Impaired Langerhans cell migration in psoriasis

Marie Cumberbatch,[1] Minal Singh,[2] Rebecca J. Dearman,[1] Helen S. Young,[2] Ian Kimber,[1] and Christopher E.M. Griffiths[2]

[1]Syngenta Central Toxicology Laboratory, Macclesfield, Cheshire, SK10 4TJ, England, UK
[2]The Dermatology Centre, University of Manchester, Salford, Manchester, M6 8HD, England, UK

We have examined whether psoriasis is associated with systemic effects on epidermal Langerhans cell (LC) function and, specifically, the migration of LCs from the skin. Compared with normal skin, the frequency and morphology of epidermal LCs in uninvolved skin from patients with psoriasis was normal. However, mobilization of these cells in response to stimuli that normally induce migration (chemical allergen, tumor necrosis factor α [TNF-α], and interleukin-1β [IL-1β]) was largely absent, despite the fact that treatment with TNF-α and IL-1β was associated with comparable inflammatory reactions in patients and controls. The failure of LC migration from uninvolved skin was not attributable to altered expression of receptors for IL-1β or TNF-α that are required for mobilization, nor was there an association with induced cutaneous cytokine expression. Although a role for altered dynamics of LC migration/turnover has not been formally excluded, these data reveal a very consistent decrement of LC function in psoriasis that may play a decisive role in disease pathogenesis.

CORRESPONDENCE
Christopher E.M. Griffiths:
Christopher.griffiths@manchester.ac.uk

Abbreviations used: LC, Langerhans cell; DPC, diphenylcyclopropenone; IL-1Ra, IL-1R antagonist; PDC, plasmacytoid DC.

Psoriasis is a common inflammatory skin disease that affects ∼2% of the population. The disease may present in several different forms, but the most common (>80% of cases) is chronic plaque psoriasis, consisting of red, heavily scaled, well-demarcated plaques on elbows, knees, scalp, and lower back, although any skin surface may be involved (1). Psoriasis may also affect the nails in 50% of patients, and a seronegative arthritis, psoriatic arthritis, occurs in ∼10%. Currently, there is no cure for psoriasis, and although rarely fatal, the disease causes considerable morbidity and impairment of quality of life (2). Early-onset psoriasis (that presenting before the age of 40) has a strong familial component. At least eight chromosomal susceptibility loci have been identified, the strongest linkage being to chromosome 6p and, in particular, to HLA-Cw6 (3). To date, no gene or gene product has been specifically linked to psoriasis. Development of the skin lesions of psoriasis is contingent on environmental triggers, including β-hemolytic streptococcal pharyngitis, stress, and various drugs, such as β blockers and lithium, in genetically predisposed individuals.

On routine histology, plaques of psoriasis show abnormal proliferation and differentiation of epidermal keratinocytes. Current thinking is that psoriasis is mediated by T cells (4). An established plaque of psoriasis is characterized by an infiltration of activated $CD45RO^+$ memory/effector T cells, with $CD8^+$ T cells predominating in the epidermis. The majority (85%) of T cells in plaques of psoriasis are cutaneous lymphocyte–associated antigen positive (5). It is thought that T cells become activated and acquire cutaneous lymphocyte–associated antigen expression at extracutaneous sites, such as the tonsil. One theory is that streptococcal M protein involved in induction of cutaneous lymphocyte–associated antigen expression on T cells may lead to cross-reaction with an as-yet-unknown epitope of epidermal keratin. Plaques of psoriasis are characterized by a predominance of Th1 cytokines, particularly IL-2 and IFN-γ, coupled with increased activity of TNF-α (6). A central role for T cells in the pathogenesis of psoriasis has been underscored further by the observation that T cell–targeted therapies such as ciclosporin (7), an IL-2 fusion toxin (8), and an anti-CD11a monoclonal antibody (efalizumab; reference 9) are effective therapies for this disease. Furthermore, the uninvolved skin of patients with psoriasis transplanted onto the flanks of SCID mice can be converted to psoriasis by injection of activated autologous peripheral blood mononuclear cells (10).

DCs are a unique population of leukocytes that regulate immune responses and have the ability to interact with and activate T cells (11). Langerhans cells (LCs) are members of this wider family of DCs. LCs reside in the epidermis, where they serve as sentinels of the immune system, their responsibilities being to sample the external environment for changes and challenges and to deliver information (antigen) to T cells within skin-draining lymph nodes. The ability of LCs to migrate from epidermis to regional lymph nodes is thus of pivotal importance to the induction of cutaneous immune responses. The initiation and regulation of LC mobilization and migration are orchestrated by cutaneous cytokines and chemokines. Collectively, the available data reveal that under normal circumstances the stimulation by chemical allergens of LC migration away from the epidermis is dependent on the local availability of IL-1β and TNF-α. We have previously shown that exposure of human volunteers either to a contact allergen (diphenylcyclopropenone [DPC]) by topical administration (12) or to TNF-α (13) or IL-1β (14) by intradermal injection results in a substantial (20–30%) reduction in the frequency of epidermal LCs. This is due to the migration of epidermal LCs to draining lymph nodes. The observation that only a proportion of human LCs is able to mobilize in response to cytokine or allergen stimulus is believed to reflect the fact that functional heterogeneity exists amongst epidermal LCs.

The role of LCs in the pathogenesis of psoriasis is poorly understood. There are conflicting reports that the frequency of epidermal LCs in the uninvolved skin of psoriasis patients is the same, increased, or decreased compared with the skin of normal volunteers (15, 16). Clinical research points to a functional impairment in LC function in patients with psoriasis. This evidence consists of impaired contact hypersensitivity to experimental allergens such as 2,4-dinitrochlorobenzene (17) and the observation that allergic contact dermatitis is relatively rare in patients with psoriasis (18). These observations led us to investigate LC function in psoriasis, particularly migration in response to the classic proinflammatory cytokines IL-1β or TNF-α and to the contact allergen DPC.

RESULTS

LC migration

We have investigated the integrity of LC migration in the uninvolved skin of patients with psoriasis. In the first series of experiments, allergen-induced LC migration was examined. 10 patients and one normal volunteer were exposed topically to the contact allergen DPC (2% in acetone) or to acetone alone, and punch biopsies were taken 17 h later for assessment of $CD1a^+$ LC numbers (Fig. 1 a). These results are shown in comparison with historical data, where LC densities were measured as a function of both CD1a (10 subjects) and HLA-DR (five subjects) expression (Fig. 1 a, bottom; reference 12). Topical application of 2% DPC to uninvolved psoriatic skin failed to induce marked LC mobilization, with only one patient responding to DPC with a decrease in LC densities (22% reduction; Fig. 1 a, top). In contrast, normal

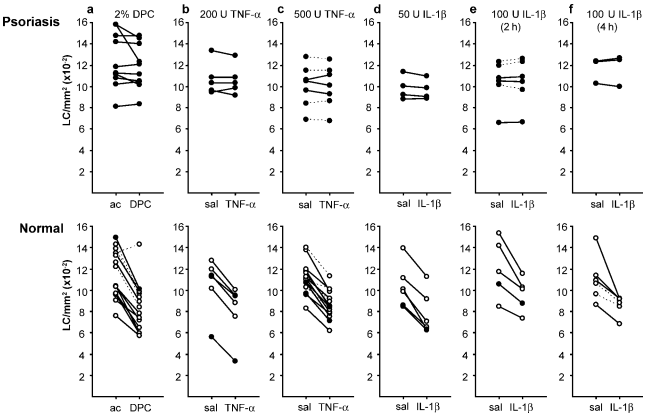

Figure 1. Impaired allergen- and cytokine-induced LC migration in the uninvolved skin of patients with psoriasis. Patients and normal healthy volunteers were treated topically with acetone (ac) or 2% DPC (a) or were injected intradermally with homologous recombinant TNF-α (b, 200 U; c, 500 U) or IL-1β (d, 50 U; e and f, 100 U), or saline (sal). Punch biopsies were taken from treated sites 17 h after treatment with DPC, 2 h after injection of TNF-α, and 2 h (e) or 4 h (f) after the administration of IL-1β. Epidermal sheets were prepared, and the density of $CD1a^+$ LCs (solid line) or $HLA\text{-}DR^+$ LCs (dotted line) was assessed after indirect immunofluorescence staining. For each volunteer, results are displayed as the mean number of LC/mm² for test (DPC or cytokine) versus control (ac or sal). Each line represents the response of a different individual. Solid circles represent data from current investigation, and open circles represent historical data (12–14).

healthy volunteers responded vigorously to chemical allergen. The response to DPC in the healthy volunteer examined concurrently (32.5% reduction) was similar to historical control values (Fig. 1 a, bottom), resulting in a significant ($P < 0.01$) reduction of 27.5% in LC densities in the skin of normal healthy volunteers exposed to DPC.

To investigate whether the observed impairment of DPC-induced LC migration was due to a lack of production of appropriate cytokines required for LC mobilization, homologous recombinant TNF-α or IL-1β were delivered intradermally into the skin of patients or normal volunteers. Treatment with 200 U TNF-α for 2 h (conditions shown previously to result in optimal LC migration in normal skin; reference 13) did not alter $CD1a^+$ LC numbers in the uninvolved skin of psoriasis patients compared with exposure to saline alone (Fig. 1 b, top). As expected, in two healthy volunteers treated concurrently, exposure to 200 U TNF-α induced LC migration that was similar to historical control data, with an overall mean reduction in $CD1a^+$ LC numbers of 24% ($P < 0.01$; Fig. 1 b, bottom). Failure to detect LC migration in uninvolved psoriatic skin was not the result of a more rapid migration (19), with intradermal administration of 200 U TNF-α to either one normal volunteer or one patient failing to stimulate LC mobilization after 30 min of exposure (unpublished data). Furthermore, LCs in psoriatic skin failed to respond to a higher dose (500 U) of TNF-α. Thus, in a further seven patients, no TNF-α (500 U)–mediated reduction in LC frequency was recorded, regardless of whether

LCs were detected on the basis of CD1a (three patients) expression or HLA-DR (four patients) staining (Fig. 1 c, top). In contrast, 500 U TNF-α caused a decrease in $CD1a^+$ LC densities in two normal volunteers tested concurrently and in historical controls comprising 13 (CD1a, 9; HLA-DR, 4) normal individuals examined under identical exposure conditions (Fig. 1 c, bottom). In total, the mean reduction in epidermal LC densities provoked by 500 U TNF-α was 22.3% ($P < 0.01$) in normal skin. In subsequent experiments, we explored the hypothesis that impaired LC migration in psoriasis patients might be due to lack of biologically active IL-1β. To address this, four patients and one concurrent normal volunteer received 50 U IL-1β intradermally or saline alone, and biopsies were taken 2 h later, conditions established previously for vigorous LC migration in normal human skin (14). In this cohort of volunteers, treatment with 50 U IL-1β was associated with no change in $CD1a^+$ LC densities in uninvolved psoriatic skin (Fig. 1 d, top). However, this dose of IL-1β stimulated the expected reduction in $CD1a^+$ LC numbers in the corresponding normal subject, and together with historical control data, a mean IL-1β–mediated reduction in $CD1a^+$ LC frequencies in normal skin of 25.4% ($P < 0.01$) was observed (Fig. 1 d, bottom). To determine whether LCs in uninvolved psoriatic skin are less responsive to IL-1β, the activity of a higher dose (100 U) of IL-1β was examined (14). In six psoriasis patients injected with 100 U IL-1β, no change in either $CD1a^+$ ($n = 3$) or $HLA\text{-}DR^+$ ($n = 3$) LC numbers was evident 2 h after exposure (Fig. 1 e, top). However, LC mobilization was observed in one healthy volunteer examined concurrently and in four historical control subjects who participated in a previous study to give an overall reduction in $CD1a^+$ LC numbers of 19.4% ($P < 0.01$; Fig. 1 e, bottom). Finally, we examined whether LCs in uninvolved psoriatic skin would respond to IL-1β with delayed kinetics compared with LCs in normal skin. Thus, a further three patients were exposed to 100 U IL-1β for 4 h. Again, LCs in the uninvolved skin of psoriasis patients failed to migrate in response to treatment with 100 U IL-1β (Fig. 1 f, top), under conditions where all normal individuals responded to this dose of IL-1β in a previous investigation with a mean reduction in LC numbers of 20.6% ($P < 0.01$; Fig. 1 f, bottom).

LC density and morphology

Baseline LC frequencies and morphology at resting (control) skin sites have been collated during these experiments. Resting LC values varied considerably between individuals but were not significantly different between psoriasis patients and control subjects. Thus, densities of $CD1a^+$ LCs ranged from 660 to 1,583 LC/mm^2 in the uninvolved skin of psoriasis patients and from 562 to 1,493 LC/mm^2 in normal volunteers, with similar values recorded for $HLA\text{-}DR^+$ LCs (Fig. 2 a). Overall, the mean (± SD) epidermal $CD1a^+$ and $HLA\text{-}DR^+$ LC values for uninvolved psoriatic skin (1,101 ± 215 LC/mm^2, $n = 27$, and 1,058 ± 222 LC/mm^2, $n = 7$, respectively) and normal skin (1,100 ± 210 LC/mm^2, $n = 43$, and 1,218 ± 168 LC/mm^2, $n = 11$, respectively) did not differ significantly. Examination of LC morphology at control-treated sites revealed no marked differences in the dendritic appearance of LCs in uninvolved psoriatic or normal skin samples, regardless of whether epidermal sheets were examined by fluorescence or confocal microscopy (Fig. 2, b and c, respectively). Similarly, there were no apparent differences in the level of expression of CD1a or HLA-DR determinants between volunteer groups. However, small focal points of cells stained more intensely for HLA-DR were observed occasionally in

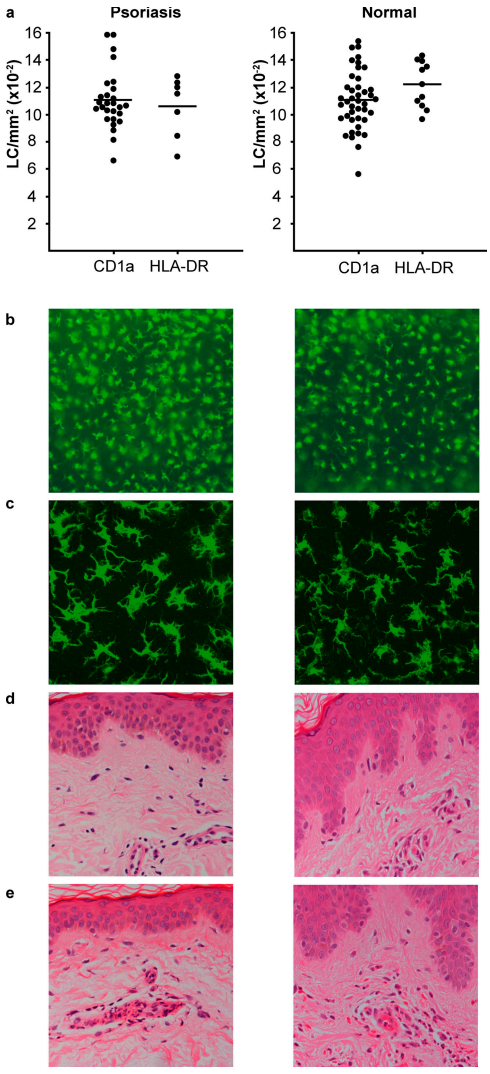

Figure 2. Epidermal LCs in uninvolved psoriatic skin appear similar to LCs in normal skin. (a) Comparison of epidermal $CD1a^+$ and $HLA\text{-}DR^+$ LC numbers at sites treated with control (acetone or saline) derived from data displayed in Fig. 1 for uninvolved psoriatic and normal skin. Solid line represents the mean LC value for each group. (b) Distribution of $CD1a^+$ LCs in epidermal sheets prepared from uninvolved psoriatic skin and normal skin biopsies observed by fluorescence microscopy. (c) Confocal images of $CD1a^+$ LCs in epidermal sheets prepared from uninvolved psoriatic skin and normal skin biopsies. (d and e) Histological examination of skin sections prepared from uninvolved psoriatic or normal skin biopsies 2 h after exposure to saline (d) or TNF-α (e).

epidermal sheets from the uninvolved skin of psoriasis patients, which were not apparent in normal skin (unpublished data) and may be due to low-level inflammation present even in the normal-appearing skin of patients with psoriasis. Although the morphology of LCs varied somewhat between subjects from both groups, clear and consistent changes in LC morphology were not observed routinely in uninvolved psoriatic skin compared with normal skin, at either control or treated (DPC or cytokine) skin sites. As such, it was not possible to form reliable conclusions regarding the maturation status of LCs under these conditions, which suggests that a more quantitative approach is required.

Clinical observations and histological assessment

We reported previously that topical exposure of normal skin to DPC results in an early inflammatory response visible after 17 h in ~50% of volunteers and a delayed, more aggressive reaction (graded 2–3) sometimes requiring treatment in >50% of individuals after 10 d (12). We report here that, in contrast, DPC failed to induce an early inflammatory reaction in uninvolved psoriatic skin in any of the patients examined, and only two patients responded with a delayed inflammatory reaction (graded 1 and 2). There was no association between inflammation and LC migration in these patients. The single normal volunteer treated concurrently with DPC in this study failed to develop an early inflammatory response but acquired a severe reaction (grade 3) after 10 d, which required treatment. Injection of TNF-α or IL-1β caused equivalent local erythematous reactions (graded 1–2) in a similar proportion of subjects in both volunteer groups. The presence of an inflammatory response to cytokine in uninvolved psoriatic skin was confirmed histologically in four patients treated with 500 U TNF-α (histological score 0–2) and five patients exposed to 50 U IL-1β (scored 1–3), the nature of the inflammatory cell infiltrate being consistent with that reported previously for normal skin (Fig. 2, d and e; reference 13). Injection of cytokine was tolerated equally in both groups of volunteers with minimal systemic side effects. Pulse rate, blood pressure, and body temperature did not change significantly over the 2–4-h period of examination.

Basal and inducible cutaneous cytokine production

To determine whether impaired cytokine-induced LC migration in uninvolved psoriatic skin is due to a failure of LCs to receive both cytokine signals for migration, we measured the level of TNF-α and IL-1β in blister fluids prepared from uninvolved psoriatic and normal skin sites after injection of each of these cytokines. In addition, we looked at other proinflammatory and antiinflammatory cytokines involved in cutaneous immune responses (IL-6, IL-8, IL-10, IL-12p70, GM-CSF, and IFN-γ). In each case, individuals received injections of cytokine (IL-1β or TNF-α) or saline alone as control, enabling comparisons of both basal and inducible cytokine production to be made (Fig. 3). We found that administration of 100 U IL-1β up-regulated TNF-α secretion in all volunteers examined; however, the response was significantly greater in psoriasis patients (3.7-fold increase; $P < 0.01$; $n = 5$) than in normal subjects (2.2-fold increase; $n = 4$; Fig. 3 a, top). Conversely, TNF-α also promoted increased IL-1β secretion in all cases, although the response in psoriasis patients (3.5-fold increase; $n = 5$) was not significantly different from that observed in normal controls (2.8-fold increase; $n = 2$; Fig. 3 a, bottom). Furthermore, mean (± SE) basal IL-1β and TNF-α levels at saline-treated sites in uninvolved psoriatic skin (IL-1β, 4.3 ± 2.0 pg/mg; TNF-α, 6.9 ± 1.2 pg/mg; $n = 10$) were equivalent to those found in normal skin (IL-1β, 3.0 ± 0.9 pg/mg; TNF-α, 6.2 ± 2.4 pg/mg; $n = 6$). A similar pattern emerged for all other cytokines examined (Fig. 3 b). Thus, basal cytokine levels in saline-treated uninvolved psoriatic skin were comparable with those found at control sites in normal skin. In addition, although some variability existed between volunteers in basal cytokine levels and the extent of enhanced cytokine production, administration of either TNF-α or IL-1β generally enhanced production of all cytokines examined for both psoriasis patients and normal volunteers. However, no significant differences were apparent for psoriasis patients versus normal subjects. To investigate the possibility that raised IL-1R antagonist (IL-1Ra) levels in patients with psoriasis negate IL-1 responses, suction blister fluids were further analyzed by ELISA for the presence of this antagonist. However, similar levels of IL-1Ra were found to be present in blister fluids taken from resting (saline treated) skin sites (normal, 1.92 ± 0.3 ng/mg, mean ± SE of $n = 6$; psoriasis, 1.86 ± 0.4 ng/mg, mean ± SE of $n = 10$) for both volunteer groups, and this did not alter with cytokine (TNF-α or IL-1β) injection (unpublished data).

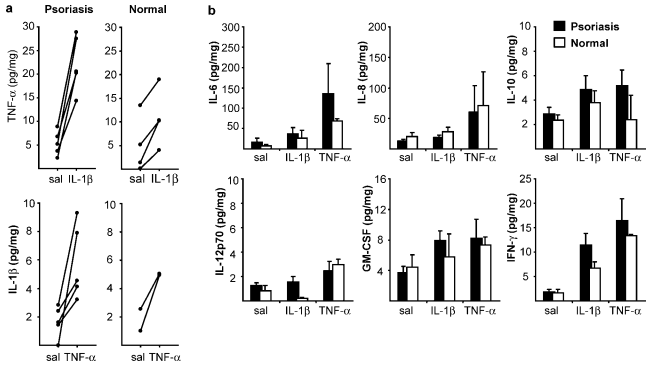

Figure 3. Comparable basal and inducible cutaneous cytokine secretion in blister fluids raised at uninvolved psoriatic and normal skin sites. Psoriasis patients and normal volunteers were exposed intradermally to 100 U IL-1β or 500 U TNF-α. Suction blister equipment was applied and blister fluid aspirated after 90–120 min for cytokine array analysis by Luminex. (a) Individual responses for IL-1β–induced TNF-α production (top) and TNF-α–induced IL-1β secretion (bottom) are displayed. (b) Mean (± SE) cytokine levels for IL-6, IL-8, IL-10, IL-12p70, GM-CSF, and IFN-γ observed after injection of saline (sal) alone, IL-1β, or TNF-α. Mean (± range) is displayed for TNF-α–induced cytokine secretion in normal skin. Cytokine levels are displayed as picogram/milligram of protein measured in blister fluid samples using a modified Lowry.

Expression by LCs of IL-1RI and TNF-R2

We next examined whether the failure to induce mobilization of LCs in uninvolved psoriatic skin might be explained at the level of IL-1RI or TNF-R2 expression, both of which are expressed by LCs in normal human skin (20). For each analysis, epidermal cell suspensions were prepared concurrently from punch biopsies taken from one normal individual and from the uninvolved skin of one patient with psoriasis. Cells were dually stained for either IL-1RI or TNF-R2, together with CD1a to identify LCs, before analysis by two-color flow cytometry. In total, seven matched comparisons were conducted for IL-1RI, three of which included concurrent measurements for TNF-R2 (Fig. 4). We found that the frequency of $CD1a^+$ LCs (1–2%) in epidermal cell suspensions did not differ between volunteer groups, which is consistent with the data presented in Fig. 1. However, although the percentage of $CD1a^+$ LCs expressing IL-1RI or TNF-R2 varied between individuals, no overall difference in either the proportion of LCs expressing these cytokine receptors or their level of expression on LCs was observed. Thus, the percentage of $CD1a^+$ LCs expressing IL-1RI ranged from 40 to 74% (mean ± SE, 63.3 ± 4.5%) for normal volunteers and from 48 to 78% (61.7 ± 4.3%) for psoriasis patients. Comparisons of TNF-R2 expression by LCs revealed that 18.4 ± 5.7% (range, 9–25%) and 18.6 ± 4.4% (range, 12–23%) of $CD1a^+$ LCs in normal and uninvolved psoriatic skin displayed this cytokine receptor, respectively. These results demonstrate that IL-1RI and TNF-R2 are present on LCs in the uninvolved skin of patients with psoriasis and to the same extent as observed in normal skin.

DISCUSSION

Collectively, these results clearly demonstrate that LC function is abnormal in uninvolved skin from patients with psoriasis. This is particularly remarkable given the observations that within nonlesional skin the numbers and morphological characteristics of epidermal LCs were found to be entirely normal. It could be argued that the significant impairment of LC function in psoriasis is a consequence of cutaneous inflammation. The observation that LC migration was either absent or impaired irrespective of the extent/severity of psoriasis is evidence that this is not the case.

It is now well established that the mobilization of epidermal LCs is dependent on both IL-1β and TNF-α (11, 13) and that if, in experimental systems, either one of these cytokines is unavailable, then migration is no longer inducible (21, 22). In humans also there is evidence that a decrement in IL-1β associated with aging (23) or an inhibition of cutaneous TNF-α production resulting from local treatment with lactoferrin (13, 14) is in each case characterized by impaired LC migration. In the current investigations, however, it is apparent, that the absence of LC mobilization is not attributable to a lack of cytokine signals because migration could not be provoked by the local administration of either IL-1β or TNF-α under conditions where these cytokines were shown to cross-induce each other and where they are known to provoke migration of LCs from the epidermis of healthy volunteers. In addition, there is no reason to doubt that these cytokines were able to deliver signals to resident LCs in uninvolved skin. Thus, expression by LCs of the relevant receptors, IL-1RI and TNF-R2 (11), were apparently normal, and in any event both cytokines were able to elicit inflammatory reactions in nonlesional psoriatic skin that were comparable with those observed in the skin of normal donors. Furthermore, patterns of cytokine production induced by local exposure to either IL-1β or TNF-α and measured in blister fluid in normal skin from healthy donors did not differ from those observed in nonlesional skin.

These observations pose questions regarding the cellular and molecular bases for impaired or absent LC migration in apparently unaffected skin from patients with psoriasis. Myriad changes have been identified in psoriatic lesions compared with the healthy skin of normal subjects. Among these are disease-related changes that might influence responses of LCs to cytokines and other stimuli, such as overexpression of IL-1Ra (24), the expression of factors such as macrophage inflammatory protein 3α (CCL20; references 25, 26), which could serve to encourage retention of LCs within inflamed skin or other bioactive proteins that have been found to be overexpressed in psoriatic skin (IL-12, IL-20, interferon regulatory factor-2, transforming growth factor-α, and various chemokines; references 27–31) and which might alone or in concert inhibit migration through as-yet-unidentified mechanisms. Although changes such as these, associated with disease progression, might be implicated as causes of the abnormal characteristics of LCs within plaques of psoriasis, it is harder to envisage how such mechanisms exert what would appear to be a systemic effect of some magnitude on epidermal LCs, including cells resident within nonlesional skin. For example, there was no evidence in such skin of increased levels of IL-1Ra compared with healthy controls.

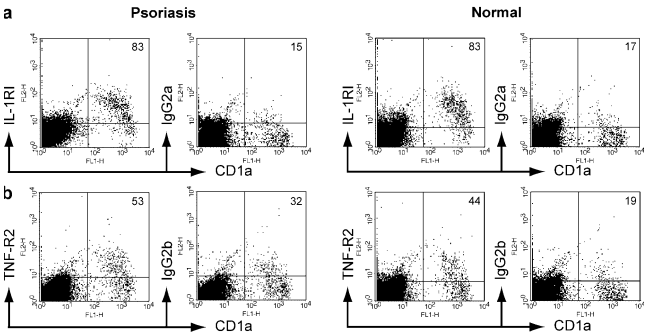

Figure 4. Detectable IL-1RI and TNF-R2 expression on LCs in epidermal cell suspensions prepared from normal healthy and uninvolved psoriatic skin. Epidermal cell suspensions were dually stained for IL-1RI/CD1a (a) or TNF-R2/CD1a (b), or appropriate isotype control combinations, and 10^5 cells were analyzed using a FACSCalibur flow cytometer. Representative results for epidermal cell suspensions stained concurrently for IL-1RI and for TNF-R2, from one normal volunteer and one patient with psoriasis, are displayed.

However, one possibility may reside in recent findings regarding the activity of plasmacytoid DCs (PDCs) that are found in increased numbers both in plaques of psoriasis and nearby uninvolved skin (32). The available evidence identifies IFN-α derived from activated PDCs as an important initiator of psoriasis (32). In this context it may be relevant that type 1 interferon (IFN-α/β) has been shown in vitro to inhibit the maturation of LCs and their migration in response to the CCR7 ligand CCL21 (33). These data raise the possibility that expression of IFN-α/β by activated PDCs in involved and uninvolved skin in psoriasis may be associated with a generalized inhibition of LC migration.

However, it should not necessarily be inferred that uninvolved skin in patients with psoriasis is comparable in every way with the normal skin of healthy subjects, and indeed it has been proposed that there exist differences (of as-yet-uncertain importance or consistency) that can be identified by global gene expression analyses (34). Nevertheless, such a dramatic functional difference as that described here has not previously been identified within nonlesional skin, and the expectation has been that the skin of susceptible subjects would, if anything, be associated with enhanced traffic of LCs and other leukocytes (35). In support of the data described herein, impaired DC mobilization was also observed in a mouse model of atherosclerosis where inflammation of the skin was evident (36). Thus, impaired DC migration may represent an inherent feature of other diseases with inflammatory pathologies. The interpretation in the context of psoriasis would be that LCs retained within the epidermis could present antigen locally to sustain or exacerbate a cutaneous inflammatory reaction. Another concept with intriguing implications for the pathogenesis of psoriasis is the observation that LCs may play a regulatory role in cutaneous immunity (37). Thus, it was reported that the afferent phase of contact hypersensitivity was enhanced in Langerin-diptheria toxin subunit A–transgenic mice, which constitutively lack epidermal LCs, raising the possibility that LCs not only serve to initiate adaptive immune responses but may also play a suppressive role. Furthermore, it was shown in these studies that the enhanced contact hypersensitivity response most likely occurred through increased priming of T cells (37). This implies that a critical determinant in psoriasis (a T cell–mediated disease) could arise from a lack of regulation from LCs early in the psoriatic process.

Another important consideration is that of dynamics of LC trafficking. Little is known about the turnover of LCs in human skin either under homeostatic or pathological conditions. As such, it is not possible at this stage to exclude the possibility that altered kinetics of LC turnover in nonlesional psoriatic skin contribute to the apparent lack of LC migration observed here in response to inflammatory stimuli. Although we have highlighted a functional defect in LCs in nonlesional psoriatic skin that may have important implications for the disease process, it is important to note that the majority of cutaneous immune responses proceed normally in these patients, suggesting that other DC populations, such as dermal DCs, are unlikely to be similarly affected.

The hypotheses we advance here are either that susceptibility to the development of psoriasis is associated with (and possibly predicated on) abnormal epidermal biology that is manifested by impaired LC mobilization or, alternatively, that disease progression is characterized by systemic changes that affect LC function. Irrespective of whether a systemic impairment of LC mobilization is related to disease predisposition or disease progression, the factors that might result in altered function, or the absence of which may compromise function, have not yet been identified. However, one potential candidate worthy of consideration is interferon consensus sequence binding protein (38).

In conclusion, we have demonstrated that psoriasis is associated with a considerable impairment of epidermal LC mobilization. These data provide clear evidence that, with respect to either cause or effect, psoriasis is characterized by very substantial changes in LC biology.

MATERIALS AND METHODS

Patients. 47 patients with a dermatologist-confirmed diagnosis of chronic plaque psoriasis (25 female and 22 male; age range, 18–58 yr; mean age, 40.9 yr) and 19 normal healthy volunteers (13 female and 6 male; age range, 20–55 yr; mean age, 38.1 yr) were recruited for study. Three of the psoriasis patients and one of the normal individuals volunteered for two different aspects of the study. An additional 40 normal volunteers involved in previous studies (24 female and 20 male; age range, 19–56 yr; mean age, 35.7 yr) were included for comparative purposes. Of these, one individual had volunteered for two different studies and three individuals also volunteered for the current investigation. Patients had been off systemic or photo therapy for psoriasis for at least 4 wk and were not using any active topical therapy, other than emollients, to the buttock or hip region at the time of the study. Clinical severity of psoriasis ranged from 1 to >70% body surface area involvement.

The study was approved by the Salford and Trafford Local Research Ethics Committee, and all subjects gave written, informed consent.

Exposure to allergen or cytokines. The skin-sensitizing chemical DPC (Calderdale and Huddersfield National Health Service Trust) was dissolved in acetone. Subjects were exposed topically at sites (each 2 cm^2 area) identified on the non–sun-exposed buttock to 50 μl of 2% (wt/vol) DPC in acetone or acetone alone. In some volunteers, DPC caused a delayed inflammatory reaction that required treatment (clobetasol propionate, 0.5% cream or Dermovate [Glaxo Wellcome]). Homologous recombinant human TNF-α (specific activity 3.6×10^7 U/mg by L929 assay; a gift from R.W. Groves, St Thomas' Hospital, London, UK) and IL-1β (specific activity $>1 \times 10^7$ U/mg; Insight Biotechnology Ltd.) were diluted in sterile normal saline to the appropriate concentrations. Subjects received 50-μl intradermal injections of cytokine diluted in saline or of sterile saline alone to sites identified on the non–sun-exposed buttock. For psoriasis patients, all treatments were delivered to sites on uninvolved skin of normal appearance >5 cm away from a plaque of psoriasis.

Biopsy. 6-mm punch biopsies were taken under local anesthesia (1% lidocaine) from each of the treated sites after various periods of exposure. Biopsies were processed immediately for either analysis of epidermal LC frequency or histological examination.

Preparation and analysis of epidermal sheets. Biopsies were processed for analysis of epidermal $CD1a^+$ or $HLA\text{-}DR^+$ LCs as described previously (12). Samples were examined by fluorescence microscopy, and the frequency of stained cells was assessed in a blinded fashion using an eyepiece with a calibrated grid (0.32×0.213 mm at 40× magnification). For each sample, 50 consecutive fields in the central portion of the biopsy were examined

and the results expressed as the mean number of cells/square millimeter. No specific staining was observed in control experiments using mouse monoclonal IgG2a (clone DAK-G05; DakoCytomation) in place of the primary antibody. Images were acquired digitally using either a laser-scanning confocal microscope (BX51; Olympus) system (Radiance 2100; Bio-Rad Laboratories) or a fluorescence microscope (BX50; Olympus) coupled with a color charge-coupled device camera (RS CoolSNAP; Photometrics) and MetaMorph Imaging System (version 4.01; Princeton Instruments).

Clinical assessment. All treated sites were assessed hourly for signs of erythema (graded on a scale of 0 [none] to 3 [severe]) and induration (graded on a scale of 0 [none] to 3 [severe]), in addition to a subjective assessment of pain. Immediately before treatment and at half-hour intervals thereafter, blood pressure, pulse, and temperature were recorded.

Histological examination. Biopsies for histological examination were placed immediately in 4% buffered formal saline for routine processing to paraffin sections and staining with hematoxylin and eosin. Sections were examined in a blinded fashion. The extent of inflammation was scored using the following 5-point scale: 0, normal, no neutrophils present; 1, diapedesis only and neutrophils mostly intravascular; 2, mild vasculitis and neutrophils both intravascular and extravascular with mild vessel wall damage, including endothelial swelling; 3, marked vasculitis and neutrophils in walls of small vessels and arterioles with marked extravasation; and 4, epidermal spongiosis, a neutrophilic infiltrate in the papillary dermis, and a lymphocytic perivascular infiltrate in the middermis.

Suction blister formation. Suction blister cups (12 mm; Ventipress Oy) were applied to treated sites and a vacuum of 250 mmHg applied (39, 40). After suction blister formation (90–120 min), the pressure was released and blister fluid aspirated using a 23-gauge needle. Fluids were centrifuged for 5 min in a microcentrifuge (MSE Micro Centaur; Fisher Scientific) and frozen immediately at −70°C.

Cytokine array analysis and protein determination. Concentrations of TNF-α, IL-1β, IL-6, IL-8, IL-10, IL-12p40, GM-CSF, and IFN-γ were measured in blister fluids using the Bio-Plex cytokine and chemokine array system according to the manufacturer's instructions (Bio-Rad Laboratories). Cytokine content was measured using a multiple analyte profiler (Luminex 100; MiraiBio Hitachi Genetic Systems). Protein content was determined using a modification of the Lowry assay (41), the Bio-Rad DC protein assay (Bio-Rad Laboratories), a colorimetric assay for determination of protein concentration. The cytokine content of blister fluid samples is expressed as picograms of cytokine/milligrams of protein.

Measurement of IL-1Ra. The IL-1Ra content of suction blister fluids was measured by ELISA (IL-1Ra Cytoscreen kit; Biosource International) according to the manufacturer's instructions, and results were expressed as nanogram of cytokine/milligram of protein.

Flow cytometric analysis of epidermal cell suspensions. Two 6-mm punch biopsies were taken from untreated non–sun-exposed buttock skin. In the case of psoriasis patients, biopsies were taken from normal-appearing untreated non–sun-exposed buttock skin >5 cm from a plaque of psoriasis. Biopsies were trimmed of underlying fat using a scalpel, washed thoroughly in PBS, and incubated for 60 min at 37°C with 0.4% trypsin and 0.2% EDTA (Invitrogen). Samples were washed thoroughly in RPMI-1640 growth medium (Invitrogen) supplemented with 25 mM Hepes, 500 μg/ml streptomycin and 500 U/ml penicillin (RPMI) containing 20% heat-inactivated FCS. Epidermal cells were separated from the underlying dermis by gentle scraping with a scalpel and a single cell suspension prepared by gentle mechanical disaggregation through 200-mesh stainless steel gauze. Cells were washed twice in RPMI containing 20% FCS and resuspended in RPMI containing 10% FCS (RPMI-FCS). For analysis of IL-1RI and TNF-R2 expression by LCs, epidermal cell suspensions were incubated first with rat anti–human IL-1RI (CD121a, clone RMHL1-1, rat IgG2a; Serotec, Oxford, UK), rat anti–human TNF-R2 (CD120b, clone hTNFR-M1, rat IgG2b; BD Biosciences), or isotype-matched control antibodies (rat IgG2a [clone R35-95] or rat IgG2b [clone R35-38]; BD Biosciences), each diluted to 10 μg/ml, followed by *R*-phycoerythrin-conjugated $F(ab')_2$ goat anti–rat IgG diluted 1:50. Subsequently, cells were blocked with mouse serum (1:10), followed by treatment with FITC-conjugated mouse anti–human CD1a (clone HI149, mouse IgG1; BD Biosciences) diluted 1:50. Cell staining was analyzed by two-color flow cytometry of 10^5 cells using a FACSCalibur flow cytometer (BD Biosciences).

Statistical analyses. Differences between LC values were considered by analysis of variance allowing for volunteer as a random effect and treatment as a fixed effect. Selected comparisons between treatment groups were compared using a two-sided Student's *t* test, based on the error mean square in the analysis of variance. TNF-α– and IL-1β–induced cytokine responses were considered by analysis of covariance.

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