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Orphan chemoattractant receptor GPR15 mediates dendritic epidermal T cell recruitment to the skin

Katharina Lahl[1,2], **Johanna Sweere**[1,2], **Junliang Pan**[1,3], and **Eugene Butcher**[1,2]

[1]Laboratory of Immunology and Vascular Biology, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305, USA

[2]The Center for Molecular Biology and Medicine, Veterans Affairs Palo Alto Health Care System, Palo Alto, CA 94304, USA

[3]Palo Alto Institute for Research and Education

Abstract

Homing of murine dendritic epidermal T cells (DETCs) from the thymus to the skin is regulated by specific trafficking receptors during late embryogenesis. Once in the epidermis, Vγ3δ1 TCR DETCs are maintained through self-renewal and participate in wound healing. We show that GPR15, an orphan G protein-linked chemoattractant receptor implicated in recruitment of regulatory T cells to the colon, is highly expressed on fetal thymic DETC precursors and on recently recruited DETCs, and mediates the earliest seeding of the epidermis which occurs at the time of establishment of skin barrier function. DETC in *GPR15*−/− mice remain low at birth, but later participation of CCR10 and CCR4 in DETC homing allow DETC to reach near normal levels in adult skin. Our findings establish a role for GPR15 in skin lymphocyte homing and suggest that it may contribute to lymphocyte subset targeting to diverse epithelial sites.

Keywords

DETC; trafficking receptor; GPR15; skin homing

Introduction

Skin and other squamous epithelia are protected by specialized lymphocyte populations that reside within the epithelium and dermis. The cutaneous epithelium in humans and mice contains specialized populations of γ/δ T cells [1]. The mouse skin harbors so-called dendritic epidermal T cells (DETCs), a unique, highly specialized subset characterized by its dendritic shape and its exclusive expression of γ3δ1 T cell receptor (also known as γ5, depending on the nomenclature used [2]), thought to recognize a self-antigen on stressed or damaged skin cells [3, 4] and to receive costimulation through Junctional Adhesion Molecule-Like protein (JAML) [5]. DETCs participate in wound healing, bacterial defense,

Correspondence should be addressed to Eugene Butcher: ebutcher@stanford.edu, VA Palo Alto Health Care Systems, 3801 Miranda Avenue, Building 101, Palo Alto, CA 94304, Phone: (650) 852-3369, Fax: (650) 858-3986.

Conflict of Interest
The authors declare no conflicts of interest.

cytokine production in response to inflammation, cytolytic activity towards tumor cells, and epidermal barrier function [1, 6–8].

DETCs mature in the fetal thymus and migrate to the skin between embryonic day 16 and 18 [9]. Thereafter, they are maintained in the epidermis through local self-renewal. The migration of DETC into the epidermis involves skin-associated trafficking receptors including ligands for vascular E-selectin [10], and chemoattractant receptors CCR4 [10] and CCR10 [11]. DETCs anchor to the apical epidermis close to keratinocyte tight junctions through engagement of an unknown ligand recognized by the γδ TCR receptor and CD103 [4, 12].

GPR15 is an orphan GPCR and HIV co-receptor with homology to leukocyte chemoattractant receptors [13, 14]. Recent studies have highlighted its role as a T cell homing receptor: Using a *gpr15* GFP knock-in model, the authors showed that GPR15 is selectively expressed by colon regulatory T cells (Tregs) under homeostatic conditions [15], and that it mediates Treg recruitment to the colon. We here show that GPR15 is required for embryonic trafficking of DETCs to the epidermal skin. Our results imply a broader role for GPR15 in lymphocyte trafficking to epithelial sites.

Results

GPR15 is expressed in fetal thymic dendritic epidermal T cell precursors

Analyses of gene expression data for mouse thymic and peripheral T cell populations revealed specifically high expression of *gpr15* by mature ($CD24^{low}$ [16]) fetal thymic Vγ3 cells, precursors of DETC (Fig. 1A) (Immgen.org [17]). Expression from the *gpr15* promoter was confirmed by flow cytometry on embryonic day 17- derived heterozygous $gpr15^{GFP/wt}$ thymic cell suspensions. The embryonic $gpr15^{GFP/GFP}$ knockout thymus harbored comparable frequencies of pre-DETCs, showing that GPR15 is dispensable for pre-DETC development (Fig. 1B). DETCs leave the thymus around embryonic day 17 to seed the epidermis. $V\gamma3^{+}$ pre-DETCs can still be identified in the thymus at day 1 after birth, although at this developmental stage they make up only a small fraction of thymic cells (Fig 1C, left panel). Only a subset of the remaining $V\gamma3^{+}$ T cells in the thymus express GFP at this time point (Fig. 1C). We observed higher GFP expression in $gpr15^{GFP/GFP}$ vs $gpr15^{GFP/WT}$ pre-DETC, probably reflecting a gene dosage effect (Fig. 1C).

GPR15 is required for skin localization of thymus-derived dendritic epidermal T cells

Since pre-DETCs exclusively seed the epidermis and GPR15 has previously been shown to be a functional homing receptor, we analyzed the efficiency of DETC recruitment in presence or absence of GPR15. The epidermis of $gpr15^{GFP/GFP}$ knockout mice lacked DETCs at day 1 after birth, while DETCs in $gpr15^{GFP/WT}$ heterozygous mice were not affected. All DETCs in $gpr15^{GFP/WT}$ mice were GFP^{+} at this early time (Fig. 2A); in contrast, by day five after birth, DETC in heterozygous mice were largely GFP^{-}, indicating that GPR15 expression is rapidly downregulated on skin resident DETCs (data not shown). Indeed, DETCs had completely lost GPR15-GFP expression in adult mice, suggesting that the receptor is not required for resident DETC maintenance (Fig. 2B). While *gpr15*

knockout neonates lacked DETCs, the frequency of DETC partially recovered over time: DETC comprised 65±11% (SEM, N=3) of total recovered epidermal $CD3^+$ cells in adult *gpr15*$^{GFP/GFP}$ knockout mice, compared with 92±1% in heterozygotes (Fig. 2B, C). Similar recovery of DETC numbers after birth has previously been shown in analyses of the role of CCR10, which is also important for DETC recruitment to the epidermis [11]. Interestingly however, CCR10 deficiency caused redistribution of $V\gamma3^+$ DETC with accumulation of DETCs in the dermis [11]. In contrast, in *gpr15*$^{GFP/GFP}$ knockout mice $V\gamma3^+$ DETC isolated from the dermal fraction were also reduced, indicating an overall diminishment of recruited DETC in the skin (Fig. 2C). The phenotype of the DETCs in the adult epidermis of *gpr15*$^{GFP/GFP}$ knockout mice was comparable to that of DETCs in *gpr15*$^{WT/WT}$ mice (Fig. 2D). In accordance with the abundance of DETCs in adult *gpr15*$^{GFP/GFP}$ mice, GPR15 deficient mice showed no significant delay in wound healing (data not shown), a result which also rules out a substantial GPR15-dependent defect in DETC functional properties. Postnatal recovery of DETC appears to be mediated by CCR4: CCR4 deficient mice have only a modest reduction in skin DETC at birth, but a greater defect in DETC numbers as adults [18]. Moreover, whereas CCR10 and GPR15 are lost on adult skin DETC ([11] and Fig. 2B), CCR4 is highly and uniformly expressed [10]. We already detect substantial numbers of DETCs in the epidermis of *gpr15*$^{GFP/GFP}$ knockout mice at day 5 after birth (data not shown). CCR4 and/or CCR10 may thus rescue DETC homing to the epidermis beginning shortly after birth, where DETC numbers rise quickly through self-renewal. Taken together these results show a clear role for the homing receptor GPR15 in targeting thymus derived DETC precursors to the skin, and suggest distinct if overlapping roles for three skin homing receptors, GPR15, CCR10 and CCR4, in this process.

Concluding Remarks

We here show that GPR15 is essential for embryonic DETC recruitment to the skin. Interestingly, GPR15 is expressed by subsets of conventional skin homing $\alpha\beta$ TCR^+ T cells in blood, and of T cells infiltrating inflamed skin in contact sensitivity models (KL and ECB, unpublished). CCR10 and CCR4, and T cell E-selectin ligands, participate not only in DETC homing during development, but also in conventional effector/memory T cell homing to skin [19–22]. It remains to be determined whether GPR15 plays a significant role in cutaneous T cell homing in the adult. Our present finding that GPR15 mediates DETC recruitment to the skin, together with its previously reported role in regulatory T cell localization to the colon, suggest an important role for GPR15-dependent homing of lymphocytes at barrier sites.

Material and Methods

Mice

GPR15$^{GFP/GFP}$ mice were from Dan Littman [23] or JAX and were maintained and bred in specific pathogen-free conditions in the animal facility at the Veterans Affairs Palo Alto Health Care Systems (VAPAHCS). To ensure age matching, we bred mice heterozygously and compared knockout and heterozygous littermates. Mice were used at 8 – 12 weeks of age unless otherwise stated.

Lymphocyte isolation

Thymic lymphocytes were isolated by removing the thymus and generating a single cell suspension by straining through a 70μm wire mesh. Skin lymphocytes were freshly isolated as previously described [24] with minor adjustments. Briefly, mouse ears were removed at the base, rinsed in 70% ethanol, air-dried and split into dorsal and ventral halves. The ear halves were placed dermal side down on 0.8% trypsin in PBS (Sigma) and incubated for 30–45 minutes at 37°C. After enzymatic digestion, epidermis and dermis were separated using forceps. Epidermal sheets were transferred into complete IMDM medium and dermal sheets were transferred into complete IMDM medium containing 2mg/ml collagenase IV (Worthington). Skin sheets were shaken for 30 minutes at 37°C and filtered through a 100 μM cell strainer. Cell suspensions were washed twice with complete IMDM medium before enrichment of lymphocytes using a 40%/70% Percoll gradient.

Flow cytometry and antibodies

Cells were first blocked with FACS buffer (PBS with 0.5% BSA) containing 1μg/ml anti CD16/CD32 (clone 93, eBioscience). The following antibodies were used for staining: CD3-PerCP Cy5.5 (145-2C11, eBioscience), TCR Vγ3-APC (536, Biolegend), TCRγ/δ-PE (GL3, BD Biosciences). Dead cells were excluded by propidium iodide staining (Sigma). FACS data was acquired on a Fortessa from BD, using the FACS Diva software. Further analysis was performed using FlowJo from Treestar. Statistics were calculated using GraphPad Prism, where the unpaired Student's t-test was employed.

Histology

Mouse ears were removed at the base and hairs were removed with Nair cream. Ears were then split into dorsal and ventral halves. The ear halves were placed dermal side down on 0.5M ammonium thiocyanate and incubated for 40 minutes at 37°C. Epidermis and dermis were separated using forceps. Epidermal sheets were mounted on microscopic slides and incubated in 4% PFA for 5 minutes. After washing, cells were blocked for 30 minutes with Fc block in PBS containing 10% FCS and 0.1% saponin, followed by incubation with anti TCRγ/δ-PE (GL3, BD Biosciences) for one hour. Slides were mounted by ProLong® Gold Antifade Reagent (Life Technologies).

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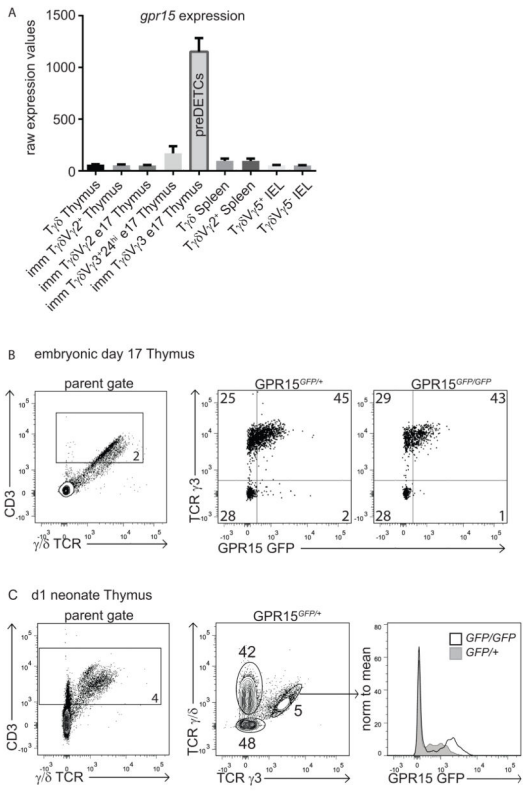

Figure 1. GPR15 is expressed in fetal thymic dendritic epidermal T cell precursors

A) Gene expression data are from the Immgen consortium (GSE15907). Mean±SD of raw expression values from three independent datasets.

B) Parent gate plot show $CD3^+$ gate on live single thymocytes from a representative embryonic day 17 *gpr15*$^{GFP/+}$ mouse. Main data plots (middle, right) show all cells from $CD3^+$ gates and are representative of at least three littermates each derived from a timed *gpr15*$^{GFP/+}$ x *gpr15*$^{GFP/GFP}$ breeding.

C) Parent plot (left) and middle main plot show the same subsets as in B) from d1 neonatal littermates. The histogram plot shows GPR15 GFP expression of thymic preDETCs. The parent gate is indicated by the arrow. Representative of two *gpr15*$^{GFP/GFP}$ mice and seven *gpr15*$^{GFP/+}$ mice.

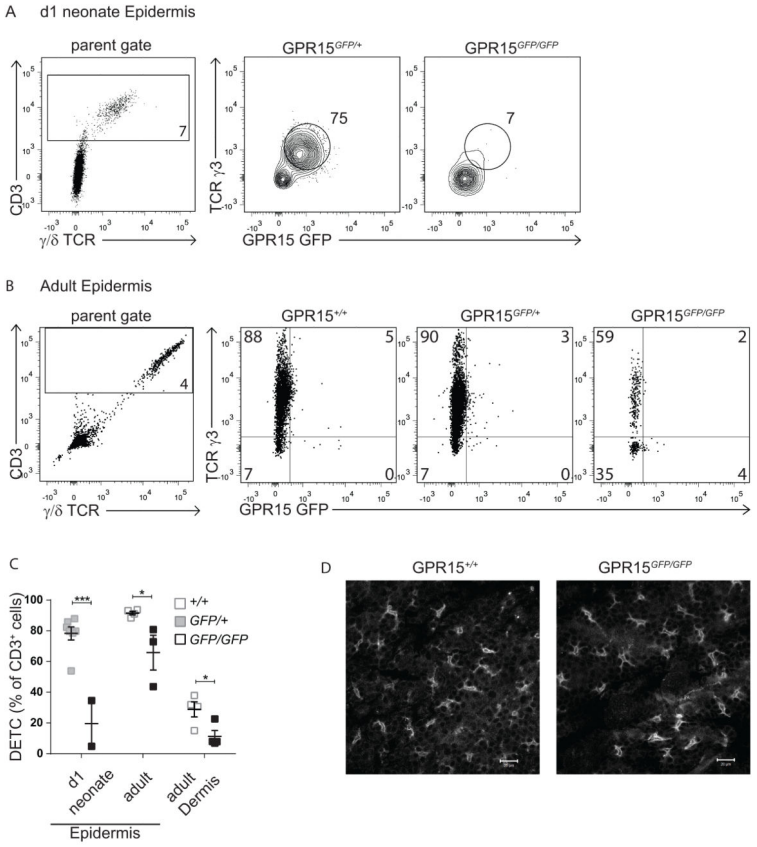

Figure 2. GPR15 is required for skin localization of thymus-derived dendritic epidermal T cells

A) Parent gate plot show $CD3^+$ gate on live single cells from the epidermis of a representative neonatal day 1 *gpr15*$^{GFP/+}$ mouse. Main data plots (middle, right) show all cells from $CD3^+$ gates. Representative of two *gpr15*$^{GFP/GFP}$ mice and seven *gpr15*$^{GFP/+}$ mice.

B) Parent gate plot show $CD3^+$ gate on live single cells from the epidermis of a representative adult *gpr15*$^{GFP/+}$ mouse. Main data plots (middle, right) show all cells from $CD3^+$ gates and are representative of four technical replicates with one to four biological replicates each.

C) DETC in epidermis of d1 mice, and in separately isolated lymphocytes from epidermal sheets and dermis of adult mice, as percent of total $CD3^+$ T cells. Scatter plots showing 2 to 7 individual mice, with SEM. Unpaired t test; *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$

D) Confocal images of γ/δ TCR stained epidermal sheets from adult *gpr15*$^{GFP/GFP}$ knockout mice versus *gpr15*$^{WT/WT}$ mice. 40x, scale bars = 20μm.