HHS Public Access

Author manuscript

J Invest Dermatol. Author manuscript; available in PMC 2013 February 01.

Published in final edited form as:

J Invest Dermatol. 2012 August ; 132(8): 2106–2109. doi:10.1038/jid.2012.71.

TGFβ/Smad3 signal pathway is not required for epidermal langerhans cell development

Ying-Ping Xu[1,2,4,5], **Yuling Shi**[1,2], **Zhi-Zhong Cui**[4], **Hong H. Jiang**[6], **Li Li**[6], **Xiao-Fan Wang**[7], **Li Zhou**[1,2,3], and **Qing-Sheng Mi**[1,2,3]

[1]Henry Ford Immunology Program, Henry Ford Hospital, Detroit, MI

[2]Department of Dermatology, Henry Ford Hospital, Detroit, MI

[3]Department of Internal Medicine, Henry Ford Hospital, Detroit, MI

[4]Department of Preventive Veterinary Medicine, College of Animal Science and Veterinary Medicine, Shandong Agriculture University, Taian, China

[5]Department of Immunology, Taishan Medical University, Taian, China

[6]Department of Internal Medicine, Wayne State University, Detroit, MI

[7]Department of Pharmacology and Cancer Biology, Duke University, Durham, North Carolina

TO THE EDITOR

Epidermal Langerhans cells (LCs) are professional antigen-presenting dendritic cells (DCs) that reside in the epidermis and form the first immunological barrier to the external environment (Romani *et al.*, 2010). Although considerable progress has been made in identifying the developmental requirement of LCs, the mechanisms that regulate LC development and homeostasis are still not fully elucidated. TGF-β1 is one of key regulators to control LC development and homeostasis. LCs are absent in mice that lack TGF-β1, owing to a failure in LC differentiation, survival or both (Borkowski *et al.*, 1996; Kaplan *et al.*, 2007; Zahner *et al.*, 2011). This finding is further supported by the dependence of LCs development on other TGFβ1-related molecules, including inhibitor of DNA binding 2 (ID2), a TGFβ1-induced inhibitor of helix–loop–helix transcription factors (Hacker *et al.*, 2003), and Runx3, specifically expressed by mature DCs and mediates their response to TGFβ1 (Fainaru *et al.*, 2004). Furthermore, TGF-β1 is also required to maintain the pool of immature LCs in the epidermis (Kel *et al.*, 2010). Although TGF-β1 is expressed by both keratinocytes and LCs, an autocrine source of TGF-β1 from LCs is required for LC development (Kaplan *et al.*, 2007). Taken together, TGF-β1 is essential for the ontogeny,

To whom correspondence should be addressed: Dr. Qing-Sheng Mi, Henry Ford Immunology Program, Departments of Dermatology and Internal Medicine, Henry Ford Health System, 1 Ford Place, Detroit, MI, United States. Tel: +1-313-876-1017; Fax: +1-313-876-1016; qmi1@hfhs.org; or Li Zhou, Henry Ford Immunology Program, Departments of Dermatology and Internal Medicine, Henry Ford Heath System, 1 Ford Place, Detroit, MI, United States. Tel: +1-313-874-4881; Fax: +1-313-874-4879; lzhou1@hfhs.org.

CONFLICT OF INTEREST

The authors state no conflict of interest

homeostasis, and function of epidermal LCs. However, the intracellular signaling pathway of TGF-β1 in epidermal LCs remains elusive.

In a well-defined classical TGF-β linear signaling pathway, once activated, TGF-β1 signals through its two cell surface receptors, TGF-β receptor 1 (TβR1) and TGF-β receptor 2 (TβR2), leading to Smad-mediated transcriptional regulation. There are eight Smads: Smad1 to Smad8. Smad2 and Smad3 are activated through carboxy-terminal phosphorylation by TGFβ1. These receptor-activated Smads (R-Smads) are released from the receptor complex to form a heterotrimeric complex with a common Smad4, and translocate into the nucleus to regulate the transcription of target genes (Derynck and Zhang, 2003; Tsukazaki *et al.*, 1998). In addition, TGF-β1 also can activate other signaling cascades, including MAPK pathways (Itoh *et al.*, 2000; Massague, 2000; Moustakas *et al.*, 2001). Unlike mice with the conventional disruptions of Smad2 and Smad4 that are lethal, mice with Smad3 deficiency are viable and can survive. However, Smad3 knockout (KO) mice develop progressive diseases, including leucocytosis and massive inflammation. The loss of Smad3 results in multiple cell defects, including T cells, neutriphils and macrophage (Werner *et al.*, 2000; Yang *et al.*, 1999). Here we used Smad3 KO mice (Datto *et al.*, 1999) to directly test if TGFβ/Smad3 signaling pathway is involved in the ontogeny and homeostasis of epidermal LCs.

During LC early development, a single wave of LC precursors recruited in the epidermis around embryonic day 18, which subsequently differentiate into LCs after birth, and LCs then undergo a massive burst proliferation during the first week of life to form a typical LC network (Chorro *et al.*, 2009). Given that TGF-β signals are required for ontogeny and maintaining of LCs in the epidermis, we first assessed if Smad3 is required for LC homeostasis in vivo. Epidermal cell suspensions prepared from ears and dorsal skin of Smad3KO mice and WT littermates, on day 0, 1, 3, 8 after birth, as well as at 3 and 5-week old, were stained with anti-Langerin and anti-MHC-II antibodies. As shown in Figure 1 a & b, we did observe a 3–4-fold expansion of LCs from 0.78 ± 0.11% on day 0 to 2.72 ± 0.14% on day 3 in WT mice. Langerin expression was undetectable in $MHCII^+$ LCs in the epidermis on day 0 and 1, but upregulated Langerin expression was detected in about 70%–80% LCs by day 3, which is consistent with recent report (Chorro *et al.*, 2009; Kel *et al.*, 2010). However, there were no significant differences on the rations of epidermal LCs between Smad3 KO and WT mice at any time points by flow cytometry (Figure 1a & b) and immunohistological staining at day 0 after born and 5 weeks old (Figure 1c & d). Thus, Smad3 is not required in the TGF-β signal pathway for oncogeny and homeostasis of epidermis LCs.

Upon activation by various stimuli, immature LCs residing in epidermis collect antigen and increase their MHC II and costimulatory molecules, including CD80 and CD86, and then migrate to T cells areas of draining lymph nodes, leading to immune responses (Romani *et al.*, 2010). However, loss of TβR1 in LCs makes LC spontaneous maturation, suggesting the TGF-β signal as an essential pathway to control the immature state of LCs (Kel *et al.*, 2010). We next tested if TGF-β/Smad3 signaling pathway is required for maintaining the immature state of epidermal LCs. As shown in Figure 2a, the frequencies of $CD80^+$ LCs and $CD86^+$LCs were comparable between Smad3 KO and WT mice. Furthermore, the

expression levels of MHCII, CD80 and CD86 on LCs, represented by mean fluorescence intensity (MFI), were not changed (Figure 2b). LC maturation in Smad3 KO mice were further evaluated after *in vitro* epidermal culture. As shown in Figure 2c, after 60h culture, both the percentages of CD86- and CD80-positive LCs and $MHCII^{high}$ LCs as well as the relative CD80, CD86, and MHCII expression levels on LCs (Figure 2d) were significantly increased in Smad3KO and WT mice compared to unstimulation condition (Figure 2a & b), but there was no significant difference between Smad3 KO and WT mice ($P > 0.05$). Thus, the lack of Smad3 signaling does not affect the immature state of LCs as well as LC maturation.

Due to their physical location, LCs acquire and process antigens. To evaluate the role of Smad3 in antigen phagocytic function of LCs, freshly isolated epidermal cells from KO and WT mice were incubated at 37°C or 4°C (as control) with FITC-Dextran for 45 minutes and then stained with anti-mouse MHC-II and CD45.2 antibodies. As shown in Figure 2e, the phagocytic capacity of LCs in Smad3 KO mice had no significant difference ($P > 0.05$) compared to the WT LCs, based on the ratio of FITC-positive LCs (Figure 2e) or MFI expression levels (Figure 2f). Thus, lack of Smad3 signal pathway does not affect LC phagocytosis.

In summary, lack of Smad3 surprisingly does not significantly interrupt the development and immature state of epidermal LCs, and Smad3-deficient LCs have normal maturation and phagocytosis. Our data suggest that Smad3 is not required in the TGF-β signal pathway for ontogeny, homeostasis, and function of epidermal LCs. Recent report indicated that Smad2 and Smad3 were redundantly essential for TGF-β–mediated induction of Foxp3-expressing regulatory T cells and suppression of IFN-γ production in $CD4^+$ T cells (Takimoto *et al.*, 2010). This raises the possibility that Smad2/Smad3 redundancy may also exist in TGF-β/Smads pathways in LCs. In addition, non-TGF-β/Smads pathways may also regulate LC ontogeny and homeostasis (Figure 2g). Further investigations are warranted to clarify the TGF-β signaling pathways through which TGF-β controls LC ontogeny and homeostasis.

ACKNOWLEDGMENTS

This work was supported in part by grant from National Institutes of Health Grant R21AR059976, RO1HL087014, and Henry Ford Health System Start-up Grant for the Immunology Program and Dermatology Research (T71016 and T71017). We thank Riqun Qi and Min Liu for their assistance, and all members of Mi and Zhou laboratories for their helpful advice and support.

REFERENCES

Borkowski TA, Letterio JJ, Farr AG, Udey MC. A role for endogenous transforming growth factor beta 1 in Langerhans cell biology: the skin of transforming growth factor beta 1 null mice is devoid of epidermal Langerhans cells. J Exp Med. 1996; 184:2417–2422. [PubMed: 8976197]

Chorro L, Sarde A, Li M, Woollard KJ, Chambon P, Malissen B, et al. Langerhans cell LC) proliferation mediates neonatal development, homeostasis, and inflammation-associated expansion of the epidermal LC network. J Exp Med. 2009; 206:3089–3100. [PubMed: 19995948]

Datto MB, Frederick JP, Pan L, Borton AJ, Zhuang Y, Wang XF. Targeted disruption of Smad3 reveals an essential role in transforming growth factor beta-mediated signal transduction. Mol Cell Biol. 1999; 19:2495–2504. [PubMed: 10082515]

Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature. 2003; 425:577–584. [PubMed: 14534577]

Fainaru O, Woolf E, Lotem J, Yarmus M, Brenner O, Goldenberg D, et al. Runx3 regulates mouse TGF-beta-mediated dendritic cell function and its absence results in airway inflammation. EMBO J. 2004; 23:969–979. [PubMed: 14765120]

Hacker C, Kirsch RD, Ju XS, Hieronymus T, Gust TC, Kuhl C, et al. Transcriptional profiling identifies Id2 function in dendritic cell development. Nat Immunol. 2003; 4:380–386. [PubMed: 12598895]

Itoh S, Itoh F, Goumans MJ, Ten Dijke P. Signaling of transforming growth factor-beta family members through Smad proteins. Eur J Biochem. 2000; 267:6954–6967. [PubMed: 11106403]

Kaplan DH, Li MO, Jenison MC, Shlomchik WD, Flavell RA, Shlomchik MJ. Autocrine/paracrine TGFbeta1 is required for the development of epidermal Langerhans cells. J Exp Med. 2007; 204:2545–2552. [PubMed: 17938236]

Kel JM, Girard-Madoux MJ, Reizis B, Clausen BE. TGF-beta is required to maintain the pool of immature Langerhans cells in the epidermis. J Immunol. 2010; 185:3248–3255. [PubMed: 20713882]

Massague J. How cells read TGF-beta signals. Nat Rev Mol Cell Biol. 2000; 1:169–178. [PubMed: 11252892]

Moustakas A, Souchelnytskyi S, Heldin CH. Smad regulation in TGF-beta signal transduction. J Cell Sci. 2001; 114:4359–4369. [PubMed: 11792802]

Romani N, Clausen BE, Stoitzner P. Langerhans cells and more: langerin-expressing dendritic cell subsets in the skin. Immunol Rev. 2010; 234:120–141. [PubMed: 20193016]

Takimoto T, Wakabayashi Y, Sekiya T, Inoue N, Morita R, Ichiyama K, et al. Smad2 and Smad3 are redundantly essential for the TGF-beta-mediated regulation of regulatory T plasticity and Th1 development. J Immunol. 185:842–855. [PubMed: 20548029]

Tsukazaki T, Chiang TA, Davison AF, Attisano L, Wrana JL. SARA, a FYVE domain protein that recruits Smad2 to the TGFbeta receptor. Cell. 1998; 95:779–791. [PubMed: 9865696]

Werner F, Jain MK, Feinberg MW, Sibinga NE, Pellacani A, Wiesel P, et al. Transforming growth factor-beta 1 inhibition of macrophage activation is mediated via Smad3. J Biol Chem. 2000; 275:36653–36658. [PubMed: 10973958]

Yang X, Letterio JJ, Lechleider RJ, Chen L, Hayman R, Gu H, et al. Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-beta. EMBO J. 1999; 18:1280–1291. [PubMed: 10064594]

Zahner SP, Kel JM, Martina CA, Brouwers-Haspels I, van Roon MA, Clausen BE. Conditional deletion of TGF-betaR1 using Langerin-Cre mice results in Langerhans cell deficiency and reduced contact hypersensitivity. J Immunol. 2011; 187:5069–5076. [PubMed: 21998450]

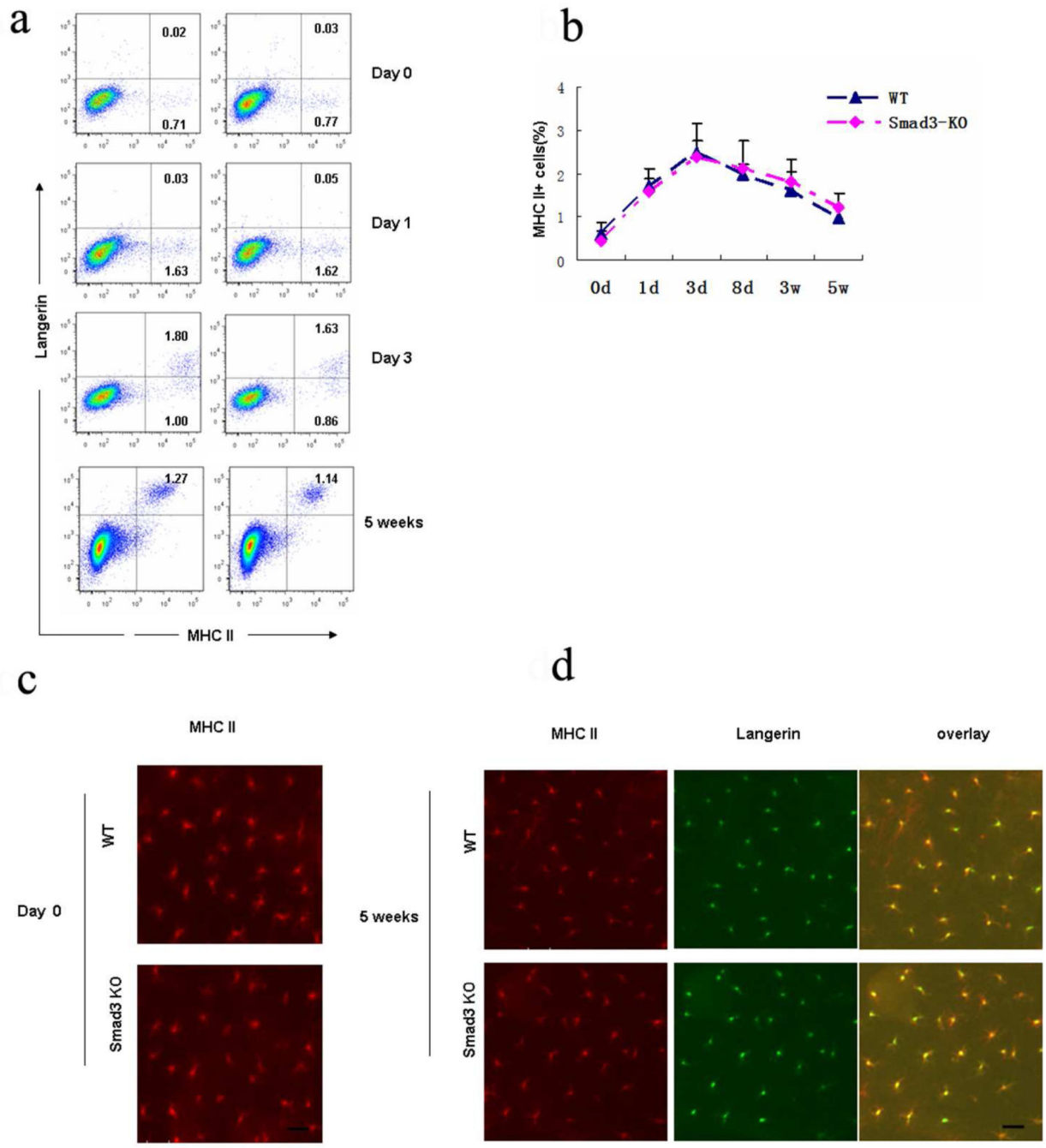

Figure 1.

Smad3 is not required for ontogeny and homeostasis of epidermis LCs. (**a**) and (**b**) Epidermal suspensions prepared from ears and trunk skin of Smad3 KO and WT littermates on day 0, 1, 3, 8 after birth, as well as at 3 and 5 weeks old, were stained with anti-Langerin and anti-MHC-II antibodies and analyzed by flow cytometry. At each time point, 3–5 mice were analyzed, $P > 0.05$; (**c**) Epidermal sheets from Smad3 KO and WT littermates at 0 day post-birth were stained with anti-MHCII. (**d**) Epidermal sheets from Smad3 KO and WT littermates at 5 weeks old were stained with anti-MHC II (red) and Langerin (green). One

representative epidermal sheet is shown from n=3 mice analyzed. Scale bar=10um, original magnification X200.

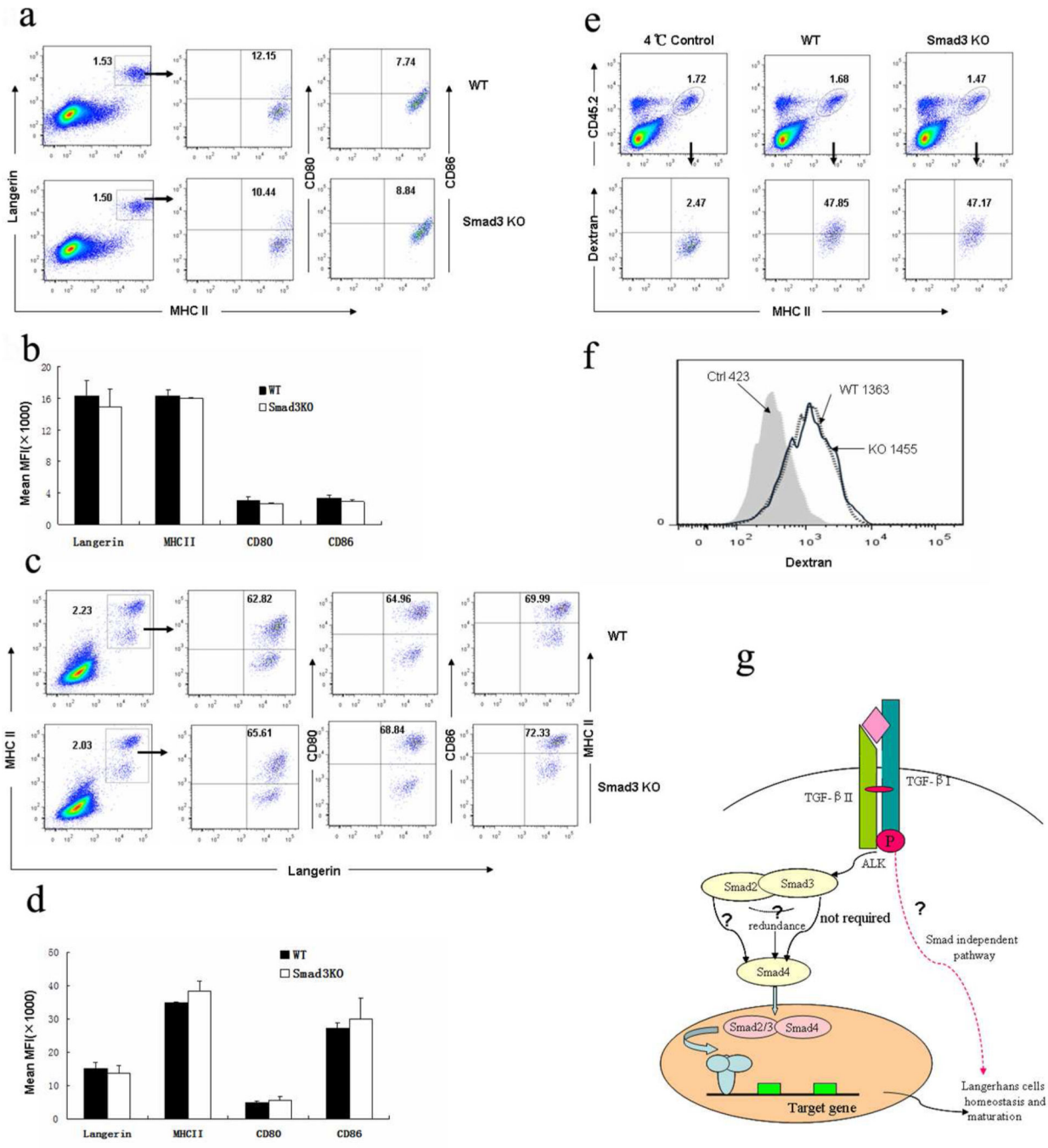

Figure 2.

Smad3 is not an essential factor to control epidermis LC maturation and LC uptaking-antigen ability. (**a**) and (**b**) Epidermal suspensions freshly isolated from ears and trunk skin of Smad3 KO and WT littermates at 5 weeks old, were stained with anti-Langerin, MHC-II, CD80, CD86 antibodies and analyzed by flow cytometry. No significant differences were observed in the ratios (a) as well as in the expression (b) (average MFI ± SD). Three to five mice were analyzed, $P > 0.05$. (**c**) and (**d**) Epidermal suspensions isolated from ears and trunk skin of Smad3 KO and WT littermates at 5 weeks old, were cultured in RPMI for 60h,

then stained with anti-Langerin, MHC-II, CD80, CD86 antibodies and analyzed by flow cytometry. No significant differences were observed in the ratios (**c**) as well as in the expression (**d**), Three to five mice were analyzed, $P > 0.05$. (**e**) and (**f**) Phagocytic ability of LCs (CD45.2 and MHCII double positive cells) was assessed by flow cytometry of FITC-Dextran phagocytosis. LCs from Smad3 KO mice were able to phagocyte FITC-Dextran as efficiently as LCs from wild-type control (**e**). Numbers in histogram indicate geometric mean fluorescence of test samples (**f**). Ctrl, control (cells incubated with FITC-Dextran at 4°C). Data represent one of at least three experiments with similar results. (**g**) The model of induction of signaling responses by TGF-β1 in skin LCs.