authors state no conflict of interest.

***Jan Kottner*[1] *and David L. Streiner*[2,3]**

[1]Clinical Research Center for Hair and Skin Science, Department of Dermatology and Allergy, Charité-Universitätsmedizin Berlin, Berlin, Germany; [2]Department of Psychiatry and Behavioural Neurosciences, McMaster University, Hamilton, Ontario, Canada and [3]Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada
E-mail: jan.kottner@charite.de

REFERENCES

Finlay AY, Khan GK (1994) Dermatology Life Quality Index (DLQI)—a simple practical measure for routine clinical use. *Clin Exp Dermatol* 19:210–6

Hongbo Y, Thomas CL, Harrison MA *et al.* (2005) Translating the science of quality of life into practice: What do dermatology life quality index scores mean? *J Invest Dermatol* 125: 659–64

Julious SA (2004) Sample sizes for clinical trials with normal data. *Stat Med* 23:1921–86

Nassar D, Sbidian E, Bastuji-Garin S *et al.* (2013) Typology of the primary outcome construction in dermatology: a systematic review of published randomized controlled trials. *J Invest Dermatol* 133:371–6

National Heart, Lung, and Blood Institute (2002) Third report of the expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (ATP III Final Report) http://www.nhlbi.nih.gov/guidelines/cholesterol/atp3_rpt.htm, accessed 4 March 2013

Shadish W, Cook T, Campbell D (2002) *Experimental and Quasi-Experimental Designs for Generilized Causal Inference* Boston: Houghton Mifflin

Streiner DL (2002) Breaking up is hard to do: the heartbreak of dichotomizing continuous data. *Can J Psychiatry* 47:262–6

Streiner DL, Cairney J (2007) What's under the ROC? An introduction to receiver operating characteristic curves. *Can J Psychiatry* 52:121–8

World Health Organization (2006) BMI classification http://apps.who.int/bmi/index.jsp?introPage=intro_3.html, accessed 2 March 2013

JID Open

Treatment-Related Restoration of Langerhans Cell Migration in Psoriasis

Journal of Investigative Dermatology (2014) **134,** 268–271; doi:10.1038/jid.2013.289; published online 25 July 2013

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

Abbreviations: FAE, fumaric acid ester; FCS, fetal calf serum; LC, Langerhans cell; PBS, phosphate-buffered saline; PASI, psoriasis area severity index; TNF-α, tumor necrosis factor-α

Accepted article preview online 28 June 2013; published online 25 July 2013

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 cytokines associated with the pathogenesis of psoriasis, such as TNF-α (adalimumab and etanercept) and IL-12/IL-23 (ustekinumab; Nestle *et al.*, 2009). Fumaric acid esters (FAEs) are an alternative systemic therapy for moderate-to-severe psoriasis, although their precise mechanism of action has yet to be elucidated (Wain *et al.*, 2010).

To facilitate a more searching examination of the potential role of compromised LC mobility in psoriasis, and its contribution to disease pathogenesis, we have developed an *ex vivo* epidermal explant model. This model is based on a modification of assays that have primarily monitored LC migration in explants as a function of LCs accumulating in the explant medium (Ratzinger *et al.*, 2002; de Gruijl *et al.*, 2006; Bond *et al.*, 1999). Development of such a model obviates recourse to intradermal injection of either IL-1β or TNF-α as has been used previously (Cumberbatch *et al.*, 2006).

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 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 media 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 ($T=0$); the remaining epidermal sheet was floated on 500 μl of culture media (10% FCS/RPMI containing 100 μg ml^{-1} streptomycin and 100 U ml^{-1} penicillin) in 12-well plates and incubated at 37 °C in a 5% CO_2 environment for 24 h ($T=24$). Following the 24-h incubation, 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 mm^2. 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:

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

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.

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). However, there was no significant LC mobilization from explants derived from uninvolved skin of patients with psoriasis solely on topical therapies (untreated; $n=5$; 2.2 ± 1.1%; Figure 1d). Thus, to this extent, at least, the explant model faithfully reflects the *in vivo* differences observed between normal subjects and those with early-onset psoriasis with regard to cytokine-(TNF-α or IL-1β) induced migration (Cumberbatch *et al.*, 2006).

To investigate the effect of systemic therapies on LC migration, LC frequencies at baseline ($T=0$) and $T=24$ from

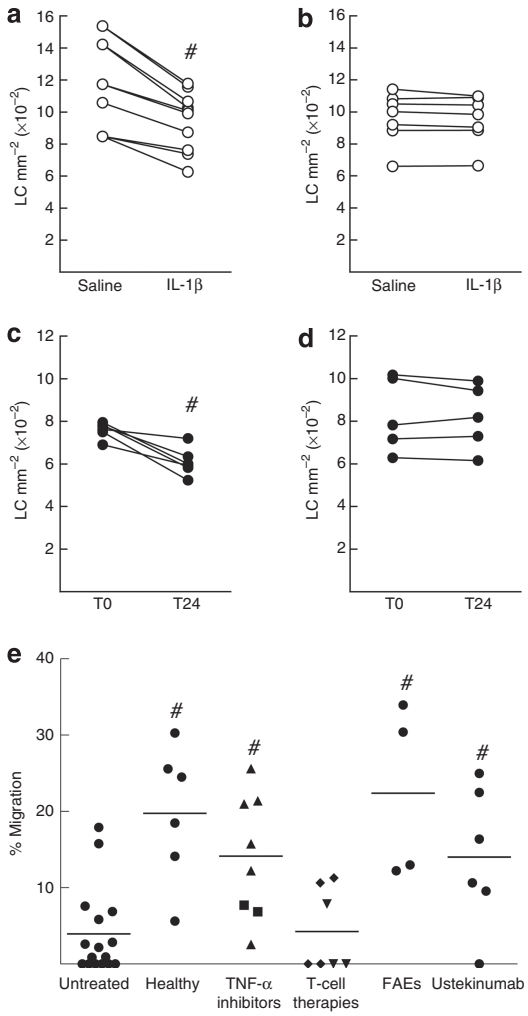

Figure 1. Explant model to investigate Langerhans cell (LC) migration in early-onset psoriasis: impact of systemic therapies. Historical data (Cumberbatch *et al.*, 2003; 2006) showing LC frequencies 2 h post *in vivo* intradermal administration of 50 or 100 U IL-1β or saline control in: (**a**) healthy 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**). $^{\#}P<0.05$.

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

impact on the ability of these cells to sense the local antigenic microenvironment and regulate cutaneous immune responses. It is also not clear why certain therapeutic interventions, but not others, are associated with a restoration of LC motility. It may be that anti-TNF and anti-IL-12/23 therapies result in a resetting of normal epidermal function, including LC mobilization. These data demonstrate the utility of the *ex vivo* explant model and provide evidence that aberrant LC mobilization is a function of the psoriatic process, rather than a predisposing phenotype.

CONFLICT OF INTEREST

CEMG has received honoraria, speaker's fees, and/or research grants from AbbVie, Actellion, Celgene, Janssen, LEO Pharma, Merck Sharpe Dohme, Novartis, Pfizer, Sandoz, and Trident. IK and RJD are currently in receipt of research grants from Novartis. The remaining authors state no conflict of interest.

ACKNOWLEDGMENTS

We are grateful to Mr Jean Bastrilles for subject recruitment and sample collection, and to our volunteers for their participation. We would also like to thank Ms Rummana Begum and Dr Laura Eaton for their technical help. This research was funded in part by the Medical Research Council (grant reference G0700292). Christopher Griffiths is an NIHR Senior Investigator.

***Frances L. Shaw*[1,3], *Kieran T. Mellody*[1,3], *Stephanie Ogden*[1,2], *Rebecca J. Dearman*[1], *Ian Kimber*[1] and *Christopher E.M. Griffiths*[2]**

[1]*Faculty of Life Sciences, University of Manchester, Manchester, UK and* [2]*Dermatology Research Centre, University of Manchester, Manchester Academic Health Science Centre, Manchester, UK*
E-mail: frances.shaw@manchester.ac.uk
[3]*The first two authors contributed equally to this work and have joint first authorship.*

REFERENCES

Bond E, Adams WC, Smed-Sörensen A *et al.* (1999) Techniques for time-efficient isolation of human skin dendritic cell subsets and assessment of their antigen uptake capacity. *J Immunol Methods* 348:42–56

Cumberbatch M, Dearman RJ, Kimber I (1997) Langerhans cells require signals from both tumour necrosis factor-alpha and interleukin-1 beta for migration. *Immunology* 92: 388–95

Cumberbatch M, Bhushan M, Dearman RJ *et al.* (2003) IL-1beta-induced Langerhans' cell migration and TNF-alpha production in human skin: regulation by lactoferrin. *Clin Exp Immunol* 132:352–9

Cumberbatch M, Singh M, Dearman RJ *et al.* (2006) Impaired Langerhans cell migration in psoriasis. *J Exp Med* 203:953–60

de Gruijl TD, Sombroek CC, Lougheed SM *et al.* (2006) A postmigrational switch among skin-derived dendritic cells to a macrophage-like phenotype is predetermined by the intracutaneous cytokine balance. *J Immunol* 176:7232–42

Ghoreschi K, Bruck J, Kellerer C *et al.* (2011) Fumarates improve psoriasis and multiple sclerosis by inducing type II dendritic cells. *J Exp Med* 208:2291–303

Gordon KB, Bonish BK, Patel T *et al.* (2005) The tumour necrosis factor-alpha inhibitor adalimumab rapidly reverses the decrease in epidermal Langerhans cell density in psoriatic plaques. *Br J Dermatol* 153:945–53

Griffiths CEM, Dearman RJ, Cumberbatch M *et al.* (2005) Cytokines and Langerhans cell mobilisation in mouse and man. *Cytokine* 32: 67–70

Gupta AK, Baadsgaard O, Ellis CN *et al.* (1989) Lymphocytes and macrophages of the epidermis and dermis in lesional psoriatic skin, but not epidermal Langerhans cells, are depleted by treatment with cyclosporin A. *Arch Dermatol Res* 281:219–26

Menter A, Griffiths CEM (2007) Current and future management of psoriasis. *Lancet* 370:272–84

Nestle FO, Kaplan DH, JNWN Barker (2009) Psoriasis. *N Engl J Med* 361:496–509

Ratzinger G, Stoitzner P, Ebner S *et al.* (2002) Matrix metalloproteinases 9 and 2 are necessary for the migration of Langerhans cells and dermal dendritic cells from human and murine skin. *J Immunol* 168:4361–71

Shaw FL, Cumberbatch M, Kleyn CE *et al.* (2010) Langerhans cell mobilization distinguishes between early-onset and late-onset psoriasis. *J Invest Dermatol* 130:1940–2

Wain EM, Darling MI, Pleass RD *et al.* (2010) Treatment of severe, recalcitrant, chronic plaque psoriasis with fumaric acid esters: a prospective study. *Br J Dermatol* 162: 427–34

Loss of *IL36RN* Function Does Not Confer Susceptibility to Psoriasis Vulgaris

Journal of Investigative Dermatology (2014) **134,** 271–273; doi:10.1038/jid.2013.285; published online 18 July 2013

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; Onoufriadis *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

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

Accepted article preview online 21 June 2013; published online 18 July 2013