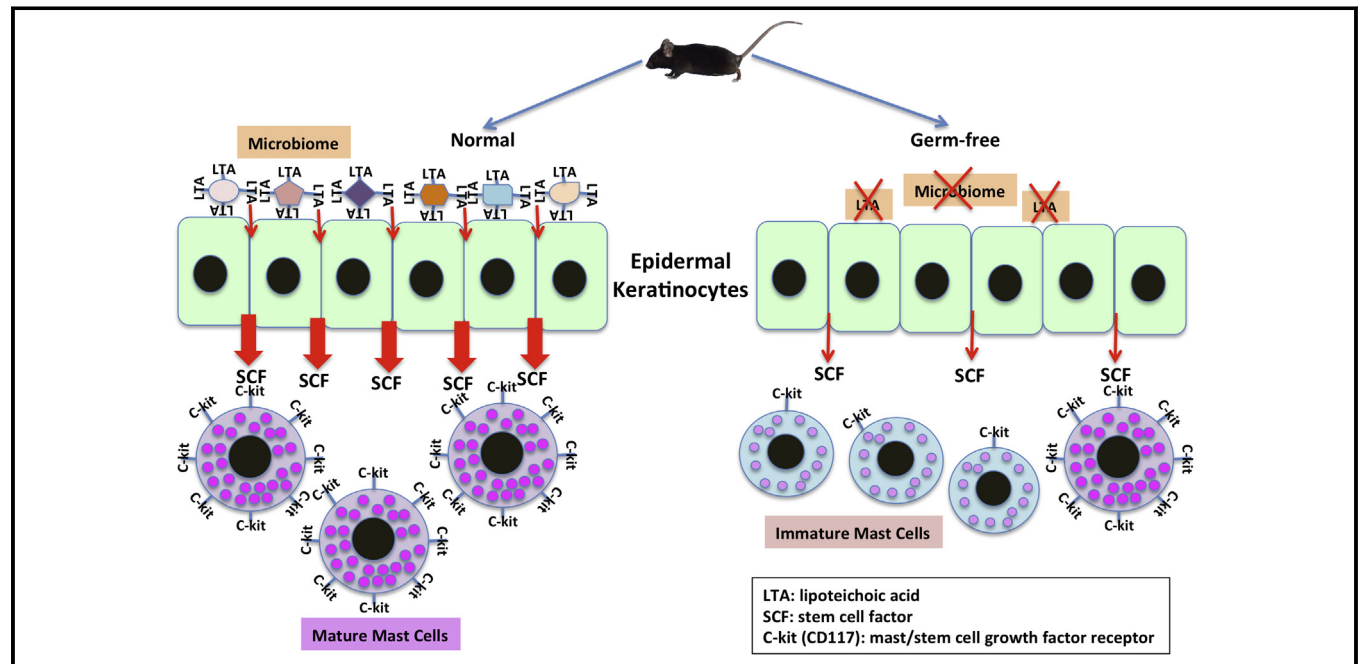


Skin microbiome promotes mast cell maturation by triggering stem cell factor production in keratinocytes

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GRAPHICAL ABSTRACT



Background: Mast cell (MC) progenitors leave the bone marrow, enter the circulation, and settle in the skin and other tissues. Their maturation in tissues is influenced by the surrounding microenvironment.

Objective: We tested the hypothesis that environmental factors play a role in MC maturation in the skin.

Methods: MCs were numerically, phenotypically, and functionally compared between germ-free (GF), specific

pathogen-free, and GF mice reconstituted with microbiota. The maturity of MCs was then correlated with skin levels of stem cell factor (SCF), a critical MC differentiation factor, and lipoteichoic acid (LTA), a Toll-like receptor 2 ligand. MCs were also evaluated in mice with keratinocyte-specific deletion of *Scf*.

Results: We found that GF mice express abnormally low amounts of SCF, a critical MC differentiation factor, and contain MCs that are largely undifferentiated. Reconstituting the GF microbiota reverted this MC phenotype to normal, indicating that the phenotype is related to ongoing interactions of the microbiota and skin. Consistent with the immaturity of GF MCs, degranulation-provoking compound 48/80 induced less edema in the skin of GF mice than in conventional mice. Our results show that the skin microbiome drives SCF production in keratinocytes, which triggers the differentiation of dermal MCs. Because the skin microbiome is a rich source of LTA, a Toll-like receptor 2 ligand, we mimicked the GF microbiome's effect on MCs by applying LTA to the skin of GF mice. We also demonstrated that MC migration within the skin depends exclusively on keratinocyte-produced SCF.

Conclusion: This study has revealed a novel mechanism by which the skin microbiota signals the recruitment and maturation of MCs within the dermis through SCF production

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by LTA-stimulated keratinocytes. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

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Changes in the microbiome have been connected with different skin diseases, such as atopic dermatitis, acne, and psoriasis.¹ Specific commensal bacteria, such as *Staphylococcus epidermidis*, have been implicated in the modulation of skin inflammation,² and recent studies suggest that the skin microbiota might play a role in the recruitment, accumulation, and function of various immune cells, such as modulation of T-cell differentiation.¹ Despite the essential role of mast cells (MCs) in several skin diseases,^{3,4} little is known about whether or how the skin microbiome modulates the differentiation, survival, and function of MCs.

MCs are localized in close proximity to the environment. They originate from CD34⁺ bone marrow progenitor cells and migrate into the circulation and subsequently into peripheral tissues, where their final differentiation takes place under the influence of cytokines in the surrounding tissue microenvironment.⁵⁻⁷ The tissue-specific influence is important for promoting MC accumulation and survival in the skin and gastrointestinal tract.⁸⁻¹⁰ Interestingly, a recent study demonstrated that the skin microbiota can extend into the dermis, thereby establishing physical contact between bacteria and various cells below the basement membrane. This contact allows normal commensal bacteria to directly communicate with dermal cells in a site previously thought to be sterile.¹¹

The gram-positive Actinobacteria phylum constitutes the majority of the skin microbiota.¹² Because the gram-positive cell wall contains a high amount of lipoteichoic acid (LTA), this Toll-like receptor (TLR) 2 ligand is one of the most abundant molecules on the surface of the skin. We have previously shown that LTA permeates the whole skin¹³ and increases the antimicrobial capacity of skin MCs to defend against viral infections.^{14,15} Thus LTA derived from the skin microbiome is a candidate agonist for modulating MC biology.

Stem cell factor (SCF) is essential for the differentiation, survival, and migration of MCs.^{6,16-19} SCF can be produced in a regulated fashion by various skin cells,^{16,20} including keratinocytes,²¹ although the relevant stimuli remain to be explored. The microbiome has primary contact with the epidermis, particularly keratinocytes,¹¹ but it is not known whether SCF production by keratinocytes can be modulated by the skin microbiome. Earlier studies have shown that murine MCs do not fully mature until 8 to 15 days after birth,^{22,23} supporting the notion that environmental factors can drive the production of SCF or other factors that allow for MC differentiation in the skin. In this study we have investigated this possibility by focusing on the role of commensal bacteria and their major product, LTA, in MC differentiation by stimulating keratinocytes to produce SCF in different conventional, gene-targeted, and gnotobiotic murine models.

METHODS

Mice

C57BL/6J wild-type control mice, K14-cre transgenic mice (a gift from Dr Gallo at the University of California, San Diego [UCSD]), *Scf^{fl/fl}* mice (a gift from Dr Morrison at the University of Texas Southwestern Medical Center), and *Tlr2^{-/-}* mice (Jackson Laboratory, Bar Harbor, Me) on a C57BL/6J

Abbreviations used

DAPI:	4'-6-Diamidino-2-phenylindole dihydrochloride
ExGF:	GF mice cohoused with SPF mice
FACS:	Fluorescence-activated cell sorting
GAPDH:	Glyceraldehyde-3-phosphate dehydrogenase
GF:	Germ-free
LCM:	Laser capture microdissection
LTA:	Lipoteichoic acid
MC:	Mast cell
qPCR:	Quantitative PCR
SCF:	Stem cell factor
SPF:	Specific pathogen-free
TLR:	Toll-like receptor
UCSD:	University of California, San Diego

background were housed at the University Research Center at the UCSD. Germ-free (GF) mice on a C57BL/6 background (Taconic Biosciences, Germantown, NY) were housed in the UCSD Gnotobiotic Mouse Facility. All animal experiments were approved by the UCSD Institutional Animal Care and Use Committee. K14-cre transgenic mice were bred with *Scf^{fl/fl}* mice for the generation of K14-cre *Scf^{fl/fl}* mice. K14-cre littermate control mice were used in all experiments. At least 3 independent experiments were performed to assess reproducibility, with at least 4 mice in each group.

MC-deficient (C57BL/*Kit^{W-sh}*) mice were a donation from Dr Peter Bemer's laboratory (Developmental Biology Program, Memorial Sloan-Kettering Cancer Center at Cornell University, New York, NY). These mice have been extensively studied since they were generated.²⁴ Adult *Kit^{W-sh}* mice had a profound deficiency in MCs in all tissues examined but normal levels of major classes of other differentiated lymphoid cells.

For microbial reconstitution experiments, GF mice were cohoused in the same cage for 5 weeks with conventional, genetically matched littermate mice under specific pathogen-free (SPF) conditions. After 5 weeks of cohousing, bacterial reconstitution in GF mice was confirmed by colony-forming unit assays of fecal homogenates.

In vivo MC activation experiment

Intraplantar injection was performed with compound 48/80 (0.5 µg per paw; 10 µL) or PBS alone in wild-type mice, GF mice, and *Kit^{W-sh}* mice. Change in hindpaw width was measured with digital calipers (±0.01 mm; Mitutoyo, Aurora, Ill) and calculated as the average of the left and right paw widths. Baseline paw widths for each mouse were taken before treatment and subtracted from posttreatment paw widths to estimate tissue edema.²⁵

Cell culture

Primary murine MCs were generated according to our previously published protocol.²⁶ Briefly, bone marrow cells were cultured in RPMI 1640 medium (Invitrogen, San Diego, Calif) supplemented with recombinant murine IL-3 (1 ng/mL; R&D Systems, Minneapolis, Minn) and recombinant murine SCF (20 ng/mL, R&D Systems). After 4 weeks, MCs were consistently generated, as confirmed by the expression of CD117 (c-Kit) and FcεRI, and cell maturation was confirmed by means of metachromatic staining with toluidine blue. The purity of MCs was greater than 98%.

Primary human MCs were derived from human cord blood CD34⁺CD45⁺ cells (Astarte Biologics, Bothell, Wash), according to Kirshenbaum and Metcalfe.²⁷ Briefly, CD34⁺CD45⁺ cells were cultured in serum-free medium (Stemline II; Sigma, St Louis, Mo) containing recombinant human SCF (100 ng/mL, R&D Systems), recombinant human IL-6 (100 ng/mL, R&D Systems), and recombinant human IL-3 (20 ng/mL, R&D Systems, first week only). After 10 weeks, human MCs were consistently generated, as confirmed by the expression of CD117 and FcεRI. Cell maturation was confirmed by

means of metachromatic staining with toluidine blue. The purity of human MCs was greater than 98%.

Dermal fibroblasts and epidermal keratinocytes

Primary normal human epidermal keratinocytes from Life Technologies (Grand Island, NY) were cultured in EpiLife medium supplemented with EpiLife Defined Growth Supplement and 60 $\mu\text{mol/L}$ CaCl_2 (Invitrogen). Subconfluent cells were seeded in 6-, 12-, or 24-well plates and grown to 80% confluence before LTA was added at different concentrations.

Primary human and mouse dermal fibroblasts from iXCells Biotechnologies (San Diego, Calif) were cultured in Dulbecco modified Eagle medium (Invitrogen) with 10% FBS (HyClone, Logan, Utah). Subconfluent cells were seeded in 6-well plates and grown to 90% confluence before we added primary murine or human MCs to the well with MC growth factors (5×10^5 MCs per well) for coculture.

K14-cre *Scf^{fl/fl}* mouse dermal fibroblasts were isolated from mouse skin, as previously described,²⁸ and cultured in 6-well plates.

Epidermal mouse keratinocytes were isolated from mice, as previously described.²⁹ Briefly, the skin was peeled off from neonates, and the dermis was gently separated from the epidermis to a single-cell solution. Cells were cultured in 6-well plates in supplemented EpiLife medium (Invitrogen). Cells were 80% confluent for all experiments.

Blocking experiments with anti-TLR2 antibodies

Isolated mouse epidermal keratinocytes and normal human epidermal keratinocytes (Life Technologies) were used. Cells were preincubated with anti-mouse TLR2 mAb (Clone: T2.5; BioLegend, San Diego, Calif), anti-human TLR2 mAb (Clone: TL2.1, BioLegend), or its isotype control IgG (BioLegend). These antibodies are useful for blocking studies and have been reported to block TLR2-specific cell activation *in vitro* and *in vivo*.³⁰⁻³²

Flow cytometry (fluorescence-activated cell sorting)

Single-cell suspensions were prepared by mincing mouse skin tissue with scissors, followed by enzymatic digestion with collagenase Type II (Worthington Biochemical, Lakewood, NJ), collagenase Type IV (Gibco, Grand Island, NY), and 0.5 mg/mL DNase I (Roche, Mannheim, Germany). Cells were stained with anti-CD117, anti-Fc ϵ RI, or anti-TLR2 mAbs (BD Biosciences, San Jose, Calif), according to the manufacturer's instructions. Cells were analyzed with the Guava EasyCyte 8HT 2-laser, 6-color microcapillary-based benchtop flow cytometer (Millipore, Temecula, Calif).

Laser capture microdissection for analysis of gene expression

After intradermal injection with 50 μL of LTA (1 mg/mL; InvivoGen, San Diego, Calif) or PBS, skin samples were collected and washed in RNAlater (Qiagen, Hilden, Germany) for 5 minutes. The samples were then immediately frozen in O.C.T. Compound (Sakura, Alphen aan den Rijn, The Netherlands) with a dry ice/ethanol bath (-72°C) and sectioned at a thickness of 8 μm in an RNase-free environment using RNaseZap spray (Thermo Fisher, Waltham, Mass). Sections were treated with RNAlater for 4 minutes and then stained with 0.1% toluidine blue (Sigma, prepared in RNase-free water) by using the laser capture microdissection (LCM) Frozen Section Staining Kit (Arcturus, San Diego, Calif), according to the manufacturer's instructions. We performed contamination-free isolation of 200 MCs and 100 pieces of epidermis from each sample by using the CellCut laser microdissection system (Molecular Machines & Industries, Munich, Germany). We extracted RNA from these samples by using the PicoPure RNA Isolation Kit (Life Technologies), and the resulting total RNA was used for cDNA synthesis by using the iSCRIPT cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, Calif). After performing preamplification PCR with pooled TaqMan Assays and the TaqMan PreAmp Master Mix Kit (Life Technologies), we performed Real-Time Quantitative RT-PCR in an ABI 7300 Real-Time PCR system (Applied Biosystems, Foster City, Calif). mRNA expression of type I Collagen alpha 1 (*Col1a1*) was

undetectable in all samples, which confirmed that there was no contamination of skin fibroblasts in the laser-captured MCs and epidermis (data not shown).

Real-time quantitative RT-PCR

Total RNA was isolated by using the RNeasy Mini Kit (Qiagen), and 0.5 μg of total RNA was used for cDNA synthesis by using the iSCRIPT cDNA Synthesis Kit (Bio-Rad Laboratories). Real-time quantitative RT-PCR was performed in an ABI 7300 Real-Time PCR system (Applied Biosystems) with TaqMan primers and probes (Life Technologies). We used the comparative $\Delta\Delta$ cycle threshold method to quantify gene expression. Target gene expression levels in the test samples were normalized to endogenous glyceraldehyde-3-phosphate dehydrogenase (GAPDH) levels and reported as fold differences relative to *GAPDH* gene expression in untreated baseline controls.³³ All assays were performed in triplicate, and the experiments were repeated at least 3 times.

ELISA

ELISA kits (R&D systems) were used to determine SCF protein expression in mouse skin and normal human epidermal keratinocytes. All protein was detected from supernatants of tissue homogenate or cell-culture media according to the manufacturer's instructions. ELISA measurements were normalized to the total weight or volume of each sample.

Histology and fluorescence staining

Animal skin samples were collected, fixed with buffered formalin, and embedded in paraffin. After deparaffinizing and rehydrating the sections, some sections were stained with toluidine blue (RICCA Chemical, Arlington, Tex) or rhodamine-avidin³⁴ to identify MCs. After antigen retrieval at 95°C to 100°C for 30 minutes with citrate buffer (pH 6.0; Enzo Life Sciences, Farmingdale, NY), immunofluorescent staining of skin sections was performed with mAbs against LTA (Abcam, Cambridge, United Kingdom), MC chymase (Abcam), the melanocyte combined markers HMB45+DT101+BC199 (Abcam), SCF (Santa Cruz Biotechnology, Dallas, Tex), TLR2 (Abcam), and c-Kit (Tonbo Biosciences, San Diego, Calif) and their isotype controls (Santa Cruz Biotechnology). Alexa Fluor 488- and Alexa Fluor 594-conjugated secondary antibodies (Life Technologies) were used according to the manufacturer's instructions. Slides were mounted in ProLong Anti-Fade reagent with 4'-6-diamidino-2-phenylindole dihydrochloride (DAPI; Molecular Probes, Eugene, Ore). We imaged the sections with a Bx51 research microscope (Olympus, Center Valley, Pa) and the X-Cite 120 fluorescence illumination system (EXFO Photonic Solutions, Mississauga, Ontario, Canada). All isotype controls showed negative staining (see Fig E1 in this article's Online Repository at www.jacionline.org).

Statistical analyses

All data are presented as means $\pm$ SDs. At least 3 independent experiments were performed to assess reproducibility. Comparisons between groups were analyzed by using the Student *t* test or ANOVA for multiple comparisons. For all statistical tests, *P* values of less than .05 were considered statistically significant.

RESULTS

GF mice have immature MCs in the dermis

To determine the importance of the microbiome in MC maturation, we stained for the presence of c-Kit⁺ MCs in the skin of GF mice, SPF conventional mice, and GF mice cohoused with SPF mice (ExGF) for 5 weeks to reconstitute their microbiome (as confirmed by bacterial plate cultures of gut microflora). We observed a significantly smaller population of c-Kit⁺ MCs in the GF mice (Fig 1, A-C) that was normalized after bacterial reconstitution in the ExGF mice (isotype antibody control

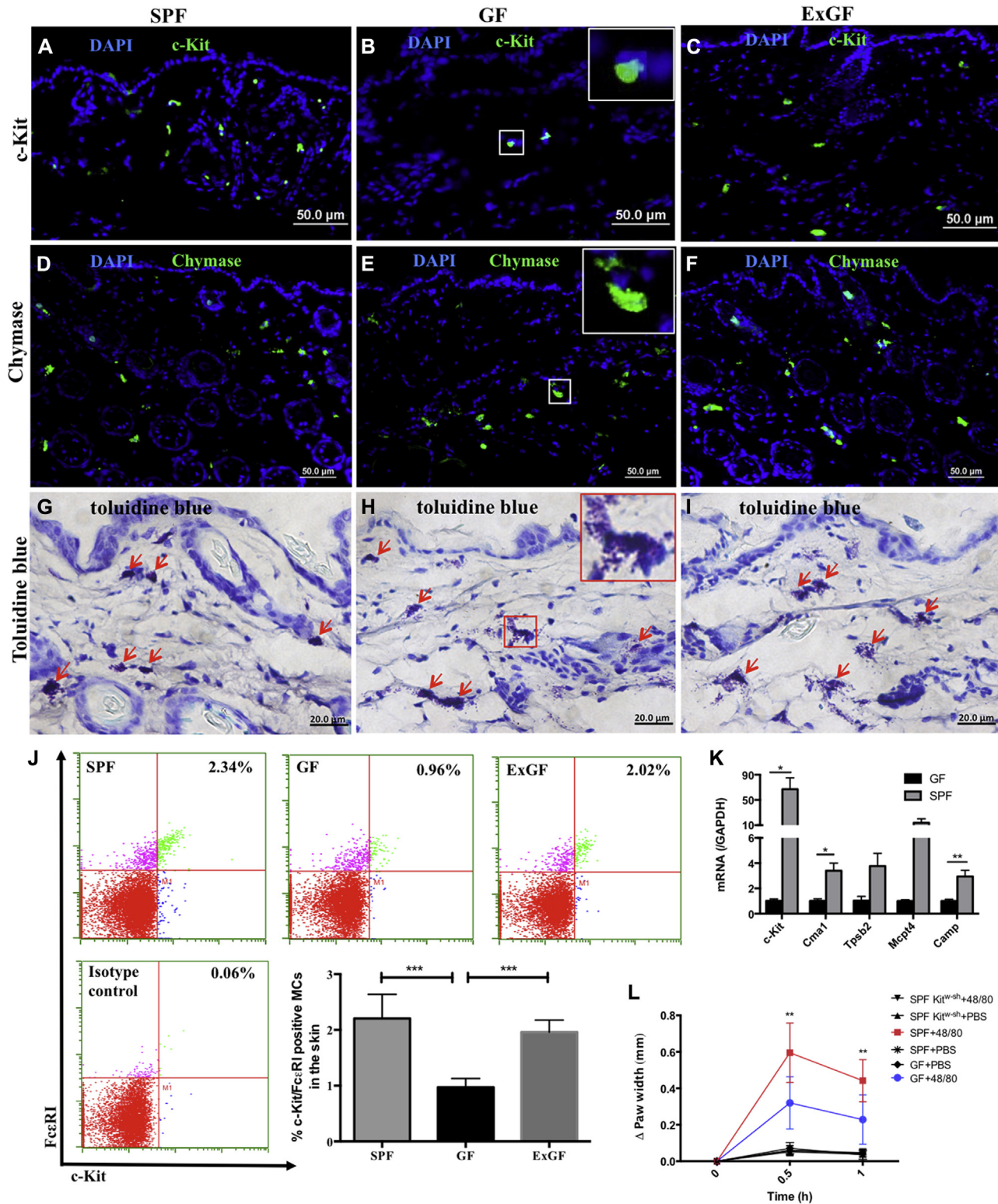


FIG 1. GF mice have immature MCs in the dermis. **A-C**, Immunofluorescent staining for c-Kit (green) and DAPI (blue) in skin from SPF, GF, and ExGF (GF cohoused with SPF for 5 weeks) mice. **D-F**, Immunofluorescent staining for chymase-positive cells (green) and DAPI (blue) in skin from SPF, GF, and ExGF mice. **G-I**, Toluidine blue staining of skin from SPF, GF, and ExGF mice. Red arrows indicate MCs, and the inset in Fig 1, B, E, and H, shows a magnification of the squared area in the image. **J**, Flow cytometric plots and enumeration of mature MC numbers in samples of whole skin from SPF, GF, and ExGF mice. **K**, qPCR analysis for MC markers in GF and SPF skin using the toluidine blue-positive MCs collected from skin sections by using LCM. **L**, Paw thickness in SPF, GF, and SPF MC-deficient *Kit^{W-sh}* mice after injection of PBS (control) or compound 48/80. * $P < .05$, ** $P < .01$, and *** $P < .001$.

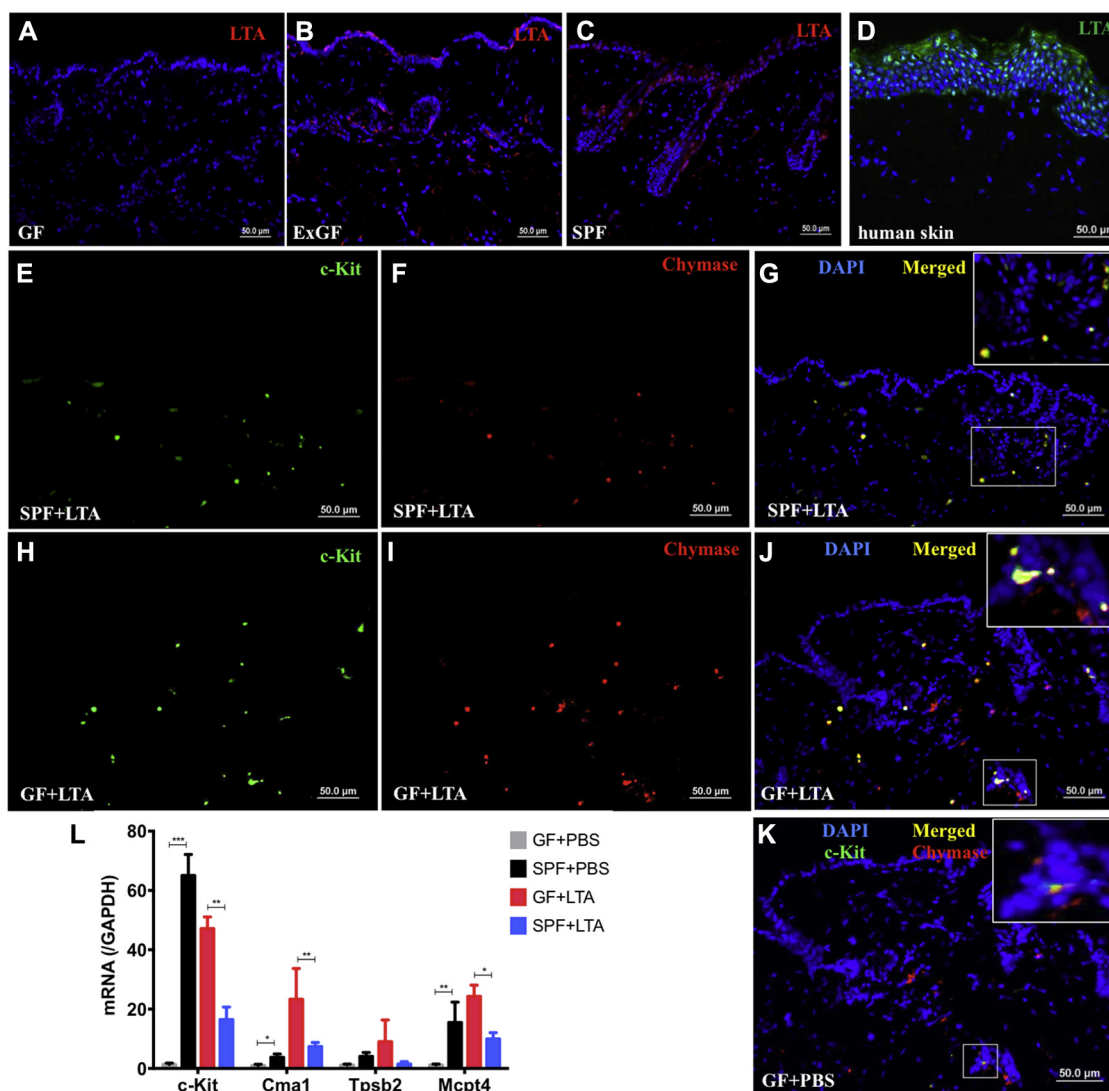


FIG 2. Staphylococcal LTA promotes MC maturation in GF mice. **A–C**, Anti-LTA (red) and DAPI (blue) staining in skin from GF, ExGF, and SPF mice. **D**, Anti-LTA (green) and DAPI (blue) staining at the human skin surface and in deeper epidermal layers. **E–J**, Immunofluorescent staining for c-Kit (green), chymase (red), and DAPI (blue) in skin sections from SPF and GF mice, as indicated, after LTA injection. The *inset* in Fig 2, G, J, and K, shows magnification of the squared area in the image. **K**, Immunofluorescent staining for c-Kit (green), chymase (red), and DAPI (blue) in the skin of GF mice after PBS injection. **L**, qPCR analysis for MC markers in GF and SPF skin using toluidine blue-positive MCs collected from skin sections by using LCM after LTA injection. * $P < .05$, ** $P < .01$, and *** $P < .001$.

staining for all of the experiments are in Fig E1, A and B). To extend this observation, dermal and epidermal cells were harvested from the skins of the 3 mouse populations, and MCs were enumerated by using fluorescence-activated cell sorting (FACS) based on the presence of both the SCF receptor (c-Kit) and FcεRI (Fig 1, J). FACS confirmed that the skin of GF mice had markedly decreased numbers of c-Kit⁺FcεRI⁺ MCs, whereas ExGF mice had normal MC numbers in the skin (Fig 1, J).

In parallel with these experiments, we stained skin sections for chymase and with toluidine blue to detect all MCs, regardless of the functional state (Fig 1, D–I). Surprisingly, these staining methods revealed similar numbers of skin MCs in GF, SPF, and

ExGF mice (Fig 1, D–I), suggesting that MCs were present in GF mice but that these cells were not positive for markers of differentiated cells, such as c-Kit. To better characterize the phenotype of the MCs from GF, SPF, and ExGF mice, we isolated toluidine blue-positive cells from skin sections of these mice by means of LCM. After RNA extraction, we assessed the mRNA expression profiles of several genes involved in and characteristic of MC maturation, including chymase (*Cma1*), tryptase β 2 (*Tpsb2*), MC protease 4 (*Mcpt4*), SCF receptor (*c-Kit*), and cathelicidin antimicrobial peptide (*Camp*). As shown in Fig 1, K, MCs from SPF mice expressed higher levels of all MC markers compared with MCs from GF mice, especially c-Kit, explaining the reduced numbers of c-Kit⁺ MCs in GF mice. Controls for

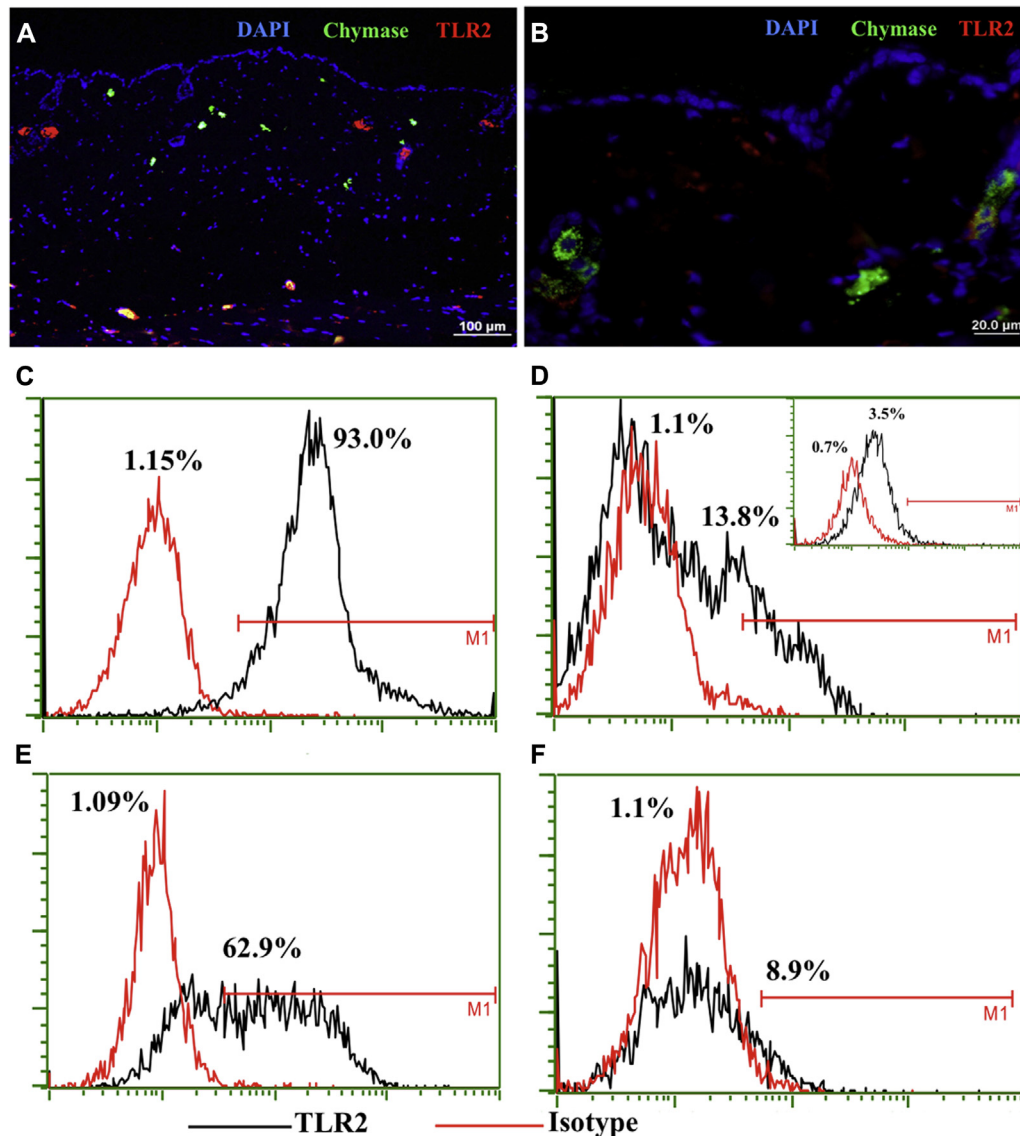


FIG 3. TLR2 MC expression in the skin. **A**, SPF mouse skin was stained with anti-chymase (green), anti-TLR2 (red), and DAPI (blue) to define TLR2 presence on dermal MCs. TLR2 is visible in the deeper layer of dermis (yellow cells at the bottom), and green cells indicate MCs that do not express TLR2. **B**, Magnification of chymase-positive MCs that do not express TLR2. **C** and **D**, FACS plots and enumeration of murine bone marrow-derived MCs that were differentiated *in vitro* without (Fig 3, **C**) or with (Fig 3, **D**) dermal fibroblasts for 7 days, stained with anti-TLR2 antibody, and then analyzed by using FACS. The *inset* in Fig 3, **D**, shows the expression of TLR2 in MCs derived from the bone marrow of *Tlr2*^{-/-} mice. **E** and **F**, Human CD34⁺ cord blood cell-derived MCs were differentiated *in vitro* without (Fig 3, **E**) or with (Fig 3, **F**) fibroblasts for 7 days, stained with anti-TLR2 antibody, and then analyzed by using FACS.

the LCM tissue sections were negative for toluidine blue, and in these sections the genes of interest were undetectable (see Fig E1, C-F). These results suggest that the skin microbiome markedly affects MC gene expression and maturation.

To assess any functional differences between MCs in GF and SPF mice, we injected 0.5 μ g (10 μ L) of compound 48/80 (a substance known to induce MC degranulation) into the hind paws of 3 mice from each population. At both 30 minutes and 1 hour after injection, paw width was measured to assess for swelling because of compound 48/80-induced MC degranulation. The paws of SPF mice swelled to a greater extent than the paws of GF

mice, suggesting that MCs in GF mice have altered functionality (Fig 1, L).

Staphylococcal LTA promotes MC maturation in GF mice

Given that LTA can have a strong influence on MC behavior in infections,^{14,26} we hypothesized that the lack of microbiome and thus the predicted lack of microbially derived LTA in GF mice might account for the observed differences in MC maturation. To test this hypothesis, by means of immunostaining, we

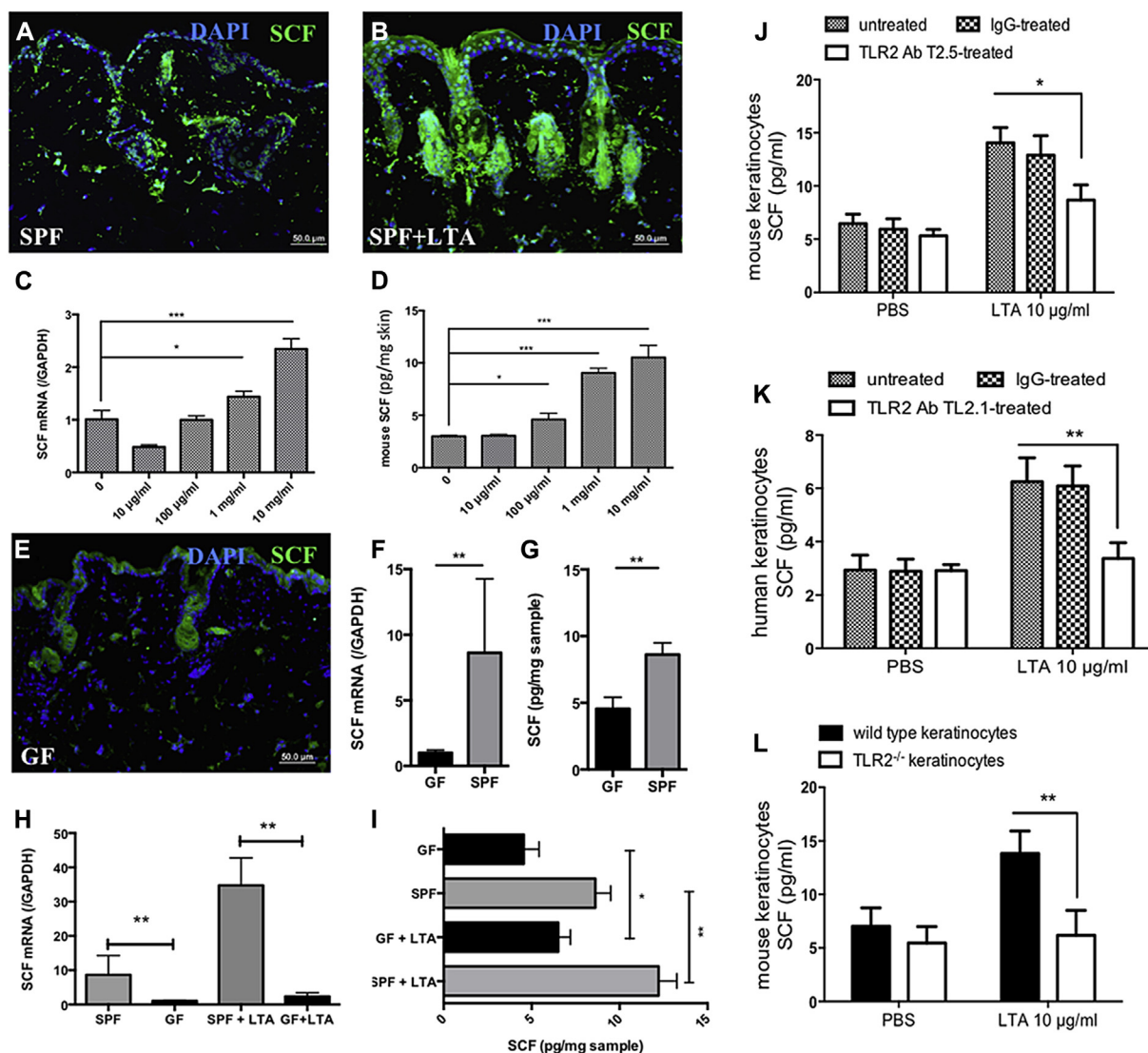


FIG 4. SCF expression in keratinocytes is low in the absence of the microbiome. **A** and **B**, Anti-SCF (green) and DAPI (blue) immunostaining of SPF mouse skin after injection of PBS (Fig 4, **A**) or LTA (Fig 4, **B**). **C** and **D**, Quantification of *Scf* expression in mouse skin by using qPCR (Fig 4, **C**) and by using ELISA (Fig 4, **D**) after LTA injection. **E**, Anti-SCF (green) and DAPI (blue) immunostaining of GF mouse skin. **F**, qPCR analysis of *Scf* in epidermis isolated from GF and SPF mice. **G**, ELISA quantification of SCF in epidermis isolated from GF and SPF mice (normalized by tissue weight). **H**, qPCR analysis of *Scf* in isolated epidermis of GF and SPF mice before and after injection of LTA. **I**, ELISA quantification of SCF in isolated epidermis of GF and SPF mice (normalized by tissue weight) before and after injection of LTA. **J**, ELISA quantification of SCF in mouse keratinocytes pretreated for 1 hour with either anti-TLR2 antibody Clone T2.5 or with the relevant isotype IgG control. **K**, ELISA quantification of SCF in human keratinocytes pretreated for 1 hour with either anti-TLR2 antibody Clone TL2.1 or with the relevant isotype IgG control. **L**, ELISA quantification of SCF in isolated mouse keratinocytes from *Tlr2*^{-/-} and wild-type mice. **P* < .05, ***P* < .01, and ****P* < .001.

first confirmed that LTA was indeed absent in the skin of GF mice (Fig 2, **A**), whereas LTA could be seen clearly in the epidermis and hair follicles of ExGF and SPF mice (Fig 2, **B** and **C**; isotype antibody control staining for these experiments is shown in Fig E1, **G**). As shown in Fig 2, **D**, in human skin LTA was not only present on the skin surface but also found in the deeper layers of epidermal keratinocytes. As a staining control, we cultured normal human epidermal keratinocytes *in vitro* with and without LTA and stained with an anti-LTA mAb. No LTA was detected in the keratinocytes unless they

were treated with LTA (see Fig E1, **H-J**). Taken together, our data suggest that the skin is positive for LTA only in the presence of an intact microbiome.

Next, to determine whether LTA can play a direct role in promoting MC maturation, we injected the backs of GF and SPF mice with 100 μ L of LTA (1 mg/mL). Immunofluorescent staining of the LTA-injected mouse skin 24 hours after injection revealed increased numbers of c-Kit⁺ MCs in both SPF and GF mice (Fig 2, **E-J**); chymase expression also changed, which is compatible with quantitative PCR (qPCR) expression in Fig 2,

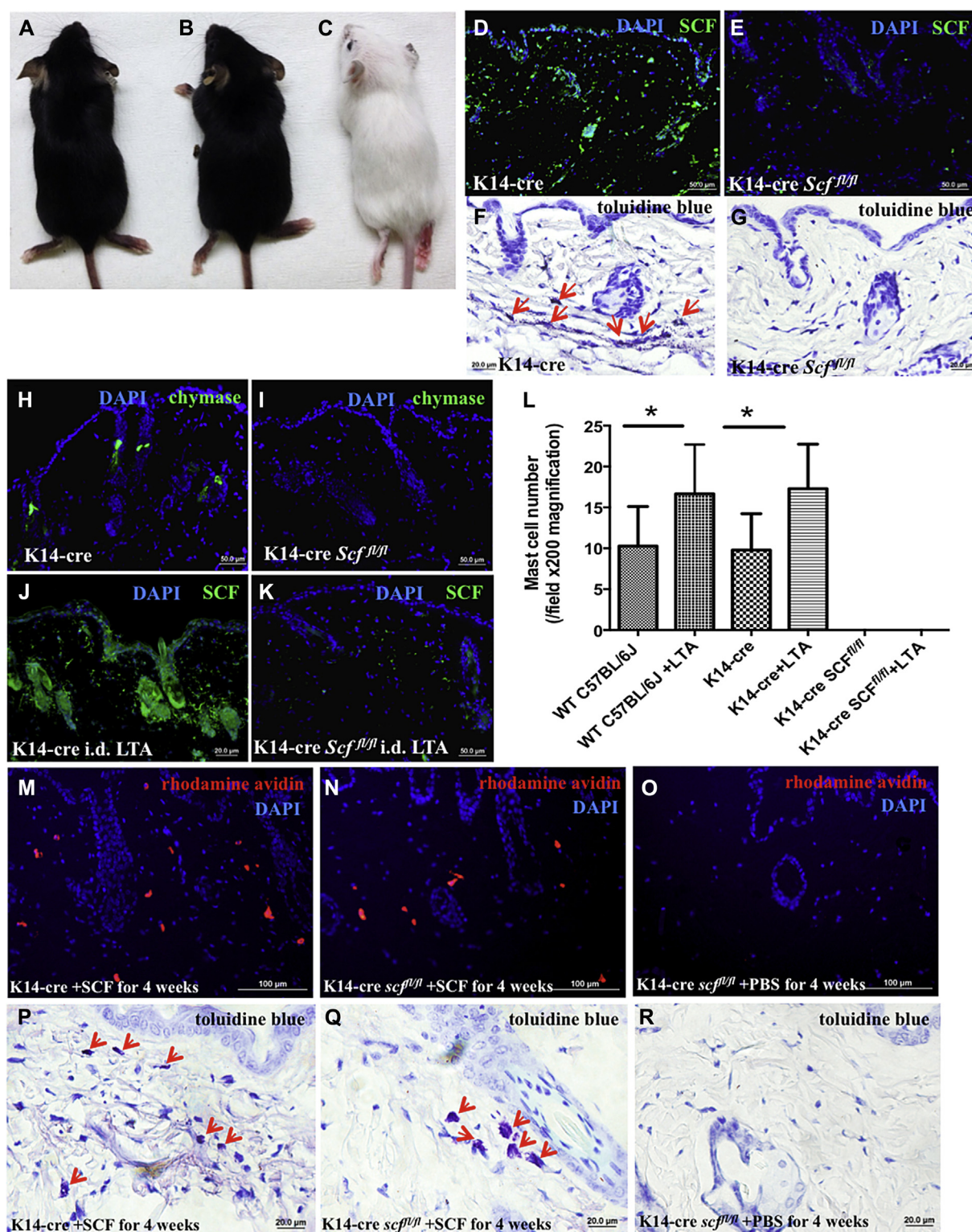


FIG 5. K14-cre *Scf^{fl/fl}* mice lack SCF in the epidermis and MCs in the dermis. **A-C**, K14-cre (Fig 5, A) and *Scf^{fl/fl}* (Fig 5, B) mice exhibit a normal phenotype, whereas K14-cre *Scf^{fl/fl}* mice are albino (Fig 5, C). **D** and **E**, K14-cre (Fig 5, D) and K14-cre *Scf^{fl/fl}* (Fig 5, E) mouse skin was immunostained with anti-SCF mAb (green) and DAPI (blue). **F** and **G**, Toluidine blue staining of the skins of K14-cre (Fig 5, F) and K14-cre *Scf^{fl/fl}* mice (Fig 5, G). Red arrows indicate MCs. **H** and **I**, Skins from K14-cre (Fig 5, H) and K14-cre *Scf^{fl/fl}* (Fig 5, I) mice were immunostained with anti-chymase mAb (green) and DAPI (blue). **J** and **K**, After injection with 100 μ L of LTA (1 mg/mL), K14-cre (Fig 5, J) and K14-cre *Scf^{fl/fl}* (Fig 5, K) mouse skins were immunostained with anti-SCF mAb (green) and DAPI (blue). **L**, Quantification of MCs by using positive toluidine blue staining shows that MC presence could not be rescued by LTA when SCF was conditionally deleted from skin

J. For comparison, staining of skin from GF mice treated only with PBS is shown in Fig 2, K. To confirm and extend the immunofluorescence studies, skin sections from the same mice were stained with toluidine blue, and toluidine blue-positive MCs were isolated by means of LCM and analyzed for changes in gene expression by using qPCR. The dermal MCs from GF mice showed dramatically increased expression of *Cmal*, *Tpsb2*, *Mcpt4*, and *c-Kit* (Fig 2, L), demonstrating that the final maturation of MCs is influenced by bacterial products from the skin environment.

Staphylococcal LTA-induced MC maturation is not dependent on MC TLR2 expression

To determine the mechanism of LTA action, we first stained for the presence of TLR2, the receptor for LTA, on MCs in SPF mouse skin. Surprisingly, TLR2 was found to colocalize with MC chymase in the deeper layers of the dermis (Fig 3, A), whereas in the upper dermal layer, MCs lacked TLR2 expression (Fig 3, A and B). Isotype antibody control staining for this experiment is shown in Fig E1, K. This observation was also true for MCs in human skin (data not shown). The downregulation of *Tlr2* in dermal mouse MCs was confirmed by means of qPCR on MCs collected by using LCM (see Fig E1, L).

Seeking to find an explanation for the observation that MCs lose their TLR2 expression when entering the skin, we tried to coculture MCs with different components of skin connective tissue, including fibroblasts (human fibroblasts with human MCs and mouse fibroblasts with mouse MCs), hyaluronic acid, collagen type I, fibronectin, or gelatin. After 7 days of coculture, we performed FACS analysis on the MCs to determine the levels of TLR2 expression. Murine MCs differentiated from bone marrow and human MCs differentiated from CD34⁺ cord blood cells both showed significant expression of TLR2 when cultured alone (Fig 3, C and E). Interestingly, after 1 week of coculture with fibroblasts, a significant percentage of both murine and human MCs lost their TLR2 expression (Fig 3, D and F), resembling MCs cultured from the bone marrow of *Tlr2* knockout mice, which we used as a control (Fig 3, D, inset). In contrast, MCs that were cultured with extracellular matrix components did not lose their TLR2 expression (see Fig E2 in this article's Online Repository at www.jacionline.org). Therefore we conclude that direct contact of MCs with fibroblasts promotes the downregulation of TLR2. Consequently, the effect of LTA on MC maturation is not through MC TLR2 expression, suggesting that LTA does not exert its effect directly on MCs but is probably acting indirectly through TLR2 on other types of cells in the skin, such as keratinocytes. Keratinocytes represent the interface with the external environment and are definitely the cells that are most exposed to the skin microbiome. To verify *Tlr2* expression in mouse keratinocytes, we isolated keratinocytes from mouse skin and showed, with qPCR analysis, that they were positive for *Tlr2* (see Fig E2, I) and that their expression of *Tlr2* was increased by exposure to LTA.

Staphylococcal LTA promotes SCF expression in the epidermis

Given that SCF is the main inducer of MC differentiation,³⁵ we hypothesized that SCF provides a possible link between LTA and MCs. To determine whether SCF is produced in the skin in response to commensal bacteria *in vivo*, we injected 100 μ L of 1 mg/mL LTA into the skins of SPF mice. A significantly enhanced SCF signal was detected by means of immunofluorescent staining 24 hours after injection (Fig 4, A and B). To extend this finding, we stimulated mice with different doses of LTA and after 24 hours dissected the skin and analyzed *Scf* (Kit ligand) mRNA expression by using quantitative RT-PCR. As shown in Fig 4, C, *Scf* expression at the site of injection increased significantly in a dose-dependent manner when compared with baseline values. Furthermore, the observed increases in *Scf* mRNA resulted in corresponding increases in SCF protein levels (Fig 4, D). The main resident cell types in the skin were also analyzed for SCF expression by using qPCR and ELISA to reveal the sources of SCF in response to LTA. We found that keratinocytes expressed high levels of *SCF* mRNA 7 hours after LTA challenge (see Fig E3, A, in this article's Online Repository at www.jacionline.org), with an accompanying increase in SCF protein levels 16 hours after LTA stimulation (see Fig E3, B). We also compared *Scf* expression in mouse fibroblasts versus keratinocytes *in vitro*. Both cell types upregulated *Scf* in response to LTA to similar degrees, but keratinocytes did so considerably faster than fibroblasts (see Fig E3, C).

GF mice were analyzed for SCF expression to further investigate the role of LTA-induced expression of SCF in the skin (Fig 4, E). Our results showed that GF mice exhibited lower expression levels of SCF protein in the skin when compared with SPF mice. To better evaluate the epidermal SCF levels in these mice, we separated epidermal cells from the dermis using dispase. SCF mRNA and protein levels in these isolated epidermal samples were much higher in SPF mice than in GF mice (Fig 4, F and G). Moreover, when challenged with LTA, epidermal samples from SPF mice showed greater increases in SCF expression compared with samples from GF mice (Fig 4, H and I).

It is known that keratinocytes express *TLR2* *in vivo* and *in vitro*,³⁶ and by using qPCR, we confirmed that keratinocytes increase *Tlr2* expression in response to LTA (see Fig E2, I); however, there are no reports that link TLR2 stimulation directly to SCF expression. Therefore, to investigate the mechanism of SCF induction by LTA in keratinocytes, we treated mouse keratinocytes and normal human keratinocytes with LTA for 24 hours in the absence or presence of neutralizing anti-human TLR2 antibodies (for human keratinocytes) or anti-mouse TLR2 antibodies (for mouse keratinocytes) and measured SCF release in the medium using ELISA. LTA stimulation significantly increased SCF secretion in keratinocytes, which was blocked by anti-TLR2 antibodies (Fig 4, J and K). In confirmation of these findings, keratinocytes from wild-type but not *Tlr2*^{-/-} mice showed induction of SCF release on LTA stimulation (Fig 4, L). These data show that

epidermis. **P* < .05. **M-O**, Rhodamine-avidin staining (red) and DAPI (blue) for MCs in the skins of K14-cre (Fig 5, M) and K14-cre *Scf*^{fl/fl} mice (Fig 5, N) subcutaneously injected with SCF (50 μ g/kg/d) or PBS (Fig 5, O) for 4 weeks. **P-R**, Toluidine blue staining for MCs (red arrows indicate MCs) in the skins of K14-cre (Fig 5, P) and K14-cre *Scf*^{fl/fl} mice (Fig 5, Q) subcutaneously injected with SCF (50 μ g/kg/d) or PBS (Fig 5, R) for 4 weeks.

TLR2 is absolutely required for LTA-induced SCF expression in keratinocytes.

SCF from keratinocytes is essential for maintaining MCs in the skin

Based on our immunofluorescent staining, LTA induces the strongest expression of SCF in keratinocytes (Fig 4, A and B); therefore we sought to determine the relative importance of SCF in supporting MCs within the skin and in delivering signals from the microbiome. For these experiments, we bred K14-cre transgenic mice that express Cre recombinase under the control of the human keratin 14 promoter with mice containing floxed *Scf* alleles (*Scf^{fl/fl}* mice) to generate K14-cre *Scf^{fl/fl}* mice, which lack *Scf* in a keratinocyte-specific manner. These mice are albino, viable, fertile, normal in size, and do not display any gross physical or behavioral abnormalities (Fig 5, A-C).

As shown in Fig 5, D and E, K14-cre *Scf^{fl/fl}* mice, unlike their K14-cre littermate control mice, have no SCF protein in their keratinocytes, even as newborns (see Fig E4, F and H, in this article's Online Repository at www.jacionline.org). In contrast, skin fibroblasts from K14-cre *Scf^{fl/fl}* mice express *Scf* normally, as shown by anti-SCF antibody staining *in vivo* (see Fig E3, E and F) and *in vitro* (see Fig E3, G and H). Furthermore, these conditional knockout mice have normal numbers of MCs and normal levels of SCF expression in the small intestinal mucosa (see Fig E4, A-D) and bone marrow cells (see Fig E4, E) and display no significant abnormalities in WBC counts in the blood (see Table E1 in this article's Online Repository at www.jacionline.org).

Toluidine blue staining (Fig 5, F and G) and chymase immunofluorescent staining (Fig 5, H and I) of mouse skin sections showed no evidence of MCs in the skin of K14-cre *Scf^{fl/fl}* mice. Notably, they lacked MCs in the dermis, even in newborn mouse pups (see Fig E4, H and I). Furthermore, K14-cre *Scf^{fl/fl}* mice lacked melanocytes (see Fig E5, A-D, in this article's Online Repository at www.jacionline.org), which are known to be dependent on normal SCF expression,³⁷ thus providing a likely explanation for the albino appearance. Together, these results demonstrate that keratinocyte-derived SCF is critical for maintaining normal numbers of MCs and melanocytes in the skin.

To evaluate whether LTA stimulation could induce recruitment of MCs into the skin in the absence of keratinocyte-derived SCF, we injected LTA into control (K14-cre) and K14-cre *Scf^{fl/fl}* mice (Fig 5, J and K). LTA did not induce SCF expression in K14-cre *Scf^{fl/fl}* mouse keratinocytes, and most importantly, it did not lead to MC recruitment (Fig 5, L). In contrast, intradermal injection of LTA increased the number of MCs in the skin of K14-cre littermate control mice (Fig 5, L). No inflammation was observed after LTA injection (data not shown), which is in accordance with our previously published data (Wang et al, 2010¹⁴). Taken together, our data show that LTA signals to MCs by inducing the expression of SCF in keratinocytes. To directly demonstrate that skin MCs are dependent on high levels of SCF, as produced by keratinocytes, we injected SCF into the dermis of K14-cre *Scf^{fl/fl}* mice and examined MC numbers. SCF injection induced MC recruitment into the skin, reaching levels similar to those seen in wild-type mice (Fig 5, M-R). For comparison, the melanocytes did not normalize on acute injection of SCF (see Fig E5, F), indicating that the melanocyte defect in the absence of SCF is likely to be developmental in nature.

DISCUSSION

The importance of SCF in promoting MC differentiation and localization has long been established; however, the specific mechanisms by which MCs are recruited to the skin to become terminally differentiated remain unknown. Thus we sought to determine whether the microbiota of the skin plays a role in dermal MC localization and development. Our studies prove that the microbiome influences MC maturation and function. We have discovered an important system of communication between the skin microbiome and MC differentiation. The mechanism that we have now established works through the production of SCF in keratinocytes, which is essential for maintaining MCs within the dermis.

At first, we investigated the phenotype and maturation level of MCs in GF mice. When gene expression analysis was performed on MCs, cells from GF mice exhibited far lower gene expression levels than required for MC maturity. More specifically, their expression of c-Kit was greatly reduced. The most impressive and important observation was that when cohousing GF mice with SPF mice, the ExGF mice not only regained their microbiome but also converted back to a normal MC phenotype in the skin. A striking and novel observation was the altered MC function in the absence of the microbiome. In fact, to further confirm that the microbiome is essential to MC maturation and function, we injected the paws of GF mice with compound 48/80 to induce *in vivo* MC degranulation, and we noticed a significantly reduced inflammatory reaction in GF mice. This discovery might be important for a better understanding of the pathogenesis of skin diseases, such as atopic dermatitis, in which changes in the microbiome might be involved in disease flare-ups and have even been proposed to be responsible for the diseases themselves.³⁸

The concept of c-Kit⁺ MCs was first proposed when assessing the MC profile of *Kit^{W-sh}* mice. Yamazaki et al demonstrated that the skin of *Kit^{W-sh}* mouse embryos maintained a high expression of c-Kit, whereas this expression was nearly abolished 5 days after birth.³⁹ However, mRNA for MC-specific carboxypeptidase A was still detected in the 5-day-old mice, confirming that c-Kit⁺ MCs might be present in the skin under certain circumstances. When we examined isolated skin MCs using FACS for the presence of c-Kit and the high-affinity IgE receptor, a significantly lower number of MCs were detected in GF mice. The data collected by using LCM indicates that c-Kit⁺ MCs are present in the skin of GF mice. These same differences between GF and SPF recorded by using FACS were observed in the qPCR analysis of LMC of MCs in GF and SPF mice, indicating that the phenotype of the cells was different in the GF mice.

Our data show that immature MCs in GF mice are capable of increasing their expression of c-Kit when a normal microbiome is re-established. Moreover, they also mature on stimulation with LTA. Immunofluorescent staining confirms that after LTA stimulation, the number of c-Kit⁺ MCs in the dermis of GF mice more closely resembles the MC composition in the skin of SPF mice. Additionally, when LTA is injected into the skin of GF mice, the expression of MC maturity markers increases drastically; however, when MCs are already exposed to a microbiome, as in SPF mice, the presence of additional LTA maintains these MC markers at a constant level. This confirms a heightened sensitivity of the GF MCs in responding to LTA. Thus the skin microbiota and, more specifically, some of their byproducts, such as LTA, are a key factor in priming MCs for proper development.

The changes that we observed after exposing GF skin with LTA is similar to what we observed with microbiome reconstitution. We chose to use LTA because we knew from our previous work that it had a positive influence on MCs during infections and because it is a defined molecule, as opposed to bacterial supernatant or living commensals that could have raised questions about the mechanism of activity on MCs.

Having established a clear association between the presence of the microbiome, LTA, and MC maturation, we sought to determine how LTA exerts this effect on MCs. A recent article¹¹ has demonstrated that the microbiota extends within the dermis, therefore enabling physical contact between bacteria and various cells below the basement membrane, establishing that normal commensal bacteria directly communicate with the host. However, we showed that MCs approaching the skin surface lose their TLR2 expression. Also, another article has suggested that MC TLR2 is internalized after MCs enter the skin,⁴⁰ and we have further confirmed that MCs significantly downregulate their expression of TLR2 on localization to the skin.⁴⁰ Moreover, we have proved that MCs need fibroblast contact to internalize TLR2. Based on the current knowledge, one might hypothesize that LTA can exert direct activity on MCs entering the skin; however, the fact that MCs cannot re-enter the skin in the absence of SCF from keratinocytes, even after injections of LTA, suggests to us that the effects of bacterial LTA signals must also be transmitted to MCs through a different ligand/receptor or through another cell type.

Because keratinocytes are the cells most exposed to the microbiome, we investigated keratinocyte expression of SCF. Our data suggest that LTA does influence MCs, not through direct interaction but by triggering the production of SCF in neighboring keratinocytes. Both our mouse models and *in vitro* assays show that staphylococcal LTA can induce a significant upregulation of SCF in keratinocytes, indicating that a physical interaction between microbes and skin cells can maintain increased SCF levels in the epidermis. Based on these findings, we used GF mice to assess whether LTA from the microbiota is necessary for SCF production and MC localization in the skin. Previous work⁴¹ has shown that commensal bacteria play a key role in promoting the migration of MCs into the intestine, which, like the skin, is home to large communities of indigenous bacteria. A few studies have briefly discussed the presence of skin MCs in GF mice,^{41,42} but none have provided specific information on their function and location in the skin. Our data demonstrate that GF keratinocytes produce SCF at lower levels compared with SPF mice, providing a connection to the incomplete skin MC maturation observed in GF mice.

On the basis of these results, we developed a mouse model that conditionally depletes SCF from the epidermis (K14-cre *Scf*^{fl/fl}). Analysis of these mice revealed a complete lack of dermal MCs, which not only suggests that SCF from keratinocytes is essential for maintaining MCs in the skin but also that it might be locally produced SCF that maintains MCs within their target organs. From these results, we have gained valuable information: (1) that LTA requires keratinocyte-SCF production to transmit the signal to MCs and (2) that without keratinocyte-SCF production, MCs cannot be present in the skin. There is previous work that has shown that overexpressing SCF in keratinocytes leads to increased numbers of MCs in the skin,¹⁹ but never (until now) has it been shown that conditionally knocking out *Scf* from keratinocytes is sufficient to prevent MCs from entering the skin. In

this sense we have developed a useful ideal model for MC knockout studies with potential application to additional organ systems, such as the gut and lung parenchyma.

Recently, other factors have been implicated in communications between the microbiome and resident immune cells of the skin. It has been shown that skin commensals modulate T-cell development and the immune response through a mechanism involving IL-1 and IL-1 receptor antagonist.⁴³ According to this study, IL-1 signaling from keratinocytes is diminished in the absence of commensals. Indeed, IL-1 α production by cutaneous cells is significantly reduced in GF relative to SPF mice, and monoassociation of GF mice with *S epidermidis* restores production of this cytokine. In contrast, keratinocytes from GF mice display increased levels of *Il1ra* mRNA relative to keratinocytes from SPF mice, and the addition of *S epidermidis* to GF mice significantly reduces IL-1 receptor antagonist levels in keratinocytes.⁴³ These results indicate that commensals control various aspects of functional IL-1 signaling. Our findings mimic this pattern of communication, starting with the microbiome, extending to keratinocytes, and ending with immune cell recruitment.

The findings of this study confirm the concept that both the microbiota and host cells work together to allow for normal skin MC physiology.

In conclusion, our results show that the skin microbiome drives SCF production in keratinocytes, which triggers the development of dermal MCs. We further demonstrate that MC localization within the skin depends exclusively on keratinocyte-derived SCF. Thus we present a novel mechanism by which the skin microbiota signals, through keratinocytes, recruitment of MCs within the dermis and conditions MC functions. This finding opens new perspectives into the possible modulation of the presence and function MCs under normal conditions and in different skin diseases.

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Key messages

- The skin microbiome influences MC maturation in the dermis, and therefore a normal microbiome is necessary for full maturation of MCs and for their antimicrobial capacity.
- Gram-positive bacterial products (LTA) enhance MC maturation by acting through epithelial cells.
- SCF produced by skin epithelial cells is necessary for correct MC localization in the dermis.
- The novel mechanism by which the skin microbiota signals the recruitment and maturation of MCs within the dermis through SCF production is important for understanding the pathogenesis of skin diseases involving MC function, such as atopic dermatitis or psoriasis, in which the role of the microbiome is still not clear.

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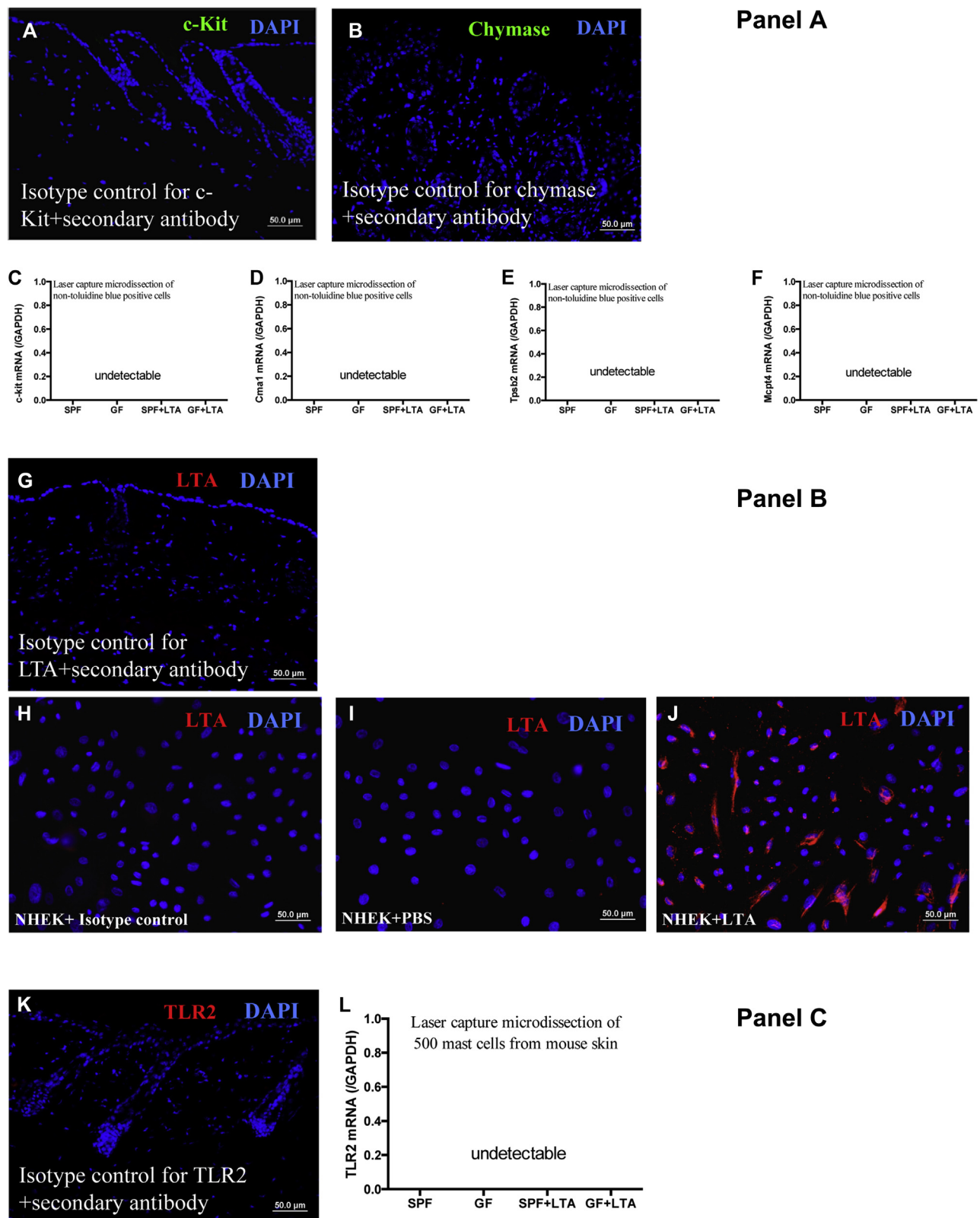


FIG E1. *Panel A* controls for Fig 1. **A** and **B**, Immunofluorescent staining for c-Kit (green), chymase (green), and DAPI (blue) in mouse skin showing the negativity of isotype control antibody staining. **C-F**, qPCR analysis of the controls of non-toluidine blue-positive cells collected by means of LCM. *Panel B* controls for Fig 2. **G**, Immunofluorescent staining for LTA (red) and DAPI (blue) in mouse skin showing the negativity of isotype control antibody staining. **H-J**, Primary normal human keratinocytes (NHEK) immunostained with anti-LTA mAb (red) and DAPI (blue). Keratinocytes were treated with isotype control (Fig E1, **H**), PBS (Fig E1, **I**), or LTA at 10 μ g/mL for 24 hours (Fig E1, **J**). *Panel C* controls for Fig 3. **K**, Immunofluorescent staining for TLR2 (red) and DAPI (blue) in mouse skin showing the negativity of isotype control antibody staining. **L**, qPCR analysis of *Tlr2* expression in MCs collected by means of LCM based on toluidine blue staining of skin from SPF and GF mice.

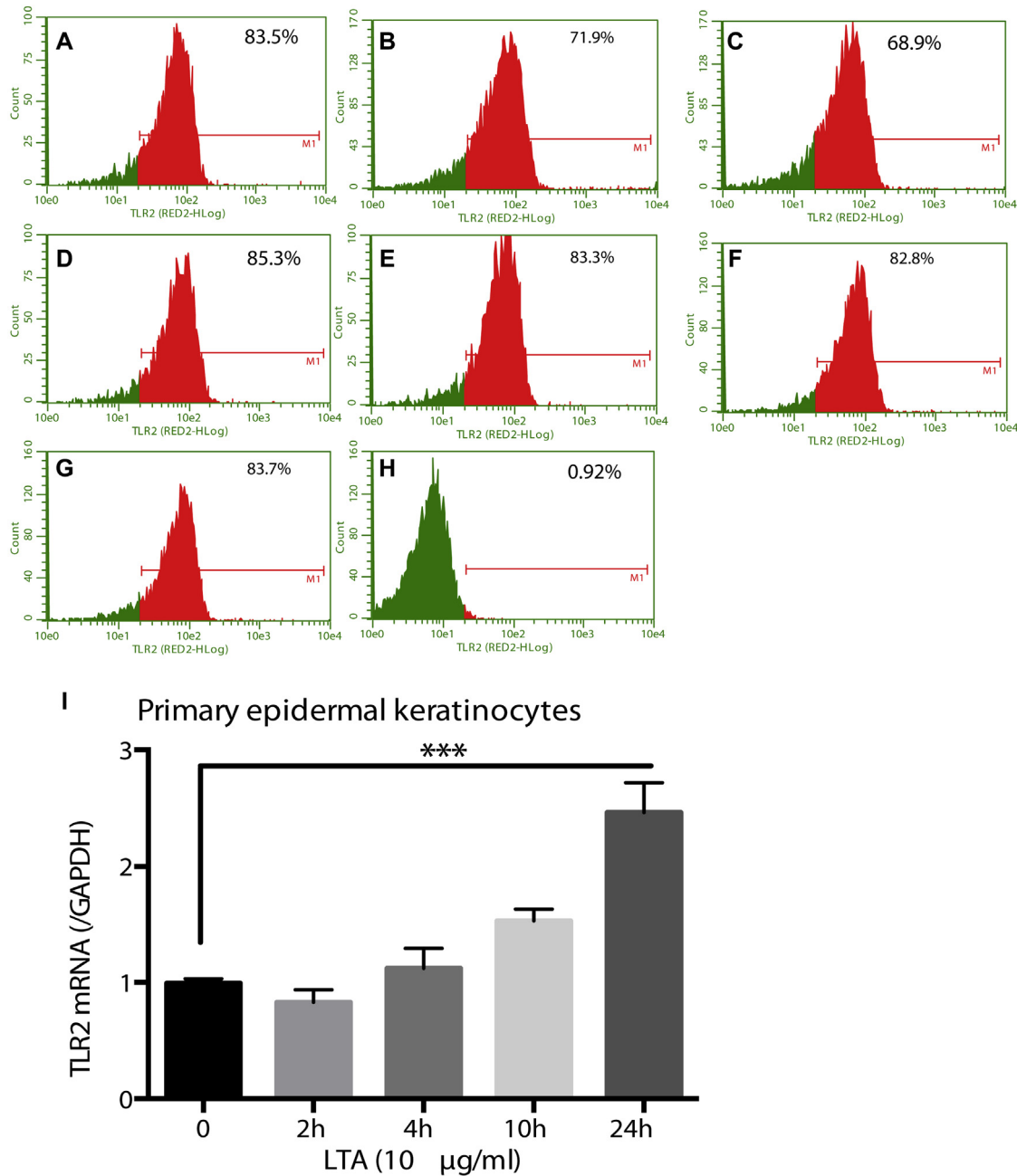


FIG E2. TLR2 expression in MCs cocultured with connective tissue components and TLR2 (LTA) and TLR4 (LPS) ligands. FACS analysis of TLR2 expression in mouse bone marrow–derived MCs after 7 days of coculture with different components of skin connective tissue and TLR2 (LTA) and TLR4 (LPS) ligands. **A–G,** Murine MCs were cultured in normal media with added PBS (Fig E2, A), 10 μ g/mL hyaluronic acid (Fig E2, B), 100 ng/mL LPS (Fig E2, C), or 10 μ g/mL LTA (Fig E2, D), or they were cultured in plates coated with collagen type I (Fig E2, E), fibronectin (Fig E2, F), or gelatin (Fig E2, G). **H,** Isotype control. **I,** *Tlr2* expression in mouse keratinocytes: qPCR analysis of *Tlr2* expression in primary isolated mouse skin keratinocytes before and after stimulation with LTA at 10 μ g/mL. *** $P < .001$.

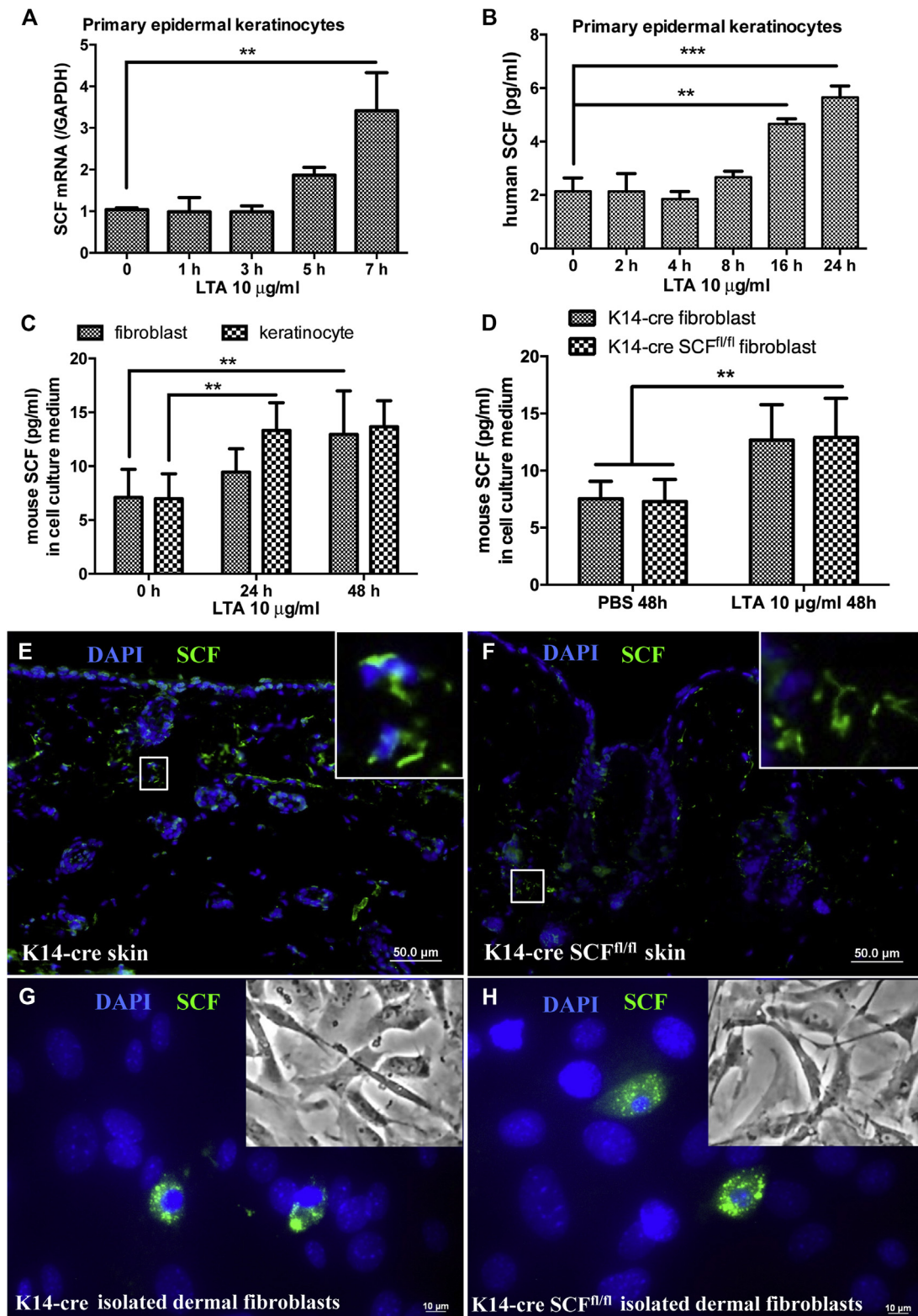


FIG E3. SCF expression in keratinocytes and fibroblasts. **A**, qPCR analysis of SCF in primary human keratinocytes after stimulation with LTA at 10 μ g/mL. **B**, ELISA quantification of SCF in primary human keratinocytes after stimulation with LTA at 10 μ g/mL. **C**, ELISA quantification of SCF in primary isolated mouse keratinocytes and fibroblasts after stimulation with LTA at 10 μ g/mL. **D**, ELISA quantification of SCF in mouse fibroblasts isolated from the skins of K14-cre and K14-cre SCF^{f/f} mice. **E** and **F**, SCF (green) and DAPI (blue) immunostaining of the skins of K14-cre (Fig E3, **E**) and K14-cre SCF^{f/f} mice (Fig E3, **F**). The inset shows a magnification of the squared area in the image. **G** and **H**, SCF (green) and DAPI (blue) immunostaining in dermal fibroblasts isolated from K14-cre (Fig E3, **G**) and K14-cre SCF^{f/f} mice (Fig E3, **H**). The inset shows that these mice have normal fibroblast morphology. ** $P < .01$ and *** $P < .001$.

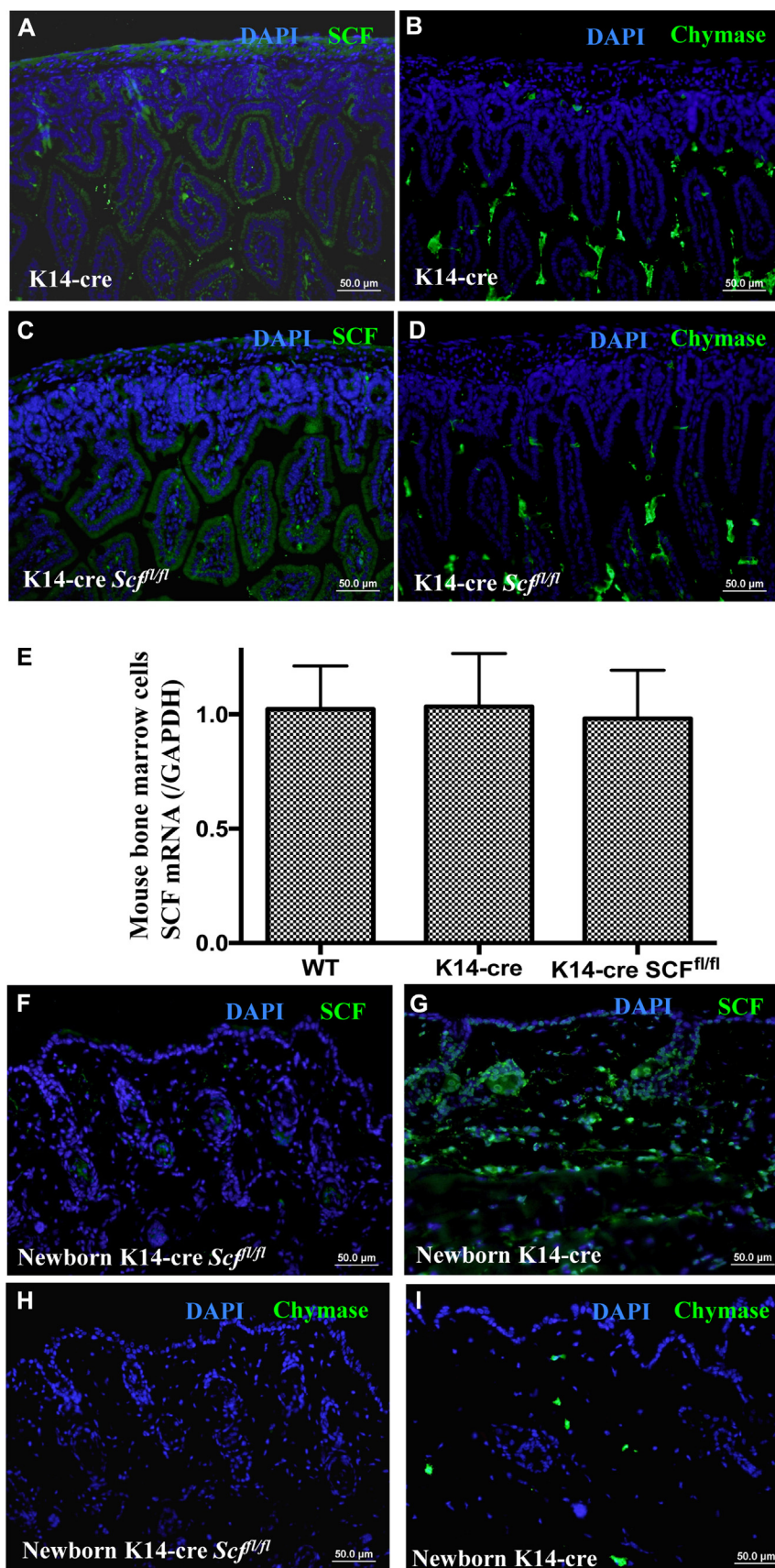


FIG E4. SCF expression in different tissues of K14-cre *Scf^{fl/fl}* mice. **A-D**, Immunofluorescent staining of SCF (green) or chymase (green) and DAPI (blue) reveals normal levels of SCF and MCs in the small intestine of K14-cre (Fig E4, **A** and **B**) and K14-cre *Scf^{fl/fl}* (Fig E4, **C** and **D**) mice. **E**, *Scf* expression was assayed by using qPCR from bone marrow cells extracted from the femurs of mice. **F** and **G**, SCF (green) and DAPI (blue) immunofluorescent staining in the skins of K14-cre *Scf^{fl/fl}* (Fig E4, **F**) and K14-cre (Fig E4, **G**) newborn pups. **H** and **I**, Chymase (green) and DAPI (blue) immunofluorescent staining in the skins of K14-cre *Scf^{fl/fl}* (Fig E4, **H**) and K14-cre (Fig E4, **I**) newborn pups.

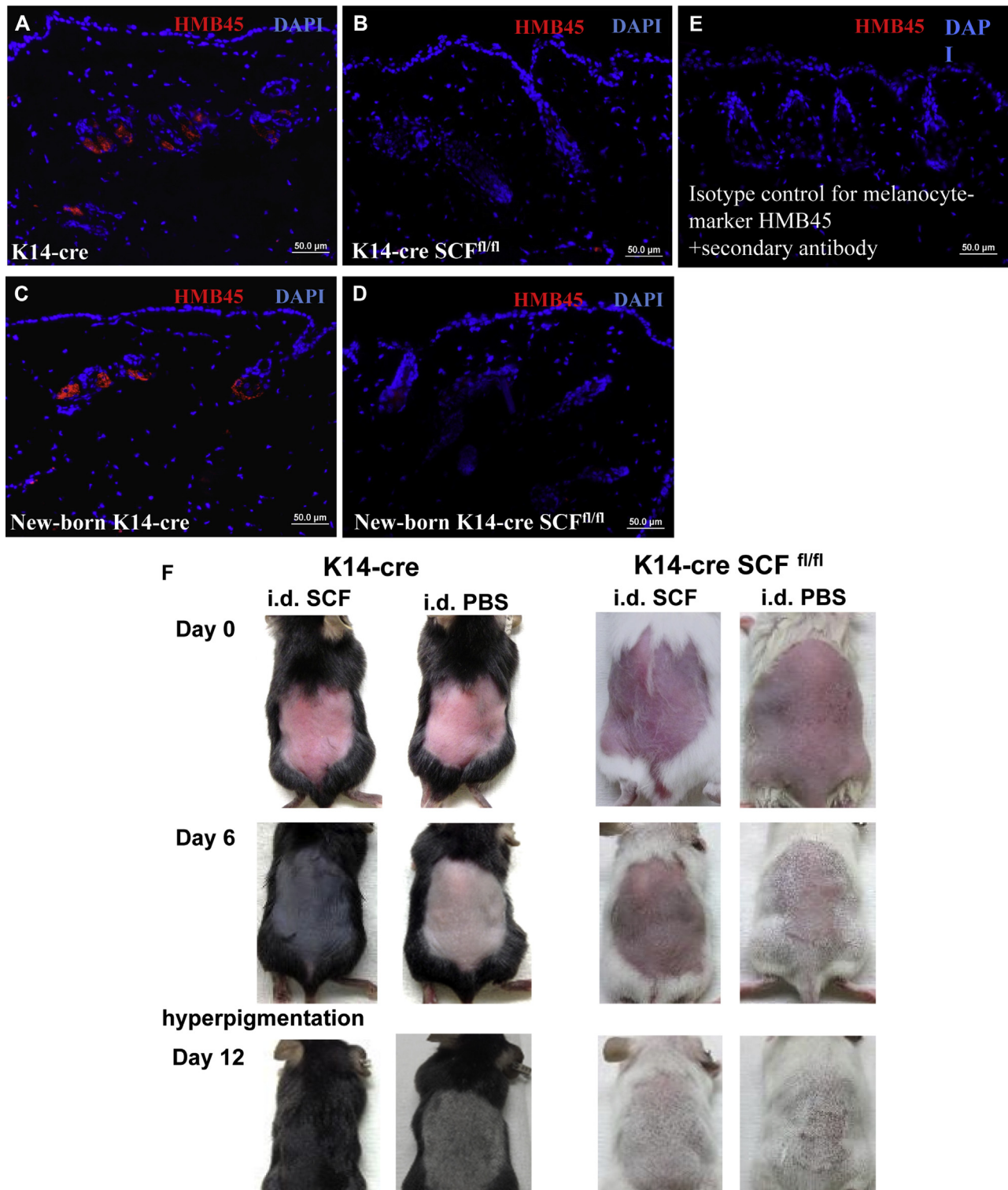


FIG E5. Melanocytes in K14-cre *Scf^{fl/fl}* mice. **A-D**, The melanocyte marker HMB45 (red) and DAPI (blue) immunofluorescent staining reveals normal numbers of melanocytes in the skins of K14-cre adults and newborn pups (Fig E5, **A** and **C**), whereas K14-cre *Scf^{fl/fl}* adults and newborn pups lack normal numbers of melanocytes in the skin (Fig E5, **B** and **D**). **E**, Isotype control for anti-HMB45 antibody plus secondary antibody. **F**, Intradermal injection (*i.d.*) of SCF (500 ng per mouse) every 2 days for 12 days induced hyperpigmentation in K14-cre mice but did not recover pigmentation in K14-cre *Scf^{fl/fl}* mice.

TABLE E1. Blood analysis: WBC and RBC counts and differentials of K14-cre *Scf^{fl/fl}*, K14-cre, and wild-type C57 mice

Species	Sample ID:	K14-cre <i>Scf^{fl/fl}</i> mice			K14-cre mice			Wild-type C57 mice		
		1	2	3	1	2	3	1	2	3
WBC	K/ μ L	6.58	6.94	6.86	7.7	7.76	9.24	9.18	9.64	9.88
NE (no.)	K/ μ L	0.33	1.11	1.02	0.68	0.66	0.6	0.6	1.06	1.07
LY (no.)	K/ μ L	5.77	5.13	5.24	6.32	6.48	8.15	8.15	7.9	8.14
MO (no.)	K/ μ L	0.44	0.55	0.46	0.68	0.61	0.44	0.4	0.64	0.62
EO (no.)	K/ μ L	0.03	0.11	0.11	0.01	0.01	0.03	0.02	0.03	0.04
BA (no.)	K/ μ L	0.01	0.03	0.03	0.01	0	0.01	0.02	0	0.01
NE (%)	%	4.99	16.03	14.89	8.88	8.46	6.54	6.5	10.97	10.84
LY (%)	%	87.68	73.97	76.33	82.11	83.46	88.25	88.74	81.98	82.34
MO (%)	%	6.75	7.9	6.72	8.82	7.89	4.74	4.33	6.67	6.32
EO (%)	%	0.45	1.65	1.59	0.13	0.15	0.36	0.26	0.36	0.44
BA (%)	%	0.12	0.45	0.46	0.07	0.04	0.1	0.17	0.02	0.07
RBC	M/ μ L	9.26	11.46	11.79	9.74	9.36	9.98	9.75	9.29	9.45
HB	g/dL	12.9	14.9	15.4	14.2	14.1	13.7	13.5	12.8	13
HCT	%	39.7	45.7	45.3	41.4	41	38.1	38.1	36.9	37.7
MCV	fL	42.9	39.9	38.4	42.5	43.8	38.2	39.1	39.7	39.9
MCH	pg	13.9	13	13.1	14.6	15.1	13.7	13.8	13.8	13.8
MCHC	g/dL	32.5	32.6	34	34.3	34.4	36	35.4	34.7	34.5
RDW	%	16.9	21.1	20.2	18.6	19.2	17.6	18.5	19.8	19.8
PLT	K/ μ L	411	644	653	925	952	881	900	1061	1158
MPV	fL	6	5.1	5	4.6	4.6	5.1	5.1	4.4	4.4

There is no significant difference in hematology between K14-cre *Scf^{fl/fl}* mice and control mice.

BA, Basophils; EO, eosinophils; HB, hemoglobin; HCT, hematocrit; K/ μ L, thousand cells per microliter; LY, lymphocytes; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MO, monocytes; M/ μ L, million cells per microliter; MPV, mean platelet volume; NE, neutrophils; PLT, platelet count; RDW, red cell distribution width.