Keratinocytes Share Gene Expression Fingerprint with Epidermal Langerhans Cells via mRNA Transfer

Qingtai Su¹ and Botond Z. Igyártó¹

The immune functions of epithelia-resident dendritic cells are influenced by epithelial-derived cytokines. Here we identified a communication form between tissue-resident dendritic cells and niche cells that allows direct intracellular material exchange between the parties. We show that many keratinocyte (KC)-specific molecules such as keratins and adhesion molecules could be detected in the epidermal-resident Langerhans cells (LCs) as mRNA and protein. Furthermore, KC-derived Cre led to genetic recombination in the LCs. We also found that LCs containing KC-derived material were more prone to migration. The KC-specific signatures were transferred from KCs to LCs through an exosome-independent mechanism that likely involved nanotubes/dendrites. The transfer of material between epithelial cells and epithelia-associated dendritic cells was not limited to mice or to KC-to-LC transfer. Taken together, these data suggest that the epithelial environment might have a long-term effect on dendritic cell biology and that genetic tools that specifically target epithelial cells also affect tissue-resident immune cells.


INTRODUCTION

Langerhans cells (LCs) are a subset of dendritic cells (DCs) that are resistant to radiation and reside in the epidermis, where they are tightly attached to the surrounding keratinocytes (KCs) (Kaplan et al., 2007; Kashem et al., 2017; Romani et al., 2010). LCs participate in promotion of self-tolerance, antifungal immunity, skin immunosurveillance, and protective humoral immune responses (Collin et al., 2013; Kaplan, 2017; Romani et al., 2010; Yao et al., 2015). In steady state, a small fraction of LCs undergoes spontaneous maturation through a mechanism that is not yet understood (Clausen and Stoitzner, 2015; Dalod et al., 2014). This homeostatic and phenotypic maturation involves the upregulation of chemokine receptor CCR7 and downregulation of E-cadherin to detach themselves from the surrounding KCs and to enable the migration of the LCs to the skin-draining lymph nodes (LNs) (Jiang et al., 2007; Ohl et al., 2004). Because LCs often can be found in the LNs carrying cellular material of epidermal origin (Breathnach and Wyllie, 1965; Hemmi et al., 2001; Wang et al., 2005; Yoshino et al., 2006), this homeostatic migration is thought to contribute to the induction and maintenance of peripheral tolerance (Steinman et al., 2003).

The tissue microenvironment, through an unknown mechanism, is capable of shaping the chromatin landscapes of macrophages, which results in tissue-specific functions of macrophages (Lavin et al., 2014). The progenitors of DC subsets are preprogrammed in the bone marrow (Schlitzer et al., 2015), but it is expected that, similar to macrophages, their tissue of residence and the local microbiome will shape their immunological fitness. DC populations in different tissues display tissue-specific diversity and functions (Pakalnisky and Schraml, 2017), and thus, it is anticipated that the close communication between DCs and the tissue microenvironment might endow them with functional diversity and plasticity.

The primary role of KCs is to form a physical barrier toward the outside world, but they also actively contribute to the immunological defense of the skin. They are equipped with different pattern recognition receptors that can mediate inflammasome activation and secretion of antimicrobial peptides and many proinflammatory cytokines, as well as chemokines (Lebre et al., 2007; Miller and Modlin, 2007; Tan et al., 2015). Thereby, KCs indirectly participate in adaptive immune regulation, via DC polarization, mobilization, and migration to skin-draining LNs.

In this study, we found that LCs bear a broad KC gene signature. Furthermore, we showed that the gene signature was not synthesized by the LCs but was instead acquired from the KCs. Our findings were extended to other mouse tissues and to human epidermis. Nanotubes/dendrites might be one of the potential mechanisms that mediates this mRNA transfer. Collectively, our data demonstrate, to our knowledge, a previously unreported dynamic communication route between LCs and their resident microenvironment, through
which immunological plasticity might be shaped and orchestrated.

RESULTS

Epidermal LCs carry KC-specific gene and protein signatures

We first determined the genes that are differentially expressed between epidermal LCs (eLCs) and the LCs that have already migrated to LNs. For this purpose, we explored the publicly accessible ImmGen microarray database and its Web-based comparative tool (ImmGen.org, 2017). We noticed that eLCs showed higher expression than LN LCs of some keratins, structural proteins normally associated with KCs (see Figure 1a). Panther GO analysis of the top 20 differentially expressed genes revealed a heavy presence of adhesion, cell junction, and cytoskeletal elements (see Figure 1a). These findings prompted us to examine the presence of other KC-specific gene transcripts in eLCs. Using a published database on KC-specific genes (Gazel et al., 2003), we further found that the eLC-enriched genes showed considerable overlap with KC-specific gene transcripts (see Supplementary Figure S1a). Based on Panther GO analysis, most of these genes encode cell adhesion molecules, cytoskeletal proteins, and structural proteins (see Supplementary Figure S1a). The expression levels of KC-specific transcripts in eLCs positively correlated with those of KCs (see Supplementary Figure S1b). The presence of mRNAs coding for these molecules in eLCs was confirmed by quantitative reverse transcription–PCR (qRT-PCR) on flow-sorted eLCs (see Supplementary Figure S1c). Analysis of the ImmGen data sets on eLCs and other DC subsets also unveiled that eLCs contain higher levels of skin-specific keratins than any other DC subset isolated from other tissues (see Figure 1b).

Pan-cytokeratin staining on epidermal cell suspension showed that the keratins were present in eLCs on protein levels as well. Multicolor fluorescent histology and flow cytometry revealed that a significant portion of the eLCs have detectable levels of cyto keratins in the cytosol (see Figure 1c–e). ImageStream examination of epidermal cell suspensions derived from KRT14-YFP mice, in which KCs are labeled with YFP, unequivocally showed YFP+ LCs (16.9% ± 7.1) (see Figure 1f). Thus, eLCs exhibit KC-specific gene and protein signatures that are proportional with the KCs’ expression profile.

eLCs receive mRNAs coding for KC-specific proteins

To further study the presence of KC-specific mRNA transcript levels in eLCs, we analyzed the expression values of KC-specific genes in eLCs by qRT-PCR and reads per kilobase of transcript per million mapped reads (RPKM) in KCs, based on published data (Reemann et al., 2014). The KC-specific gene products found in eLCs positively correlated with the expression levels in the surrounding KCs (see Figure 2a). In contrast, fibroblast-specific genes did not show correlation with eLCs (see Figure 2b). This observation prompted us to examine whether eLCs acquire mRNAs from KCs. To determine whether genes coding for KC-specific proteins are transcribed/synthesized by the LCs or whether they receive them from the surrounding KCs, we sorted the eLCs and KCs by flow cytometry and performed the Assay for Transposase-Accessible Chromatin sequencing (ATAC-seq) on these cells. ATAC-seq can be used to interrogate whether or not the genes are accessible for transcription. We found that most KC-specific genes were not accessible for transcription in eLCs but were in KCs (see Figure 2c, and Supplementary Figure S2a). Principal component analysis also confirmed that the chromatin status of the KCs is distinct from other immune cell populations, whereas the chromatin status of eLCs clustered with other immune cells (see Figure 2d and Supplementary Figure S2b). To bring further experimental evidence to material transfer from KCs to eLCs, we generated a new mouse model. We bred the KRT14-Cre Rosa26 flox-Stop-flox eYFP (KRT14-YFP) mice to the Langerin-DTA (DTA) mice to generate mice (KRT14-YFP-DTA) that lack LCs and whose KCs are labeled with YFP. The lack of LCs in combination with YFP expression by KCs was determined by flow cytometry, and the genotype was confirmed using standard PCR (data not shown). Next, we irradiated these mice along with control DTA mice and reconstituted them with congenically (CD45.1+) marked wild-type (WT) bone marrow. The scientific rationale for this experiment was that the WT eLCs repopulating the empty YFP+ dermis will not be able to turn on YFP, and if they turn YFP+, the YFP signal (protein/mRNA) had to be acquired from the surrounding KCs. Two to 4 months after reconstitution, ~40% (40.25% ± 2.88) of the repopulating donor-derived WT eLCs contained detectable levels of YFP in KRT14-YFP-DTA mice (see Figure 2e). qRT-PCR analysis on the sorted eLCs revealed the presence of mRNA that codes for YFP in YFP+ eLCs but not in YFP- eLCs or dendritic epidermal T cells (DETCs) (see Figure 2f). ImageStream examination of epidermal cell suspensions derived from KRT14-YFP-DTA chimeric mice unequivocally showed YFP+ LCs but not DETCs (see Figure S2c). Thus, these data show that eLCs receive KC-specific gene transcripts, and probably proteins, directly from KCs.

KC-derived Cre leads to genetic recombination in eLCs

Various keratin promoter-driven Cre expression constructs such as KRT14-Cre and KRT5-Cre are widely used to selectively turn on/off genes in the KCs. Because mRNAs and keratin proteins from KCs are also abundantly present in the eLCs, we sought to determine whether KRT14-promoter-driven Cre protein/mRNA from KCs could be transferred in big enough quantities to eLCs to lead to genomic recombination in the eLCs. To test this, we sorted KCs, DETCs, and YFP+ and YFP eLCs from KRT14-YFP mice using flow cytometry. A representative gating strategy and YFP expression by different cell populations are presented in Figure 3a. Both conventional PCR and qRT-PCR analysis on sorted YFP+ and YFP− eLCs confirmed the genetic recombination in the YFP+ eLCs (see Figure 3b and c, and Supplementary Figure S3). Of note, about half of the YFP+ eLCs underwent genomic recombination, which might indicate that the remaining half of the cells received the YFP from KCs in form of mRNA or protein. In accordance with our flow data, DETCs showed no significant levels of

2314 Journal of Investigative Dermatology (2019), Volume 139
genetic recombination (see Figure 3c). The YFP signal in the LNs of KRT14-YFP mice was also limited to the langerin-expressing migratory DCs (see Figure 3d). Thus, these data suggest that the epithelial environment might have a long-term effect on DC biology and that genetic tools specifically targeting epithelial cells also affect tissue-resident immune cells.

Inflammation preferentially drives the migration of YFP+ eLCs
Analysis of the steady-state KRT14-YFP epidermis revealed that YFP+ LCs had significantly higher side scatter, CCR7, and slightly higher levels of major histocompatibility complex II (MHC-II) than YFP− LCs (see Figure 4a). Langerin was not significantly different between the two LC populations (see...
Figure 4a). Next, we sought to determine the effect of inflammatory stimuli on eLCs containing KC-derived molecules. One side of the back flank skin of the KRT14-YFP mice was left untreated, whereas the other side was shaved and gently tape-stripped. As expected, tape stripping led to the migration of eLCs (see Figure 4b). Using a paired t-test, we found that the percent of YFP+ LCs presented with a significant decrease, whereas the YFP- LCs showed a trend but did not reach significance (see Figure 4b). Together, our data suggest that YFP+ LCs have a preactivated phenotype that might react faster to the environmental insults than the YFP- LCs.

eLCs acquire KC-derived molecules through an exosome-independent mechanism that might involve nanotubes

Next, we aimed to determine the mechanism by which LCs acquire KC-derived cellular material. Our imaging data (cytospin and ImageStream) showed the keratin and YFP signals in the cytosol of eLCs, which argues against a possible acquisition through phagocytosis of dead cells (a fluorescent signal limited to certain area[s] of the cytosol would be expected). Exosomes have been shown to aid intercellular protein and even RNA (mostly microRNA) transfers (Ratajczak and Ratajczak, 2016; Tkach and Théry, 2016). Thus, we first tested the role of exosomes in the intercellular communications. The mRNAs of epidermal cells from CD45.1+ mice were labeled with mRNA-selective dye. These cells were then cocultured with unlabeled epidermal cell suspensions from CD45.2+ mice with or without the use of the Transwell system. We found no significant mRNA transfer to unlabeled LCs when the cells were physically separated by a membrane with 0.4-µm pore size, but almost all of the LCs became fluorescent when direct cellular contact was possible. The mRNA transfer was limited to LCs (see Figure 5a, and Supplementary Figure S4a). Thus, these data suggest that free mRNA, exosomes, other forms of extracellular vesicles, and cell debris might play a minimal role in mRNA transfer from KCs to LCs. These negative data prompted us to turn our attention toward nanotubes. An almost unlimited cell content exchange can take place through nanotubes between different cell types that are in relatively close proximity (Gerdes et al., 2007). The ImmGen microarray database, qRT-PCR, and flow cytometry all showed that eLCs are the only cells in the epidermis that express TNFaip2, a tumor necrosis factor-α–induced protein 2 (see Figure 5b), which is indispensable for the generation of nanotubes (Hase et al., 2009; Kimura et al., 2016). To test whether nanotubes can form between the cells of the epidermis, we labeled epidermal single-cell suspensions with red (Vybrant DiD) fluorescent dye, cultured them in vitro for 48 hours, and then analyzed them by fluorescence microscopy. Fluorescent imaging revealed the formation of nanotubes between the cells (see Figure 5c). To determine which epidermal cells can communicate with each other, we sorted...
the KCs, DETCs, and eLCs and labeled them with different dyes (DiD, DiI, and DiO). To investigate the communication between these cells, all combinations of two populations were cocultured together at the original sorting ratios for 2 days. Our fluorescence-activated cell sorting results indicated that material transfer is highly active between eLCs and KCs/DETCs, but the communication between DETCs and KCs is limited (Figure 5d). Similar results were obtained when differentially labeled unsorted cells were cocultured (see Supplementary Figure S4b). To further dissect the material transfer from KCs to LCs, we performed time-lapse imaging. Epidermal cell suspension prepared from DTA mice (no LCs) was labeled with mRNA dye and cocultured with epidermal cell suspension from WT mice where the eLCs were stained with fluorochrome labeled anti–MHC-II antibodies. The cultures were imaged for 8 hours. The live-imaging data clearly show that the eLCs are highly motile and scan the labeled KCs and slowly extract the fluorescently labeled mRNAs.
mRNA from them (see Supplementary Movie S1). Thus, our data support the idea that tunneling nanotubes/dendrites might be one of the mechanisms through which LCs acquire KC-derived cellular materials.

LCs also transfer cellular material to both KCs and DETCs

To test whether material transfer between KCs and LCs is unidirectional, we examined the epidermal cells of huLangerin-Cre-YFP (Lang-YFP) mice. In these mice, YFP expression is limited to LCs (Kaplan et al., 2007). We found that, in the steady state, significant numbers of KCs and, to a lesser extent, DETCs contained YFP (see Supplementary Figure S5a and b). Our ATAC-seq data showed that the chromatin region around Cd207 (langerin) is transcriptionally inactive in KCs (see Supplementary Figure S5c). Therefore, these suggest that KCs receive YFP from LCs and that LCs can communicate with both KCs and DETCs bidirectionally.

Epithelial material transfer is universal to all tissue-resident DCs and is not limited to mice

The mRNA transfer between eLCs and KCs prompted us to examine whether this phenomenon exists in other tissue-resident DCs. In addition to eLCs, other DC subsets such as the CD103- DETCs in the lung or CD11b DCs in the liver are epithelia-associated DCs. Epithelial cells show organ-specific expression of keratin subtypes. For example, lung epithelia express K19 and K13. In the liver, K8 and K18 are the dominant keratins expressed (Moll et al., 2008). A search in the ImmGen microarray database uncovered that DC subsets residing in these epithelia carry the corresponding epithelial gene signatures (see Supplementary Figure S6a).

To determine the translatability of our mouse data to humans, we analyzed freshly sorted human eLCs for the presence of a keratin signature using qRT-PCR. We found that, similar to mice, freshly isolated human eLCs contain relatively high levels of K14-encoding mRNA, but the expression significantly decreased 3 days after in vitro cultures (see Supplementary Figure S6b). These findings suggest that the material transfer between epithelial cells and DCs is a species-independent, universal phenomenon.

DISCUSSION

In this study, we showed that eLCs exhibited a KC-specific gene signature. We further demonstrated that the mRNAs of these...
KC genes were not synthesized de novo by LCs. mRNAs were produced by and then transferred from KCs because the genomic loci of the KC-specific genes are in the closed conformation in eLCs. KC-derived material transfer led to genetic recombination in LCs. The genetic material transfer from epithelial cells was not limited to eLCs, as other DC subsets also bore the specific genetic fingerprint of the local epithelial cells. We propose the tunneling nanotubes/dendrites as the potential route by which DCs capture mRNA from surrounding cells. This study reveals, to our knowledge, a previously unreported active transport of mRNA from KCs to LCs.

This project started with an observation that eLCs contained high levels of mRNAs coding for keratins, structural proteins associated with KCs and other epithelial cells. At first glance, we thought that this might be because of contamination with KCs. Despite the odds, we followed up on this observation, and here, we bring direct and indirect experimental evidence that a simple contamination with KCs cannot account for our observations, for the following reasons:

1. Epidermal cytospins clearly showed significant numbers of eLCs with cytosolic cytokeratin staining.
2. ImageStream analysis of KRT14-YFP or KRT14-YFP-DTA chimeric epidermal cell suspension showed cytosolic YFP distribution in eLCs but not DETCs.
3. In KRT14-YFP-DTA chimeric mice, only the donor-origin WT eLCs contained YFP mRNA and not the DETCs.
4. YFP+ LCs could also be detected in the LNs.
5. KC-derived Cre only led to significant amounts of genetic recombination in eLCs and not in DETCs.
6. ATAC-seq data showed closed chromatin conformation for keratin genes in eLCs.

Although we cannot completely rule out at this time the possibility that other avenues such as exosomes or phagocytosis of apoptotic cells could contribute to the material transfer, the data presented here support the role of an alternative mechanism, a possible nanotube involvement. Nanotubes can be considered highways with almost unrestricted bidirectional traffic in which the exchanged content is protected from the harsh extracellular environment (Buszczak et al., 2016). Intact mRNA transfer by the phagocytosis of apoptotic KCs is highly unlikely because the apoptotic cells shut down their mRNA synthesis, and the phagosomes would fuse with lysosomes that would ultimately lead to the degradation of the phagolysosomal content. Furthermore, material taken up by phagocytosis and endocytosis would lead to fluorescent signals that would be associated with well-defined intracellular structures. Contrary to this, our cytospin, ImageStream, and live imaging
showed cytosolic distribution of keratins, YFP, and mRNA in LCs. Exosomes have been shown to carry different microRNAs but no significant amounts of intact mRNA (Batagov and Kurochkin, 2013). In addition, in a recent article, it was suggested that nanotubes and not exosomes favor intercellular mRNA transfer (Haimovich et al., 2017). Our Transwell experiments also support a dominant, exosome-independent route. The fact that the material transfer does not happen between all of the cells found in the epidermis also argues against exosomal transfer and supports the concept of controlled communication through nanotubes or a previously unidentified mechanism. The role of gap junctions in the material transfers should also be considered (Pang et al., 2009; Saccheri et al., 2010), though their contribution is unlikely; because of their sheer sizes, nucleic acids and proteins are usually precluded from crossing through these channels. Although in vitro experiments are vital for studying the intercellular communication pathways, they could also be a source of artifacts; thus, the real challenge will be to image and document the material transfer in an in vivo setting.

The biology underlying the mRNA transfer and protein expression is still under investigation. In the steady state, LCs are thought to sample the surrounding environment, promoting self-tolerance to skin antigens (Mutyambizi et al., 2009). The tolerogenic potential for DCs has long been recognized (Morelli et al., 2001). The internalization of apoptotic melanocytes by LCs was proposed to explain the

Figure 5. KCs and LCs might communicate through nanotubes. (a) Epidermal cells from CD45.1+ mice were stained with mRNA-selective dye and cultured with unlabeled CD45.2+ epidermal cells or without the use of the Transwell system. CD45.2+ LCs are shown. Control: unlabeled CD45.2+ epidermal cells. Data from one representative experiment out of two are shown with three mice pooled. (b) Left: Tnfaip2 expression comparison between eLCs and Other DCs (ImmGen Datagroup, DCs). Middle: Tnfaip2 expression in epidermal cells (KC, DETC, and LC) determined by qRT-PCR. Right: Tnfaip2 in KCs and eLCs by FACS (gray: isotype, red: Tnfaip2). Data from one representative experiment out of two are shown with three mice pooled. (c) Epidermal cell suspensions were labeled with red fluorescent dye and cultured for 2 days. Arrows indicate the formation of tunneling nanotubes between the cells. One representative experiment out of three is shown. Scale bar = 20 μm. (d) FACS analysis of flow-sorted epidermal cells 2 days after dye labeling and coculture. KCs, DETCs, and eLCs were labeled with DiD, Dil, and DiO, respectively, and cultured at the original sorting ratios. The cells were then identified based on CD45 and MHC-II expression and their dye content determined using flow cytometry. One representative experiment out of three is shown. DC, dendritic cell; DETC, dendritic epidermal T cell; eLC, epidermal Langerhans cell; FACS, fluorescence-activated cell sorting; KC, keratinocyte; LC, Langerhans cell; MHC-II, major histocompatibility complex II; qRT-PCR, quantitative reverse transcriptase–PCR.
presence of melanin in these cells (Breathnach and Wyllie, 1965; Hemmi et al., 2001; Yoshino et al., 2006). However, in our study, the abundance of KC-specific mRNA in LCs implies transport from live KCs. We speculate that this biological process might contribute to the maintenance of peripheral tolerance in steady state. It will be important to determine whether melanoma or other skin cancers could exploit this pathway to promote immunological tolerance against cancer antigens and metastasis to LNs (Lou et al., 2012, 2017). The material exchange between eLCs and other epidermal cells is bidirectional. However, what role that could play in the skin homeostasis also remains to be exploited. Our unpublished observation that, in the absence of LCs, KCs have decreased levels of MHC-II (both transcript and protein) suggests that LCs, by sharing MHC-II with KCs, could endow KCs with accessory antigen-presenting cell properties. Antigen presentation by KCs in the absence of costimulation could support generation of anergic/regulatory CD4 T-cell responses and thus further contribute to maintain tolerance.

Inflammation triggers the migration of LCs and changes their tolerogenic properties into properties that are immunogenic in nature (Romani et al., 2003). Our study indicates that the eLCs are highly heterogeneous, and LCs that acquired detectable levels of KC-derived materials are more responsive to stimuli. These LCs showed higher granularity, MHC-II, and CCR7 content than the LCs that did not contain KC-derived material, which is consistent with a more activated phenotype. Thus, it will be important to compare the contribution of these subsets in regulating adaptive immune responses. Steady-state LCs have dual origins: the yolk sac and fetal liver (Hoeffel et al., 2012). It remains to be determined whether the LCs that contain YFP belong to either one of these subsets. We only found detectable levels of YFP in a fraction of LCs in the KRT14-YFP mice, but pan-cytokeratin staining showed a much wider distribution. These findings could simply mean that our techniques are not sensitive enough and that either all of the LCs have KC-derived molecules or only a subset of LCs with a specific origin can acquire this function. However, chimera experiments do not support this latter hypothesis because bone marrow–derived LCs were also capable of acquiring KC-specific cellular material. Thus, LCs that contain KC-derived material at detectable levels could simply be at a certain stage of their differentiation or life cycle. It is quite possible that LCs containing high levels of KC-derived molecules spent more time in the epidermis than LCs with lesser amounts and thus accumulated more material, and they are ready to exit the epidermis as part of their homeostatic migration.

Whether this material transfer between LCs and niche cells also contributes to antigen presentation in the pathological state, such as autoimmune skin diseases (e.g., pemphigus, where desmoglein mRNAs are present in eLCs), remains to be determined. On the other hand, the acquired structural proteins might play an important role in the motility and cellular structure of eLCs and could also serve to maintain epithelial integrity. The material transfer from KCs could aid the transformation of the hematopoietic LC-precursors into epithelial-like cells. This transformation would be a temporary phase where eLCs, without the need of profound genetic remodeling, would be able to synthesize KC-specific structural proteins to prevent the formation of weak points in the epidermis and thus maintain epithelial integrity/rigidity (Kubo et al., 2009). Upon activation and migration to the LNs, the LCs would slowly lose the epithelial-like features and revert to the more motile hematopoietic phenotype (Seré et al., 2012).

DCs form dendrites that are thought to facilitate efficient scanning of the intercellular environment for intruders (Merad et al., 2013). Dendrites are cell protrusions that can be of different sizes. Based on our time-lapse imaging data, we propose that besides scavenging the intercellular space, the dendrites can function as nanotubes/sampling devices to maintain connections and collect real-time intracellular information directly from the surrounding cells. This can be especially true in the case of the epidermis, where the LCs are faced with a very packed environment formed by KCs. The LCs adapt by squeezing between KCs and forming very long and intricate, interconnected dendrite/nanotube webs. In this way, all of the LCs can almost instantly be alerted of environmental insults, danger signals (viral and bacterial genomic material), and pathogens that have been taken up by more abundant structural cells such as the KCs. Moreover, in the epidermis, only LCs express TNFαip2, which is indispensable for nanotube formation (Hase et al., 2009). This suggests that LCs serve as an information hub in collecting, interpreting, and distributing data. This could be functionally analogous to the nervous system and is an interesting point in light of Paul Langerhans’ original observations that described the LCs as nerve cells (Langerhans, 1868). DETCs are also located in the epidermis, but compared to LCs, their dendrites are wider and lamellipodia-like, ending in cup-like structures (Chodaczek et al., 2012; Heath and Carbone, 2013). These and the lack of TNFαip2 could explain the limited material transfer between KCs and DETCs. The exclusive role of LCs in information collection from other cells might have evolved to put the decision-making power regarding environmental insults into the hands of the professional antigen-presenting cells.

The use of keratin-driven Cre to delete or activate genes in KCs or other epithelial cells is a widely accepted technique (Mohammed et al., 2016). Our observation, however, raises a major concern for all of the studies in which the keratin promoter–driven Cre expression was used to delete genes specifically in KCs. Our data indicate that epithelia-resident DCs are also affected. Thus, results regarding the function of KCs and other epithelial cells in regulating skin-related immune responses have to be interpreted in this context. Cellular material transfer was not limited to the skin, which implies that gut epithelia–specific Cre could have similar effects on local immune cells. Whether DCs that are localized to other tissues communicate with the local fibroblasts, reticulocytes, endothelial cells, and others through dendrites/nanotubes and bear their gene signatures remains to be determined. It also will be important to determine whether similar intercellular material transfer can contribute to cross-presentation of antigens and thymic selection. Our principal component analysis comparison of mouse gut DC populations’ gene expression profile (Bain et al., 2017) with that of human fibroblasts isolated from different tissues (Higuchi...
et al., 2015) revealed that only fibroblasts derived from human gut lamina propria clustered closer with the gut DC populations (unpublished observation). These data therefore suggest that material exchange between nonhematopoietic and immune cells is widespread and not limited to epithelium.

In summary, here we show a communication form between DCs and their resident microenvironment, through which immunological plasticity might be shaped and orchestrated.

**MATERIALS AND METHODS**

An extended description of materials and methods is given in Supplemental Materials and Methods online.

**Mice**

The Baylor Institutional Care and Use Committee approved all mouse protocols. For details on all mouse strains used, see Supplemental Materials and Methods (online).

**Immunofluorescent staining**

For epidermal cytopin, the dissociated single cells were spun onto microscope slides, stained, and imaged as described in the Supplemental Materials and Methods.

**Panther GO Analysis**

We used the http://www.pantherdb.org/ online tool to perform these analyses. The top enriched eLC gene list was analyzed using the http://www.pantherdb.org/ Web tool. The protein class annotation data are presented.

**qRT-PCR**

Flow-sorted cells were analyzed as presented in Supplemental Materials and Methods.

**ATAC-sequencing**

Cellular nuclei were harvested, processed, and analyzed as described in Supplemental Materials and Methods.

**Flow cytometry**

Single-cell suspensions of skin and LNs were obtained and stained as previously described (Kaplan et al., 2005). All of our stainings included viability dye (Fixable Viability Dye eFluor780, eBio-science, Thermo Fisher Scientific, Waltham, MA), and stringent doublet discrimination gating was used to exclude possible contaminants. See Supplemental Materials and Methods for details.

**Ex vivo epidermal cell cultures**

After flow sorting, the different cells were stained with fluorescescent dyes according to the protocol from the manufacturer (V22889, Thermo Fisher Scientific), cultured, and imaged as described in the Supplemental Materials and Methods (online).

**Data availability statement**

Datasets are included with the manuscript and/or available at ImmGen: https://www.immgen.org/.

**CONFLICT OF INTEREST**

The authors state no conflict of interest.

**ACKNOWLEDGMENTS**

We thank Gerard Zurawski and Sandra Zurawski for sharing reagents with us and for thoughtful discussions. We are also very grateful for the help that we received from the Yin Lin lab (Yin Lin, Kenian Chen, and Lauren Metangi) in performing the ATAC-seq experiments and subsequent analysis. We thank the animal facility, flow core, and genomics core for their help and support. Special thank you to George J. Snipes for the human skin samples. We also thank Carson Harrod for editing the manuscript. The Baylor Scott & White Health Foundation supported this work.

**AUTHOR CONTRIBUTIONS**

Conceptualization: BZI; Data Curation: BZI, QS; Formal Analysis: QS, BZI; Funding Acquisition: BZI; Investigation: BZI, QS; Visualization: QS, BZI; Writing - Original Draft Preparation: BZI, QS; Writing - Review and Editing: BZI, QS

**SUPPLEMENTARY MATERIAL**

Supplemental material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2019.05.006.

**REFERENCES**


Batagov AO, Kurochkin IV. Exosomes secreted by human cells transport largely mRNA fragments that are enriched in the 3’-untranslated regions. Biol Direct 2013;8:12.


SUPPLEMENTARY MATERIALS AND METHODS

Mice
Langerin-DTA (DTA) (Kaplan et al., 2005) and huLangerin-Cre-YFP (Lang-YFP) (Kaplan et al., 2007) mice have been previously described. KRT14-Cre mice were purchased from the Jackson Laboratory and bred to Rosa26 flox-Stop-flox eYFP (KRT14-YFP) mice. The resulting mice were then bred to Langerin-DTA mice to generate mice (KRT14-YFP-DTA) that lack Langerhans cells (LCs) and in which the keratinocytes (KCs) are labeled with YFP. The generated mice were characterized using PCR and flow cytometry. B6 Cd45.1, Pep Boy (Cd45.1) wild-type (WT) mice were purchased from the Jackson Laboratory and bred in house. All experiments were performed with 6- to 16-week-old age-matched mice. Mice were housed in microisolator cages and fed autoclaved food and acidified water. The Baylor Institutional Care and Use Committee approved all mouse protocols.

Immunofluorescent staining
For epidermal cytospin, the dissociated single cells were spun onto microscope slides at 500 g for 20 minutes, followed by cold methanol fixation for 5 minutes. The glass slides were then rinsed with cold 0.1% Triton X-100 phosphate buffered saline and stained with pan-keratin (AE1/AE3; eBioscience, Thermo Fisher Scientific, Waltham, MA), major histocompatibility complex II (MHC-II) (clone M5/114.15.2; BioLegend, San Diego, CA), and langerin antibodies (clone 4C7; BioLegend) at the concentrations recommended by the suppliers. For in vitro culture experiments, the epidermal cell suspensions were cultured in 8-well EZ slides (Millicell, MilliporeSigma, Burlington, MA) for 2 days in complete RPMI culture medium (10% FBS, 1% P/S, 1% NEAA, 0.1% 2-ME, 1% HEPES, 1% NaPv, 1% VIT). The cells were then fixed with methanol before mounting with cover slips. Pictures were taken using an epifluorescent microscope (Axio Imager M2, Zeiss, Oberkochen, Germany) at the indicated magnification.

Panther GO Analysis
We used the http://www.pantherdb.org/ online tool to perform these analyses. The top enriched epidermal LC (eLC) gene list was analyzed using the http://www.pantherdb.org/ web tool. The protein class annotation data are presented.

qRT-PCR
Flow-sorted cells were centrifuged and resuspended in the cell lysis buffer to extract mRNA. mRNA extraction was performed according to the manufacturer’s instructions (Purelink RNA Mini Kit, Ambion, Foster City, CA). mRNA was eluted into nuclease-free water, and the concentration was determined by NanoDrop. Thereafter, 10–50 ng of mRNA was synthesized into cDNA following the manufacturer’s instructions (ArrayScript AM2049, Ambion), and 1 ng of cDNA was used for quantitative reverse transcriptase in real time for each well using the SsoAdvanced Universal SYBR Green Supermix (1725271, Bio-Rad, Hercules, CA). Each sample was run in duplicate, and the expression levels were determined using the ΔΔCt method. Primer sequences are shown in Supplementary Table S1.

ASSAY FOR TRANSPOSASE-ACCESSIBLE CHROMATIN SEQUENCING (ATAC-SEQ)
A total of 50,000 cells were obtained by flow sorting. Cellular nuclei were harvested after quick cell lysis followed by a 10-minute centrifugation at 500g. Nuclei were then transferred to transposition reaction mix (Nextera DNA Library Preparation Kit, FC-121-1030) at 37 °C for 30 minutes. DNA fragments were purified using the MinElute PCR Purification Kit (Qiagen 28004, Hilden, Germany) and eluted in 14 μl Buffer EB. Purified DNA was quantified using the Qubit dsDNA HS Assay Kit (Fisher Q32851). PCR amplification was carried out on the transposed DNA, followed by the size selection procedure (SPRSelect, Beckman Coulter Q32851). Fragment lengths between 200 bp and 600 bp were recovered. The quality of each library was assessed using the Agilent High Sensitivity DNA Kit (Agilent, Santa Clara, CA). Individual libraries were quantitated via quantitative reverse transcriptase in real time using the KAPA Library Quantification Kit, Universal (KAPA Biosystems, Wilmington, MA). These DNA fragments were sequenced on a HiSeq 2500 with paired-end 76-base read lengths.

Processing of ATAC-seq data
The 3′ ends of the ATAC-seq reads often contain adaptor sequences that affect read mapping if they are not trimmed. We used an iterative mapping strategy to solve this problem. In brief, reads were trimmed to a starting length (e.g., 30 bases) from the 3′ end and mapped to the mm9 reference genome using Bowtie (version 0.12.7) with the parameters –m2000 and –m1, as previously described (Buenrostro et al., 2013). Reads that were mapped to multiple locations because of the short read-length were extended toward the 3′ end with a small step size (e.g., one base) and mapped again. The procedure was repeated until the reads could not be extended further. Using this mapping method, a maximum mapping rate was achieved, and all of the informative reads were retained. After mapping, reads that originated from the mitochondria were discarded. All of the reads that were mapped to the “+” strand were offset by +4 bp, and all reads that were mapped to the “−” strand were offset by −5 bp to represent the center of the transposon binding events, as previously described (Buenrostro et al., 2013).

Principal component analysis of ATAC-seq data
To compare the chromatin accessibility landscape of different cell lineages with our samples, we downloaded the ATAC-seq data (GEO: GSE77695) from the O’Shea Laboratory. Reads were mapped to the mm9 reference genome, as described above. Normalized (to 10 million total reads) ATAC-seq read counts were calculated using HOMER (Heinz et al., 2010) and were log2-transformed. The transposed matrix was supplied as the input for the prcomp function in the R software package to perform the principal component analysis.

Flow cytometry
Single-cell suspensions of skin and lymph nodes (LNs) were obtained and stained as previously described (Kaplan et al., 2005). Cell suspensions were directly labeled with fluorescently conjugated antibodies for cell surface markers anti-MHC-II (see above), anti-CD45.2 (clone 104; BioLegend), anti-CD45.1 (clone A20; BioLegend), and
anti-langerin (see above). All of our stainings included viability dye (Fixable Viability Dye eFluor780, eBioscience), and stringent doublets discrimination gating was used to exclude possible contaminants. Staining was performed on ice in the dark for 40–60 minutes. Fixation of epidermal cell suspensions was performed by 5 minutes of cold acetone treatment before the staining first with anti-TNFAip2 antibody (clone F6, Santa Cruz Biotechnology, Santa Cruz, CA) and anti—pan-keratin antibody (see above) and then for other cell surface markers. Data were acquired using an LSRll or LSR Fortessa flow cytometer (Becton Dickinson, Franklin Lakes, NJ) with DIVA software. FlowJo software (Tree Star, Ashland, OR) was used for data analysis. For ImageStream imaging, cell suspensions of the epidermis were stained with the indicated cell surface markers (anti-CD45.2 PE and anti-MHC-II AF647 for KRT14-YFP mice; anti—MHC-II PE and anti-CD45.1 AF700 for KRT14-YFP-DTA mice) and run on an Amnis ImageStream instrument (EMD Millipore). Images were compensated and analyzed on IDEA software.

Ex vivo epidermal cell cultures

Epidermal cells were harvested from the C57BL/6 mice as described previously. After flow sorting, the LCs were stained with Vybrant DiO, DETCs with Dil, and KCs with DiD fluorescent dye, according to the protocol from the manufacturer (V22889, Thermo Fisher Scientific). After 5 minutes of staining, the epidermal cells were washed with complete RPMI culture medium twice, seeded in the 12-well plate, and cultured in a CO2 incubator for 2 days (cell ratios were based on in situ ratios determined by flow cytometry). As for the unsorted epidermal cell suspensions, they were stained with either DiD or DiO, mixed at a 1:1 ratio, and cultured for 2 days. For the fluorescent imaging, the cell mixtures were seeded in Millicell EZ slides (PEZGS0816 Millipore) for 2 days. After a brief wash, the glass slides were sealed with Aqua-Poly/Mount (Polysciences, Inc, Warrington, PA) and imaged immediately. SYTO RNASelect Green Fluorescent Dye (Molecular Probes S32703) was used to stain the cell suspensions or flow-sorted cells, according to the manufacturer’s instructions. Stained (CD45.1+ mice) and unstained (CD45.2+ mice) cells were mixed at a 1:1 ratio and seeded in a 24-well plate (1 million cells in total) in complete RPMI medium. The cell mixture was harvested 2 days after seeding and examined for mRNA transfer based on the CD45.2 congenic marker. A Transwell insert with 400 nm pores (Corning 353095) was used to study communication through exosomes; 0.5 million cells stained by mRNA-selective dye were seeded on top of the Transwell membrane, whereas 0.5 million unstained cells were seeded in the bottom of a 24-well plate. The bottom CD45.2+ cells were phenotyped by fluorescence-activated cell sorting 2 days later. For live cell imaging, epidermal cell suspensions were generated from Langerin-DTA and WT mice, as described previously. The cell suspension from Langerin-DTA mice was stained with SYTO RNASelect Green Fluorescent Dye, whereas cell suspension from WT mice was stained with anti—MHC-II-PE (as above). After two washes with cell culture medium, cells were mixed at ratio 1:1 and seeded into a glass bottom microwell dish (P35GCOL-0-14-C, MatTek). Two hours after seeding, the cells were imaged with Andor Spinning Disk Confocal Microscope 100X objective (Nikon, Minato, Japan) for 8 hours at 10-minute intervals. Cells were maintained in 37 °C 5% CO2 atmosphere. Images were processed by Metamorph, and channels were adjusted and merged using Fiji software (Schindelin et al., 2012).

Generation of bone marrow chimeras

Bone marrow cells were harvested from the tibia and femur of CD45.1+ congenic donor mice, as previously described (Yao et al., 2015). Recipient mice (DTA-YFP and KRT14-YFP-DTA) were irradiated with a sublethal dose of gamma radiation (700 mGy) 6 hours before the bone marrow transfer and kept on antibiotic water for 2 weeks after irradiation. A total of 15–20 million cells were adoptively transferred into the recipient mice via the tail vein. Cells from the epidermal layer were dissociated 2–4 months after transplantation and stained with corresponding markers. Cells from skin-draining LNs were stained to determine the rate of chimerism.

Tape stripping

The back skin of the mice was either shaved using surgical prep blades (No. 74-0001, Personna) or left untouched. The shaved side of the flank was further stripped 4–5 times with adhesive tape (FisherBrand labeling tape, Thermo Fisher Scientific). After 24 hours, the mice were killed, and skin from both sides were harvested and processed, as described above.

Preparation and culture of human epidermal cell suspensions

Fresh human skin samples were obtained from trauma surgery. The skin was cut into narrow strips (≈5 mm) and incubated with dispase II (Roche, Basel, Switzerland) solution (2 U/ml in RPMI/PBS solution) at 4 °C overnight. The epidermis was peeled off of the dermis and chopped into small pieces using a razor blade. The chopped epidermis was then incubated in trypsin-DNase solution (0.1 mg/ml DNAse in 0.25% trypsin) for another 2 hours at 37 °C. The digestion was quenched with excess complete RPMI culture medium. The cells were filtered, counted, and then stained with the following markers: anti-CD45 (clone HI30; BD Biosciences), anti-HLA-DR (clone L243; BD Biosciences), and anti-langerin (clone 4C7; BioLegend). Cell sorting was performed on an Aria II cytometer (Becton Dickinson). The sorted cells were either directly lysed for mRNA extraction (as described previously) or cultured in complete RPMI medium for 3 days, followed by mRNA extraction.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 7.0 software (GraphPad, La Jolla, CA). Comparison between two groups was made by Student’s t test unless otherwise noted. A P-value of < 0.05 was considered statistically significant.
Supplementary Figure S1. Epidermal LCs express keratinocyte-specific gene signatures. (a) KC signature genes shared with eLCs. The eLC gene list was from ImmGen and the KC-specific gene list from the data set of a previous study (Gazel et al., 2003). The table includes both FC and FD. The right graph shows the PANTHER GO analysis of this gene list. (b) The FC and FD were plotted, and Pearson correlation was performed. (c) The expressions of some KC-specific genes listed in (a) were determined in flow-sorted KCs, LCs, and DETCs by qRT-PCR. DETC: dendritic epidermal T cell; eLC, epidermal Langerhans cell; FC, fold change; FD, fold difference; KC, keratinocyte; qRT-PCR, quantitative reverse transcriptase–PCR.
Supplementary Figure S2. ATAC-seq profiling of KCs and LCs. (a) ATAC-seq illustrations of Krt1, Krt5, Krt15, and Krt16 in KCs and LCs. (b) Left: principal component analysis of ATAC-sequencing data (distal peaks) for various immune cell populations and KCs (KeraC). Right: ATAC-sequencing profile clustering of different immune cells and KCs based on distal peak counts shown as a heatmap. (c) Representative images for YFP⁺ LC, YFP⁻ LC and DETC of KRT14-YFP-DTA mice by ImageStream. Scale bar = 7 μm. YFP expression in LCs was validated by FACS from the same sample. ATAC-seq, Assay for Transposase-Accessible Chromatin sequencing; DETC, dendritic epidermal T cell; FACS, fluorescence-activated cell sorting; KC, keratinocyte; LC, Langerhans cell.
Supplementary Figure S3. *KRT14*-driven Cre leads to genomic recombination in LCs of *KRT14-YFP* mice. Conventional PCR was used to detect the presence of recombination (blue) and nonrecombination (red) in the genomic DNA of flow-sorted epidermal cell populations from *KRT14-YFP* mice. Primer colors correspond to Figure 3b. LC, Langerhans cell.

Supplementary Figure S4. Material transfer between LCs and other cells. (a) Epidermal cells from CD45.1+ mice were stained with mRNA-selective dye and cultured with unlabeled epithelial cells from CD45.2+ mice. Two days later, the mRNA transfer was determined by FACS. Control: only epidermal cells from CD45.2+ mice. (b) Similar experiment as in (a) except membrane dyes were used. FACS analysis (MHC-II and CD45.2) was performed after 2 days coculture. FACS, fluorescence-activated cell sorting; LC, Langerhans cell; MHC-II, major histocompatibility complex II; nd, not detectable.
Supplementary Figure S5. LCs transfer cellular material to both KCs and DETCs. (a) FACS analysis of YFP expression in epidermal cells of Lang-YFP and control mice. (b) Quantification of YFP+ cell percentages within each population. (c) Chromatin accessibility of Cd207 in KCs and LCs is shown. One representative experiment out of three is shown (n = 3). DETC, dendritic epidermal T cell; FACS, fluorescence-activated cell sorting; KC, keratinocyte; LC, Langerhans cell.
Supplementary Figure S6. Epithelial material transfer is universal to all epithelia-resident DCs, and it is not limited to mice. (a) Krt13, Krt19, Krt8, and Krt18 expression comparisons among DC populations from ImmGen database interface. (b) KRT14 expression in freshly isolated human KCs and LCs or 3 days after isolation and in vitro culture. Internal housekeeping gene: GAPDH. One representative experiment out of two is shown. DC, dendritic cell; KC, keratinocyte; LC, Langerhans cell.
### Supplementary Table S1a. Mouse qRT-PCR Primers

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krt5</td>
<td>Fwd</td>
<td>TGAGATGAACCGAATGATCCAG</td>
</tr>
<tr>
<td>Krt5</td>
<td>Rev</td>
<td>GCTGTGTCTCGGATCTTTGAG</td>
</tr>
<tr>
<td>Lgals7</td>
<td>Fwd</td>
<td>CCAAGAACAAGCAGAATTGGG</td>
</tr>
<tr>
<td>Lgals7</td>
<td>Rev</td>
<td>GGGGGAAGGAGGAGTTTGGG</td>
</tr>
<tr>
<td>Desmoplakin</td>
<td>Fwd</td>
<td>AGCCAGCTCTGGTGAAAGT</td>
</tr>
<tr>
<td>Serpinb5</td>
<td>Fwd</td>
<td>CGGGATACCTGCAACTCAAG</td>
</tr>
<tr>
<td>Krt16</td>
<td>Fwd</td>
<td>GATCAGGAATCTAGGAGTGAG</td>
</tr>
<tr>
<td>Lgals7</td>
<td>Rev</td>
<td>GCTGTTTCTGGCATCTTTGAG</td>
</tr>
<tr>
<td>Lgals7</td>
<td>Rev</td>
<td>GCCTGTATCCTTGCTCAG</td>
</tr>
<tr>
<td>Serpinb5</td>
<td>Rev</td>
<td>GCAAGGACAGAATGATAGAG</td>
</tr>
<tr>
<td>Krt16</td>
<td>Rev</td>
<td>CTATCGTGATCTGCTGCTG</td>
</tr>
<tr>
<td>Lgals7</td>
<td>Fwd</td>
<td>CCAAGAACAAGCAGAATTGGG</td>
</tr>
<tr>
<td>Serpinb5</td>
<td>Rev</td>
<td>GCAAGGACAGAATGATAGAG</td>
</tr>
<tr>
<td>Krt16</td>
<td>Rev</td>
<td>CTATCGTGATCTGCTGCTG</td>
</tr>
<tr>
<td>Lgals7</td>
<td>Rev</td>
<td>GCTGTTTCTGGCATCTTTGAG</td>
</tr>
<tr>
<td>Serpinb5</td>
<td>Rev</td>
<td>GCAAGGACAGAATGATAGAG</td>
</tr>
</tbody>
</table>

### Supplementary Table S1b. Human qRT-PCR Primers

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRT14</td>
<td>Fwd</td>
<td>GAAGTGAAGATCCGTGACTGG</td>
</tr>
<tr>
<td>KRT14</td>
<td>Rev</td>
<td>GCAGAAGGAGACCAGTGATAGAG</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Fwd</td>
<td>CTTTGTCAAGCTCATTTCAG</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Rev</td>
<td>TCTTTCCTCTCAGTGCTCTCC</td>
</tr>
</tbody>
</table>

### Supplementary Table S1c. Mouse Genomic qPCR Primers

<table>
<thead>
<tr>
<th>qPCR Target</th>
<th>Primer Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>YFP</td>
<td>YFP Fwd (genomic rec)</td>
<td>AGGCCGCTCTAGAATCAGT</td>
</tr>
<tr>
<td>YFP</td>
<td>YFP Rev (genomic rec)</td>
<td>CGACGGTATCAGCTGATAG</td>
</tr>
<tr>
<td>Cre</td>
<td>Cre Fwd</td>
<td>TGAGCTGAGGAGAATGAT</td>
</tr>
<tr>
<td>Cre</td>
<td>Cre Rev</td>
<td>GCTACACAGAGCAGAATC</td>
</tr>
</tbody>
</table>

### Supplementary Table S1d. Mouse Genomic Conventional PCR Primers

<table>
<thead>
<tr>
<th>PCR Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>YFP Fwd</td>
<td>GGTGTAAGGAGAAACTCTCC</td>
</tr>
<tr>
<td>YFP Rev</td>
<td>GGCACAAAGGAAAGGAGA</td>
</tr>
</tbody>
</table>

Abbreviations: RT-PCR, quantitative reverse transcriptase—PCR. Blue and red colors correspond to primer sets on Figure 3b.