Effects of depilation methods on Imiquimod-induced skin inflammation in mice

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Abbreviations

IMQ – Imiquimod
HF – hair follicle
IF - Infundibulum
IFE – interfollicular epidermis
DETC – dendritic epidermal T cells
Imiquimod (IMQ) acts as a Toll-like-receptor-7/8 agonist and its topical application is used as a model for proriasiform skin inflammation. In mice and humans skin inflammation is characterized by infiltration of several immune cells (Drobits et al., 2012; Kalb et al., 2012; Palamara et al., 2004) and activation of the IL-23/IL-17/IL-22-axis (Riol-Blanco et al., 2014; vanderFits et al., 2009). MHC-II upregulation on keratinocytes, hyperproliferation, parakeratosis and increased vascularization are also observed recapitulating psoriatic hallmarks (Flutter and Nestle, 2013).

Hair removal is essential before IMQ is applied to the murine skin. Different laboratories use various depilation strategies like razor shaving, depilation cream or wax stripping. Shaving does not affect hair follicles (HF), whereas wax stripping results in depletion of HF stem cells leading to anagen entry (Paus et al., 1994; Amberg et al., 2016). The effects of depilation cream have not carefully been investigated yet.

We tested if these three different depilation strategies affect the inflammatory response by applying IMQ for 7 consecutive days on the back skin of late telogen (65 days-old) C57BL/6 mice 1 day after hair removal. All IMQ-treated mice exhibited splenomegaly as previously reported, which was not seen in untreated or vehicle-treated controls (Palamara et al., 2004) (Suppl. Fig. 1a). As it is important to distinguish infundibulum (IF) thickening from rete ridges found in human psoriatic skin, we analyzed IF and interfollicular epidermis (IFE), since both compartments harbor stem cells that can replenish injured epidermis. All treatment groups responded to IMQ with a similar increase in IFE thickening (Fig. 1a, Suppl. Fig. 1b). The IF was already longer in creamed and waxed mice compared to shaved mice and increased further after IMQ application in shaved and creamed, but not in waxed mice (Fig. 1b). These results demonstrate that waxing masks the effects of IMQ on the IF.

We next investigated whether these distinct effects of hair removal methods were specific for IMQ or applied also to other triggers of skin inflammation such as rmIL-23 injection. Similar to IMQ treatment we found acanthosis of the IFE following intradermal rmIL-23 injection.
although waxing induced significantly less IFE thickening than shaving or creaming (Fig.1c, Suppl.Fig.1c). IF length was already increased by PBS injection in creamed and waxed compared to shaved mice (Fig.1d). Except for creamed mice, the IF increased further upon rmIL-23 injection (Fig.1d). These results show that different hair removal methods affect skin morphology in two skin inflammation models and that the effects on IFE are less strong with rmIL-23 when compared to IMQ.

We recently showed that telogen HFs from shaved mice treated with IMQ started to enter anagen (Amberg et al., 2016). Analysis of HF stem cell activation by staining for the proliferation marker Ki67 or the M-phase marker PSer10H3 revealed that telogen HFs of shaved skin remained quiescent, whereas all HFs of creamed or waxed skin had proceeded to mid-anagen (Fig.1e, Suppl.Fig.2a). Since creaming and waxing per se already induced anagen, there was no additional effect induced by IMQ (Suppl.Fig.1b,2a). A significant increase in proliferation was observed in the IFE and IF of all IMQ-treated mice (Fig.1f-g, Suppl.Fig.2b-c). However, skin exposed to depilation cream showed the lowest increase in proliferation upon IMQ treatment (Fig.1f-g). Different depilation methods also affected other psoriatic hallmarks like microvessel density following IMQ treatment, whereby the most pronounced vessel increase was observed in waxed mice by staining with MECA-32 (Suppl.Fig.2d-e).

FACS analysis showed that all groups responded to IMQ with elevated numbers of CD45+ cells (Fig.2a). In contrast to shaving and creaming, waxing did not increase MHC-II+ dendritic cells after IMQ treatment (Suppl.Fig.3a). Surprisingly, dendritic epidermal T cells (DETC) (TCRδhi) were significantly lower in waxed compared to shaved or creamed mice and did not decrease further after IMQ treatment (Fig.2b), showing that waxing alone leads to DETC activation and emigration. In line, TCRδlo cells did not change in waxed mice after IMQ treatment, but where increased in shaved and creamed mice (Fig.2c). TCRβ+ cells were not affected (Suppl.Fig.3b). Depilation cream induced an increase in dermal CD45+ cells in
untreated and IMQ treated mice (Suppl.Fig.3c). Macrophages increased after IMQ treatment in shaved and waxed mice, but remained low in creamed mice (Fig.2d). Waxed mice showed reduced neutrophils and CD11b⁺/Ly6C⁹⁹ monocytes after IMQ treatment, compared to shaved mice (Fig.2e-f). Mast cells were already elevated in waxed compared to shaved and creamed mice, but were less activated in waxed mice despite their numerical increase following IMQ treatment (Suppl.Fig.3d-f).

The changes in the inflammatory infiltrate following rmIL-23 injection under different depilation regimens were similar to what observed with IMQ (Fig.2g-l), except for TCRδ⁻⁰ cells, which were increased in waxed skin (Fig.2i). Moreover, PBS injection alone led to increased macrophages and monocytes in mice depilated by cream or wax, but not by shaving indicating that injection injury is per se an inducer of these cells in combination with the respective depilation method (Fig.2j,l). Furthermore, unlike in IMQ-treated skin, we did not observe differences in monocyte numbers between shaved or waxed IL-23-treated skin (Fig.2l). However, due to the small area of skin treated with IL-23, a separate analysis of epidermis and dermis was not possible, which might account for the differences compared to IMQ treatment. Expression analysis of cytokines typical for psoriasiform skin inflammation by qRT-PCR showed that \textit{IL-6} and \textit{Cxcl-1} were induced in IMQ-treated creamed or waxed skin. A similar trend could be observed for \textit{TNFα}, \textit{IL-23p19} and \textit{IL-17a} (Fig.2m-q).

In summary, hair removal by waxing showed the most prominent differences compared to shaving following IMQ or IL-23 treatment, possibly caused by removal of the stratum corneum through waxing (Mohammed \textit{et al.},2012). This extra irritation likely alters skin homeostasis. Some of these differences were also observed using depilation cream. However, we cannot exclude that ingredients of the depilation cream are responsible for the observed effects. Based on our results, we suggest standardizing the depilation methods between different laboratories using IMQ to allow better comparison of experiments. Our recommendation is to apply topical IMQ on razor-shaved back skin during late telogen. This
condition not only exhibits the greatest differences in HF stem cell activation, but also in immune cell infiltration and psoriatic hallmarks.

Conflict of Interest

The authors declare no conflict of interest.

Animals

C57BL/6 mice were purchased from Jackson Laboratories and kept in the animal facility of the Medical University of Vienna for several generations in accordance with institutional policies and federal guidelines. Animal experiments were approved by the Animal Experimental Ethics Committee of the Medical University of Vienna and the Austrian Federal Ministry of Science and Research.

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References


Figure 1, Amberg et al 2016
Figure 2, Amberg et al 2016