Journal of Advanced Veterinary Research

https://advetresearch.com

Establishment of Psoriasis in BALB/c Murine Model using Imiquimod: A Pilot Study

Aina Akmal Mohd Noor[1], Nurul Khaiza Yahya[1], Maryam Azlan[2], Norhanani Mohd Redzwan[1*]

[1]*Department of Immunology, School of Medical Sciences, Universiti Sains Malaysia, 16150, Kubang Kerian, Kelantan, Malaysia*
[2]*School of Health Sciences, Universiti Sains Malaysia, 16150, Kubang Kerian, Kelantan, Malaysia*

ARTICLE INFO

Original Research

Received:
16 January 2021

Accepted:
08 March 2021

Keywords:

Autoimmunity,
BALB/c mice, Imiquimod,
Skin inflammation, Psoriasis

ABSTRACT

Psoriasis is an immune-mediated inflammatory disease exploiting the skin which is triggered by many possible factors concerning the epidermal keratinocytes and autoimmune cells. To study psoriasis in vivo, imiquimod (IMQ) topical application is used as it is a ligand of TLR7 in mouse to induce an acute psoriatic murine model. In obtaining the expected outcomes, one must understand the fundamental procedures to successfully initiate psoriasis in mouse. As IMQ is an acute psoriasis model inducer, it has been proposed that the condition can be progressed up to seven days after application. Aldara cream containing 5% imiquimod and Vaseline as its control was topically applied for seven days consecutively on the shaved back of the BALB/c mice. Modified Psoriasis Area and Severity Index (PASI) score was used to score the inflammation severity of the induced area. The exacerbation of the diseased skin in the mouse was seen to be more severe as it developed. The increasing PASI score of the psoriatic mouse from 2 to 7 by days suggested that psoriasis establishment had made evidence which consists of progressed erythema, skin thickening and scaling. The spleen in the psoriatic mouse portrayed splenomegaly in a gross observation compared to the control spleen. Hence, using this method with such dosage further agrees that it is the most suitable dosage and can provide a good immunological framework for establishing psoriasis in vivo.

Introduction

Psoriasis is one of the immune-meditated inflammatory diseases with unclear aetiology presented with impaired immune cells such that T cell, macrophages, dendritic cells (DCs) and neutrophils, which work in tandem with activated and hyperproliferative keratinocytes (Cai *et al*., 2012). It affects two to three percent of the global population (Mohd Affandi *et al*., 2018). It is typically presented with epidermal acanthosis, dermal immune-cell infiltration and neovascularisation over the elbows, knees, scalp and back trunk (Woo *et al*., 2017). It is substantial to establish a groundwork especially in studying psoriasis *in vivo* settings since this disease is practically involved in systemic networks. The systemic networks are recommended to be studied thoroughly to explore the overall cellular connectivity; hence *in vivo* studies can counterbalance the entire responses, which may lead to new exploration.

Several ways have been discovered for researchers to study this disease *in vivo* using murine models i.e. induced, xenotransplanted, transgenic and knockout (Swindell *et al*., 2017). One of it, is by using repeated topical application of imiquimod (IMQ) (induced model) (Rodríguez-Martínez *et al*., 2017). This chemical acts as a Toll-like receptor (TLR)-7 and TLR-8 in mouse and human respectively to cause a chain reaction among immune cells, hence igniting psoriasis (El Malki *et al*., 2013). The first published report article in 2009 describes that IMQ-induced psoriatic laboratory animal is a method in a preclinical context whereby skin inflammation is initiated through TLR-7 activation. The observation can be evaluated by erythema and inflammatory cell invasion (Hawkes *et al*., 2018).

Further findings exploit this technique since IMQ-induced psoriasis mice are low-cost and easy to monitor the lesion. It can induce psoriasis in mice at any desired age or specific time point. Other than that, this technique is considered as convenient with simple execution, which does not require any specific training in prior. Topical application, as its execution method, is the route of administration of IMQ since it has a small chemical structure and lipophilicity properties (Dorjsembe *et al*., 2019). Moreover, it offers the most resemblance to human psoriasis in terms of inflammation, neovascularisation patterns and hyperproliferative cellular activity (Luo *et al*., 2016; Rodríguez-Martínez *et al*., 2017). To understand psoriasis in-depth, it is highly recommendable to study its deviant cytokine characteristics. Therefore, this acute model using BALB/c mouse is suitable to study its maximum irregular responses of immune cells in a range of three to four days.

*Corresponding author: Norhanani Mohd Redzwan
E-mail address: hanani.redzwan@usm.my

ISSN: 2090-6277/2090-6269/

Materials and methods

Ethical approval was obtained from USM Animal Ethics Committee [USM/IACUC/2019/(120)(1029)].

The mice were supplied and maintained at the Animal Research and Service Center, USM Health Campus. In this study, eight to ten weeks old male BALB/c mice weighing 25–30 g were used. The mice were kept in polypropylene cages filled with corn cob granules bedding (Chipsi, Germany) and the cage was closed with stainless steel wire top clips. A standard mice pellets (Gold Coin Feedmils, Malaysia) was given, considering one mouse would receive 10 g pellets daily. Unlimited supply of tap water ad libitum was given as well. The surrounding temperature was maintained approximately at 22.0±3.0℃, the humidity within 50-60% and steady 12 h of light and dark cycle (light 0700-1900). The working area was sanitised. All mice were subjected to three days of acclimatization period before the commencement of the experiment.

Psoriasis initiation

By using clean weighing plates, 62.5 mg of Aldara cream containing 5% of IMQ and Vaseline (Unilever, United Kingdom) were weighed. For anaesthesia, the mice were continuously supplied with 4% isoflurane inhalation for three minutes and then maintained in 1.5-2% in an air-tight glass box. Then, on the rostral area of the mice, the fur was trimmed and shaved. By using a clean cotton bud, Aldara cream was topically applied thoroughly on the exposed skin. The control mouse was treated similarly but with a control vehicle cream; Vaseline in an appropriate amount. The mice were left in an individual cage and they were left to confirm its regained consciousness. The topical application for both mice respectively were repeated daily throughout the experiment.

Physical and behavioural changes in mice

The mice were visually observed daily. This included its skin appearance and any remarkable behavioural changes (withdrawn action, pain responses). The mice were daily observed for its skin appearance and any remarkable behavioural changes such as withdrawn action and pain responses. The modified PASI score (Luo *et al*., 2016) was used; the factors in the measure consist of erythema, thickening and scaling as shown in Table 1. Each factor was graded independently on a scale from 0–4 (0, none and 4, very marked). The cumulative score ranged from 0–12. All appearances were confirmed by a veterinarian physician.

Mice euthanasia and harvesting samples

Sodium pentobarbital (200 mg/kg) (Alfasan®, Holland) was injected intraperitoneally and waited until the mouse had fully unconscious. The mouse was quickly decapitated through its neck and blood was collected in a microcentrifuge tube. Each paw was punctured with needles on the working tray. The mouse was dissected and its skin and spleen were harvested.

Results

The development of psoriasis was monitored daily following the daily topical application of 5% IMQ on the shaved back of the mouse. The skin gradually formed some rough scaly patches simultaneously with redness indicating erythema. The thickness of the skin could be felt using fingers and by gentle pinching of the effected skin. The overall development of psoriasis in the mouse is shown in Fig. 1.

At the beginning of the experiment, both mice showed curiosity to learn and were physically active although it would be rather subtle to be observed. These characteristics were portrayed as they were actively moving around their cage besides constantly eating, drinking and grooming. Approaching to day 4, the skin of the psoriatic mouse observed has changed. Moreover, unusual behaviours were noticed in psoriatic mice as it was quite lethargic, has an abnormal posture and showed convulsion and pain when handled as shown in Fig. 2.

To quantify the psoriatic severity assessment and thus further proven the observation in mouse, a modified PASI score adapted from Luo *et al*., (2016) was used. The scores were independently recorded and the plotted graph used the daily cumulative scores. Noted that only the IMQ-induced mouse plotted a gradually increased scoring compared to the control mouse indicating that the exacerbation of psoriasis evaluated by erythema, skin thickening and scaling had occurred.

Table 1. The modified PASI score used to evaluate psoriasis in mouse.

PASI Score	Parameters
Score	Erythema
0	Normal skin
1	Blood capillary visible
2	Scattered erythema
3	Erythema on a whole back skin
4	Dense erythema
Score	Thickness
0	Normal skin
1	Keratoplasia
2	Keratoplasia appear on half of back skin OR skin lesions significantly overtop the normal skin
3	Thickness on whole back skin OR skin lesions significantly overtop the normal skin
4	Skin lesions sclerosis
Score	Scales
0	Normal skin
1	Scales visible
2	Scales appear on half of back skin
3	Scales appear on whole back skin
4	Dense desquamation

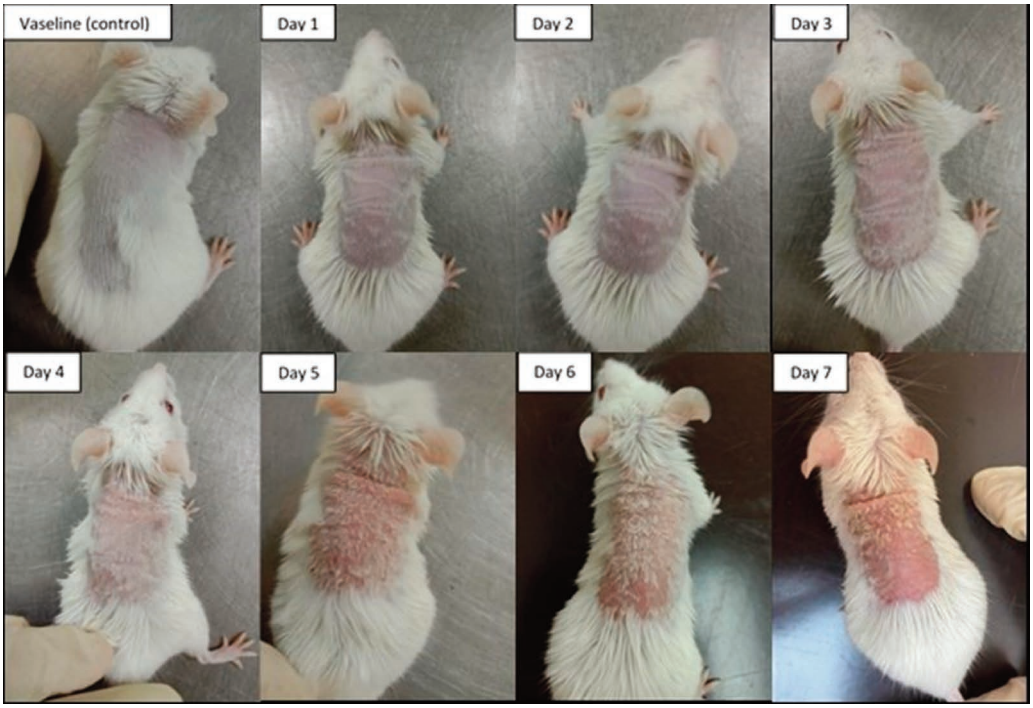

Fig. 1. The progression of the mouse with a daily application of 5% IMQ. The shaved areas of mice were topically applied with 5% IMQ for seven days. Note that the skin of the mouse applied with IMQ gradually exacerbated with psoriatic lesions by days. The lesions included redness, thickness and scales formation.

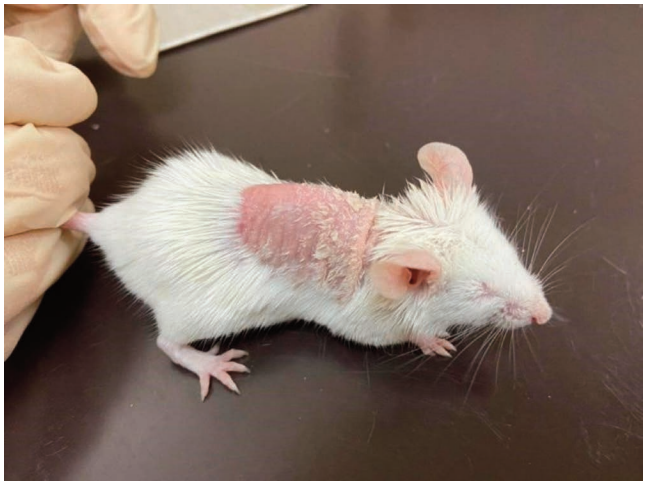

Fig. 2. Psoriasis-induced mouse showed pain signs when handled. The mouse tended to avoid being handled, squinted eyes, showed moderate pain and distress. The coat was not properly groomed and generally looked uncomfortable.

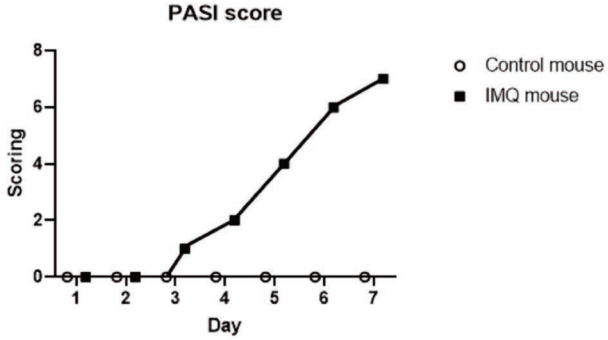

Fig. 3. The modified PASI score of the mice using erythema, skin thickness and scaling as scoring parameters. During the daily evaluation, the scoring would be assessed and calculated to produce the cumulative scores (range 0 to 12). Note that the increasing trend by days was observed in IMQ mouse compared to the control mouse.

Spleen is the target organ in this study since it plays major roles in regard to immune cells. The gross examination upon harvesting the spleens was performed and assembled as pictured in Fig. 4. The spleen in IMQ-induced mouse had increased its size or splenomegaly. This event was possible due to increased cellular proliferation within the spleen because of disturbed immune cells regulation.

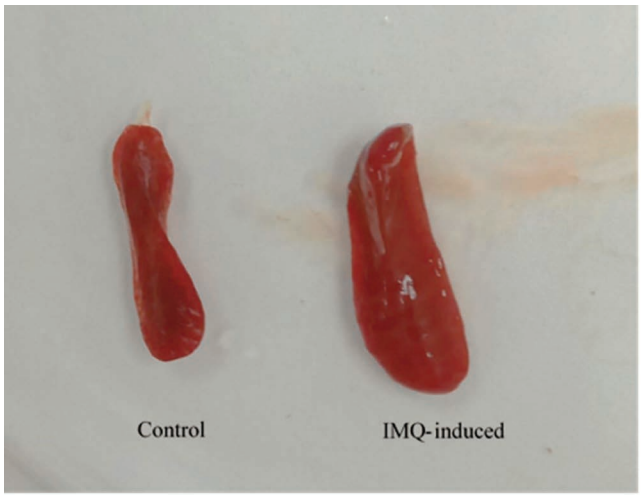

Fig. 4. Mouse spleen harvested from control (left) and IMQ-induced mice (right). IMQ mouse had a significantly bigger spleen compared to control mouse.

Discussion

Psoriasis is a type of chronic inflammatory skin condition, which characterised with hyperplastic epidermal keratinocytes and hyperactivity of epidermal immune cells (Schaefer *et al*., 2015). IMQ is a cream, clinically used for actinic keratosis, basal cell carcinomas and warts caused by human papillomavirus. Long term application of IMQ in a particular dosage can eventually elicit psoriasis (Madsen *et al*., 2018).

In mice, IMQ can bind on TLR-7 on its macrophages and

DCs. Subsequently, these cells are aggregated to invade to the inflammatory areas and express its proinflammatory cytokines and chemokines such as IL-1α, IL-6, IL-23 and interferon (IFN)-α. This event matures DCs to launch T helper cells (Th)1/Th7 pathways, which are commonly related with psoriasis induction pathway (Furiati *et al*., 2019). Keratinocytes simultaneously undergo hyperproliferation ordinarily via MyD88 independent pathway; adenosine receptor-mediated 3',5'-cyclic monophosphate (cAMP) pathway. During the early phase of inflammation, keratinocytes are deficit in TLR7/8 receptors. Through the mentioned pathway, MyD88 is activated for the downstream target of TLR7/8 primarily. This action is mediated by IL-23 and IFN-α produced by CD11c+ myeloid DCs and plasmacytoid DCs respectively (Rabeony *et al*., 2014; Ueyama *et al*., 2014). This secreted IL-23 liaises γδ T cells to produce IL-17 and IL-22 to further proliferate keratinocytes using inflammasome complex to quantify S100 proteins and LL-37 (Schön *et al*., 2006; Costa *et al*., 2017).

Therefore, the successful keys for IMQ to elicit psoriasis especially in mice are infiltration of plasmacytoid dendritic cells (pDCs) and type 1 interferon activity (Schaefer *et al*., 2015). Cytokines regulatory patterns are also similar to human especially in the most recent referred axis; interleukin (IL)-17/IL-23 (Gilliet *et al*., 2004; El Malki *et al*., 2013). Thus, it is well known to provide a great amount of IL-17 production which provokes neovascularisation and keratinocytes hyperproliferation (Van Belle *et al*., 2012).

In one previous experiment had utilized 62.5 mg and 25.0 mg of IMQ cream to induce psoriasis. By using 25.0 mg of IMQ still can undeniably induce psoriasis but more on as a chronic model of murine psoriasis. Although both dosages could be used for murine psoriatic induction, the observation for skin thickness by evaluating its histological assessment differentiation would be quite similar (Horváth *et al*., 2019). Thus, this finding further concurs the former dosage of IMQ to initiate preferable psoriatic mice.

Daily topical application of IMQ on targeted skin area in mice induces psoriatic signs such as eventual intense of skin erythema and scaling, keratinocytes changes, thickened epidermis and invasion of immune cells in the epidermis (Sakai *et al*., 2016). Herein, seven days of 62.5 mg IMQ application was adequate to produce such signs and symptoms. Collectively, these phenotypes are closely mimicking the psoriasis pathogenesis in human but not literal translating (Nadeem *et al*., 2015; Vinter *et al*., 2016). It is true that daily topical of IMQ on mice, which is one of the procedures to study preclinical psoriasis, does not completely translate into clinical findings, but this method can trigger cytokine expression pattern, histopathological changes and cellular trafficking which are seen in human patients (Flutter and Nestle, 2013; Yoshiki *et al*., 2014). Therefore, the variable cytokine expression pattern by different time points can be further evaluated by flow cytometry analysis.

When the psoriatic mouse been handled, it often showed a strong withdrawn manner with painful face expression. According to a guideline provided (Burkholder *et al*., 2012), these behaviours collectively proposed that the induced mouse experienced mild or anticipated pain and distress. It is not recommended to execute this study for more than eight days (El Malki *et al*., 2013; Luo *et al*., 2016). Hence, it is appropriate to conduct an introductory study to monitor and observe the overall timeline for the progression of induced psoriasis in the mouse.

Since psoriasis is also characterized by impaired keratinocytes, which leads to hyperproliferation and parakeratosis. Hence, by observation of naked eyes and passing a finger on the affected area, it could be indeterminate that the skin of the induced mouse had thicker skin with scaly conditions. This is possible since the infiltration of the dermis and epidermis by activated immune responses had occurred (Nestle *et al*., 2009; Jeon *et al*., 2017). Acanthosis is a probable event because the number of keratinocytes has increased basal cell layer due to striking responses by IL-22 and IL-17 (Jeon *et al*., 2017). Respectively, changed epidermal differentiation gives rise to parakeratosis condition which leads to the scaling of the skin in psoriasis (Van der Fits *et al*., 2009; Albanesi *et al*., 2018). Moreover, a modified PASI score is used as a guideline to measure the severity and extend of psoriasis (Luo *et al*., 2016; Wu *et al*., 2019). The psoriatic mouse scored 2 on Day 4 and steadily increased to a maximum of 7 on Day 7 (Fig. 3). The increasing trend of the psoriatic severity in IMQ induced mouse could also be suggested as the increased skin thickness. However, to evaluate this parameter precisely and better, Vernier callipers measurement and histological findings must be carried out.

Spleen is a type of lymphoid organ where it filters blood and a draining area of administered compounds. Numerous populations of B and T cell lymphocytes host the organ and hence, it is advantageous to assess its histopathology or biochemical evaluation (Elmore, 2006). In this study, from a gross examination, splenomegaly was observed in an IMQ induced mouse (Fig. 4). This event was possible due to expanded cellular proliferation in the spleen which was established by an inflammatory immune reaction such as the expansion of CD4+ T cell percentage in the composition of the immunocytes (Zhang *et al*., 2016).

Choosing BALB/c murine model in establishing psoriasis is highly coveted as it is one of the most five prominent inbred strains to study particularly in immunology and infectious disease fields (Nakamura, 2013). In comparison with B6 mouse strain, BALB/c produced psoriatic lesions rapidly in regard to IMQ repeated application (Van der Fits *et al*., 2009; Flutter and Nestle, 2013). Executing this method would undertake the desirable induced acute psoriatic BALB/c mouse model with its exceptional post-treatment cytokine responses.

Researchers who are interested in studying cytokine profiling and exploring psoriatic treatment can implement this work. Moreover, histologic exacerbation of psoriasis in BALB/c can easily be assessed using techniques such as immunohistochemistry and haematoxylin and eosin. As for our downstream studies, following this preliminary technique, we would investigate the relationship between innate and adaptive immune cells, DCs and B cells respectively in order to establish fundamental interpretation predominantly in psoriasis pathogenesis. So here, we intended to provide the foundation of ideas relating to the response of the immune cells since it is crucial to explore the whole process to further tackle this disease.

Conclusion

Topical application of 62.5 mg of IMQ is an optimum dosage to induce the psoriasis-like disease. In addition, the physical examination, altered behavioural observation and increasing PASI score were depicted that psoriasis-like disease has proficiently exacerbated in mice. Moreover, splenomegaly that has been found in induced mice might also represent the proliferation of immune cells such as T and B cells. The imiquimod (IMQ)-induced psoriasis-like mouse model using this specific dosage might provide a good framework to proceed with other downstream studies in understanding the pathophysiology of psoriasis and in future could path the way for potential therapeutic approach for better treatment of psoriasis.

Acknowledgement

We would like to thank Universiti Sains Malaysia for the research support and Animal Research and Service Centre (ARASC), USM for the animal facilities. This study was financially supported by the Fundamental Research Grant Scheme, Ministry of Higher Education, Malaysia (FRGS/1/2019/SKK06 /USM/03/3, grant no. 203.PPSP.6171243).

Conflict of interest

The authors declare no conflict of interest.

References

Albanesi, C., Madonna, S., Gisondi, P., Girolomoni, G., 2018. The interplay between keratinocytes and immune cells in the pathogenesis of psoriasis. Front. Immunol. 9, 1549.

Burkholder, T., Foltz, C., Karlsson, E., Linton, C.G., Smith, J.M., 2012. Health evaluation of experimental laboratory mice. Curr. Protoc. Mouse Biol. 2, 145-165.

Cai, Y., Fleming, C., Yan, J., 2012. New insights of T cells in the pathogenesis of psoriasis. Cell Mol. Immunol. 9, 302-309.

Costa, S., Marini, O., Bevilacqua, D., DeFranco, A.L., Hou, B., Lonardi, S., Vermi, W., Rodegher, P., Panato, A., Tagliaro, F., Lowell, C.A., Cassatella, M.A., Girolomoni, G., Scapini, P., 2017. Role of MyD88 signaling in the imiquimod-induced mouse model of psoriasis: focus on innate myeloid cells. J. Leukoc. Biol. 102, 791-803.

Dorjsembe, B., Ham, J.Y., Kim, J.C., 2019. The imiquimod induced psoriatic animal model-scientific implications. BJSTR. 13, 9722-9724.

El Malki, K., Karbach, S.H., Huppert, J., Zayoud, M., Reißig, S., Schüler, R., Nikolaev, A., Karram, K., Münzel, T., Kuhlmann, C.R.W., Luhman, H.J., von Stebut, E., Wörtge, S., Kurschus, F.C., Waisman, A., 2013. An alternative pathway of imiquimod-induced psoriasis-like skin inflammation in the absence of interleukin-17 receptor a signaling. J. Invest Dermatol. 1332, 441-451.

Elmore, S.A., 2006. Enhanced histopathology of the spleen. J. Toxicol. Pathol. 34, 648-655.

Flutter, B., Nestle, F.O., 2013. TLRs to cytokines: mechanistic insights from the imiquimod mouse model of psoriasis. Eur. J. Immunol. 43, 3138-3146.

Furiati, S.C., Catarino, J.S., Silva, M.V., Silva, R.F., Estevam, R.B., Teodoro, R.B. Rodrigues, D.B., 2019. Th1, Th17, and Treg responses are differently modulated by TNF-α inhibitors and methotrexate in psoriasis patients. Sci. Rep. 9, 1-11.

Gilliet, M., Conrad, C., Geiges, M., Cozzio, A., Thürlimann, W., Burg, G., Nestle., F.O., Dummer, R., 2004. Psoriasis triggered by toll-like receptor 7 agonist imiquimod in the presence of dermal plasmacytoid dendritic cell precursors. Arch. Dermatol. 140, 1490-1495.

Hawkes, J.E., Adalsteinsson, J.A., Gudjonsson, J. E., Ward, N. L., 2018. Research techniques made simple: murine models of human psoriasis. J. Invest. Dermatol. 138, e1-e8.

Horváth, S., Komlódi, R., Perkecz, A., Pintér, E., Gyulai, R., Kemény, Á., 2019. Methodological refinement of Aldara-induced psoriasiform dermatitis model in mice. Sci Rep. 9, 1-8.

Jeon, Y.J., Sah, S.K., Yang, H.S., Lee, J. H., Shin, J., Kim, T.Y., 2017. Rhododendrin inhibits toll-like receptor-7-mediated psoriasis-like skin inflammation in mice. Exp. Mol. Med. 49, e349-e349.

Luo, D.Q., Wu, H.H., Zhao, Y.K., Liu, J.H., Wang, F., 2016. Different imiquimod creams resulting in differential effects for imiquimod-induced psoriatic mouse models. Exp. Biol. Med. 241, 1733-1738.

Madsen, M., Hansen, P.R., Nielsen, L.B., Cardoso, R.M., van Eck, M., Pedersen, T.X., 2018. Imiquimod-induced psoriasis-like skin lesions do not accelerate atherosclerosis in low-density lipoprotein receptor–Deficient mice. Am. J. Pathol. 188, 1486-1496.

Mohd Affandi, A., Khan, I., Ngah Saaya, N., 2018. Epidemiology and clinical features of adult patients with psoriasis in Malaysia: 10-year review from the Malaysian Psoriasis Registry (2007–2016). Dermatol. Res. Pract. 2018, 4371471.

Nadeem, A., Al-Harbi, N.O., Al-Harbi, M.M., El-Sherbeeny, A.M., Ahmad, S.F., Siddiqui, N., Ansari, M.A.Z., Al-Sharary, S.D., 2015. Imiquimod-induced psoriasis-like skin inflammation is suppressed by BET bromodomain inhibitor in mice through RORC/IL-17A pathway modulation. Pharmacol. Res. 99, 248-257.

Nakamura, H., 2013. BALB/c mouse. Brenner's Encyclopedia of Genetics. pp. 290–292.

Nestle, F.O., Kaplan, D.H., Schon, M.P., 2009. Barker J. Psoriasis. N. Engl. J. Med. 361, 496-509.

Rabeony, H., Petit-Paris, I., Garnier, J., Barrault, C., Pedretti, N., Guilloteau, K., Jegou, J.F., Guillet, G., Huguier, V., Lecron, J. C., Bernard, F.X., Morel, F., 2014. Inhibition of keratinocyte differentiation by the synergistic effect of IL-17A, IL-22, IL-1α, TNFα and oncostatin M. PloS ONE 9, e101937.

Rodríguez-Martínez, S., Cancino-Diaz, J.C., Martínez-Torrez, I., Pérez-Tapia, S.M., Cancino-Diaz, M. E., 2017. Psoriatic animal models developed for the study of the disease. Psoriasis: An Interdisciplinary Approach. 75.

Sakai, K., Sanders, K.M., Youssef, M.R., Yanushefski, K.M., Jensen, L., Yosipovitch, G., Akiyama, T., 2016. Mouse model of imiquimod-induced psoriatic itch. Pain 157, 2536.

Schaefer, C.P., Cappelleri, J.C., Cheng, R., Cole, J.C., Guenthner, S., Fowler, Johnson, S.J., Mamolo, C., 2015. Health care resource use, productivity, and costs among patients with moderate to severe plaque psoriasis in the United States. J. Am. Acad. Dermatol. 73, 585-593.

Schön, M.P., Schön, M., Klotz, K.N., 2006. The small antitumoral immune response modifier imiquimod interacts with adenosine receptor signaling in a TLR7-and TLR8-independent fashion. J. Invest. Dermatol. 126, 1338-1347.

Swindell, W.R., Michaels, K.A., Sutter, A.J., Diaconu, D., Fritz, Y., Xing, X., Sarkar, M.K., Liang. Y., Tsoi, A., Gudjonsson, J.E., Ward, N.L., 2017. Imiquimod has strain-dependent effects in mice and does not uniquely model human psoriasis. Genome Med. 9, 1-21.

Ueyama, A., Yamamoto, M., Tsujii, K., Furue, Y., Imura, C., Shichijo, M., Yasui, K., 2014. Mechanism of pathogenesis of imiquimod-induced skin inflammation in the mouse: A role for interferon-alpha in dendritic cell activation by imiquimod. Int. J. Dermatol. 41, 135-143.

Van Belle, A.B., de Heusch, M., Lemaire, M.M., Hendrickx, E., Warnier, G., Dunussi-Joannopoulos, K., Fouser, L.A., Renauld, J.C., Dumoutier, L., 2012. IL-22 is required for imiquimod-induced psoriasiform skin inflammation in mice. J. Immunol. 188, 462-469.

Van der Fits, L., Mourits, S., Voerman, J.S., Kant, M., Boon, L., Laman, J.D., Cornelissen, F., Mus, A.M., Florencia, E., Prens, E.P., Lubberts, E., 2009. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. J. Immunol. 182, 5836-5845.

Vinter, H., Kragballe, K., Steiniche, T., Gaestel, M., Iversen, L., Johansen, C., 2016. Tumour necrosis factor-α plays a significant role in the Aldara-induced skin inflammation in mice. Br. J. Dermatol. 174, 1011-1021.

Woo, Y.R., Cho, D.H., Park, H.J., 2017. Molecular mechanisms and management of a cutaneous inflammatory disorder: psoriasis. Int. J. Mol. Sci. 18, 2684.

Wu, D.H., Zhang, M.M., Li, N., Li, X., Cai, Q.W., Yu, W.L., Liu, L., Zhu, W., Lu, C.J., 2019. PSORI-CM02 alleviates IMQ-induced mouse dermatitis via differentially regulating pro-and anti-inflammatory cytokines targeting of Th2 specific transcript factor GATA3. Biomed. Pharmacother. 110, 265-274.

Yoshiki, R., Kabashima, K., Honda, T., Nakamizo, S., Sawada, Y., Sugita, K., Yoshioka, H., Ohmori, S., Malissen, B., Nakamura, M., 2014. 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. J. Invest. Derm. 134, 1912-1921.

Zhang, S., Liu, X., Mei, L., Wang, H., Fang, F., 2016. Epigallocatechin-3-gallate (EGCG) inhibits imiquimod-induced psoriasis-like inflammation of BALB/c mice. BMC Complement Altern. Med.16, 1-11.