IL-23 from Langerhans Cells Is Required for the Development of Imiquimod-Induced Psoriasis-Like Dermatitis by Induction of IL-17A-Producing γδ T Cells

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Psoriasis is a common chronic inflammatory skin disease that involves dysregulated interplay between immune cells and keratinocytes. Recently, it has been reported that IL-23 induces CCR6+ γδ T cells, which have the pivotal role in psoriasis-like skin inflammation in mice of producing IL-17A and IL-22. Langerhans cells (LCs) are a subset of dendritic cells that reside in the epidermis and regulate immune responses. The role of LCs has been extensively investigated in contact hypersensitivity, but their role in psoriasis remains to be clarified. In this study, we focused on Th17-related factors and assessed the role of LCs and γδ T cells in the development of psoriasis using a mouse psoriasis model triggered by topical application of imiquimod (IMQ). LC depletion by means of diphtheria toxin (DT) in Langerin DT receptor–knocked-in mice suppressed hyperkeratosis, parakeratosis, and ear swelling in the IMQ-treated regions. In addition, LC-depleted mice showed decreased levels of Th17-related cytokines in IMQ-treated skin lesions. Moreover, the IMQ-treated skin of LC-depleted mice showed a decreased number of IL-17A-producing CCR6+ γδ T cells. These results suggest that LCs are required for the development of psoriasis-like lesions induced by IMQ in mice.

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INTRODUCTION

Psoriasis is an inflammatory epidermal hyperproliferative skin disease that affects 2–3% of the population in Caucasians. In this disease, patients develop erythematous papules and scaly plaques over the skin surface. Several lines of evidence, such as the presence of activated CD4+ and CD8+ T cells in psoriatic plaques (Ferenczi et al., 2000), the results of studies on human skin xenografts in mice (Boymann et al., 2004), and the therapeutic efficacy of T cell–targeted drugs (Di Cesare et al., 2009), have suggested the involvement of T cell–mediated immune responses in this disease. In addition, the expression levels of IL-1, tumor necrosis factor (TNF), IL-12, IL-17, IL-22, and IL-23 are elevated in psoriatic skin in humans (Di Cesare et al., 2009), and IL-23R gene polymorphisms are associated with psoriasis (Capon et al., 2007). These findings suggest the involvement of these cytokines in the pathogenesis of this skin disease.

Recent studies have demonstrated that the IL-23/Th17 pathway is linked to a number of inflammatory diseases, including psoriasis and animal models of multiple sclerosis, arthritis, and inflammatory bowel disease (Cua et al., 2003). The most persuasive evidence for the role of IL-23/Th17 in psoriasis comes from clinical studies. Treatment with an anti–IL-12/IL-23p40 Ab (ustekinumab) is effective against psoriasis (Krueger et al., 2007). TNF-α-neutralizing antibodies are also widely used to treat psoriasis and significantly improve psoriasis area severity index scores. Notably, inhibition of TNF-α by a soluble TNF-α receptor–neutralizing antibody (etanercept) was associated with reduced Th17 responses (Zaba et al., 2007). As Th1 responses were not affected by the administration of etanercept, this finding suggests that Th17 cells are particularly important in driving psoriasis. Consistently, amelioration of psoriasis has been associated with reduced Th17 responses (Zaba et al., 2007). Th17 cytokines, principally IL-17A and IL-22, exert profound effects on keratinocytes (Nogales et al., 2008). In psoriasis lesions, keratinocytes are activated and proliferate at
substantially faster rates than normal keratinocytes (Iizuka, 1994). IL-17A has been shown to induce cytokine and chemokine production by keratinocytes, whereas IL-22 induces antimicrobial peptide production by keratinocytes and is a direct potent stimulator of keratinocyte growth (Nogales et al., 2008).

More recently, topical treatment with imiquimod (IMQ), a ligand for Toll-like receptor (TLR) 7 and TLR8, was reported as a novel mouse model for psoriasis-like skin inflammation inducing acanthosis, parakeratosis, and a mixed inflammatory infiltrate (van der Fits et al., 2009). IMQ is used in humans for topical treatment of genital and perianal warts caused by human papillomavirus, actinic keratosis, and superficial basal cell carcinoma. Clinical application of IMQ can induce psoriasis or exacerbate the disease in patients with well-controlled psoriasis (Fanti et al., 2006). In mice, IMQ-induced dermatitis development is critically dependent on the IL-23/IL-17 axis (van der Fits et al., 2009). Moreover, administration of IL-23 into mouse skin results in the epidermal recruitment of CCR6+ cells, a subtype of γδ T cells that produces IL-17A (Mabuchi et al., 2011), and IMQ-treated mice show increased numbers of IL-17-producing γδ T cells (Roller et al., 2012). However, it remains unclear how these IL-17-producing γδ T cells are induced in the IMQ-treated skin, specifically in regard to the involvement of skin resident–dendritic cells (DCs).

Recent immunological studies have demonstrated that there are Langerhans cells (LCs), Langerin+ dermal DCs (dDCs), and Langerin+ dDCs in murine skin (Ginhoux et al., 2007). LCs are a subset of skin-resident DCs that form a dense network in the epidermis (Romani et al., 2010; Egawa and Kabashima, 2011). The role of LCs has been well studied in contact hypersensitivity, the induction of which is a classical technique permitting the examination of cutaneous adaptive immune responses (Honda et al., 2012; Kaplan et al., 2012). Transgenic mice with an inducible or constitutive ablation of LCs showed enhanced contact hypersensitivity (Kaplan et al., 2005), whereas other LC ablation mouse models developed reduced or unaffected contact hypersensitivity (Bennett et al., 2007).

In this study, we explored the role of LCs in a mouse model in which psoriasis-like skin inflammation is triggered by topical application of IMQ. LC-depleted mice showed reduced ear swelling and decreased levels of Th17-related cytokines in IMQ-treated skin regions. LC-depleted IMQ-treated skin showed decreased IL-17A production and expression of CCR6 from skin γδ T cells. We also found that LCs produce IL-23 in IMQ-applied skin regions and that IL-23-producing cells were reduced at draining lymph nodes after IMQ application. These results suggest that LCs are required for generation of psoriasis-like lesions in response to IMQ in mouse models.

RESULTS
LC-depleted mice show reduced IMQ-induced psoriasis-like skin inflammation

Consistent with a previous report (van der Fits et al., 2009), topical application of IMQ on the skin of wild-type mice for 5 days induced psoriasis-like lesions featuring redness, scales, and crust formation. To evaluate the function of LCs in the pathogenesis of psoriasis, LCs were depleted with diphtheria toxin (DT) in Langerin-DTR–knocked-in mice. LCs were completely ablated from the epidermis within 24 h after injection of DT. We also investigated whether Langerin+ dDCs are involved in IMQ-induced psoriasis-like skin inflammation. It has been reported that Langerin+ dDCs recolonize in the dermis in 5 days or less after DT injection (Bursch et al., 2007). Ten days after DT injection, LCs are still absent in the epidermis but dDCs are present (Supplementary Figure 1a online). IMQ cream was applied on both ears of LC/Langerin+ dDC–depleted mice or on ears of LC-depleted mice for 5 consecutive days. When the absolute number of each LC/DCC subset per skin specimen was calculated, the subset of Langerin+ dDCs absolutely disappeared by IMQ application, but that of LCs and Langerin– dDCs was not affected (Supplementary Figure 1a, b, d, e, f online). In addition, the IMQ application increased the number of Gr1+ cells regardless of the depletion of LCs or Langerin+ dDCs (Supplementary Figure 1c, g online). Ear thickness was measured on day 6 after the start of IMQ application. Compared with IMQ-treated non-DT control mice, LC-depleted and IMQ-treated mice showed milder increases in ear thickness (Figure 1a and b). To exclude the involvement of newly differentiated LCs, we applied a daily DT treatment and IMQ application for 5 consecutive days to ensure efficient ablation of LC throughout the course of disease. A daily DT treatment and IMQ application did not affect the result of the ear thickness change (Supplementary Figure 2 online). Microscopic evaluation of skin sections from mice treated with IMQ revealed changes characteristic of psoriasis-like lesions, such as acanthosis, parakeratosis, desquamation, and dermal infiltration of immune cells. In contrast, IMQ administration had qualitatively and quantitatively fewer effects on Langerin+ cell (including LCs and Langerin+ dDCs)–depleted mice (IMQ application 1 day after DT treatment; Day 1) or LC-depleted mice (IMQ application 10 days after DT treatment; Day 10) (Figure 1a). Hyperproliferation of keratinocytes in IMQ-induced psoriatic regions was also monitored by immunohistochemical staining of Stat3 and Ki67. IMQ-treated skin keratinocytes of control C57BL/6 mice were highly positive for Stat3 and Ki67 as compared with IMQ-treated Langerin+ cell–depleted (Day 1) or LC-depleted mice (Day 10) (Figure 1a). These findings indicate that LCs are critical for the induction of IMQ-induced psoriasis-skin inflammation.

LC-depleted skin shows decreased IL-12/23p19, IL-12/23p40, IL-17A, IL-22, and TNF-α mRNA induction by IMQ

Recently, the roles of Th1 and Th17 cytokines in the pathogenesis of IMQ-induced psoriasis-like skin inflammation have been demonstrated (van der Fits et al., 2009). To characterize immune responses in IMQ-treated skin of LC-depleted mice, the expression of cytokines was measured by real-time PCR. IMQ cream was applied on both ears of LC/Langerin+ dDC–depleted mice or only LC-depleted mice for 5 consecutive days. On day 6, the mice were killed and mRNA from both ears was extracted. In control C57BL/6j mice,
application of IMQ induced IL-12/23p19, IL-12/23p40, IL-17A, IL-22, and TNF-α mRNA expression (Figure 2a–e), whereas expression of IL-12p35 mRNA was not detected by real-time PCR (data not shown). In LC-depleted and IMQ-treated mice, the induction of IL-12/23p19, IL-12/23p40, IL-17A, IL-22, and TNF-α mRNAs was suppressed on both day 1 and day 10 (Figure 2a–e). As IL-23 maintains IL-17-producing cells, the above findings suggest that IMQ stimulates the production of IL-23, but not of IL-12, in LCs, which in turn promotes Th17 in IMQ-treated skin lesions.

IMQ induces IL-23 production from LCs after application of IMQ

Recently, it was postulated that IL-23, a cytokine driving the development of IL-17- and IL-22-producing Th17 cells, is functionally involved in the pathogenesis of psoriasis. Expression of IL-23 is increased in psoriasis lesional skin (Lee et al., 2004), and increased numbers of Th17 cells are present (Lowes et al., 2008). Application of IMQ leads to hyperplasia of the epidermis. In the same fashion, intradermal injection of IL-23 in mouse skin results in erythema, a mixed inflammatory infiltrate, and epidermal hyperplasia (Chan et al., 2006). However, the source of IL-23 in psoriasis is still unclear. To investigate the source of IL-23 in IMQ-treated psoriasis-like skin, we first examined the expression of TLR7, a receptor for IMQ, in the skin-composing cells. In the steady state, LCs and Langerin+ dDCs expressed TLR7, whereas γδ T cells, Gr1+ cells, and keratinocytes were TLR7 negative irrespective of IMQ application (Figure 3a and b). Recently, it has been reported that the IMQ-containing
IMQ was applied to non-DT-treated mice, increased expression of TLR7-mRNA; however, only Langerin\(^+\) dDCs showed high expression of TLR7-mRNA (Figure 3c). As shown in Supplementary Figure 1b online, Langerin\(^+\) dDCs disappeared from IMQ-applied skin and expression of TLR7 from dDCs could not be measured. We next examined the IL-23-producing cells in the IMQ-applied skin by means of flow cytometry. IMQ-applied skin contained more IL-23-producing cells than did control mice (Figure 3d and e). To identify which cells produced IL-23, IMQ applied skins were analyzed by flow cytometry stained by EpCAM, Langerin, \(\gamma\delta\)TCR, and Gr1. Only both EpCAM- and Langerin-positive cells or LCs produced IL-23 after IMQ application to the skin (Figure 3f). In control skin, \(\sim 10\%\) or less of LCs produce IL-23. However, \(\sim 80\%\) of LCs produced IL-23 in IMQ-applied skin (Figure 3g). These results suggest that IMQ administration induces IL-23 production in LCs.

**Decreased infiltration of IL-17A-producing CCR6\(^+\) \(\gamma\delta\) T cells in LC-depleted skin after IMQ application**

It has been reported that IL-23-responsive dermal \(\gamma\delta\) T cells are the major IL-17 producers in the skin (Cai et al., 2011), and were revealed to express CCR6 in a murine model of psoriasis (Mabuchi et al., 2011). We next investigated the production of IL-17A and CCR6 expression from \(\gamma\delta\) T cells in IMQ-treated skin. IMQ cream was applied on both ears of LC/Langerin\(^+\) dDC-depleted mice or on ears of only LC-depleted mice for 5 consecutive days. On day 6, epidermal-cell and dermal-cell suspensions were prepared from IMQ-treated skin. When IMQ was applied to non-DT-treated mice, increased numbers of IL-17A-producing \(\gamma\delta\) T cells were detected in the epidermis, which were reduced in LC-depleted mice (Figure 4a and b). On the other hand, similar numbers of IL-17A-producing \(\gamma\delta\)TCR mid\(^+\) cells were observed in the dermis of IMQ-treated skin regardless of the presence of LCs (Figure 4c and d). These IL-17A-producing \(\gamma\delta\)TCR mid\(^+\) cells were V\(\gamma\)4 and CCR6 positive (Supplementary Figure 3 online). We also analyzed the IL-17A positive cells in the CD4\(^+\) T-cell subset. The number of IL-17A-producing CD4\(^+\) T cells increased in the IMQ-treated epidermis and dermis. The number of IL-17A-producing CD4\(^+\) cells was decreased in the dermis of LC- or Langerin\(^+\) dDC-depleted skin (Figure 4e–h). These data indicate that LCs induce infiltration of IL 17A-producing CCR6\(^+\) \(\gamma\delta\) TCR mid\(^+\) cells into epidermis and psoriasis-like inflammation after IMQ application.

**LC-depleted and IMQ-treated mice show decreased numbers of IL-12/23p40-positive cells and IL-17A-producing \(\gamma\delta\) T cells at regional lymph nodes**

To analyze IL-23-producing cells and IL-17A-producing cells in the draining lymph nodes, we next performed flow cytometric analysis of regional lymph node cells at the end of a 5-day course of IMQ treatment. Compared with control mice, mice that had just received 5 days of IMQ treatment exhibited increased numbers of IL-23-producing major histocompatibility complex (MHC) class II\(^+\) cells in the draining lymph nodes. On the other hand, Langerin\(^+\) cell–depleted or LC-depleted mice did not show any significant change in the number of IL-23-producing cells (Figure 5a and c). These IL-23-producing MHC class II\(^+\) cells were positive for EpCAM and Langerin, whereas IL-23-non-producing MHC class II\(^+\) cells were negative for EpCAM and Langerin (Figure 5b). Surprisingly, almost all LCs in draining lymph nodes produce IL-23. These results indicate that IL-23-producing MHC class...
IL-17A-producing γδ T cells was significantly increased in the IMQ-treated mice, and decreased in the Langerin+ cell–depleted or LC-depleted mice (Figure 5d and e). We also studied IL-17A-producing CD4+ T cells. The number of IL-17A-producing CD4+ T cells was increased by treatment with IMQ, which was decreased by depletion of Langerin+ cells or only LCs (Figure 5f and g). Of note, the total number of IL-17A-producing CD4+ T cells was less than a tenth of IL-17A-producing γδ T cells. Thus, γδ T cells were the main source of IL-17A in the draining lymph nodes of IMQ-treated mice, and LC abrasion led to reduced numbers of IL-17A-producing γδ T cells. These results suggest that LCs are required for the IL-23 pathways that lead to IL-17 production by γδ T cells in the draining lymph nodes following IMQ treatment.

IL-23 from skin-derived LCs induces psoriatic skin inflammation

On the basis of the indication that IL-23 from LCs may increase IL-17 production by γδ T cells in the draining lymph nodes, we further explored a role of IL-23 from LCs by transplanting bone marrow (BM) cells of IL-23-deficient (IL23KO) mice into X-ray-irradiated wild-type (WT) mice or vice versa. As LCs are resistant to X-ray irradiation (Merad et al., 2004), IL-23-producing LCs existed in the skin of X-ray-irradiated WT mice. Two months after transplanting BM cells of WT or IL23KO mice into X-ray-irradiated WT or IL23KO mice, mice received topical applications of commercially available IMQ cream on both ears for 5 consecutive days. Ear thickness was measured on day 6 after the start of IMQ application. Compared with IMQ-treated WT mice in which both donors and recipients were WT, IL23KO mice, in which both donors and recipients were IL23 KO mice, showed a decrease in ear thickness (Figure 6). When transplanting BM cells of WT mice into X-ray-irradiated IL23KO mice ear thickness significantly decreased, whereas ear thickness did not show any change compared with WT mice when transplanting IL23KO BM cells into irradiated WT mice (Figure 6). In Figure 6, WT control showed less swelling than

Figure 3. LCs produce IL-23 after application of IMQ. IMQ cream was applied to mice ears for 5 consecutive days and whole-skin suspension was analyzed by real-time PCR. (a) TLR7 was expressed on Langerin−/dDCs by application of IMQ. (b, c) Whole-skin suspension was stained with PE-conjugated IL-23 for flow cytometry. LC-depleted skin did not show an increase in the number of IL-23-producing cells. Data points represent individual frequencies. (d, e) CD45+ MHC class II–positive cells were gated by EpCAM and Langerin. Other CD45-positive cells were gated by γδTCR and Ly-6G (Gr1). Only LCs showed IL-23 production by IMQ application (f, g). Data are representative of four independent experiments and each group consisted of more than five mice. dDCs, dermal dendritic cells; DT, diphtheria toxin; IMQ, imiquimod; KCs, keratinocytes; LCs, Langerhans cells; MHC, major histocompatibility complex; NS, not significant; TLR, Toll-like receptor.
mice shown in Figure 1b. This difference was possibly due to the procedures of X-ray irradiation and BM transplantation. WT mice in Figure 6 were X-ray irradiated once and then transplanted with WT BM cells. Hence, total numbers and distribution of lymphocytes in BM-transplanted WT mice in Figure 6 are supposed to be different from those in normal WT mice shown in Figure 1. Taken together, these data indicated that IL-23 from LCs has a critical role in the IMQ-induced skin inflammation, possibly that of modulating IL-17-producing \( \gamma \delta \) T cells in the draining lymph nodes.

**DISCUSSION**

In this report, we analyzed the pathomechanisms of psoriasisiform dermatitis caused by IMQ in Langerin-DTR–knocked-in mice. The IMQ-treated mouse skin resembles human plaque-type psoriasis with respect to erythema, skin thickening, scaling, and epidermal alterations (acanthosis, parakeratosis), but these findings were not observed in a LC-depleted state. In addition, LC-depleted mice showed decreased levels of Th17-related cytokines in IMQ-treated skin lesions. Moreover, the IMQ-treated skin of LC-depleted mice showed a decreased
number of IL-17A-producing CCR6<sup>+</sup> γδ<sup>+</sup> T cells. These results suggest that LCs are required for the development of murine psoriasis-like lesions induced by IMQ.

The importance of T cells in the pathogenesis of psoriasis is supported by the response of patients to treatment with agents that affect T-cell functions, such as cyclosporine. Several cytokines have been implicated in the pathogenesis of psoriasis, including TNF-α, IFN-γ, IL-12, IL-17, IL-22, and IL-23 (van der Fits <i>et al.</i>, 2009). It has recently been reported that IL-23 expression is elevated in psoriatic skin lesions (Lee <i>et al.</i>, 2004), IL-23 induces psoriasis-like dermatitis (Chan <i>et al.</i>, 2006), polymorphisms in IL-23p19, IL-12/23p40, and IL-23R are associated with the development of psoriasis (Capon <i>et al.</i>, 2007), and antibody against IL-12/23p40 can ameliorate psoriasis in human skin (Krueger <i>et al.</i>, 2007). IL-23 is essential for the differentiation and survival of IL-17-producing cells (Diveu <i>et al.</i>, 2008), and IL-17 expression is augmented in psoriatic skin. In addition, anti-IL-17 antibody administration ameliorates psoriasis, indicating that IL-17 has an important role in the pathogenesis of psoriasis (Di Cesare <i>et al.</i>, 2009).
IMQ application has been shown to induce IL-17A-producing γδ T cells in the regional lymph nodes (Roller et al., 2012) and IL-23p19 mRNA in mice (van der Fits et al., 2009). However, the source of IL-23 in IMQ-treated areas remained unclear. Here, we clearly demonstrated that LCs were the major DC subset to produce IL-23 in the IMQ-treated skin, and that IL-23 induced the infiltration of IL-17-producing Vγ4+ γδTCR mid⁺ cells to the epidermis. These findings are consistent with the recent studies, which demonstrated that LCs are required for induction of IL-17-producing cells in Candida albicans-infected skin lesions (Igyarto et al., 2011). Recently, Wohn et al. (2013) reported that Langerin-negative dDCs, rather than LCs, are responsible for IMQ-induced psoriatic plaque formation. Their conclusion was seemingly contrary to ours in which LCs are indispensable for IMQ-induced psoriatic plaque formation. These two apparently contradictory experimental results using Langerin-DTR mice may be caused by the different IMQ-induction methods used by the two laboratories. Wohn et al. (2013) applied IMQ on the back of mice, whereas we painted IMQ on the ears of mice. In human beings, psoriasis is predisposed to take place on knees and elbows. This predisposition may reflect the difference in nature and the number of LCs and Langerin-negative dDCs depending on the body sites. Moreover, time course of DT application is different between the two laboratories. Wohn et al. applied DT on day −3 and painted IMQ, whereas we applied DT on day −1 and applied IMQ. The time course of the DT application affects the number of LCs as shown in Supplementary Figure 1 online. These differences in the IMQ-applied areas and the timing of DT application possibly gave rise to the different results. It remains unclear whether IMQ directly acts on LCs in vivo. Recently, it has been reported that Aldara cream, which contains IMQ, induces inflammation largely independently of TLR7, and keratinocyte death and IL-1 release also occur in response to the vehicle cream in the absence of IMQ (Walter et al., 2013). However, the role of IMQ for psoriatic skin inflammation is still unclear. Intriguingly, the expression level of TLR7 is rather high in dermal DCs compared with LCs. We examined the production of IL-23 from LCs or dermal DCs by stimulating them with IMQ in vitro. However, we could detect no increase in IL-23 production from LCs or dermal DCs (data not shown). Therefore, there may be other pathways besides TLR7 that are involved in upregulating IL-23 production from LCs. Therefore, it should be clarified whether IMQ directly acts on LCs to produce IL-23 through TLR7 or indirectly induces LC activation possibly through other immune cells, such as plasmacytoid DCs or keratinocytes in the future.

Recently, CCR6 was shown to be a marker of peripheral IL-17A-expressing γδ T cells (Cua and Tato, 2010). These γδ T cells are observed following IL-23 intracutaneous injections (Mabuchi et al., 2011). We now demonstrate that the accumulation of IL-17A-producing CCR6⁺ γδ T cells in the epidermis and the draining lymph nodes is decreased in the LC-depleted mice. This finding supports the hypothesis that LCs are the main source of IL-23 for the induction of IL-17A-producing CCR6⁺ γδ T cells in the IMQ-induced dermatitis model. It was reported that topical application of IMQ induces migration of LCs from treated skin into the draining lymph nodes (Suzuki et al., 2000). In line with this, we demonstrated the accumulation of IL-23-producing LCs in the draining lymph nodes after IMQ application and the induction of IL-17A-producing CCR6⁺ γδ T cells in the lymph node cell suspensions by IL-23. Therefore, LCs may function in two ways. First, IMQ treatment induces the accumulation of IL-23-producing LCs in the draining lymph nodes for the induction of IL-17A-producing γδ T cells. These γδ T cells migrate to the epidermal area of the IMQ-treated skin, possibly via CCR6, where LCs may enhance the functions or survival of IL-17A-producing γδ T cells for the development of psoriatic skin lesions.

Although IMQ-induced psoriasis-like skin lesions in mice share some clinical and histological characteristics with human psoriasis (Chan et al., 2006), there are several differences between them. Most notably, the human psoriatic skin lesion contains predominantly γδ T cells and not many γδ T cells. However, it has recently been reported that dermal IL-17A-producing γδ T cells were significantly
increased in human psoriatic skin (Cai et al., 2011), and that production of the local chemokine CCL20, a ligand for CCR6, is also increased in human psoriatic skin (Kryczek et al., 2008). The increased frequency of γδ T cells in psoriatic skin suggests that γδ T cells may be crucial in the development of psoriasis not only in mice but also in humans.

In conclusion, we demonstrate that IMQ treatment induces IL-23 production by LCs in mice, and that LC depletion resulted in attenuated IMQ-induced psoriasis-like dermatitis with decreased numbers of infiltrating IL-17A-producing γδ T cells. In addition, these IL-17A-producing γδ T cells were induced by IL-23 in the draining lymph nodes. These findings suggest that LCs seem to be the main cutaneous DC subset in the development of IMQ-induced psoriasis.

MATERIALS AND METHODS

Mice and treatments

Seven 10-week-old C57BL/6J mice were obtained from SLC (Shizuoka, Japan). Mice were maintained on a 12-hour light/dark cycle under specific pathogen-free conditions. Langerin DTR–knocked-in mice were generated (Kissenpfennig et al., 2005). To deplete Langerin+ cells, mice were injected intraperitoneally with DT (200 ng each; Sigma-Aldrich, St Louis, MO). IL23KO mice were obtained from Keio University (Hasegawa et al., 2013). Protocols were approved by the Institutional Animal Care and Use Committee of the University of Occupational and Environmental Health. Mice at 8–11 weeks of age received a daily topical dose of 62.5 mg of commercially available IMQ cream (5%) (Mochida Pharmaceutical, Tokyo, Japan) on both ears for 5 consecutive days.

Generation of bone marrow chimera mice

To generate bone marrow chimera mice, recipient WT C57BL/6J mice or IL-23 KO mice (C57BL/6J background) underwent 9 Gy total body irradiation on day –1, and received bone marrow single-cell suspensions from WT C57BL/6J mice or IL-23 KO mice on day 0 intravenously through the tail vein.Recipient mice were treated with oral antibiotics for 2 months after transplantation.

Culture medium

RPMI-1640 (Gibco BRL Life Technology, Grand Island, NY) was supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 5 × 10–5 M 2-mercaptoethanol, 10–5 M sodium pyruvate, 25 mM HEPES, 1% nonessential amino acids, 100 units/ml penicillin, and 100 µg/ml streptomycin (all from Gibco).

Preparation of skin suspensions

Skin sheets from mouse ears were floated in 0.2% of trypsin in PBS (pH 7.4; Sigma-Aldrich) for 30 min at 37 °C as described previously (Tokura et al., 1994). The epidermis was separated from the dermis with forceps in PBS supplemented with 10% fetal calf serum. Both epidermis and dermis were minced and incubated for 1 h at 37 °C in PBS with collagenase II (Sigma-Aldrich). The obtained cells were filtered through a 40-µm filter.

Flow cytometry

Cells were immunostained with various combinations of fluorescence-conjugated mAbs and analyzed with a FACSCanto flow cytometer (BD Biosciences, San Diego, CA) and FlowJo software (Tree star, Ashland, OR). The expression levels of cell surface or intracellular molecules and intracytoplasmic cytokines were analyzed using the following antibodies: Alexa Fluor 488-conjugated anti-γδTCR (clone; eBioGL3), PE-conjugated anti-IL-12/23p40 (clone; C17.8), IL-17A (clone; eBio17B7), IL-22 (clone; 1H8PWSR), TNF-α (clone; MP6-XT22); PerCP-conjugated CD45 antibody (clone; 30-F11); biotin-conjugated anti-mouse CD207 (Langerin) (clone; eBioL31); and PE-Cy7-conjugated anti-Ly-6G (clone; RB6-8C5) (eBioscience, San Diego, CA) and anti-epithelial cell adhesion molecule (EpCAM, clone; G8.8), Vγ4 (clone; UC3-10A6), Vγ5 (clone; 536) (Biolegend, San Diego, CA), and anti-TLR7 antibody (clone; IMG4G6) (IMGENEX, San Diego, CA); APC-conjugated CCR6 antibody (clone; 140706) (BD Biosciences). All mAbs were used at a concentration of 1–5 µg per 106 cells, and each incubation was performed for 30 min at 4 °C, followed by two washes in PBS supplemented with 5% fetal calf serum and 0.02% sodium azide. Viable cells were identified by 7-AAD uptake. For intracellular cytokine staining, cells were incubated in the presence of Goldi Stop (BD Biosciences) for 2 hours. Intracytoplasmic cytokines were detected in permeabilized cell suspensions using a BD cytofix/cytoperm Plus Kit (BD Biosciences).

Histology and immunohistochemistry

Paraffin-embedded skin specimens were prepared using routine methods. The sections were stained with H&E. For immunohistochemistry, sections were deparaffinized and hydrated by washing sections in xylene followed by graded alcohol series. To unmask antigens, sections were incubated in 10 mM citric acid (pH 6) at 95 °C for 30 min. Sections were blocked with normal serum for 60 min at room temperature followed by incubation with primary antibodies (Stat3, Ki67; DAKO, Glostrup, Denmark) overnight at 4 °C. Samples were washed and incubated for 60 min with secondary antibodies.

Real-time PCR

Total RNA was extracted from skin with the SVTotal RNA isolation system (Promega, Madison, WI) according to the manufacturer’s protocol. Murine IL-23p19, IL-12/23p40, IL-17A, IL-22, and TNF-α gene expression levels were quantified in a two-step RT-PCR. cDNA was reverse transcribed from total RNA samples using the TaqMan RT reagents (Applied Biosystems, Foster City, CA). Target gene expression was quantified using TaqMan Gene Expression Assay (Applied Biosystems) in the ABI PRISM 7000 sequence detection system (Applied Biosystems). The probe was synthesized with VIC as a reporter dye and Tamra as the quencher dye. The forward primer, reverse primer, and Taqman probe were purchased from Applied Biosystems (IL-23p19: Mm01160011_g1, IL-12/23p40: Mm00434174_m1, IL-17A: Mm00439619_m1, IL-22: Mm00444241_m1, TNF-α: Mm00443258_m1, TLR7: Mm00446590_m1). As an endogenous control for these PCR quantification studies, GAPDH gene expression was measured using the TaqMan rodent GAPDH control reagents (Applied Biosystems). Gene expression was calculated using the ΔΔCt method.

CONFLICT OF INTEREST

The authors state no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid
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