The Snowballing Literature on Imiquimod-Induced Skin Inflammation in Mice: A Critical Appraisal

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Since 2009, the imiquimod- or Aldara-induced (3M Pharmaceuticals, St. Paul, MN) model of acute skin inflammation has become the most widely used mouse model in preclinical psoriasis studies. Although this model offers researchers numerous benefits, there are important limitations and possible confounding variables to consider. The imiquimod model requires careful consideration and warrants scrutiny of the data generated by its use. In this perspective, we provide an overview of the advantages and disadvantages of this mouse model and offer suggestions for its use in psoriasis research.

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INTRODUCTION

Since its initial description (van der Fits et al., 2009), the imiquimod (IMQ)- or Aldara (3M Pharmaceuticals, St. Paul, MN)-induced model of acute skin inflammation has become the most widely used murine model for preclinial studies of psoriasis. This can be seen by the dramatic increase in the number of publications using this model since 2009 (Figure 1). In this model, daily application of IMQ to the skin of mice induces localized skin and systemic inflammation primarily through the activation of toll-like receptor (TLR) 7/8 (van der Fits et al., 2009). The inducible inflammatory events of this model mirror aspects of human psoriasis, including the induction of psoriasis-like histologic features, activation of proinflammatory signaling pathways central to human psoriasis, and the recruitment of similar cellular infiltrates (Chamcheu et al., 2016; Grine et al., 2015; Ha et al., 2014; van der Fits et al., 2009; Vinter et al., 2016).

The IMQ mouse model offers scientists several distinct advantages. However, its limitations are also frequently understated and/or merely overlooked. This model of acute skin inflammation deserves careful attention and requires critical evaluation. The intent of this perspective article is to discuss the strengths and weaknesses of the IMQ model and encourage its appropriate use in ongoing dermatology research.

ADVANTAGES

Easy to use

The straightforwardness of the IMQ application for this mouse model likely accounts for its increasing use in preclinical studies. Hair on the back of anesthetized adult mice (frequently BALB/c or C57BL/6 genetic backgrounds) is shaved or depilated (by chemical or wax), followed by topical application of commercially available IMQ (5%) cream, which is contained in individual 250-mg packets. One quarter (62.5 mg) of each IMQ packet is then applied daily to the backs and/or ears of the mice for approximately 5–7 days (van der Fits et al., 2009). Treated mice can be housed together and do not require specific pathogen-free conditions or special diets. As such, set-up for this model requires minimal laboratory expertise and materials, allowing it to be easily incorporated into ongoing laboratory protocols.

Acute inflammatory response

Another advantage of the IMQ-induced mouse model is the relatively quick and reproducible inflammatory skin response. Within 12–24 hours after IMQ application, the induction of multiple inflammatory markers (e.g., type I IFN-responsive genes, S100A8/A9, and IL-23) and microscopic alterations in the epidermis (e.g., compromised epidermal integrity, keratinocyte apoptosis, and Munro's microabscesses) can be observed (Grine et al., 2015, 2016; Walter et al., 2013). After 2–3 days, mice begin to develop signs of acute skin inflammation (i.e., erythema, scaling, and skin thickening/induration), which gradually worsens with continued treatment (van der Fits et al., 2009). Similar findings are observed when IMQ is applied to the ears of mice. The short duration between topical application and skin inflammation in this model allows investigators to generate significant amounts of data in a relatively short period of time.

Convenient and cheap

Another benefit of topically applied IMQ is that it can be easily combined with existing or novel genetically modified mouse strains. This has tremendous practical implications due to the significant cost and time (e.g., 6–12 months) required to generate new transgenic mouse models and the technical expertise needed to perform xenotransplantation experiments.

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Abbreviations: IMQ, imiquimod; TLR, toll-like receptor

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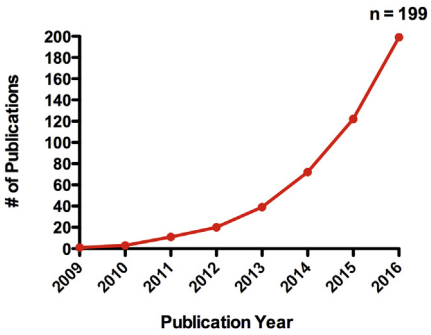

Figure 1. IMQ publication trend since 2009. Trend in the number of publications involving the use of the imiquimod-induced mouse model of skin inflammation between its initial description in 2009 and October 2016.

Overall, the IMQ model is a relatively straightforward, inexpensive, convenient model of acute skin inflammation. Its appeal to many scientists is the short treatment duration required to elicit acute skin inflammation in transgenic and knockout mice created on a variety of genetic backgrounds. The IMQ model, like other proposed mouse models of psoriasis, also has the distinct advantage of modeling aspects of the complex interplay between the skin, immune system, and other tissues that are lacking in simplified in vitro or monoculture models.

LIMITATIONS

Unintended consequences of topical treatment

IMQ is a potent activator of the immune response. Its proinflammatory properties in the skin are well documented in patients and have been exploited to treat precancerous skin lesions, warts, and nonmelanoma skin cancers (Grillo-Ardila et al., 2014; Tzellos et al., 2014). However, the IMQ-induced immune response is not restricted to the skin. In phase I clinical trials for oral IMQ in cancer patients, dose-related toxicities including hematologic abnormalities and flu-like symptoms were common (Savage et al., 1996; Witt et al., 1993). The consequences of IMQ ingestion are particularly problematic in the IMQ-induced mouse model because of the grooming behaviors of mice, especially for co-housed animals. One study demonstrated this effect by showing that the localized and systemic effects observed in the IMQ-induced model were largely ameliorated when treated mice were also placed in "Elizabethan collars" (Grine et al., 2016). Grooming and scratching behaviors can also result in significant excoriation and thickening of the epidermis, as it does in humans. Further, how oral ingestion of IMQ alters the gut microbiome and the inflammatory skin phenotype of this particular mouse model is largely unknown (Zanvit et al., 2015). Therefore, restraint must be exercised before drawing definitive conclusions about the cause of systemic inflammation (e.g., splenomegaly and lymphadenopathy) or phenotypic differences observed in this model.

Overuse with limited validation studies

The favorable features of the IMQ-induced model have led many investigators to choose this in vivo system by default for preclinical studies of psoriasis. This is implied by the rapid increase in the number of publications involving use of the IMQ mouse model shown in Figure 1. As a consequence of its accessibility and ease of use, IMQ is being applied to hundreds of permutations of transgenic mouse models to generate large amounts of phenotype information. Although the information generated from these experiments have proven informative regarding IMQ-induced skin inflammation, most experimental data have not been validated in human tissues, thus limiting the generalizability of this information. For example, only a small number of the modified genes in mice being used for IMQ-induced experiments have been identified in genome-wide association studies of human psoriasis (e.g. *IL23A, IL36RN, TRAF3IP2, STAT3*). Moreover, most IMQ experiments have been completed in either C57BL/6 or BALB/c background strains, each strain having intrinsic limitations related to cytokine and immune cell bias (Hsieh et al., 1995; Watanabe et al., 2004).

Unclear mechanism of action and confounding

The mechanism by which IMQ induces acute skin inflammation in mice is poorly understood. Its primary effect in the skin cannot be solely due to activation of TLR receptors, given that keratinocytes lack expression of TLR7/8 (Lebre et al., 2007). Several studies have underscored the TLR7-independent responses in the skin after topical IMQ treatment, including the development of acanthosis in TLR7-knockout mice (Walter et al., 2013), activation of the keratinocyte inflammasome by the Aldara vehicle itself (Walter et al., 2013), and variable histologic features between different brands of IMQ cream (Luo et al., 2016). These studies highlight the multifactorial mechanism of IMQ-induced skin inflammation. They also inform investigators of the possible confounding effects of ingredients contained in the vehicle of IMQ and/or depilatory creams (Amberg et al., 2016) and reinforce the indispensable importance of thoughtful experimental controls.

Misinterpretation of histologic findings and observer bias in skin inflammation scoring

Although the histologic features observed in the IMQ-induced model can be useful as surrogate markers of skin inflammation, they are frequently misinterpreted and overrelied on. This occurs most often when assessing epidermal thickness (acanthosis). Microscopic assessment of acanthosis in this model is most accurately measured in microns, rather than epidermal cell layer thickness, and requires that the mouse skin be horizontally sectioned to avoid artificial increases in acanthosis due to tangential sectioning of the epidermis and follicular ostia (Figure 2). Histology or acanthosis measurements are also frequently omitted from publications using this particular model. Furthermore, the histologic data provided reflect overall ear thickness at the gross level, as measured using a Vernier caliper. Similarly, the modified clinical Psoriasis Area and Severity Index proposed for scoring IMQ-induced skin inflammation (van der Fits et al., 2009) is also susceptible to observer bias and is likely dependent on an investigator's experience, as seen among physicians scoring human psoriasis (Langley and Ellis, 2004). Indeed, many publications citing phenotype improvements in their transgenic mouse model of choice show rather modest reductions in acanthosis or changes in other epidermal characteristics such as parakeratosis and neutrophilic infiltration. Complete elimination of the inflammation elicited by IMQ is challenging and rarely demonstrated.