ORIGINAL ARTICLE

Effects of Imiquimod on Hair Follicle Stem Cells and Hair Cycle Progression

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Topical imiquimod (IMQ) application is widely used as a model for psoriasiform-like skin inflammation in mice. Although the effects on the epidermis are well characterized, it is unclear how IMQ affects hair follicles and cycling. Here we investigated how IMQ affects hair follicle stem cells and whether the timing of IMQ application influences the immune infiltrate. Our results show that IMQ application at mid and late telogen activated hair follicle stem cells leading to premature hair cycle entry (anagen), which was accompanied by massive infiltration of inflammatory macrophages and gamma delta T cells, whereas the number of the respective resident populations decreased. Interestingly, high resident macrophage numbers were present in Rag2$^{-/-}$ mice and were maintained after IMQ treatment explaining why IMQ-induced anagen was reduced. This could be rescued after macrophage depletion suggesting that resident macrophages inhibit whereas inflammatory infiltrating macrophages stimulate hair follicle stem cell activation. The expression of the anagen-inhibiting factor BMP-4 was reduced by IMQ treatment as well as the activating factors Wnt showing that IMQ-induced hair follicle stem cell activation occurs by a Wnt-independent mechanism involving inflammatory cytokines such as CCL2 and TNF-α. On the basis of our findings, we recommend conducting experiments with IMQ during mid and late telogen as the biggest differences in immune cell composition are observed.

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INTRODUCTION

Imiquimod (IMQ) is a synthetic immune-response modifier acting as a toll-like receptor 7/8 agonist (Hemmi et al., 2002). In humans, the 5% IMQ cream formulation Aldara is frequently used for the treatment of genital warts, actinic keratosis, and superficial basal cell carcinomas with very high response rates (Bath-Hextall et al., 2014; de Macedo et al., 2015; Gollnick et al., 2005; Peris et al., 2014). In mice and humans, topical application of IMQ results in the activation of the interfollicular epidermis (IFE), upregulation of major histocompatibility complex-II (MHC-II) on keratinocytes, hyperproliferation, and parakeratosis (Flutter and Nestle, 2013), as well as a strong skin inflammation characterized by the infiltration of several immune cells such as plasmacytoid dendritic cells (Drobits et al., 2012; Kalb et al., 2012; Palamara et al., 2004), mast cells (Heib et al., 2007), monocytes (Terhorst et al., 2015), and T cells and activation of the IL-23/IL-17/IL-22 axis (Heib et al., 2007; Riol-Blanco et al., 2014; van der Fits et al., 2009). Langerhans cells (LCs) and gamma delta T cells (gd T cells) are activated in response to IMQ, leading to emigration of LCs (Flacher et al., 2014; Palamara et al., 2004; Pantelyushin et al., 2012). In the past few years, IMQ has been widely used by several researchers as a model for psoriasiform-like skin inflammation (Perera et al., 2014; Schonthaler et al., 2013; Tortola et al., 2012; van der Fits et al., 2009), as, similar to psoriasis, epidermal thickening is induced by IL-22 released from infiltrating immune cells (Van Belle et al., 2012).

Despite IMQ's known effects on the IFE, its effects on hair follicle (HF) and hair follicle stem cells (HFSCs) are unknown. The HF is a highly organized structure and is subdivided into the infundibulum (IF)/junctional zone at the top, the isthmus, the bulge (Bu), and the lowest part, the secondary hair germ (2°HG) (Amberg et al., 2015; Arwert et al., 2012; Jaks et al., 2010). Each of these regions of the HF harbors defined stem cells, which under homeostatic conditions serve to renew their compartment. Following HF morphogenesis after birth, renewal of the HF occurs in a largely synchronized manner (hair cycle) in the mouse until 90 days after birth and is divided into three stages, the resting phase (telogen), active phase (anagen), and destructive phase (catagen). Toward the end of telogen, quiescent stem cells from the 2°HG and later from the whole Bu become activated and proliferate to initiate anagen to generate new hair (Blanpain and Fuchs, 2009; Fuchs, 2007). At the end of anagen, progenitor cells and transit-amplifying cells stop proliferating and undergo apoptosis (catagen), leading to HF shortening and entrance into the quiescent telogen phase (Greco et al., 2009; Ito et al., 2004; Mesa et al., 2015; Tumbar et al., 2004).

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Abbreviations: Bmp, bone morphogenetic protein; Bu, bulge; DETC, dendritic epidermal T cells; gd T cells, gamma delta T cells; HF, hair follicle; 2°HG, secondary hair germ; HFSC, hair follicle stem cell; IF, infundibulum; IFE, interfollicular epidermis; IMQ, imiquimod; LCs, Langerhans cells; MHC-II, major histocampatibility complex-II; P, postnatal day; TNFα, tumor necrosis factor-α

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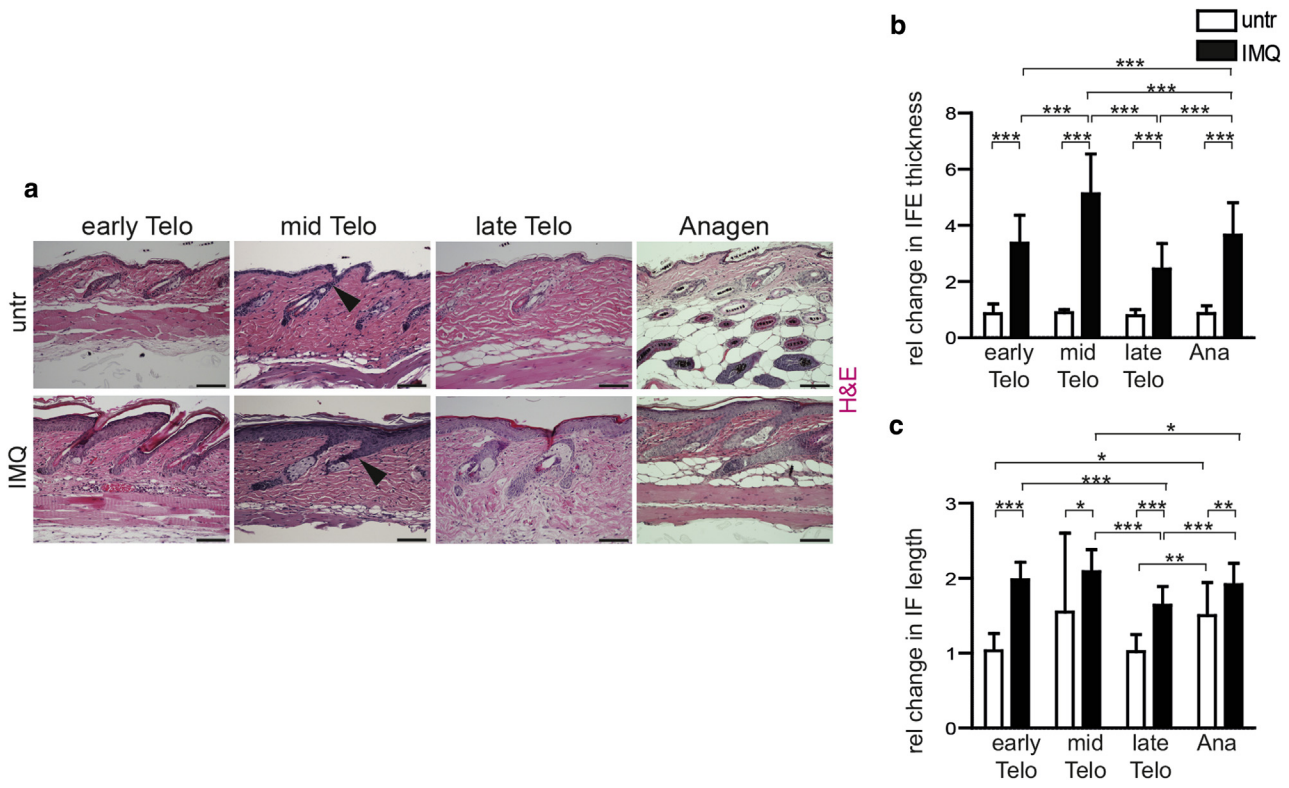

Figure 1. Imiquimod (IMQ) treatment during late telogen induces hair follicle stem cell activation. (**a**) Representative images of hematoxylin and eosin (H&E) stainings of back skin sections from mice treated with or without IMQ at indicated time points. Black arrows indicate the infundibulum (IF). Scale bar: 100 μm. Quantification of (**b**) the thickness of the interfollicular epidermis (IFE) and (**c**) the length of the IF at indicated time points and treatments.

HFSC activity is highly controlled, not only by stem cell-derived factors, which can be either inhibitory or activating, but also by immune cell- or other dermal cell-derived factors, such as Bmp2, Bmp4, Wnt10, Dkk1, follistatin, noggin, Sfrp4, and TNFα (Botchkarev, 2001; Castellana et al., 2014; Chen et al., 2014, 2015; Plikus et al., 2008). Because IMQ leads to infiltration of different immune cell populations into the skin, it is very likely that they affect HFSC and hair cycle progression. Recently, it was shown that HF cells are responsive to immune cells, such as macrophages, which under steady-state conditions produce factors either inhibiting (e.g., Bmp4) or activating (e.g., Wnts) HFSCs, depending on the telogen stage (Castellana et al., 2014). Upon depletion of skin-resident macrophages by clodronate-mediated apoptosis, the activating factors were released and HFs entered anagen. Also dendritic epidermal T cells (DETC) and dermal mast cells were shown to be present at different concentrations during the distinct hair cycle stages, emphasizing that there is crosstalk between HFs and immune cells (Maurer et al., 1995, 1997; Paus et al., 1994).

In this study, we aimed at investigating the effects of IMQ on HF and whether IMQ-induced immune responses are affected during the different hair cycle stages. Because IMQ is a widely used model of skin inflammation to study psoriasis-like skin diseases, these parameters are important to consider when planning experiments with IMQ to guarantee and increase reproducibility and avoiding variances between different laboratories.

RESULTS

IMQ treatment during late telogen induces HF stem cell activation and premature hair cycle entry

Experiments with topical IMQ are usually performed with 6- to 12-week-old mice when the second and third hair cycle occur. We therefore investigated whether IMQ application results in different effects on the skin when applied at different stages of the hair cycle. Wild-type C57BL/6 mice were shaved on the dorsum and 1 day later topically treated with Aldara for 7 consecutive days starting either at postnatal day 50 (P50) (early telogen), at P57 (mid telogen), at P65 (late telogen), or at P80 (anagen) (see Supplementary Figure S1a online). All IMQ-treated mice responded to treatment irrespective of the hair cycle stage, as indicated by the increase in spleen size, which results from a systemic inflammatory response that is typically observed in mice after Aldara treatment (see Supplementary Figure S1b) (Palamara et al., 2004). IFE thickness and IF length (taken as a readout for skin inflammation) were increased to various degrees by IMQ treatment during the different hair cycle stages (Figure 1a–c). The increase in IFE diameter was lowest in mice receiving IMQ treatment in late telogen, whereas it was comparable during early telogen and anagen (Figure 1b). IF length already revealed differences in untreated mice and was significantly increased in anagen when compared with the other stages (Figure 1c).

We next analyzed whether IMQ also activates 2°HG and HF Bu stem cells by performing immunofluorescence staining for the epidermal-dermal boundary marker β4