

Technical Advance: Langerhans cells derived from a human cell line in a full-thickness skin equivalent undergo allergen-induced maturation and migration

Krista Ouwehand,* Sander W. Spiekstra,* Taco Waaijman,* Rik J. Scheper,[†]
Tanja D. de Gruijl,[‡] and Susan Gibbs*,¹

Departments of *Dermatology, [†]Pathology, and [‡]Medical Oncology, VU University Medical Centre, Amsterdam, The Netherlands

RECEIVED JUNE 29, 2010; REVISED MAY 25, 2011; ACCEPTED MAY 31, 2011. DOI: 10.1189/jlb.0610374

ABSTRACT

In this report, the construction of a functional, immunocompetent, full-thickness skin equivalent (SE) is described, consisting of an epidermal compartment containing keratinocytes, melanocytes, and human LCs derived from the MUTZ-3 cell line (MUTZ-LC) and a fibroblast-populated dermal compartment. The CD1a⁺Langerin⁺HLA-DR⁺ MUTZ-LCs populate the entire epidermis at a similar density to that found in native skin. Exposure of the SE to subtoxic concentrations of the allergens NiSO₄ and resorcinol resulted in LC migration out of the epidermis toward the fibroblast-populated dermal compartment. A significant dose-dependent up-regulation of the DC maturation-related CCR7 and IL-1 β transcripts and of CD83 at the protein level upon epidermal exposure to both allergens was observed, indicative of maturation and migration of the epidermally incorporated LC. We have thus successfully developed a reproducible and functional full-thickness SE model containing epidermal MUTZ-LC. This model offers an alternative to animal testing for identifying potential chemical sensitizers and for skin-based vaccination strategies and provides a unique research tool to study human LC biology in situ under controlled in vitro conditions. *J. Leukoc. Biol.* 90: 1027–1033; 2011.

Introduction

Animal models provide a useful tool to investigate the skin immune-defense system and are currently used as the gold standard for allergenicity testing of chemicals. However, the replacement, reduction, and refinement of the use of test animals in general are now strongly advocated [1]. In addition to

ethical considerations, human models and in particular, excised skin may be more relevant to study the skin immune-defense system. However, a major drawback of using excised skin for research purposes and in vitro assays is the regular need for large amounts of fresh skin, which potentially creates logistical obstacles and high donor variation. An in vitro model containing defined cell types (e.g., keratinocytes, melanocytes, fibroblasts, and LCs) from allogeneic sources or cell lines would reduce the donor variability. Amplification and cryostorage of cell lines from cryopreserved stocks would overcome logistical obstacles. The aim of this study was to develop a human LC-SE.

To develop a LC-SE in which LCs can mature and migrate upon exposure to maturation-inducing agents (e.g., allergens), it is important to incorporate the essential cell types involved in this process. LCs are professional APCs in the epidermis, which play a key role in the skin immune-defense system. LCs capture exogenous antigens and migrate through the dermis to the regional LNs to induce an adaptive immune response [2, 3]. Recently, we identified a pivotal role for dermis-derived CXCL12/stromal cell-derived factor 1 in the migration of activated LCs from epidermis to dermis [4]. This chemokine is constitutively secreted by dermal fibroblasts, and its secretion is enhanced upon exposure to stress factors (e.g., chemicals, hypoxia, irradiation, and burns) [4–7]. This increased secretion most probably is the result of proinflammatory cytokines released within the epidermis, such as keratinocyte-derived IL-1 α and TNF- α and LC-derived IL-1 β . These cytokines play an essential role in LC activation and their subsequent migration to draining LNs [8]. Thus, it is essential that immunocompetent SE models contain a fibroblast-populated dermis and a keratinocyte-rich epidermis, as the interaction between epidermal and dermal cells has been proven to be important for maturation and migration of LCs in vivo. Another impor-

Abbreviations: Bteb=basic transcription element-binding protein, DNFB=dinitrofluorobenzene, KGF=keratinocyte growth factor, LC-SE=LC-containing skin equivalent, MUTZ-LC=LCs derived from the MUTZ-3 cell line, NiSO₄=nickel sulfate, qPCR=quantitative PCR, SE=skin equivalent

1. Correspondence: Dept. of Dermatology, VU University Medical Centre, De Boelelaan 1117, 1081 HV, Amsterdam, The Netherlands. E-mail: S.Gibbs@vumc.nl

tant consideration when developing a LC-SE is the source of LC. Blood-derived LCs create availability and logistical issues and introduce biological variability into the model system. Therefore, in this study, we aimed to integrate readily available MUTZ-LCs into our full-thickness SE model described previously, containing all major skin residential cell types (i.e., keratinocytes, melanocytes, and dermal fibroblasts) [9]. This SE model was chosen for our current study, as it provides a working test model within only 2 weeks after starting culture and also as a result of its robust reproducibility in our laboratory and in other laboratories [10, 11].

MUTZ-3 is a human AML cell line, which can be differentiated in a TGF- β -dependent manner into fully functional LCs expressing Langerin and bearing LC-associated Birbeck granules [12, 13]. Upon stimulation with maturation-inducing factors (e.g., allergens), MUTZ-LCs express characteristic maturation markers, such as CD83, CXCR4, and CCR7, and acquire the ability to migrate in response to CXCL12 [4, 14, 15], thus highly resembling their *in vivo* counterparts.

Here, we describe a reproducible LC-SE model consisting of a fully differentiated, reconstructed epidermis containing keratinocytes, melanocytes, and MUTZ-LC on a fibroblast-populated dermal matrix. The density of MUTZ-LC in the epidermis was comparable with *in vivo* skin, and the LC-SE was fully functional in that upon exposure to the allergens—NiSO₄ and resorcinol—MUTZ-LCs underwent maturation (shown by increased IL-1 β and CCR7 mRNA levels and by CD83 protein expression) and migrated from the epidermis into the dermis.

MATERIALS AND METHODS

Cell culture

Human neonatal foreskin was obtained from healthy donors (with written, informed consent) undergoing circumcision and was used within 48 h after the surgical procedure. The VU University Medical Centre (Amsterdam, The Netherlands) approved the experiments described in this paper. The study was conducted according to Declaration of Helsinki Principles. Dermal fibroblasts were isolated from neonatal foreskins and cultured in DMEM (ICN Biomedicals, Irvine, CA, USA) containing 1% UltrosorG (Biossepra S.A., Cergy-Saint-Christophe, France) and 1% penicillin-streptomycin (Invitrogen, Paisley, UK) at 37°C, 5% CO₂. Fibroblasts (p3) were incorporated into collagen gels (1 \times 10⁵ cells/ml), essentially as described by Spiekstra et al. [9]. Epidermal keratinocytes/melanocytes were isolated from neonatal foreskins as described previously [16]. The coculture was cultured in DMEM/Ham's F12 (Invitrogen; 3/1), supplemented with 1% UltrosorG, 1% penicillin-streptomycin, 1 μ M hydrocortisone, 1 μ M isoproterenol, 0.1 μ M insulin, and 2 ng/mL KGF and used to construct SE models (p1 or p2). The MUTZ-3 progenitor cell line was maintained as described previously [12]. MUTZ-3 were differentiated into LCs by culturing at 2 \times 10⁵ cell/mL in minimal essential media α (Gibco, Grand Island, NY, USA), supplemented with 20% v/v heat-inactivated FCS (Hyclone Laboratories, Logan, UT, USA), 1% penicillin-streptomycin, 2 mM L-glutamine (Invitrogen), 50 μ M 2-ME (Merck, Whitehouse Station, NY, USA), 100 ng/mL human rGM-CSF (Biosource International, Camarillo, CA, USA), 10 ng/mL TGF- β (Biovision, Mountain View, CA, USA), and 2.5 ng/mL TNF- α (Strathmann Biotec, Hamburg, Germany) for 7 days.

SE culture

Reconstruction of the human SE-containing MUTZ-LC was achieved by co-seeding MUTZ-LC (0.5 \times 10⁶ cells) with keratinocytes (0.5 \times 10⁶ cells) onto

fibroblast-populated collagen gels. Cells were submerged for 3 days in DMEM/Ham's F12 (3/1), supplemented with 1% UltrosorG, 1% penicillin-streptomycin, 1 μ M hydrocortisone, 1 μ M isoproterenol, 0.1 μ M insulin, and 1 ng/mL KGF. To induce epidermal differentiation, the constructs were cultured for 7 days at the air-liquid interface in DMEM/Ham's F12 (3/1), supplemented with 0.25% UltrosorG, 1% penicillin-streptomycin, 1 μ M hydrocortisone, 1 μ M isoproterenol, 0.1 μ M insulin, 2 ng/mL KGF, 1 \times 10⁻⁵ M L-carnitine, 1 \times 10⁻² M L-serine, and 50 μ g/mL ascorbic acid. Unless otherwise stated, all additives were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Medium was renewed two times/week, and all experiments were performed in duplicate unless stated otherwise.

Chemical exposure

Finn Chamber filter paper discs of 11-mm diameter (Epitest, Oy, Finland) were impregnated with vehicle (H₂O) or allergens NiSO₄ 6H₂O or resorcinol; Sigma Chemical Co.), dissolved in H₂O. The chemicals or H₂O (vehicle control) were applied topically to the stratum corneum of the LC-SE cultures for 16 h at 37°C, 5% CO₂.

Immunohistochemical staining

For morphological (H&E staining) and immunohistochemical analysis of Vimentin and Bteb, paraffin-embedded sections (5 μ m) were deparaffinized and rehydrated. Prior to staining, antigen retrieval was performed in citrate buffer, pH 6, at 100°C for 30 min. Subsequently, paraffin sections were stained with mouse mAb against Vimentin (V9; 1:200, Dako Cytomation, Denmark) or Bteb (NK1; 1:400, Monosan, The Netherlands). Isotype control to assess nonspecific binding was 10 μ g/mL mouse IgG1 (Dako Cytomation). Immunohistochemical analysis of CD1a (OKT6; 1:500, Ortho Diagnostics Systems, Rochester, NY, USA), CD207 (Langerin, DCGM4; 1:2500, Beckman Coulter, Fullerton, CA, USA), and HLA-DR (TAL.1B5; 1:400, Dako Cytomation) on frozen cryostat sections (5 μ m) and of CD1a⁺ LCs in epidermal sheets was performed as described previously [4]. The CD1a fluorescence intensity in epidermal sheets (background fluorescence subtracted) was quantified with the aid of NIS-Elements AR, Version 2.10 software (Nikon Instruments Europe B.V., Amstelveen, The Netherlands). Immunostaining of CD83⁺ LCs in epidermal sheets and collagen gels was performed by incubating intact epidermis or gel separately with CD83-PE (mouse IgG1, HB15e, BD PharMingen, Franklin Lakes, NJ, USA) in a 1:10 dilution in PBS, 0.1% BSA, or isotype mouse IgG1-PE (BD PharMingen) for 120 min.

Flow cytometry

The phenotype of MUTZ-3 and MUTZ-LC prior to epidermal incorporation or after migration into the dermis was determined by flow cytometry, as described previously [4]. Cell staining was performed using mAb to CD207 (Langerin)-PE (IgG1, Immunotech, Marseille, France), CD1a-FITC (IgG1, BD PharMingen), CD34-PE (IgG1, BD PharMingen), CD14-FITC (IgG2a, Miltenyi Biotec, Bergisch Gladbach, Germany), and isotype controls to assess nonspecific binding [mouse IgG1-PE, IgG1-FITC (BD PharMingen), mouse IgG1-PE (Immunotech), and mouse IgG2a-FITC (Miltenyi Biotec)]. The percentage of migrated LCs in dermal suspensions was determined based on 50,000 cell counts.

Real-time PCR

Epidermis was removed from dermis by peeling off intact sheets with piners. Total RNA was isolated from epidermis and dermis of the LC-SE model using the RNeasy mini kit with an on-column DNase I digestion (Qiagen, Basel, Switzerland) and stored at -80°C prior to use for RT-PCR analysis, which was performed as described previously [17]. cDNA was amplified by PCR using the following primer kits: RT² qPCR primer assay for human protein tyrosine phosphatase receptor type C (CD45), RT² qPCR primer assay for human integrin α X (complement component 3 receptor 4 subunit; CD11c), RT² qPCR primer assay for human IL-1 β (IL-1 β), and

RT² qPCR primer assay for human CCR7; and as a housekeeping gene, RT² qPCR primer assay for human RPL13A.

Quality control

Before chemical exposure, a single culture was harvested and underwent histological examination (H&E staining of 5 μ m tissue section) to confirm that a fully differentiated epidermis with stratum corneum had formed on top of a fibroblast-populated collagen gel. During chemical exposure experiments, epidermal sheets of unexposed cultures were assessed in parallel by CD1a immunohistochemical staining to ensure that LCs were incorporated within the epidermis of LC-SE [minimum CD1a fluorescence intensity (background subtracted) ≥ 25 was required to pass assessment]. Of the 10 batches of LC-SE constructed during this study, nine batches passed the quality assessment. Cytotoxicity, as determined by histological analysis of tissue sections and by release of lactate dehydrogenase (kit supplied by Roche Applied Science, Mannheim, Germany) into culture supernatants, was determined after chemical exposure. No detrimental tissue damage was observed, and cytotoxicity was below 20% compared with vehicle-exposed LC-SE. In this study, the lifespan of LC-SE was at least 1 week.

Statistical analysis

Differences between human control skin and full-thickness SE were compared using the Student's *t* test. Two-way ANOVA with Dunnett's post-test for dose-response and kinetic calculation was performed using GraphPad Prism, Version 4.00 for Windows (GraphPad Software, San Diego, CA, USA). Differences were considered significant when *P* < 0.05.

RESULTS AND DISCUSSION

The construction of immunocompetent SE-containing MUTZ-LC

In this study, we describe a novel, tissue-engineered SE model containing LC-like cells that structurally and functionally resemble native human skin. Currently, only two groups have succeeded in introducing LC into an epidermal equivalent [18–21], and just one group was able to introduce DCs into a dermal equivalent [22]. Neither of these groups reconstructed a full-thickness SE, rendering these models unfit to study LC migration from the epidermis to the dermis. Only one group has so far succeeded in reconstructing a full-thickness SE containing keratinocytes, fibroblasts, endothelial cells, dermal DC-specific ICAM-3-grabbing nonintegrin-positive DCs, and epidermal LCs containing Birbeck granules [23]. This model was shown to be functional and suited to study LC activation and migration [24]. However, all of these models require fresh blood-derived precursor cells as their source of LCs. This renders them susceptible to donor variability in their response to chemicals and also logistically difficult to construct. DC donor variability has been described to have a large impact on whether DCs in vitro will respond to a chemical [18]. Therefore, a SE-containing blood-derived DC is not reliable as an in vitro research tool with the purpose of distinguishing sensitizers from nonsensitizers. Our immunocompetent SE is the first tissue-engineered, full-thickness SE to contain fully functional human cell line-derived LCs in the suprabasal cell layers of the epidermis. Here, we show that MUTZ-LCs are capable of maturing and migrating upon ex-

posure to classical allergens in a similar manner to LCs in freshly excised skin [4].

MUTZ-3 progenitor cells have high surface expression of CD34 and low expression of CD14 and do not express Langerin and CD1a (Fig. 1, upper panels). Upon differentiation for 7 days in the presence of GM-CSF/TGF- β /TNF- α , the cells lost their CD34 expression but increased their CD14 expression, which is a recognized LC precursor marker [25]. They also acquired the characteristic LC markers Langerin and CD1a (Fig. 1, lower panels). These 7-day-cultured and partially differentiated cells were subsequently used for incorporation of LCs into SE. The resulting LC-SE closely resembled native human skin. H&E staining showed a fully differentiated epidermis on a fibroblast-populated dermal matrix (collagen gel). The epidermis had a stratum corneum, stratum granulosum, stratum spinosum, and a compact basal layer (Fig. 2A, left panel). Melanocytes were localized throughout the basal layer of the epidermis (Fig. 2A, middle panel). To determine the presence and localization of MUTZ-LC in the SE model, tissue sections were immunostained for typical LC markers. CD1a, Langerin, and HLA-DR were detected throughout the epidermal layer, similar to human control skin (Fig. 2B). In human control skin, CD1a⁺ and HLA-DR⁺ cells were also observed in the dermis and represent dermal DCs and/or macrophages. Our results clearly demonstrate that MUTZ-LCs populate the reconstructed epidermis of the SE in a similar manner to native human skin.

Density of MUTZ-LC in SE is comparable with normal skin

To determine whether the morphology and density of MUTZ-LC in the SE epidermis resembled their in vivo counterparts, immunofluorescent staining was performed on normal skin- and SE-derived epidermal sheets. Results show stellate CD1a⁺ LC in epidermal sheets of the LC-SE, similar to human control skin, whereas no staining was observed in SE without LC (Fig. 3A). Furthermore, MUTZ-LCs were distributed evenly throughout epidermal SE sheets at a slightly lower but not significantly reduced density as compared

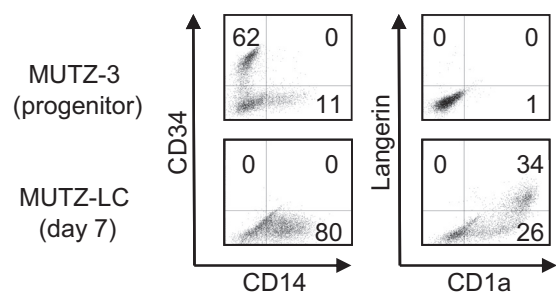


Figure 1. Characterization of MUTZ-LC. Phenotypic characterization of MUTZ-3 progenitor cells and 7-day-differentiated MUTZ-LC. FACS dotplots for CD14 (x-axis) and CD34 (y-axis) and CD1a (x-axis) and Langerin (y-axis) percentages of stained cells in each of the quadrants are shown for MUTZ-3 progenitors (upper panels) and partially differentiated MUTZ-LC (lower panels).

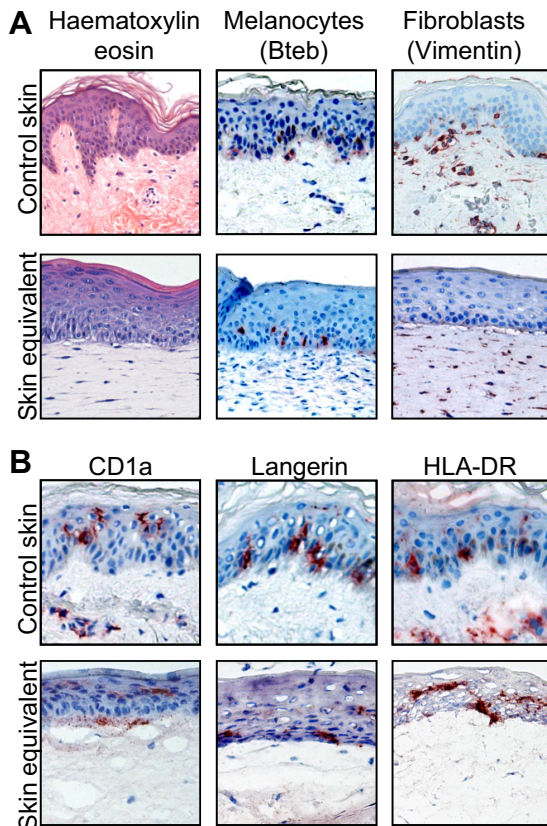


Figure 2. Characterization of the LC-SE. (Immuno)histochemical staining of human control skin and a representative full-thickness LC-SE. (A) H&E, Vimentin (fibroblast), and Bteb (melanocyte) staining results. (B) Staining results for LC markers CD1a, Langerin, and HLA-DR. No staining was observed with the corresponding isotype (IgG1) control (data not shown). Data are representative of five independent experiments performed in duplicate (original magnification: upper panels, 100 \times ; lower panels, 200 \times).

with that found in epidermal sheets derived from human skin (Fig. 3B). Based on these data, a minimum fluorescence intensity of 25 units was considered for positive assessment of LC-SE for allergenicity testing (see Materials and Methods). The two groups that succeeded in reconstruction of an epidermal equivalent containing CD34 blood-derived LCs showed similar epidermal LC density results to ours [18–21]. However, the only report describing a full-thickness SE comprising monocyte-derived LCs and dermal DCs stated three to five times less LCs next to one to three times less dermal DCs compared with normal human skin [23, 24].

MUTZ-LC in full-thickness SE mature and migrate after allergen exposure

We next determined whether MUTZ-LC could mature and migrate in the LC-SE upon exposure to a maturation-inducing stimulus. LC-SE were topically exposed to nontoxic concentrations of the allergens NiSO₄ and resorcinol—chemicals known to induce epidermis-to-dermis migration and

maturation of LCs [4]. Exposure to up to 19 mM NiSO₄ and 30 mM resorcinol for 16 h did not result in any detrimental changes in tissue architecture (Fig. 4A). LDH assay revealed >80% viability of the cultures after topical exposure to concentrations of NiSO₄ (6.5 mM and 19 mM) and resorcinol (15 mM and 30 mM) as compared with nonexposed controls (data not shown). Based on these data, the concentrations of NiSO₄ and resorcinol used in this study were considered to be nontoxic. To determine the effect of NiSO₄ and resorcinol exposure on LC density in the epidermis, the total fluorescence of CD1a⁺ MUTZ-LC in epidermal sheets (Fig. 4B, left panel) and the percentage of CD1a⁺ cells migrated into the dermis (Fig. 4B, right panel) were determined. Decreased LC densities in the epidermis and increased number of LC in the dermis were consistent with an allergen-induced, epidermis-to-dermis migration of the LC in the SE. Exposure to water vehicle control did not result in a significant change in the number of LCs as compared with unexposed LC-SE (Fig. 4B and C). Exposure of LC-SE to both allergens resulted in retracted dendrites (i.e., rounder LC morphology), most likely a result of E-cadherin down-regulation [26]. This in turn allows migration of rounded CD1a⁺ cells out of the epidermis (Fig. 4D). The group of Facy [18] also showed the retraction of dendrites upon exposure to allergens (e.g., DNFB and NiSO₄) and UV irradiation, but they were unable to study migration of LCs. This was most probably a result of the lack of a fibroblast-populated dermis providing the necessary chemokine gradient [18]. Upon UV irradiation, the monocyte-derived LCs in the full-thickness model described by Bechetoille et al. [24] did become activated and did migrate into the dermal compartment.

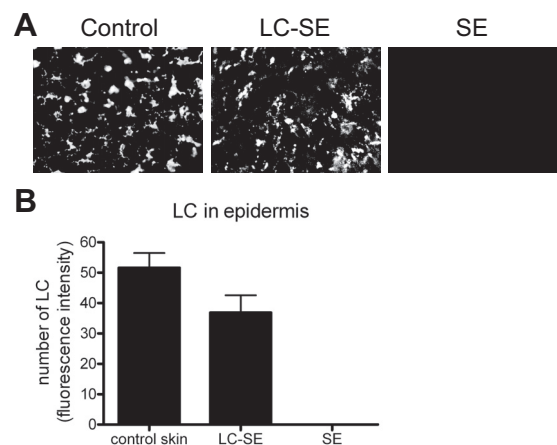


Figure 3. LC density in epidermal sheets isolated from human control skin and MUTZ-3-derived LC-SE. (A) Representative staining of CD1a-positive cells in epidermal sheets derived from human control skin and LC-SE. Control SE without LC shows total absence of fluorescently stained CD1a⁺ LC (original magnification, 200 \times). (B) Quantification of LC density by CD1a⁺ fluorescence intensity in epidermal sheets of human control skin, LC-SE, and SE. Data represent the average of five independent experiments each performed in duplicate \pm SEM. Student's *t* test was performed to calculate statistical differences.

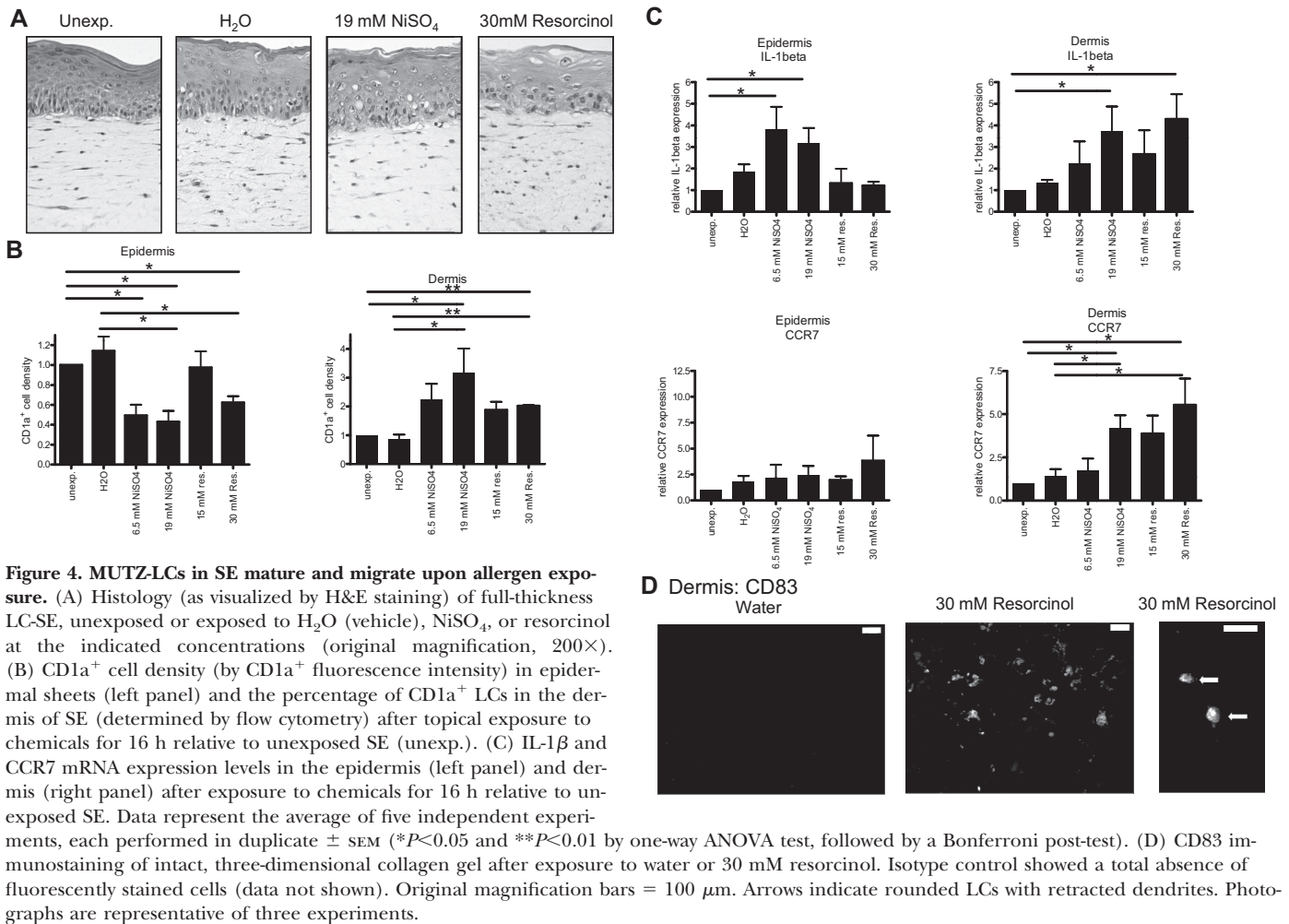


Figure 4. MUTZ-LCs in SE mature and migrate upon allergen exposure. (A) Histology (as visualized by H&E staining) of full-thickness LC-SE, unexposed or exposed to H₂O (vehicle), NiSO₄, or resorcinol at the indicated concentrations (original magnification, 200×). (B) CD1a⁺ cell density (by CD1a⁺ fluorescence intensity) in epidermal sheets (left panel) and the percentage of CD1a⁺ LCs in the dermis of SE (determined by flow cytometry) after topical exposure to chemicals for 16 h relative to unexposed SE (unexp.). (C) IL-1β and CCR7 mRNA expression levels in the epidermis (left panel) and dermis (right panel) after exposure to chemicals for 16 h relative to unexposed SE. Data represent the average of five independent experiments, each performed in duplicate ± SEM (**P* < 0.05 and ***P* < 0.01 by one-way ANOVA test, followed by a Bonferroni post-test). (D) CD83 immunostaining of intact, three-dimensional collagen gel after exposure to water or 30 mM resorcinol. Isotype control showed a total absence of fluorescently stained cells (data not shown). Original magnification bars = 100 μm. Arrows indicate rounded LCs with retracted dendrites. Photographs are representative of three experiments.

Allergen-induced expression of the LC maturation markers IL-1β, CCR7, and CD83

To further confirm that the observed decrease in epidermal LC density was a result of maturation-induced migration and not cell death as a result of chemical cytotoxicity, the expression of two DC maturation-related genes, i.e., IL-1β and CCR7, and CD83 maturation-related protein was determined upon exposure for 16 h to the chemical allergens.

An early event following skin sensitization is the increased expression of IL-1β mRNA (within 15 min). IL-1β is produced by LC in murine epidermis [27]. Indeed, IL-1β is required for the initiation of LC migration from the epidermis [28]. Based on this knowledge, we examined the effect of NiSO₄ and resorcinol on the IL-1β mRNA expression in the reconstructed, full-thickness MUTZ-LC containing SE. IL-1β mRNA was up-regulated significantly in the epidermis after 16 h exposure to as little as 6.5 mM NiSO₄. After water or resorcinol exposure, only a nonsignificant, slight increase was observed. In the dermis, increased IL-1β was detected after exposure to 19 mM NiSO₄ and also after exposure to 30 mM resorcinol (Fig. 4C), suggesting that the rising IL-1β expression levels may be related to sensitizer potency and indicating an early onset of LC maturation in the epider-

mis, detectable for strong sensitizers prior to migration into the dermis. These results are in agreement with the findings of Facy et al. [19], who also showed an increase in IL-1β expression in the epidermis after topical exposure of epidermal equivalents to strong allergens (e.g., DNFB and NiSO₄).

In contrast to IL-1β, CCR7 mRNA expression was not detectably increased in the epidermis but did show dose-dependent up-regulation in the dermis, indicative of CCR7 up-regulation following LC migration into the dermis after exposure to both allergens compared with unexposed or water-exposed cultures (Fig. 4C). This is consistent with the acquired ability of matured LCs to subsequently migrate from the dermis to draining LNs [4, 29–35]. In agreement with this finding, Bechettille et al. [24] found CCR7-positive LCs in the dermis of their model but not in the epidermis after UV irradiation.

Exposure to water vehicle did not result in a significant up-regulation of IL-1β or CCR7. Of note, IL-1β and CCR7 were not expressed in SE cultures without LCs and were not inducible after allergen exposure (data not shown). In contrast to our findings, Facy et al. [19] showed constitutive IL-1β expression in the untreated, LC-free epidermal equiv-

alent, which was up-regulated further upon allergen exposure. This seems to point to a high basal activation level of their applied keratinocytes, leading to IL-1 β release. This discrepancy with our findings may relate to differences in the methodologies used to construct the different SE. Still, in our model, when keratinocytes are cultured together with LCs, they might contribute to IL-1 β expression. To further confirm that LCs do indeed mature and migrate in the LC-SE model, we next immunostained intact, isolated epidermal sheets and collagen gels after water or resorcinol exposure. CD83 protein expression was only observed on single, rounded cells after allergen exposure in the collagen gel (Fig. 4D).

In summary, we describe the first in vitro tissue-engineered skin model, which contains fully functional LCs derived from a human cell line (i.e., MUTZ-LC). MUTZ-LCs constitute a readily available source of LCs able to capture, process, and present antigens to T cells, resulting in efficient T cell stimulation [36]. To stimulate T cell responses, LCs need to mature and acquire the ability to migrate toward CXCL12 and CCL19/21, all of which are traits shared by MUTZ-LCs [4, 36]. Our first results presented here clearly demonstrate that the LC-SE responds to the model allergens NiSO₄ and resorcinol in a comparable manner with primary LCs in fresh, full-thickness skin explants. The use of MUTZ-LC provides a solution to the poor availability of blood-derived CD34⁺ LC precursors and avoids the high interdonor variability associated with the use of blood-derived precursors. Further research will determine to what extent the LC-SE will be able to replace animal tests for estimating the sensitizing potential of new chemicals and skin-based vaccination strategies. Above all, it provides an excellent in vitro research tool to study the biology of LCs under controlled conditions, which closely mimic the natural tissue composition.

AUTHORSHIP

K.O., S.W.S., and T.W. were responsible for carrying out experiments and interpreting the data under the daily supervision of coprincipal investigators, S.G. and T.D.G. R.J.S. provided intellectual input and an important advisory role throughout. K.O., S.G., T.D.G., and R.J.S. were involved in writing the manuscript.

ACKNOWLEDGMENTS

This study was funded by the VUmc Institute for Cancer and Immunology (V-ICI), Amsterdam, and in part by a grant from the European Commission as part of the integrated project, "Novel Testing Strategies for In Vitro Assessment of Allergens (Sens-it-iv), LSHB-CT-2005-018681". We thank H. W. van Essen for technical assistance.

REFERENCES

- Schechtman, L. M. (2002) Implementation of the 3Rs (refinement, reduction, and replacement): validation and regulatory acceptance considerations for alternative toxicological test methods. *ILAR J.* **43** (Suppl.) S85–S94.
- Tamaki, K., Stingl, G., Katz, S. I. (1980) The origin of Langerhans cells. *J. Invest. Dermatol.* **74**, 309–311.
- Moll, H., Flohe, S., Rollinghoff, M. (1995) Dendritic cells in *Leishmania major*-immune mice harbor persistent parasites and mediate an antigen-specific T cell immune response. *Eur. J. Immunol.* **25**, 693–699.
- Ouwehand, K., Santegoets, S. J., Bruynzeel, D. P., Scheper, R. J., De Gruijl, T. D., Gibbs, S. (2008) CXCL12 is essential for migration of activated Langerhans cells from epidermis to dermis. *Eur. J. Immunol.* **38**, 3050–3059.
- Avniel, S., Arik, Z., Maly, A., Sagie, A., Basst, H. B., Yahana, M. D., Weiss, I. D., Pal, B., Wald, O., Ad-El, D., Fujii, N., Arenzana-Seisdedos, F., Jung, S., Galun, E., Gur, E., Peled, A. (2006) Involvement of the CXCL12/CXCR4 pathway in the recovery of skin following burns. *J. Invest. Dermatol.* **126**, 468–476.
- Ponomarev, T., Peled, A., Petit, I., Taichman, R. S., Habler, L., Sandbank, J., Arenzana-Seisdedos, F., Magerus, A., Caruz, A., Fujii, N., Nagler, A., Lahav, M., Szyper-Kravitz, M., Zipori, D., Lapidot, T. (2000) Induction of the chemokine stromal-derived factor-1 following DNA damage improves human stem cell function. *J. Clin. Invest.* **106**, 1331–1339.
- Askari, A. T., Unzek, S., Popovic, Z. B., Goldman, C. K., Forudi, F., Kiedrowski, M., Rovner, A., Ellis, S. G., Thomas, J. D., DiCorleto, P. E., Topol, E. J., Penn, M. S. (2003) Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischemic cardiomyopathy. *Lancet* **362**, 697–703.
- Cumberbatch, M., Dearman, R. J., Antonopoulos, C., Groves, R. W., Kimber, I. (2001) Interleukin (IL)-18 induces Langerhans cell migration by a tumor necrosis factor- α - and IL-1 β -dependent mechanism. *Immunology* **102**, 323–330.
- Spiekstra, S. W., Toebak, M. J., Sampat-Sardjoepersad, S., van Beek, P. J., Boersma, D. M., Stoof, T. J., von Blomberg, B. M., Scheper, R. J., Bruynzeel, D. P., Rustemeyer, T., Gibbs, S. (2005) Induction of cytokine (interleukin-1 α and tumor necrosis factor- α) and chemokine (CCL20, CCL27, and CXCL8) alarm signals after allergen and irritant exposure. *Exp. Dermatol.* **14**, 109–116.
- Boelsma, E., Tanojo, H., Bodde, H. E., Poncet, M. (1996) Assessment of the potential irritancy of oleic acid on human skin: evaluation in vitro and in vivo. *Toxicol. In Vitro* **10**, 729–742.
- El-Ghazal, A., Gibbs, S., Lamme, E., Van Blitterswijk, C. A., Poncet, M. (2002) Effect of fibroblasts on epidermal regeneration. *Br. J. Dermatol.* **147**, 230–243.
- Masterson, A. J., Sombroek, C. C., De Gruijl, T. D., Graus, Y. M., van der Vliet, H. J., Loughheed, S. M., van den Eertwegh, A. J., Pinedo, H. M., Scheper, R. J. (2002) MUTZ-3, a human cell line model for the cytokine-induced differentiation of dendritic cells from CD34⁺ precursors. *Blood* **100**, 701–703.
- Santegoets, S. J., Masterson, A. J., van der Sluis, P. C., Loughheed, S. M., Fluitsma, D. M., van den Eertwegh, A. J., Pinedo, H. M., Scheper, R. J., De Gruijl, T. D. (2006) A CD34(+) human cell line model of myeloid dendritic cell differentiation: evidence for a CD14(+)CD11b(+) Langerhans cell precursor. *J. Leukoc. Biol.* **80**, 1337–1344.
- Larsson, K., Lindstedt, M., Borrebaeck, C. A. (2006) Functional and transcriptional profiling of MUTZ-3, a myeloid cell line acting as a model for dendritic cells. *Immunology* **117**, 156–166.
- Ouwehand, K., Spiekstra, S. W., Reinders, J., Scheper, R. J., De Gruijl, T. D., Gibbs, S. (2010) Comparison of a novel CXCL12/CCL5 dependent migration assay with CXCL8 secretion and CD86 expression for distinguishing sensitizers from non-sensitizers using MUTZ-3 Langerhans cells. *Toxicol. In Vitro* **24**, 578–585.
- Waijman, T., Breetveld, M., Ulrich, M., Middelkoop, E., Scheper, R. J., Gibbs, S. (2010) Use of a collagen-elastin matrix as transport carrier system to transfer proliferating epidermal cells to human dermis in vitro. *Cell Transplant.* **19**, 1339–1348.
- van der Eerden, B. C., Emons, J., Ahmed, S., van Essen, H. W., Lowik, C. W., Wit, J. M., Karperien, M. (2002) Evidence for genomic and nongenomic actions of estrogen in growth plate regulation in female and male rats at the onset of sexual maturation. *J. Endocrinol.* **175**, 277–288.
- Facy, V., Flouret, V., Regnier, M., Schmidt, R. (2004) Langerhans cells integrated into human reconstructed epidermis respond to known sensitizers and ultraviolet exposure. *J. Invest. Dermatol.* **122**, 552–553.
- Facy, V., Flouret, V., Regnier, M., Schmidt, R. (2005) Reactivity of Langerhans cells in human reconstructed epidermis to known allergens and UV radiation. *Toxicol. In Vitro* **19**, 787–795.
- Régner, M., Staquet, M. J., Schmitt, D., Schmidt, R. (1997) Integration of Langerhans cells into a pigmented reconstructed human epidermis. *J. Invest. Dermatol.* **109**, 510–512.
- Schaerli, P., Willmann, K., Ebert, L. M., Walz, A., Moser, B. (2005) Cutaneous CXCL14 targets blood precursors to epidermal niches for Langerhans cell differentiation. *Immunology* **23**, 331–342.
- Guironnet, G., Dezutter-Dambuyant, C., Gaudillere, A., Marechal, S., Schmitt, D., Peguet-Navarro, J. (2001) Phenotypic and functional outcome of human monocytes or monocyte-derived dendritic cells in a dermal equivalent. *J. Invest. Dermatol.* **116**, 933–939.

23. Dezutter-Dambuyant, C., Black, A., Bechetoille, N., Bouez, C., Marechal, S., Autexfans, C., Cenizo, V., Pascal, P., Perrier, E., Damour, O. (2006) Evolutive skin reconstructions: from the dermal collagen-glycosaminoglycan-chitosane substrate to an immunocompetent reconstructed skin. *Biomed. Mater. Eng.* **16**, S85–S94.
24. Bechetoille, N., Dezutter-Dambuyant, C., Damour, O., Andre, V., Orly, I., Perrier, E. (2007) Effects of solar ultraviolet radiation on engineered human skin equivalent containing both Langerhans cells and dermal dendritic cells. *Tissue Eng.* **13**, 2667–2679.
25. Larregina, A. T., Morelli, A. E., Spencer, L. A., Logar, A. J., Watkins, S. C., Thomson, A. W., Falo Jr., L. D. (2001) Dermal-resident CD14⁺ cells differentiate into Langerhans cells. *Nat. Immunol.* **2**, 1151–1158.
26. Schwarzenberger, K., Udey, M. C. (1996) Contact allergens and epidermal proinflammatory cytokines modulate Langerhans cell E-cadherin expression in situ. *J. Invest. Dermatol.* **106**, 553–558.
27. Enk, A. H., Angeloni, V. L., Udey, M. C., Katz, S. I. (1993) An essential role for Langerhans cell-derived IL-1 β in the initiation of primary immune responses in skin. *J. Immunol.* **150**, 3698–3704.
28. Cumberbatch, M., Dearman, R. J., Kimber, I. (1998) Characteristics and regulation of the expression on interleukin 1 receptors by murine Langerhans cells and keratinocytes. *Arch. Dermatol. Res.* **290**, 688–695.
29. Ohl, L., Mohaupt, M., Czeloth, N., Hintzen, G., Kiafard, Z., Zwirner, J., Blankenstein, T., Henning, G., Forster, R. (2004) CCR7 governs skin dendritic cell migration under inflammatory and steady-state conditions. *Immunity* **21**, 279–288.
30. Gunn, M. D., Kyuwa, S., Tam, C., Kakiuchi, T., Matsuzawa, A., Williams, L. T., Nakano, H. (1999) Mice lacking expression of secondary lymphoid organ chemokine have defects in lymphocyte homing and dendritic cell localization. *J. Exp. Med.* **189**, 451–460.
31. Förster, R., Schubel, A., Breitfeld, D., Kremmer, E., Renner-Müller, I., Wolf, E., Lipp, M. (1999) CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell* **99**, 23–33.
32. Kellermann, S. A., Hudak, S., Oldham, E. R., Liu, Y. J., McEvoy, L. M. (1999) The CC chemokine receptor-7 ligands 6Ckine and macrophage inflammatory protein-3 β are potent chemoattractants for in vitro- and in vivo-derived dendritic cells. *J. Immunol.* **162**, 3859–3864.
33. Saeki, H., Moore, A. M., Brown, M. J., Hwang, S. T. (1999) Cutting edge: secondary lymphoid-tissue chemokine (SLC) and CC chemokine receptor 7 (CCR7) participate in the emigration pathway of mature dendritic cells from the skin to regional lymph nodes. *J. Immunol.* **162**, 2472–2475.
34. Kimber, I., Cumberbatch, M., Dearman, R. J., Bhushan, M., Griffiths, C. E. (2000) Cytokines and chemokines in the initiation and regulation of epidermal Langerhans cell mobilization. *Br. J. Dermatol.* **142**, 401–412.
35. Sozzani, S., Allavena, P., Vecchi, A., Mantovani, A. (2000) Chemokines and dendritic cell traffic. *J. Clin. Immunol.* **20**, 151–160.
36. Santegoets, S. J., Bontkes, H. J., Stam, A. G., Bhoelan, F., Ruizendaal, J. J., van den Eertwegh, A. J., Hooijberg, E., Scheper, R. J., De Gruijl, T. D. (2008) Inducing antitumor T cell immunity: comparative functional analysis of interstitial versus Langerhans dendritic cells in a human cell line model. *J. Immunol.* **180**, 4540–4549.

KEY WORDS:

MUTZ-3 · in vitro · keratinocyte · fibroblast · sensitization · vaccination strategies

Technical Advance: Langerhans cells derived from a human cell line in a full-thickness skin equivalent undergo allergen-induced maturation and migration

Krista Ouwehand, Sander W. Spiekstra, Taco Waaijman, et al.

J Leukoc Biol 2011 90: 1027-1033 originally published online June 22, 2011

Access the most recent version at doi:[10.1189/jlb.0610374](https://doi.org/10.1189/jlb.0610374)

References

This article cites 36 articles, 8 of which can be accessed free at:
<http://www.jleukbio.org/content/90/5/1027.full.html#ref-list-1>

Subscriptions

Information about subscribing to *Journal of Leukocyte Biology* is online at
http://www.jleukbio.org/site/misc/Librarians_Resource.xhtml

Permissions

Submit copyright permission requests at:
http://www.jleukbio.org/site/misc/Librarians_Resource.xhtml

Email Alerts

Receive free email alerts when new an article cites this article - sign up at
<http://www.jleukbio.org/cgi/alerts>
