Is CCR6 Required for the Development of Psoriasiform Dermatitis in Mice?

TO THE EDITOR

Intradermal injection of IL-23 and topical application of imiquimod (IMQ) are two widely adopted murine models of psoriasis. Both models result in psoriasiform dermatitis (PsD) in mice that resembles human psoriasis (van der Fits et al., 2009; Zheng et al., 2007). CCR6 is important for epidermal trafficking of IL-17/22—producing cells (Mabuchi et al., 2013) and is required for the development of PsD in the IL-23 injection model since CCR6-deficient knockout (CCR6KO) mice fail to show significant dermal inflammation (Hedrick et al., 2009). Using the IMQ model, however, Cochez et al. (2017) recently reported that CCR6 KO mice were still able to develop psoriatic lesions after application of a specific version of IMQ called Aldara (3M Pharmaceuticals, Maplewood, MN). Although epidermal IL-22 production was decreased, the authors found relatively little morphological change in the skin in CCR6KO versus wild-type (WT) control.

Other recent reports, however, suggest that CCR6 is critical for development of IMQ-mediated PsD. First, Robert et al. (2017) show that monoclonal antibodies to human CCR6 in a knock-in genetic model effectively block Aldara-mediated PsD. Second, using a small molecule antagonist of CCR6, Campbell et al. (2017) found that this inhibitor is effective in both IL-23— and IMQ-mediated PsD models in blocking skin inflammation. In the latter report, a generic version of IMQ (Fougera, Melville, NY) was used.

The possibility exists that the reason for the apparent differences in the importance of CCR6 lies in other ingredients in different commercial preparations of IMQ cream. For example, the Aldara vehicle alone can induce caspase-1-dependent pyroptosis of keratinocyte and concomitant pro-inflammatory cytokines secretion (Walter et al., 2013). To test this possibility and explore the role of CCR6 in the IMQ model, we applied a non-Aldara preparation of IMQ cream to CCR6KO mice and assessed the degree of PsD by histologic and immunologic criteria.

Mice were treated with 5% IMQ cream (Taro Pharmaceuticals, Hawthorne, NY) from day 0 to day 6 on each ear and then euthanized on day 7 (Figure 1a). Ear swelling, a marker for dermal inflammation and corresponding edema (Hedrick et al., 2009), was consistently reduced in CCR6KO versus WT mice throughout the entire course of the experiment (Figure 1a). Ear skin epidermal hyperplasia in the CCR6KO mice was reduced by 80% versus WT mice (Figure 1b—1c). Quantitative reverse transcriptase PCR showed that mRNA expression of IL-17A, IL-17F, and IL-22 was also markedly suppressed in CCR6KO animals (Figure 1d). We have previously shown that γδ low (GDL) T cells account for the majority of production of IL-17A and IL-22 in the IL-23 PsD model (Mabuchi et al., 2011). Flow cytometry

Abbreviations: GDL, γδ low; IMQ, imiquimod; KO, knock out; PsD, psoriasiform dermatitis; WT, wild-type

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revealed an approximately 75% reduction in accumulation of epidermal GDL T cells following topical IMQ in CCR6KO (vs. WT) mice (Figure 2 a, 2b). Moreover, production of IL-17A and IL-22 by GDL T cells was strikingly inhibited (Figure 2 c, 2d). In summary, contrary to data provided by Cochez et al. (2017), every tested histologic and immunologic feature of PsD was significantly reduced in CCR6KO versus WT mice when IMQ (Taro Pharmaceuticals) was used topically instead of Aldara.

Our experiments clearly demonstrate upregulation of key T-helper type 17 cytokines and robust epidermal hyperplasia in skin after exposure to this formulation of IMQ. In contrast to the results obtained using Aldara (Cochez et al., 2017), our experiments demonstrated that CCR6 is required for maximal development of PsD. Of note, not only recruitment, but also function, of GDL T cells was impacted by the absence of CCR6. Although it is not yet clear how CCR6 impacts the expression of cytokines such as IL-17A by GDL T cells, this result using IMQ validates a similar conclusion we made using the IL-23 injection model (Mabuchi et al., 2013).

Others have also observed that IMQ preparations from different sources can lead to differential experimental effects in murine psoriatic models (Alvarez and Jensen, 2016; Luo et al., 2016). Interestingly, toll-like receptor 7 KO mice also showed dermatitis upon Aldara cream application, despite abolished production of IL-23, suggesting other ingredients in IMQ cream induce dermatitis in a toll-like receptor 7-independent manner (Ueyama et al., 2014). Isostearic acid, the second most abundant component in Aldara vehicle, can induce inflammatory activation and may partially account for the differential results (Walter et al., 2013). To test the hypothesis that origins of IMQ cream account for different effects, we performed a direct comparison study between the Aldara and IMQ (Taro Pharmaceuticals) formulations. The results showed IMQ (Taro Pharmaceuticals) induced significantly greater ear swelling in WT mice than CCR6KO mice, while there was no difference between WT mice and CCR6KO mice in terms of Aldara-induced ear swelling (Supplementary Figure S1a, S1b online). Quantitative histologic analysis showed that the epidermis of IMQ (Taro Pharmaceuticals)—treated WT mice was significantly thicker than that of IMQ (Taro Pharmaceuticals)—treated CCR6KO mice on day 5, while there was no difference in epidermal thickness between Aldara-treated WT and CCR6KO mice (Supplementary Figure S1c, S2 online). Epidermal collections of
neutrophils (Munro's microabscesses), a well-recognized feature of human psoriasis, were also notably reduced in a number of CCR6KO mice treated with IMQ (Taro Pharmaceuticals) compared to Aldara-treated WT and CCR6KO mice, suggesting a qualitative reduction in the extent of psoriasiform inflammation in these animals (Supplementary Figure S2).

It should be noted that the specific bacterial and sanitary conditions in different facilities may account for differential responses to IMQ, especially because this agent acts on pathogen recognition receptors (eg, toll-like receptors). While indeed bacteria and the microbiome may influence the development of inflammatory skin disease, as illustrated with atopic dermatitis in dogs (Bradley et al., 2016), our direct side by side comparison of Aldara versus generic IMQ was performed on mice housed under identical conditions.

In conclusion, CCR6 remains crucial for the development of PsD in both the IL-23 and IMQ murine models, although to different degrees, depending on the specific methodology of testing, as suggested by our current results and results of others (Campbell et al., 2017; Cochez et al., 2017; Robert et al., 2017). Notably, in both the IL-23 and IMQ models, the function of GDL T cells in terms of producing IL-17A and IL-22 in vivo is dependent on CCR6 expression. While topical application of IMQ is a convenient and reproducible model for PsD, the preparation and source of IMQ must be considered when interpreting data obtained from these models. Additional validation with additional systems, such as the IL-23 injection model, may be prudent in order to make the most generalizable interpretations.

CONFLICT OF INTEREST
STH and YI have intellectual property rights related to a molecule that blocks the CCR6 receptor. The other authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL
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REFERENCES


IFN-γ Promotes, but Dexamethasone Dissociates, Toll-Like Receptor 2/1-Induced Host Responses in Human Macrophages


TO THE EDITOR

Activation of toll-like receptor (TLR) 2/1 on macrophages is pivotal to the innate defense response against intracellular pathogens, such as mycobacteria (Liu et al., 2006). IFN-γ promotes TLR2/1-mediated host defense against intracellular infection (Edfeldt et al., 2010; Krutzik et al., 2003). Recently, it was also suggested that glucocorticoids (GCs) ready innate immune response by enhancing expression of TLR2 on epithelial and dendritic cells (Homma et al., 2004; Imasato et al., 2002; Rozkova et al., 2006). Given the central role of TLR2 and its binding partner TLR1 in activating macrophage host defense, we asked whether both IFN-γ and GCs can promote TLR2/1 responses in human monocyte-derived macrophages (MDMs).

We stimulated MDMs with IFN-γ dexamethasone, or media alone for 20 to 24 hours and measured TLR1 and TLR2 mRNA by quantitative reverse transcriptase in real time PCR and intracellular as well as cell surface protein expression by FACS. Compared to media control, IFN-γ induced TLR1 mRNA expression (Figure 1a, P < 0.05) and intracellular protein expression (Figure 1b, P < 0.001), but had no effect on surface expressed TLR1 (Figure 1b). In contrast, dexamethasone affected neither expression of TLR1 mRNA (Figure 1a) nor surface protein expression (Figure 1b), and inhibited intracellular TLR1 expression (Figure 1b, P < 0.05). Regarding TLR2, IFN-γ did not significantly affect mRNA expression (Figure 1a), intracellular expression, or surface protein expression (Figure 1b). However, dexamethasone increased TLR2 mRNA expression (Figure 1a, P < 0.01) as well as surface expression (Figure 1b, P < 0.05), but had no effect on TLR2 intracellular expression (Figure 1b). To gain a broader insight into the IFN-γ pathway, such as IκBκ, downregulated the NF-κB pathway (Figure 1c). Furthermore, IFN-γ and dexamethasone oppositely regulated gene expression of key components of the TLR signaling cascade (MYD88, TICAM1, TOLLIP, and RIPK1) and of the NF-κB pathway (IKBKE, NFκB1, RELA, and TBK1) and upregulated the NF-κB inhibitor IκBβ (Figure 1c). Of note, dexamethasone also induced multiple members of the TLR receptor family (TLR2 to TLR5, TLR7 and TLR8), while IFN-γ promoted gene expression of TLR1, TLR3, TLR7, and TLR8 and suppressed TLR5 (Supplementary Figure 1 online). Taken together, our data show that IFN-γ promotes TLR1 expression and key members of the TLR signaling cascade. In contrast, dexamethasone slightly enhances TLR2 expression, yet suppresses key molecules of the TLR signaling cascade.

One important consequence of macrophage stimulation via TLR2/1 is the secretion of cytokines. To investigate whether IFN-γ and dexamethasone treatment of macrophages regulates their TLR2/1 ligand (TLR2/1L)-induced cytokine response, we