Discussion

Psoriasis, an inflammatory proliferative skin condition, is multifactorial in nature with no obvious single etiology. It is accepted that psoriasis pathogenesis involves, in genetically susceptible individuals, a dysregulated innate and adaptive immune response to an unknown antigen **(Brotas et al., 2012; Burden and Kirby, 2016; Coimbra et al., 2012; Lee et al., 2012; Mahil et al., 2016; Mitra et al., 2013).**

In vivo studies are essential for understanding the mechanisms of psoriasis development and testing different therapeutics. Only human develops psoriasis spontaneously with only two reports in monkeys **(Swindell et al., 2011)**. This need for in vivo model of psoriasis, closely resemble the actual process takes place in human, urged scientists to develop models for psoriasis development. Because of the multifactorial nature of the disease - genetic background, different immune dysregulation, vascular phenomenon, keratinocyte abnormal hyperproliferation - different models were developed. Each model tries to focus on a certain dysregulation in the pathway of psoriasis development **(Gudjonsson et al., 2007; Swindell et al., 2011)**. Animal models of psoriasis include endothelial- specific receptor tyrosine kinase overexpression in basal keratinocytes (KCs) (K5-Tie2), the human amphiregulin overexpression in the basal epidermal layer (K14-AREG), basal KC-specific constitutively active mutant of signal transducer and activator of transcription 3 overexpression (K5-Stat3C), the latent form of transforming growth factor beta 1 overexpression in basal KCs (K5-TGFβ1) **(Swindell et al., 2011)**, The vascular endothelial growth factor overexpression in basal keratinocyte (K14-VEGF) **(Kang et al., 2016)**, a xenograft model with human lesional or non lesional psoriatic skin get transplanted into severe combined immune deficiency mouse **(Gudjonsson et al., 2007)**, intradermal IL-23 injected mouse **(Jiang et al., 2013)** and imiquimod mouse model of psoriasis **(Van der Fits et al., 2009)**.

For a psoriasis mouse model to be ideal, it should show well recognizable epidermal hyperproliferation, thickening with altered differentiation of the epidermis, an inflammatory infiltrate that includes T- cells and dendritic cells, altered cutaneous vascularity, and responsiveness to current antipsoriatic therapeutics **(Swindell et al., 2011)**.

Having different options to choose from, the point investigated in the pathway of psoriasis development will direct the choice of optimal mouse model. K5-Stat3C and K14-AREG specifically address the abnormal keratinocyte homeostasis in psoriasis pathogenesis. While K5-TGFβ1 & K5-Tie2 add to addressing the abnormal keratinocyte homeostasis abnormal vascular phenomena, oxidative stress and basement membrane degradation. K14-VEGF model address the vascular phenomena of psoriasis and the STAT3 pathway since it signals through it **(Gudjonsson et al., 2007; Swindell et al., 2011)**. The best models mainly address the immune dysregulation are IL-23 & Imiquimod induction models. IL23 is a central key cytokine in psoriasis development induces with IL6 the differentiation of Th17 cells a central cell in psoriasis pathogenesis **(Jiang et al., 2013)**. However this model doesn't address the early steps in psoriasis pathogenesis involving dendritic cell activation and TLR role in psoriasis. IL23- model of psoriasis is a promising yet quite recent model with little work done on it in the literature.

Imiquimod, 1-(2-methylpropyl)-1H-imidazo[4,5-c] quinolin-4-amine, is an immune response modifier known for its antiviral and antitumor effects. It is an FDA approved therapy for anogenital warts, actinic keratosis, and superficial basal cell carcinomas. The induction or exacerbation of psoriasis is a known side effect for Imiquimod use **(Hanna et al., 2016)**. Van der Fits and coworkers were first to use Imiquimod in the induction of psoriasis in mice in 2009 **(Van der Fits et al., 2009)**. The induced lesions were clinically and histopathologically very similar to psoriasis and on molecular basis it was mediated by IL17/23 pathway activation **(Van der Fits et al., 2009)**. The known effects of Imiquimod explain its success in inducing psoriasis. Imiquimod is TLR7/8 agonist recruiting pDCs, activating NFκB and inducing the secretion of several cytokines including, IFN-α, TNF-α, IL-1, IL-1RA, IL-6, IL-8, IL-12 among others **(Hanna et al., 2016)**. Being well-established model since 2009, Imiquimod psoriasis model has been utilized in a lot of publication addressing the pathogenesis and therapeutics of psoriasis **(Callahan et al., 2013; Chamcheu et al., 2017; Lai et al., 2015; van der Fits et al., 2009; Zanvit et al., 2015)** For all these reasons we decided to use Imiquimod induced psoriasis model for assessment of the efficacy of MBL in treatment of psoriasis.

We successfully induced psoriasiform lesions in the back skin of C57Bl female mice.

The clinical examination showed significant increase in the erythema scaling and thickness between normal and 6 days imiquimod groups, (**Figure 25)** (*P = 0.001*).

To our knowledge this is the first time dermoscopy is used in experimental dermatology for psoriasis animal model induction and follow up of therapeutic response. In human, psoriasis dermoscopic criteria include homogenous background erythema with homogenously distributed dotted vessels along with white silvery scales **(Lallas et al., 2012; Micali et al., 2011)**. Imiquimod induced psoriasiform lesions showed dermoscopic criteria of psoriasis seen in human, **(Figure 26).** For the assessment of treatment response we developed a scoring system for the degree of background erythema, dotted vessels and silvery scaling graduated from 0 = normal, 1= mild, 2= moderate and 3= sever, **(Figures 17 - 24)**. Using dermoscopy has aided the clinical score and made it more precise specifically in erythema assessment. Successful psoriasis induction was proved histologically with cardinal features of psoriasis detected as hyperkeratosis, acanthosis (increased epidermal thickness, *P = 0.001*), parakeratosis and inflammatory infiltrate of the skin **(Figure 27).**

On successful induction of psoriasis, we evaluated the efficacy of MBL in psoriasis treatment. To understand psoriasis development, it is important to take in consideration that to maintain skin homeostasis, there is a balance between proinflammatory state, which is needed as a protective immune response when skin is preached, and the anti-inflammatory state to fine tune the immune response to avoid exaggerated immune response leading to tissue damage **(Miller et al., 2005)**. The dysregulation of such mechanisms ends up in abnormal continuous inflammatory response as seen in psoriasis.

Mannose binding lectin is a pattern recognition molecule, involved in innate immune response against microorganisms, apoptotic cells as well as the modulation of inflammatory response **(Dommett et al., 2006; Takahashi, 2011)**. It is a serum protein, synthesized and secreted mainly by liver. MBL is an acute phase reactant, its production is enhanced by inflammatory stimuli and is recruited from blood stream to sites of inflammation **(Tsutsumi et al., 2006)**. Functional MBL presents in the serum as multimers. Trimmers, and tetramers are the most common functional forms **(Dommett et al., 2006; Takahashi, 2011)**.

MBL acts as an immune response regulator with an anti-inflammatory activity protecting the body against excessive, unneeded, immune responses that would cause excess tissue damage **(Downing et al., 2003; Downing et al., 2005; Wang et al., 2011a)**.

Based on the known literature, MBL can play a role in psoriasis development through its immune modulatory function. MBL binds to monocytes and other immune cells at inflammatory loci **(Downing et al., 2005).** At high concentration, MBL directly binds to and inhibits the LPS induced maturation of immature moDCs and consequently MBL significantly inhibits LPS-induced TNF-α and IL-12 production from mature moDcs and subsequent T cell activation and proliferation **(Wang et al., 2011b)** MBL binds to TLR4 of monocytes inhibiting LPS induced NF-κB pathway activation and cytokine release (TNFα & IL12). This interaction between MBL and TLR4 functions as a safeguard against excess damage of the body in inflammatory conditions (LPS tolerance) **(Wang et al., 2011a)**. In monocytes also, MBL inhibits CpG induced TLR9 signaling and TNFα & IL-6 secretion **(Tang et al., 2015).** MBL inhibits double stranded RNA mediated TLR3 signaling in monocytes and subsequent IL-6 & TNF-α secretion **(Liu et al., 2014).** TLR3 has been recently associated with keratinocyte expression of the shared p40 subunit of IL23 & IL12 **(Ramnath et al., 2015)**. MBL null mice died of cytokine storm resulted in septic shock when infected with *S. aureus* infection **(Nadesalingam et al., 2005)**. Interestingly MBL role in cutaneous inflammatory response to injury was addressed in MBL null mice subjected to burn (thermal injury). The MBL null mice showed abnormal response with reduced sloughing of eschar (dead skin) and interestingly abnormal epidermal acanthosis compared to wild type mice **(Moller-Kristensen et al., 2007; Takahashi, 2011)**. Antagonists of TLRs - anti TLR7, 8 & 9 - showed efficacy in psoriasis management **(Jiang et al., 2013)**. Monomethyl fumarate, an immunotherapy for psoriasis, inhibits NF-κB activation, decreases IL-12 production, and modulates moDCs polarization through interfering with LPS induced TLR4 signaling in dendritic cells. In other words monomethyl fumarate, a therapeutic of psoriasis, has the same effect of MBL on LPS induced TLR4 signaling pathway as proved by Wang and coworkers **(Hari et al., 2010; Wang et al., 2011a)**. Retinoids, another effective therapeutic for psoriasis, exerts their anti-inflammatory & immunomodulatory activity through inhibition of TLR4/

NFκB signaling blocking various cytokine secretion including IL1β, IL 6, IL12 and TNF-α **(Kim et al., 2013; Gu et al., 2010).** Ultraviolet B (UVB), phototherapy of psoriasis, recruits MBL to the skin **(Lokitz et al., 2005)**. In Turkish population, the *B* allel of *mbl* gene is more frequent in psoriatics than normal **(Turan et al., 2014).** Although this wasn't proved in Egyptian population yet psoriatic patients with homozygous *AA* or heterozygous *AB* genotypes have better response to therapy than homozygous *BB* genotype. Patients with *AB & BB* genotypes responded well to Red Sea climatotherapy, albeit temporarily which can be attributed to MBL recruitment to skin on UVB exposure on Safaga climatotherapy **(Nofal, 2014)**. Thus we hypothesize that MBL can be an effective therapeutic in treatment of psoriasis especially that it targets an early step in psoriasis pathogenesis.

In our work the induced psoriasiform lesions were successfully treated by intradermal injection of recombinant mouse MBL (3 µg/ day for 4 consecutive days). The reversal of psoriasiform lesions was evident clinically ($P < 0.05$) **(Figures 29, 30, 31 & 32)**, dermoscopically ($P= 0.045$) **(Figure 33 & 34)**, skin fold thickness **(Figure 32)**, epidermal thickness on histopathological assessment **(Figure 35 & 36)**.

MBL treated mice gained weight on MBL treatment while the control group received only PBS continued to express psoriasiform lesions and to loose weight **(Figure 28).**

The areas distant to the site of injection showed also improvement like the injected areas **(Figure 37, Table 13, 14 & 15)**.

The spleen histopathology of PBS control group showed expansion of the white pulp indicating an immune reactivation process, however the spleen of MBL treated mice was of normal histology **(Figure 38)**. The weight of spleen of MBL & PBS groups was significantly higher than normal spleen. Yet, splenic weight didn't differ significantly in between both groups. This can be attributed to the anti-inflammatory effect of MBL reversing the immune reactivation process with normal splenic histopathology yet the weight wasn't reduced either due to enlarged connective tissue stroma of the spleen or due to the weight gain of MBL treated mice to which splenic weight correlates.

The improvement of distant areas of skin and of the histopathologic features of spleen indicate that the used dose of MBL exerted systemic effect which raise the possibility of

using smaller doses in future studies.

MBL is a TLR4, 9 and 3 antagonist blocking NFκB activation with subsequent reduction of several cytokine secretion as TNFα, IL-6, IL-12. IL-6 and IL-12 are key early players in psoriasis development. IL-6 plays central role in the establishment and maintenance of Th17 profile. IL-12 establishes the production of IFN-γ from Th-1 cells early in psoriasis development. We tried to identify the possible down stream targets of MBL. As expected MBL treatment reduced IL-6 expression in MBL treated group back to normal level while its expression was significantly higher in PBS group **(Figure 40)**. IL 12 expression didn't differ between PBS control group and normal **(Figure 41)** indicting that imiquimod doesn't alter IL12 expression profile. IL12 expression profile was reduced in MBL treated group compared to its production in both normal and PBS control group, albeit statistically insignificant, such observation indicates that MBL reduced the IL12 below normal levels so in human psoriasis MBL can play role through blocking IL12 as in Ustekinumab, a monoclonal antibody against shared p40 subunit of IL23 & IL12, used for psoriasis therapy.

Conclusions & Recommendations:

In conclusion, Imiquimod animal model of psoriasis is a representative and feasible model for early immune mediated psoriasis pathway. MBL represent a promising therapeutic for psoriasis, being an immune response modulator secreted at the site of inflammation to safeguard against excessive unneeded tissue damage. MBL targets many steps in psoriasis pathway including early steps of antigen presentation via TLRs and dendritic cell maturation. MBL dependent reversal of psoriasiform lesions induced by Imiquimod is associated with reduction of IL-6 expression and to some extent to IL12.

We recommend more studies to identify the efficacy of MBL in preventing the development of psoriasis lesions and to evaluate the efficacy of MBL in treating psoriasiform lesions in other animal models e.g. IL23 induced psoriasis model. Modification of the dose can be done to identify the least effective dose. Clinical trials of MBL treatment in psoriatic patients either as systemic therapy or intra-lesional can be started given the availability of MBL which has been successfully extracted from human plasma and used for clinical trials in chemotherapy patients **(Table 1)**. Nanotechnology aided MBL delivery may enhance the delivery and decrease the dose of recombinant human MBL.