6-1-2017

Mannose binding lectin in the treatment of imiquimod induced psoriasis mouse model

Hagar Nofal

The American University in Cairo

School of Sciences and Engineering

MANNOSE BINDING LECTIN IN THE TREATMENT OF IMIQUIMOD INDUCED PSORIASIS MOUSE MODEL

Thesis Submitted to

Biotechnology Graduate Program

In partial fulfillment of the requirements for the degree of Master of Science in Biotechnology

By

Hagar Osama Nofal

M.B., B.Ch.(2009), M.Sc. in Dermatology (2014), Zagazig University

Under the supervision of

Dr. Ahmed Abdellatif

Assistant Professor, Biology Department
The American University in Cairo

May/ 2017

Dedication

To Ahmed Saeed The Light Allah sent to guide me through life.

Acknowledgment

My sincere gratitude goes to my supervisor, **Dr. Ahmed Abdellatif**, who I had wonderful learning and life experience working with him for the past 2 years. I have learnt a lot, not only in science but also in life. His continued guidance, giving and support is inspiring.

To my Family, **Mom, Dad, Rofida, Sara, Mohamed, Nofal & Ahmed** who always stood by me, supported, encouraged, endured a lot and provided for me.

To Dr. **Eman Abdel Zaher** for her guidance and helpful advice in the pathology work.

To Dr. **Suher Zada** who supported and advised me sincerely through rough times.

To my friends who helped, supported and gave me very good lab tips through my journey**, Nahla Mousa, Nahla Hussien, Salma Elshafee, Sara Kamel, Sara Sami, Youmn Abdallah, Hana Heiba, and Menna El Far**, to all of you Many Thanks!

A special gratitude goes to the Al Alfi Fellowship fund for helping and providing fund for my master in AUC.

I would like to express my appreciation for AUC research grants for funding this research

A special thank you to all lab staff, who I enjoyed working with and learnt a lot as well. A special thank you for **Mr. Ahmed Soliman** & **Mr. Zein Abdeen** from the Biology Department who were very helpful.

Thanks for all your encouragement!

Abstract

Psoriasis is a chronic inflammatory skin disorder affecting around 4 % of the world population. Psoriasis develops upon exposure to an unknown antigen in a genetically susceptible individual due to dysregulated innate and adaptive immune responses. Mannose binding lectin (MBL), an innate immune player, is an immune response modulator through its effect on Toll like receptors (TLRs) of monocytes and dendritic cells, both are activated early during psoriasis development. In this study, we investigated the efficacy of MBL treatment in Imiquimod psoriasis animal model and the involved molecules in the observed response. The induction and treatment were evaluated clinically, dermoscopically, histopathologically and Interleukin (IL)-6 & IL-12 expression level using quantitative real time polymerase chain reaction (qPCR). Psoriasis was successfully induced in C57Bl mice using Imiquimod. The successfully induced psoriasis improved significantly on MBL treatment ($P < 0.05$). On the other hand, the control group didn't show any significant improvement ($P > 0.05$). IL-6 expression level was reduced to normal on MBL treatment while it was significantly higher in PBS ($P > 0.05$). IL 12 was slightly lower in MBL treated group than both normal and PBS group but wasn't statistically significant ($P>0.05$). These results support a role for MBL in the treatment of psoriasis through lowering IL-6 level and potentially IL-12. Both cytokines are involved in early psoriasis development with establishment of T helper (Th) 17 & Th1 pathways, respectively. We recommend further investigation of MBL as a promising therapeutic of psoriasis in other psoriasis mouse models and in clinical trials.

TABLE OF CONTENTS

List of Abbreviations

AMPs	Antimicrobial peptides and proteins
APCS	Antigen Presenting Cells
CCL	Chemokine Ligand
CCR	Chemokine receptor
CK6	Cytokeratin 6
CLA	Cutaneous lymphocyte-associated antigen
CXCL	Chemokine (C-X-C motif) ligand
DCs	Dendritic Cells
dsRNA	double-stranded RNA
EGF-R	Epidermal growth factor receptor
ERAP1	Endoplasmic reticulum aminopeptidase 1
GM-CSF	Granulocyte/macrophage colony-stimulating factor
GWAS	Genome Wide Association Studies
H&E	Hematoxylin and Eosin
HLA	Human leucocyte antigen
HMGB1	High mobility group box1
hβD	human β defensing
ICAM	Intracellular adhesion molecule
IGF-1	Insulin like Growth Factor-1
IL	Interleukin
IMQ	Imiquimod
INF	Interferon
IκB	Inhibitor of κB
JAK	Janus kinases
KGF	Keratinocyte growth factor
LCs	Langerhans cells
LFA	Leukocyte function associated antigen-1
LL37	Human Cathelicidins
LPS	lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MASPs	MBL-associated serine Proteases
MBL	Mannose Binding Lectin
mDcs	myeloid DCs
MHC	Major histocompatibility complex
moDCs	Monocyte derived Dendritic Cells
MTHFR	Methylene tetrahydrofolate reductase
MyD88	Myeloid differentiation factor 88
NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-alpha
NFκB	Nuclear Factor κB
PBS	Phosphate Buffer saline
PCR	Polymerase chain reaction

pDCs	Plasmacytoid dendritic cells
PDGF	Platelet derived growth factor
qPCR	Quantitative real time PCR
ROR	Retinoic acid related Orphan Receptor
SLE	Systemic Lupus Erythematosus
SNPs	Single nucleotide polymorphisms
ssRNA	single-stranded RNA
STAT	Signal Transducers and Activators of Transcription
T-bet	T- box expressed in T cells
Tc	T cytotoxic
TCR	T cell receptor
TGF	Transforming Growth Factor
Th	T helper
Tie2	Tyrosine kinase with immunoglobulin-like and EGF-like domains 1
TLRs	Toll like Receptors
TNFAIP3	Tumor Necrosis Factor Alpha-induced protein 3
TNFRSF1B	TNF receptor superfamily 1 B
TNF-α	Tumor necrosis factor- α
TNIP1	TNFAIP3- interacting protein 1
TRAF	TNF receptor (TNFR)-associated factor
TRAF3IP2	TRAF3-interacting protein 2
Treg	T regulatory cells
TRIF	Toll IL-1 receptor (TIR) domain containing adaptor inducing IFN-β
UTR	Untranslated Region
UVB	Ultraviolet B
VCAM	Vascular cell adhesion molecule
VEGF	Vascular endothelial growth factor
VLA-1	Very late antigen 1

List of Tables

List of Figures

Introduction

Psoriasis is an inflammatory skin disease affecting 3 - 4% of world population. Psoriasis can be disfiguring and disabling in severe cases. The disease affects patients' psychology and their life quality. Moreover, psoriasis has been recently associated with other comorbidities as diabetes, cardiovascular risk, and other elements of metabolic syndrome **(Cai et al., 2013; Griffiths and Barker, 2010; Mahil et al., 2016).**

In 2014's Sixty-seventh World Health Assembly of the World Health Organization (WHO) a resolution on psoriasis was passed. All state members have committed to increase the awareness about the disease and to fight stigmatization associated with it. WHO recognized that psoriatic patients suffered needlessly across the globe inadequate treatment options among other causes. With the upward trend psoriasis incidence and prevalence seems to follow, the high global burden the disease, according to WHO, equals twice as acute hepatitis C, there is flare in psoriasis therapeutic market **(World Health Organization [WHO], 2016)**. It is expected that the psoriasis market grow from $5 B market in 2014 to $13.3 B in 2024.

The exact cause of psoriasis is still unknown; however there is evident dysregulated immune response in genetically susceptible individuals to unknown stimuli **(Mahil et al., 2016; Mitra et al., 2013)**.

Innate and adaptive immune responses show dysregulation in psoriasis patients. Of the innate immune system key players, both mannose binding lectin (MBL) and toll like receptors (TLRs) have been linked to psoriasis **(Hari et al., 2010; Heitzeneder et al., 2012).** MBL is an inflammatory response modulator and is linked to autoimmune diseases e.g. systemic lupus erythematosus, rheumatic fever **(Heitzeneder et al., 2012)**. MBL has a protective role against extensive unneeded inflammatory reaction leading to tissue damage as occurs in psoriasis pathogenesis **(Downing et al., 2005; Wang et al., 2011a).** MBL levels and function depend on its gene *mbl2*. It has 6 polymorphisms in the promoter and exon 1 regions leading to varying degrees of loss of MBL function **(Heitzeneder et al., 2012).** The B variant of *mbl* gene has been associated with psoriasis in Turkish population **(Turan et al., 2014).**

Effective treatment of psoriasis needs to consider attacking a central, early point in the psoriasis development pathway without compromising the immunity to avoid the major side effects of the biologic therapy available today. MBL replacement restores the normal tolerance of the skin lost during psoriasis without impairing the immune response to infection or malignant transformation.

The aim of this work was to evaluate the efficacy of MBL, as an immune response modulator, in the treatment of psoriasiform inflammation induced in mice as an animal model for psoriasis.

Review of Literature

Psoriasis is a chronic proliferative skin condition **(Griffiths and Barker, 2010)**. It is a common disease affecting about 3 – 4 % of world's population **(Cai et al., 2013).** The condition is disfiguring and affects patient's quality of life **(Pollock et al., 2011)**.

Epidemiological studies showed that both sexes are equally affected by psoriasis. It usually first develops around 20 years old or sixth decade of life. Type I psoriasis begins before age 40 and represents about 75% of all psoriasis patients. Type I patients usually suffer more severe course, with limited success of treatment, increased prevalence of specific human leucocyte antigen (HLA)-types and stronger hereditary ties **(Mitra et al., 2013; Sabat et al., 2007)**.

Furthermore, a distinct group of disorders have been identified by some epidemiological studies to frequently associate with psoriasis, including colitis, rheumatoid arthritis, diabetes, metabolic syndrome and hypertension **(Sabat et al., 2007).**

Psoriasis has different clinical subtypes with plaque psoriasis, the most common variant affecting almost 80-90% of cases. Typically lesions are well-demarcated red scaly plaques with characteristic salmon pink color and white silvery scale the distribution is usually on the extensor surface of the limbs and the sacral area, (**Figure 1) (Burden and Kirby, 2016)**. Other clinical types include pustular, erythrodermic, unstable psoriasis and guttate psoriasis (**Burden and Kirby, 2016; Sticherling, 2005**).

Psoriasis may also affect specific sites e.g. psoriasis of the scalp, nail psoriasis, flexural, genital psoriasis and palmoplantar psoriasis. Psoriasis of pregnancy (dermatitis herpitiformis) is a specific entity affecting pregnant females either arises in a known psoriatic patient or de novo during pregnancy, and it can be associated with placental insufficiency. Psoriatic arthropathy is an oligoarticular seronegative arthropathy, which affects both axial and peripheral joints along with soft tissue swellings. It may or may not be associated with cutaneous lesions and it affects 30 % of patients with cutaneous psoriasis **(Ariza et al., 2013; Hébert et al., 2012).**

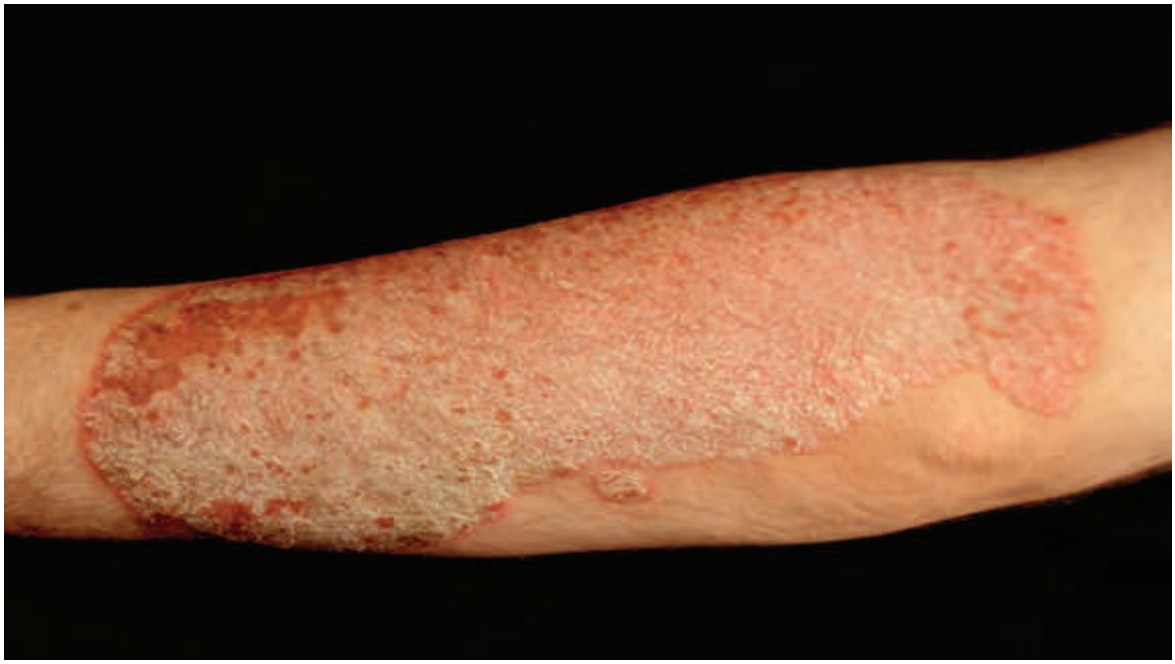


Figure 1: Plaque Psoriasis. Red salmon pink well demarcated plaque on the extensor surface of the arm covered with white silvery scale.
Adopted from Burden and Kirby, 2016

Microscopically (**Figure 2)** psoriasis is composed of three distinct components; the epidermal component, the vascular component and the inflammatory component **(Sticherling, 2005)**. Psoriatic lesions exhibit epidermal acanthosis (diffuse epidermal hyperplasia) with elongation of rete ridges, parakeratosis (retention of the nuclei in stratum corneum) along with lymphocytic infiltration with loss of granular cell layer. The dermal papillae shows dilated elongated and tortuous blood vessels along with inflammatory infiltrate with predominant T cell population, neutrophils and dendritic cells **(Burden and Kirby; 2016; Cai et al., 2013; Lever & Elder, 2009).**

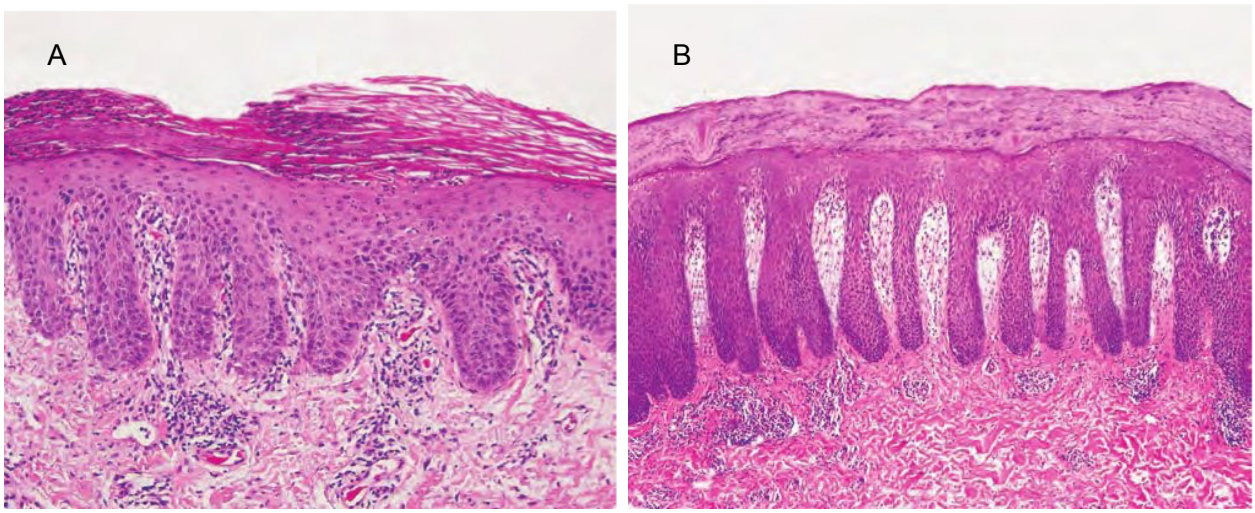


Figure 2: Psoriasis Pathology. **A)** Early Papillary dermal vasodilatation and edema with leukocyte infiltration followed by epidermal changes in the form of thinning of the granular layer along with mild epidermal hyperplasia, scattered parakeratosis, and in the lower half of the epidermis leukocytic infiltration within foci of spongiosis with formation of Spongiform Pustule of Kogoj. **B)** Fully developed plaque psoriasis with more evident parakeratosis with focal orthokeratosis, Munro micro-abscesses formation (accumulation of neutrophils in the stratum corneum), near absence of the granular layer, marked hyperplasia with elongation of rete ridges and supra-papillary epidermal thinning giving the characteristic club shaped, branched and sometimes fused at bases rete ridges. Mononuclear leukocyte infiltrates in the lower part of the epidermis are still evident. **Adopted From Lever & Elder, 2009.**

Despite the knowledge gathered regarding the molecular mechanisms behind psoriasis, major questions are still asked. Is the primary nature of psoriasis is keratinocyte disorder or an immunologic one? Is the disease autoimmune in nature or auto-inflammatory or both? Is it systemic or cutaneous disorder with comorbidities? Which has the upper hand regarding disease initiation, progression and therapeutic response the environmental or the genetic factors? **(Nestle et al., 2009).**

ETIOPATHOGENESIS OF PSORIASIS

Psoriasis is multifactorial in nature with no obvious single etiology. With the contribution (**Figure 3**) of genetic background, environmental and endogenous factors clinical psoriasis develops **(Mahil et al., 2016; Sticherling, 2005).**

The paradigm of psoriasis etiopathogenesis has evolved from divine power in ancient records passing through unknown infectious organism to a pure keratinocyte proliferation disorder and finally as a dysregulated immune system mediated disease **(Mitra et al., 2013).**

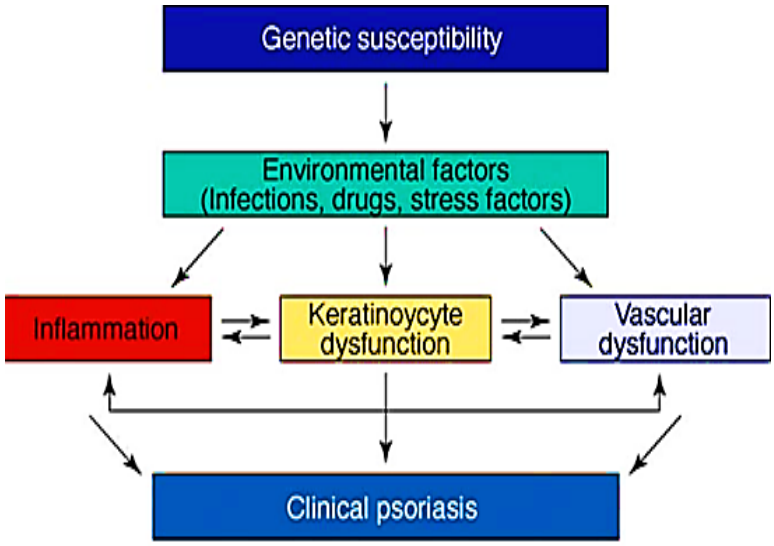


Figure 3: Etiopathogenesis of Psoriasis. Psoriasis is considered to develop due to co-occurrence of inflammatory dysregulation, keratinocyte dysfunction and vascular dysfunction on a genetic basis in response to environmental initiating stimuli eventually resulting in epidermal & vascular hyperplasia along with dermal and epidermal inflammation. **Adopted from Sticherling, 2005.**

I. Genetics of psoriasis:

The genetic background is well-recognized in psoriasis (**Figure 3**), however it has to be triggered by environmental factors for psoriasis to develop **(Brotas et al., 2012; Sticherling, 2005).**

The prevalence of psoriasis is much greater among 1st and 2nd degree relatives than healthy controls. Also the susceptibility to develop psoriasis is 14% if one parent is psoriatic, elevated to 41% if both are, and drops to 6% if one sibling is affected compared to only 2% if there is no family history of psoriasis. The concordance rate

in twins ranges from 64% to 73% in monozygotic twins and 15% to 20% in dizygotic twins **(Griffiths and Barker, 2010; Nestle et al., 2009)**. Moreover, in monozygotic twins, when both siblings develop psoriasis, the distribution, severity and age of onset are similar which illustrates the role of genetics **(Nestle et al., 2009)**.

Genome Wide Association Studies (GWAS) highlighted the association of 20 different loci with psoriasis development **(Hébert et al., 2012; Stuart et al., 2012)**. Of these genes, endoplasmic reticulum aminopeptidase 1 (*ERAP1)* gene highlighted the importance of innate immune system in the pathogenesis of psoriasis. *ERAP1*gene encodes for aminopeptidase regulating the peptide ligands of MHC- class I encoded by HLA-C **(Strange et al., 2010; Hébert et al., 2012).** Moreover, GWAS attracted the attention to the Th17/ IL23 key role in psoriasis. Polymorphisms of genes involved in regulating this pathway as *IL12B, IL23R (PSORS 7) and IL23A* have been associated with psoriasis **(Hébert et al., 2012).** Other genes - identified by GWAS- defined the important role of nuclear factor kappa (NFκB) pathway in psoriasis development e.g. Tumor Necrosis Factor Alpha-induced protein 3 *(TNFAIP3*), *TNIP1* (TNFAIP3- interacting protein 1), reticuloendotheliosis viral oncogene homolog (v-rel), tyrosine kinase 2 (*TYK2*), TRAF3-interacting protein 2 *(TRAF3IP2*) and nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-alpha *(NFKBIA***) (Hébert et al., 2012).**

Mannose-binding lectin (*mbl*) gene polymorphism analysis in psoriatic patients showed increased prevalence of B variant in psoriasis patients compared to healthy control subjects **(Turan et al., 2014).** *mbl* gene encodes for MBL a pattern recognition molecule with inhibitory effects on TLR 4 & TLR9.

II. Environmental factors:

As stated before psoriasis develops in a genetically susceptible individual on exposure to certain environmental conditions that trigger dysregulated innate immune response **(Mahil et al., 2016; Sticherling, 2005).** The environmental factors accused in psoriasis development range from infection, trauma to psychogenic factors, cigarette smoking, excessive alcohol use and sunlight exposure **(Burden and Kirby, 2016)**.

GWAS highlighted the role of the environment and the microbiome in psoriasis pathogenesis. For example, the discovery of the association of 3 susceptibility variants in late cornified envelope (*LCE*) gene cluster*; LCE3A*, *LCE3C* and *LCE3D* with psoriasis highlighted the role of skin barrier in disease development **(Hébert et al., 2012).** Another GWAS discovered the association of psoriasis with high copy number of beta-defensin genes on chromosome 8**.** β defensins are peptides with antimicrobial activity expressed in epidermis to guard against microorganisms and are highly expressed in psoriatic plaques. This high level of β defensins with their cytokine-like properties may precipitate an inappropriate inflammatory response after minor skin injury, infection or other environmental trigger leading to clinical psoriasis **(Hollox et al., 2008; Hébert et al., 2012).** Moreover, interferon induced with helicase C domain 1(*IFIH1*) gene polymorphism has been shown to be associated with psoriasis. *IFIH1* encodes for pattern recognition receptor plays a key role in sensing viral infection and subsequent activation of a cascade of antiviral responses including type I interferons and pro-inflammatory cytokines induction **(Strange et al., 2010; Hébert et al., 2012)**.

a. Infection:

Infection has been identified as a trigger for psoriasis vulgaris development and exacerbation along with the guttate variant **(Sticherling, 2005).** β hemolytic streptococcal infection and α streptococci have been accused in the development and exacerbation of different types of psoriasis **(Sigurdardottir et al., 2013).**

Two models are proposed trying to explain the role of infection in the pathogenesis of psoriasis. 1st model suggests cross reactivity between antigens of streptococci and epidermal keratinocytes. Thus, on exposure to infection primed T cells attack self-epidermal keratinocytes starting chronic inflammatory response. The 2nd model increments streptococcal super-antigens which bridge HLA-antigen and T-cell receptor without the presence of specific antigens within the receptor complex and subsequently activate polyclonal T-cells and induce massive cytokine release with subsequent immunological processes **(Sticherling, 2005; Sigurdardottir et al., 2013)**.

b. Psychological factors:

Stress is a proven provocateur of psoriasis development or exacerbation of active disease. 60% of psoriatic patients mentioned stresses to be associated with development of psoriasis and /or its exacerbation. Furthermore, stress management programs shorten the time needed to achieve clearance with standard therapies **(Griffiths and Barker, 2010; Sticherling, 2005).**

Recent advances in neuro-immunology relate psychological and neurological processes to immunological ones **(Sticherling, 2005).** Furthermore, pathologically worrier psoriatic patients have less response to photo therapy **(Griffiths and Barker, 2010).**

koebner phenomenon in psoriasis can be seen in this prospective; trauma as mechanical stress inducing psoriasis within days to weeks **(Sticherling, 2005)**

III. Immunity & psoriasis:

Immune cells have been introduced as players in the pathogenesis of psoriasis to complement the soul keratinocyte proliferative disorder as the primary pathogenesis of psoriasis **(Burden and Kirby, 2016)**. The evidence is compelling, specifically, the increased numbers of immune cells, specifically dendritic and T cells, in psoriatic lesions, the functional role of T cells and their cytokines in models of psoriasis, the therapeutic efficacy of drugs directed at the immune system, the findings that some psoriatics get cured on receiving bone marrow transplantation and that psoriatic transplant donor can transfer psoriasis to the recipient and that top hits in the whole-genome scans of genes and mRNA are immune-related **(Mahil et al., 2016; Nestle et al., 2009).**

The pathogenesis is now believed to involve dysregulated innate and adaptive immune responses in the skin to unknown antigens in a genetically susceptible individual; replacing the older concept of being a pure keratinocyte proliferation disorder with the occurrence of T cell activation and skin infiltration as a secondary event. Currently, both psoriasis and psoriatic arthritis are looked at as autoimmune disorders **(Brotas et al., 2012; Coimbra et al., 2012; Girolomoni et al., 2012; Lee et al., 2012; Mitra et al., 2013).**

Skin has been viewed as the second large innate immune organ in human body providing both a physical barrier and an immune-surveillance system **(Møller-Kristensen et al., 2007)**. This surveillance system is composed of APCs, epidermotropic T cells, cytokine-secreting keratinocytes, dermal capillary endothelial cells, mast cells, tissue macrophages, fibroblasts, granulocytes, lymph nodes, and non-Langerhans cells. All these cells communicate together by cytokine production and accordingly respond upon stimulation by chemicals, ultraviolet light, bacteria, and other irritating factors **(Coimbra et al., 2012**).

In psoriasis, there is interaction between the innate and adaptive immune systems cells and resident cells in the skin, including keratinocytes, fibroblasts, mast cells and endothelial cells **(Girolomoni et al., 2012, Mitra et al., 2013).**

In brief, antigen presenting cells - immature dendritic cells of the skin – on exposure to a still unknown antigen are activated, migrate to lymph nodes, present this antigen to T cells starting their activation and differentiation **(Coimbra et al., 2012; Lee et al., 2012**). Interestingly dendritic cells in psoriasis lesions are eight times more capable of activating T cells than those of uninvolved or normal skin **(Nestle et al., 1994).** However, the event causing the initial activation of dendritic cells has not been fully recognized yet **(Coimbra et al., 2012; Lee et al., 2012**).

Innate and adaptive immune response in psoriasis involves activation of three inflammatory pathways; IL-12/Th1, IL-23/Th17 and IL-22/Th22 **(Mitra et al., 2013)**.

a- Antigens:

The primary event in psoriasis development is the uptake and presentation of - still unknown - antigen by antigen presenting cells (APCs). Such hypothetical antigen may be an exogenous antigen; streptococcal antigen is the most accused one as the exacerbation of psoriasis or induction of the disease usually follows streptococcal pharyngitis or tonsillitis this typically occurs in guttate psoriasis **(Sticherling, 2005).**

Some evidences indicate the presence of self-antigens, although their nature is still unknown. Psoriasis is believed to result from self-continued activation of auto-reactive T cells due to exposure to keratinocyte-derived autoantigens **(Albanesi et al., 2010).**

b- Antimicrobial peptides and proteins (AMPs):

They are short (＜100 amino acids) positively charged molecules. They bind to and interact with the negatively charged membranes of microorganisms and kill. The exact mechanisms of killing are still not fully understood. AMPs may form pores in the membrane of microorganisms leading to lysis of the pathogenic threat **(Büchau and Gallo, 2007).** Antimicrobial peptides are upregulated in the skin during infection, inflammation and injury. They are expressed by keratinocytes, cells of eccrine gland, mast cells, and infiltrating immune cells (e.g. de-granulated neutrophils, natural killer cells) **(Büchau and Gallo, 2007; Reinholz, et al., 2012).**

Recent studies showed that AMPs not only function as simple antibiotics but also function as chemokines, proteinase inhibitors, and neuropeptides. Moreover, AMPs are involved in wound healing, induction of pro-inflammatory cytokine response, vascularization and skin differentiation processes. AMPs expression in epidermal keratinocytes is induced by high Ca^{+2} concentration, retinoid acid, and 1,25 (OH)-vitamin D3 **(Büchau and Gallo, 2007; Reinholz, et al., 2012)**. Due to these versatile functions of AMPs as activators of adaptive immunity and inflammation the are called ‘‘alarmins’’ **(Peric et al., 2009).**

Many evidences link AMPs with psoriasis pathogenesis. 1st AMPs are highly expressed in psoriasis lesions. 2nd the Koebner phenomenon, development of a psoriatic plaque after trauma of unaffected skin, could be attributed in part to the AMPs. On skin injury AMPs are upregulated and could be the trigger for the development of a psoriatic plaques in a genetic predisposed subject **(Büchau and Gallo, 2007).**

Many AMPs have been studied in psoriasis:

1. Human β-defensins (HβDs):

Human β-defensins (hβDs) are secreted by neutrophils among other cells e.g. epithelial cells. hβD-1 is constitutively expressed in epithelia while hβD-2 and hβD-3 are highly upregulated in inflamed skin including psoriasis. hβD- 1, 2 & 3 have antimicrobial activity. hβD-2 and hβD-3 are expressed in psoriatic skin under the influence of TNF-α, interferon- γ (INF-γ), IL-1, IL 22 via STAT 3 activation and by

IL-17A through JAK and (Nuclear Factor κ B) NF-κB **(Büchau and Gallo, 2007; Nomura et al., 2003; Peric et al., 2009).**

2. Human Cathelicidins (LL37):

Cathelicidin is secreted by epithelial cells and neutrophils. In human healthy skin it is present in very low levels however upon injury or infection its production is induced in these cells**.** Vitamin D is a potent inducer of LL-37 in cultured human keratinocytes **(Büchau and Gallo, 2007; Peric et al., 2009; Reinholz et al., 2012).**

Cathelicidin (LL-37) has wide antimicrobial activity against bacteria, viruses and fungi. Along with this antimicrobial function, LL-37 enhances cytokine and chemokine secretion from local cells e.g. keratinocytes, and mast cells. Furthermore, it influences angiogenesis, increases the proliferation of endothelial cells and suppresses induction of apoptosis in keratinocytes **(Peric et al., 2009; Reinholz et al., 2012).**

In psoriatic plaques, there is increased level of LL-37 expression **(Büchau and Gallo, 2007).** It is believed that it plays a role in starting the auto inflammatory cascade of psoriasis as LL-37 forms complexes with self DNA activating dermal plasmacytoid dendritic cells (pDCs) through its TLR 9. So LL-37 transforms the inert self-DNA into a potent trigger of interferon liberation by pDCs in psoriatic lesion; pro-inflammatory **(Reinholz et al., 2012).**

3. S100 proteins:

S100 proteins are of low molecular weight (9–13 kDa). Fourteen of the 21 S100 genes are present in the epidermal differentiation complex on chromosome 1. Thirteen S100 proteins are synthesized in normal and/or diseased epidermis. Specific S100 proteins are upregulated in psoriasis, during wound healing, inflammation, cellular stress, arthritis, skin cancer and metastasis **(Büchau and Gallo, 2007).**

S100A7, S100A8 (calgranulin A), S100A9 (calgranulin B), S100A12 (calgranulin C) and S100A15 have a putative innate immune role **(Büchau and Gallo, 2007).**

Along with the antimicrobial activities, multiple S100 proteins are involved in the control of epidermal maturation and are highly upregulated in psoriatic plaques. S100A7 & S100A15 have been shown to be strongly overexpressed in keratinocytes

on stimulation with high Ca^{+2} and pro-inflammatory cytokines **(Büchau and Gallo, 2007).**

c- Dendritic cells:

Dendritic cells (DCs) are the key sentinels of the immune system which bridge the gap between the innate and the adaptive immune systems. Dendritic cells are not only antigen-presenting cells but activate T cells present in the dermis and epidermis, as well **(Coimbra et al., 2012; Nestle et al., 2009).** Dendritic cell subtypes include myeloid (mDCs) and plasmacytoid (pDCs)**(Ueno et al., 2011).**

It is interesting to know that DCs outnumber T cells in psoriatic lesions. This highlights their role in psoriasis pathogenesis. pDCs represent 16% of the dermal infiltrate in lesional psoriatic skin, on the contrary they don't exist in normal skin **(Hari et al., 2010; Zaba et al., 2009)**.

Of myeloid DCs (mDcs), only Langerhans cells (LCs) are seen in the epidermis, while two DC subsets $^{CD1a+}$ DCs and $^{CD14+}$ DCs are found in the dermis **(Ueno et al., 2011).** Langerhans cells (LCs) are the DCs of the epidermis and other stratified squamous epithelia. They express a Langerin/CD207 marker. **(Steinman & Idoyaga et al., 2010)**

Langerhans cells express TLR 1, 2, 3, 6, and 10. They produce only few cytokines, including IL-15, a cytokine known to enhance CD8+ T cell responses. **(Ueno et al., 2011)**

In 2005 Nestle and coworkers identified pDCs and its cytokine IFN-α as an important upstream initiators for psoriasis development. Moreover, they proposed that targeted therapy against pDCs and their IFN-α should be taken in consideration for the prevention and early intervention in psoriasis **(Nestle et al., 2005).** One of pDCs activators is stressed keratinocytes which under environmental triggers as infections release self-DNA and self-RNA complexed with the cathelicidin (LL-37). Both complexes (self-DNA-LL37 & self-RNA-LL37) activate pDCs to secrete IFN-α initiating psoriatic lesions **(Denadai, 2013; Lande et al., 2007; Prignano et al., 2012)**. High amounts of IFN-α produced by activated pDCs induce a strong activation of the local immune system; mainly myeloid dendritic cells - secreting TNF-α, IL12

and IL23- and potentially autoreactive T cells which secrete cytokines to further promote the pathogenic inflammatory cascade **(Denadai, 2013; Nestle et al., 2009)**.

Sun exposure rapidly reduces pDCs in psoriatic lesional dermis preceding clinical response which suggests that sun induced clinical improvement can be partly attributed to its effect on pDCs and their products **(Denadai, 2013).**

Farkas and Kemény proposed a new model for psoriasis pathogenesis involved not only pDCs and mDCs but also what is known as monocyte derived dendritic cells (moDCs) **(Figure 4)**. These moDCs have the combined phenotype of pDCs and mDCs along with the characteristics of natural killer (NK) cells and Toll-like receptors (TLRs) **(Farkas and Kemény, 2012).** They are derived from blood monocytes which migrate into skin and differentiate into moDCs under the combined effect of pDCs's INF-α and Granulocyte monocyte colony stimulating factor (GM-CSF) produced by lymphocytes, fibroblasts, mast cells, neutrophils, macrophages and keratinocytes. Other cytokines e.g. IL-1β, TNF-α, IL-6 and IFN-γ have an important role in tuning phenotypes and functional properties of IFN-α-primed moDCs as monocytes may also receive differentiation signals from other cell types such as lymphocytes NK cells, NK T cells, macrophages, fibroblasts and keratinocytes **(Farkas and Kemény, 2012)**.

A very interesting fact is that mannose binding lectin (MBL) inhibits lipopolysaccharide (LPS) induced maturation of immature moDCs and subsequently cytokine production or T cell activation & proliferation **(Wang et al., 2011 b).**

d- Monocytes and macrophages:

The important role of monocytes /macrophages in psoriasis is not clearly defined **(Lee et al., 2012).** The TNFα, IL6, IL1 produced from peripheral blood monocytes of active psoriatic patients is higher than produced from same cells of inactive psoriatics or healthy controls. This infers that peripheral blood monocytes may be the major source of elevated TNFα in psoriasis **(Mizutani et al., 1997; Turan et al., 2014)**.

Furthermore, monocytes are the source of moDCs recruited & differentiated in the skin under the effect of INF-α and GM-CSF **(Farkas and Kemény, 2012).**

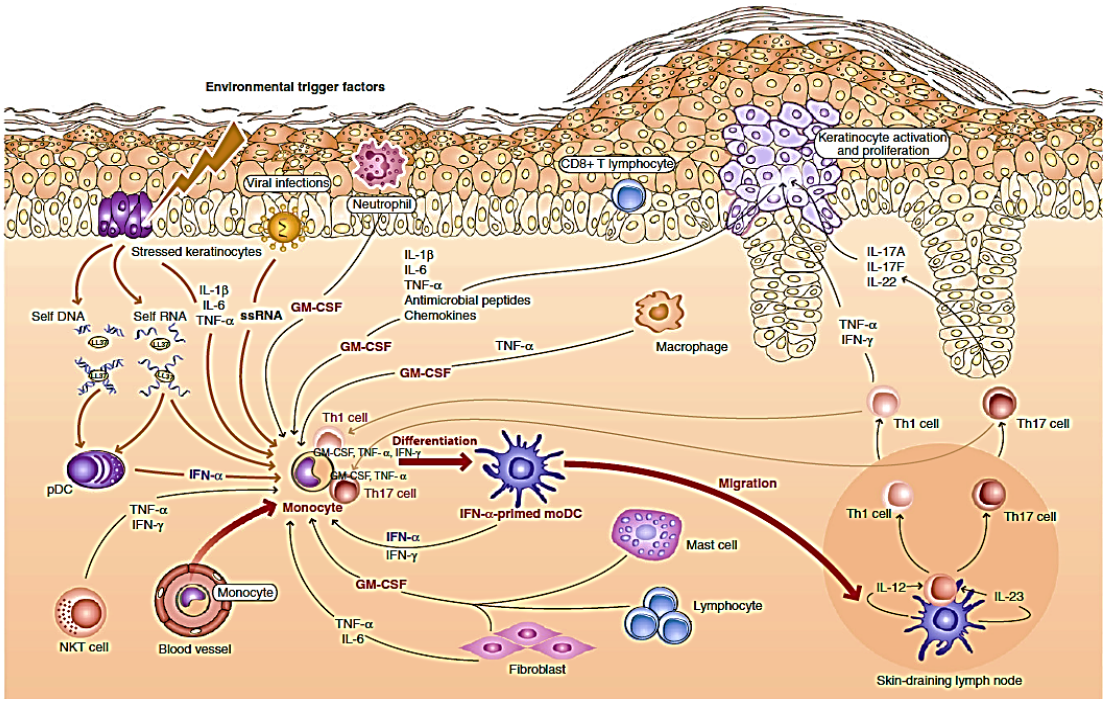


Figure 4: Farkas and Kemény psoriasis pathogenesis model. In this model the role of IFN-α-primed moDCs, TLR stimulation and T lymphocytes is highlighted.

moDC, monocyte-derived dendritic cell; IFN, interferon; GM-CSF, granulocyte/macrophage colony-stimulating factor; IL, interleukin; NK, natural killer; LL37, cathelicidin antimicrobial peptide; pDC, plasmacytoid dendritic cell; Th, T-helper; ssRNA, single-stranded RNA; TNF, tumour necrosis factor. **Adopted from Farkas and Kemény, 2012**

e- T cells

Psoriasis is a disease of dysregulated immune system with Th1/Th17 cells predominance (**Figure 5**). Many observations support such belief; an increase in Th1 cytokine profile (IFN γ, TNF α, IL 12, and IL 2) is observed in psoriasis plaques along with patient's serum, inferring a systemic release of cytokines towards Th1 dominance. Moreover, the circulating levels of IFN γ, TNF α and IL 12 correlates significantly with disease severity and the clinical success of classical therapeutics is associated with the deviation from a Th1 to a Th2 profile **(Coimbra et al., 2012**; **Lee & Hwang, 2012)**. Interestingly, psoriatic lesions cured on the accidental receiving of psoriatic patients a hematopoietic stem cell transplant from a non-psoriatic donor. On the contrary, psoriasis frequently develops in recipients of bone marrow transplants from a psoriatic donor, the recipient also frequently develops psoriasis **(Sabat et al., 2007)**. The major evidence of the key role of Th1/Th17 cells and their cytokine in psoriasis is the success of biologic therapy of psoriasis **(Brotas et al., 2012; Coimbra et al., 2012; Sabat et al., 2007)**.

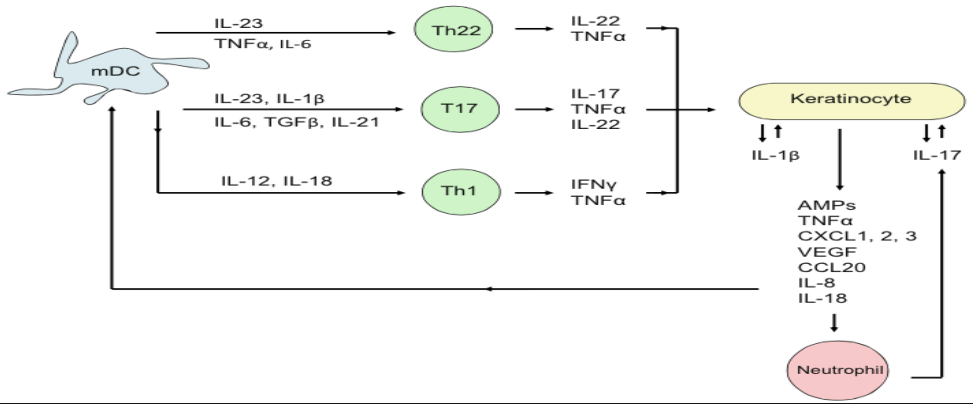


Figure 5: T cell differentiation in psoriasis.

Naïve T cell differentiates to various T cells depending on the stimulus. Naïve T cells differentiate to Th1 & Th17 in psoriasis. INFγ, IL17A, IL17B, IL 21 & IL22 all enhance keratinocyte proliferation. mDC, monocyte derived dendritic cells **Adopted from Mahil et al., 2016**

In psoriasis, on antigen presentation, T lymphocytes enter the circulatory system and, through cell-cell interactions with the endothelial cell lining of blood vessels (**Figure 6**), they migrate to inflamed skin and accumulate around the dermal blood vessels **(Coimbra et al., 2012; Jiaravuthisan et al., 2007)**.

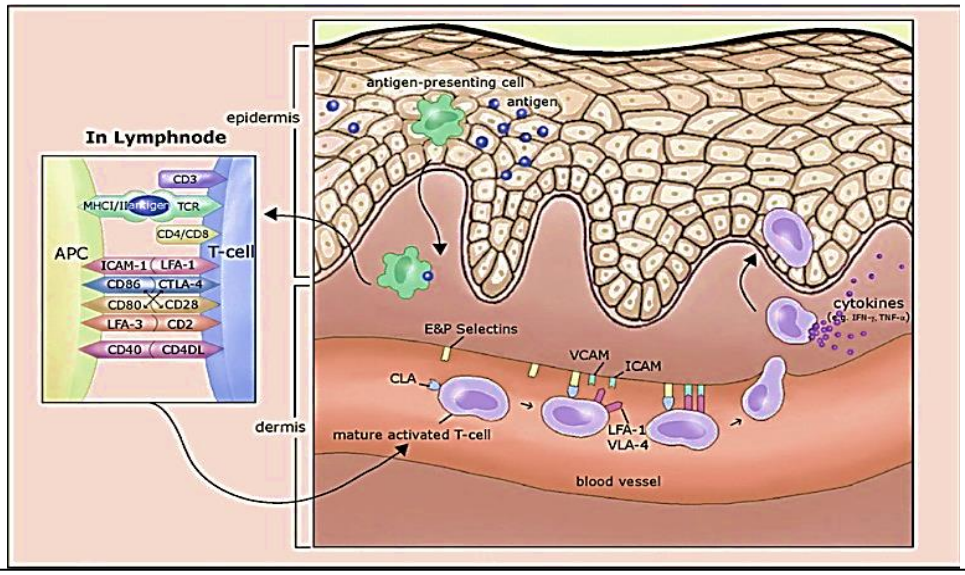


Figure 6: Immunopathogenesis of psoriasis.

The antigen (Ag) is recognized and engulfed by APCs which migrate to lymph node where T cell differentiation occurs. Differentiated T cells get back to skin. On blood vessel wall VCAM, ICAM and E& P selectins of endothelial cells binds to VLA4, LFA1 and CLA on T lymphocytes. Ag, Antigen; APCs, Antigen presenting cells; ICAM, Intercellular Adhesion Molecule; VCAM, Vascular cell adhesion protein; VLA-4, Very Late Antigen-4; LFA-1, Lymphocyte function-associated antigen 1; CLA, Cutaneous Lymphocyte Antigen. **Adopted from Jiaravuthisan et al., 2007**

The naïve T helper cells differentiate into effector cells such T helper 17 cells (Th17) and T helper 1 cells (Th1) **(Nestle et al., 2009) (Figure 5).**

T helper cells differentiate into **Th1** under the influence of IFN-γ which increases IL-12 specific receptor chain expression. IL12 drives Th1 cell-mediated immune response development and locally IL12 plays a role in induction of new psoriatic lesions **(Coimbra et al., 2012; Lee et al., 2012).** Th1 cytokine profile includes TNF-α and INF-γ.

The differentiation of naïve T helper cells into **Th17** happens under the influence of IL6, IL1 and TGF β. They induce the activated memory Th17 cells differentiation from naive $^{CD4+}$ T cells and stimulate the expression of IL17A and IL23 receptors. IL-23 drives Th17 cell proliferation and enhances more IL17A production along with other cytokines e.g. IL 17F, IL 21and IL 22 **(Coimbra et al., 2012**; **Girolomoni et al., 2012; Nestle et al., 2009).**

The orphan nuclear receptor (Retinoic acid related Orphan Receptor) RORγt is the first specific transcription factor expressed in Th17 cells. IL 6 in combination with TGF-β, amplify this Th17 cell RORγt-dependent differentiation **(Ivanov et al., 2006; Coimbra et al., 2012).** Moreover, IL 6 induces IL 21 expression with more induction of IL 21 & IL 23 receptors in naive $^{CD4\,(+)}$ T cells **(Coimbra et al., 2012).** IL 6 and IL 21, via signal transducer and activator of transcription 3 (STAT3), promote Th17 cell differentiation. STAT3 also promotes RORγt expression. IL 21 and IL 23 induce RORγt, which, in synergy with STAT3, promote IL 17 expression **(Mathur et al., 2007; Coimbra et al., 2012).**

T helper 17 cytokine profile includes IL 17, IL 22 and IL 21.

γδ T cells function as innate-like cells; involved in immune surveillance of the skin with immediate response capabilities and they amplify Th17 cell immune response upon their activation by IL 23, IL 1β or danger signal stimulation **(Cai et al., 2013; Laggner et al., 2011).**

γδ T cell role in psoriasis development has been illustrated both in animal models and human. In T cell receptor δ deficient (TCRδ $^{-/-}$) mice, the epidermal acanthosis, inflammation and IL-17 levels were significantly lowered. In humans, γδ T cells are detected at high frequency in psoriatic lesions and are capable of producing large amounts of IL-17 in response to IL-23 stimulation **(Cai et al., 2013).** Skin-homing Vγ9Vδ2 T cell subset is the subset of γδ T cells detected in psoriatic skin, the exact source of which yet to be identified. Some studies claims that they are redistributed from blood to psoriatic skin based on their low peripheral blood level in psoriatics compared to healthy control. This level gets normalized with successful therapy using psoriasis-targeted therapeutic **(Cai et al., 2013; Laggner et al., 2011).** Other suggests local expansion in the skin under inflammatory condition **(Cai et al., 2013).**

The cytokine profile of γδ T cells includes TNF-α, IFN-γ and IL-17A, along with pro-inflammatory chemokines such as IL-8, CCL4, CCL3, and CCL5. They are important for the recruitment of key immune effector cells to the skin **(Cai et al., 2013; Laggner et al., 2011).**

Many studies addressed the role of newly discovered T cell subset; **Th 22** in the pathogenesis of psoriasis. Kagami and coworkers proved increased circulating Th 22

cells in psoriatic patients among other T cells subsets **(Kagami et al., 2010).** Nograles and coworkers showed that the main source of IL22 secretion in psoriatic plaques is Th 22 and Th 17 **(Nograles et al., 2009).** The role of Th 22 in psoriasis pathogenesis is conducted through its IL22 production discussed in detail later on.

Despite the evident and major role of T cells in the pathogenesis of psoriasis, the cause of this sustained T cell response is need to be identified and whether it is due to recognition of autoantigens in psoriatic plaque or due to proliferation of memory T cells on exposure to cytokines in an antigen – independent way **(Girolomoni et al., 2012).**

f- Cytokines:

Cytokines are small (8-80 kD), biologically active proteins. They regulate cell growth, differentiation and function. They also aid in steering the immune response and inflammation. On cellular activation, they get released in brief, self-limited events. The actions of cytokines are usually redundant and pleiotropic meaning they are able to act on different cell types **(Brotas et al., 2012; Coimbra et al., 2012)**.

Cytokines are divided into groups based on their biological actions; mediators of innate immunity, mediators of adaptive immunity, and stimulators of hematopoiesis. They are also classified based on the structural similarities in between and the similarities in their receptors sequence. Regardless of the classification, different cytokines share receptor subunits, hence network and functional redundancy **(Brotas et al., 2012)**.

The psoriatic lesion results from interactions between many different cell types. Such strong interdependence between these cells is only available through a complex network of growth factors, various chemokines and pro-inflammatory cytokines (**Figure 7**), such as interleukins, IFN γ, and TNF α (**Brotas et al., 2012; Coimbra et al., 2012)**.

Psoriasis is accepted as a Th1/Th17 inflammatory disease, hence is associated with an increase in Th1 and Th17 cytokines **(Coimbra et al., 2012)**. Different cytokines involved in the pathogenesis of psoriasis **(Figure 7)** (**Brotas et al., 2012)**.

1. *Type I Interferons:*

Type I IFNs include IFN α, β, ω, δ, τ, κ subtypes. IFN α is the dominant type I IFN in psoriatic skin **(Yao et al., 2008; Zheng et al., 2007; Baliwag et al., 2015).**

Plasmacytoid dendritic cells (pDCs) are the major professional INF α secreting cells in the body **(Denadai, 2013; Farkas and Kemény, 2012; Prignano et al., 2012; Zheng et al., 2007).**

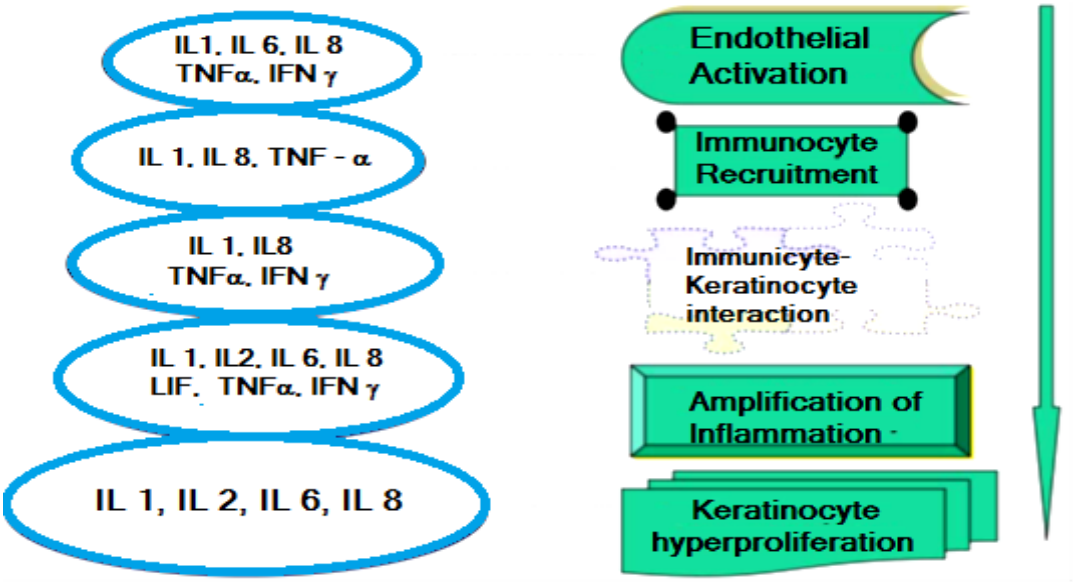


Figure 7: Cytokines of psoriasis. Various cytokines, and their role in Psoriasis development. The main cytokines are grouped based on the processes they affect. Pleiotropism, redundancy and induction of these cytokines are evident. **Modified from Brotas et al., 2012**.

IFN-γ is a type II IFN, mainly secreted by activated Th1 cells, dendritic cells, and NK cells **(Coimbra et al., 2012; Yao et al., 2008)**. Early in psoriasis and under the influence of IFN-α naïve T cells differentiate into Th1 cells producing IFN-γ and TNF-α. The secretion of IFN-γ is under the influence of IL2 from T cells, IL12 from Langerhans cells and IL18 which synergizes the action of IL12 **(Fantuzzi et al., 1999; Coimbra et al., 2012).** IFN-γ induces several chemokines in keratinocytes including CCL2, CXCL2, CXCL9, CXCL10 and CXCL11 which increase the chemotaxis of immune cells. IFN-γ activates monocytes/macrophages, dendritic cells, and endothelial cells. IFN-γ inhibits keratinocytes apoptosis, so in part it contributes to keratinocyte hyperproliferative state observed in psoriatic lesions, and it also stimulates epidermal cell proliferation **(Coimbra et al., 2012; Sabat et al., 2007)**.

3. *Tumor Necrosis Factor-α:*

Tumor necrosis factor α (TNF- α) is a key player in psoriasis development. Its expression level is increased both locally in lesional skin and systemically in the serum of the patients (**Brotas et al., 2012)**. Macrophages & monocytes are the main source of TNF α, yet it get secreted by a various immune and non-immune cells as well including neutrophils, dendritic cells, natural killer cells, endothelial cells, keratinocytes, melanocytes and fibroblasts **(Coimbra et al., 2012)**.

In psoriasis, TNF-α affects different cells, and increases the ICAM 1 expression on dermal endothelial cells with subsequent enhanced infiltration of T cells and monocytes into skin (**Figure 6**) **(Brotas et al., 2012**; **Jiaravuthisan et al., 2007)**. TNF-α upregulates IL-6 expression which in turn drives Th17 differentiation. IL 6 also stimulates keratinocyte proliferation **(Brotas et al., 2012**; **Coimbra et al., 2012).** IL-8, a chemotactic cytokine, is induced by TNF-α. TNF-α increases, the keratinocyte proliferation marker, Cytokeratin 6 (CK6) expression which stimulates keratinocyte hyperplasia **(Brotas et al., 2012; Coimbra et al., 2012; Lee et al., 2012).** TNF α enhances the NF-κB inhibitory protein, I-κB, degradation. NF-κB is a transcription factor of TNF-α, IL-6, IL-8, E-selectin, VCAM1 and ICAM1. TNFα also enhances receptors of vasoactive intestinal peptide (VIP) resulting in keratinocyte proliferation. **(Coimbra et al., 2012; Lee et al., 2012; Brotas et al., 2012**) Induction of Vascular Endothelial derived growth factor (VEDGF) by TNF-α enhances angiogenesis. TNF-α is the facilitator of LCs migration from epidermis initiating the immune response (**Figure 7**). This happens through inhibition of epidermal intercellular adhesion molecules, E-cadherins, **(Brotas et al., 2012).**

g- Interleukins:

Interleukins are cytokines produced by leukocytes and act on them **(Brotas et al., 2012)**

1. *Interleukin 1(IL-1):*

IL-1 is called a primary cytokine as it independently initiates a number of mechanisms capable of triggering inflammation. **(Brotas et al., 2012)**

In psoriasis, IL-1 increases adhesion molecules expression, stimulates angiogenesis, activate T cells, induce IFN-γ, TNF-α, IL-8, IL-6 and GM-CSF synthesis and it promotes keratinocyte proliferation. Koebner phenomenon can be

attributed to IL-1 release, when skin is subjected to trauma, keratinocyte release cytokines locally, leading to the development of new psoriatic lesions in some patients. IL-1 regulates almost 400 genes associated with cell adhesion, proteolysis, signal transduction, and abnormal epidermal differentiation processes observed in psoriasis (**Figure 7**) **(Brotas et al., 2012; Yano et al., 2008).**

2. *Interleukin 2:*

IL-2 (**Figure 7**) is a growth factor for lymphocytes and is increased in psoriatic plaques. Its receptors are expressed in activated T lymphocytes and their level in patients' plasma coincides with the severity of the disease and response to therapy **(Brotas et al., 2012)**.

3. *Interleukin 6:*

Typically IL-6 acts as a regulator of the expression of other cytokines. It is involved in cellular proliferation and differentiation. It induces acute-phase proteins in the inflammatory reaction. IL-6 (**Figure 7**) is detected in normal human skin in basal keratinocytes, fibroblasts endothelial cells and mononuclear cells **(Brotas et al., 2012).** In psoriasis it is expressed the most in the superficial vascular plexus and at the top of dermal papillae **(Brotas et al., 2012).**

IL-6 is an autocrine mitogen; induce mitosis in cells producing it. In psoriatic epidermis, IL-6, in synergy with IL-1 and TNF-α, contribute to keratinocyte hyperproliferation via its action on the epidermal growth factor receptor (EGF) **(Mitra et al., 2013; Brotas et al., 2012)**.

Moreover, IL-6, secreted by DCs, with IL1β and TGF-β induce Th17 response. IL-6 blocks regulatory T cell response as well **(Mahil et al., 2016; Kupetsky et al., 2013)**.

The efficacy of etanercept, a fusion protein binds to TNF-α, in psoriasis treatment has been associated with reduction of IL-6 level even more than the reduction observed in TNF-α.

Elevated IL-6 level has been found to coincide with arthritis, diabetes and obesity in psoriasis, which highlights the role of IL-6 in the systemic inflammatory nature of the disease. The relationship between increased IL-6 level and obesity and diabetes can be explained in part by the observation of decreased insulin effect on adipocytes and hepatocytes after exposure to IL-6 which matches with the finding of increased

IL-6 level to 2 – 3 times the control in plasma of diabetics and obese **(Brotas et al., 2012)**.

4. *Interleukin 8:*

IL-8 (CXCL8) (**Figure 7**) is a chemokine typically responsible for neutrophil chemotaxis, promotes keratinocyte proliferation and stimulates angiogenesis. Its level is increased in psoriasis both in psoriatic plaques and serum of the patients. IL-8 mobilizes as well as activates T lymphocytes, basophils and natural killer cells in psoriasis. IL-8 is a common ligand for both CXCR1 and CXCR2 **(Brotas et al., 2012; Coimbra et al., 2012; Lee and Hwang, 2012).**

5. *Interleukin 12:*

IL-12 is the key cytokine driving of Th1 development. IL-12 is formed of two subunits (p40 & p35) linked by disulfide bond **(Coimbra et al., 2012; Zheng et al., 2007).** Activated macrophages and DCs are the major source of IL-12 upon microbial stimulation **(Coimbra et al., 2012; Zheng et al., 2007).**

IL-12 activates STAT 4 which in turn increases IFN-γ expression. IFN-γ through STAT 1 activation enhances T-bet (T -box expressed in T cells) signaling leading to further increase in IFN-γ production and down regulation of Th 2 cytokines. IL-12's main function is fighting bacterial and intracellular parasites **(Mitra et al., 2013).**

6. *Interleukin 23:*

A heterodimer consist of a p19 subunit and a shared p40 subunit with IL-12. IL-23 is produced by dendritic cells and by macrophages **(Coimbra et al., 2012; Mitra et al., 2013; Zheng et al., 2007).** IL 23 receptor chains are expressed on T cells and NK cells **(Zheng et al., 2007)**. According to Coimbra and coworkers IL-23 is a key master cytokine regulator in psoriasis pathogenesis **(Coimbra et al., 2012).**

The role of IL 23 in psoriasis is evident in genetic association studies identifying a SNP in the 3'-UTR of the *IL12B* gene and two SNPs in the *IL23R* gene to be associated with psoriasis. In culture, keratinocytes extracted either from normal or lesional psoriatic skin express constitutively subunits of IL 23. The mRNA of p40 & p19 IL 23 subunits are upregulated in psoriatic plaques. Furthermore, improved

psoriatic lesions which received narrow-band ultraviolet-B (NB-UVB) phototherapy was found to be associated with reduction in IL-23 expression in lesional skin. Moreover, there is the proven efficacy of Ustekinumab, a monoclonal antibody against the shared p40 subunit of IL 23 and IL12, in psoriasis therapy (**Coimbra et al., 2012; Mitra et al., 2013; Zheng et al., 2007).**

The role of IL-23 in psoriasis includes Th 17 cells activation, survival, proliferation and terminal differentiation **(Coimbra et al., 2012; Mitra et al., 2013).** Furthermore it induces keratinocyte hyperplasia via its downstream mediators IL-22 and IL-17 as proved by Rizzo and coworkers. They proved that both IL-17 and IL-22 are essential for IL-23 mediated epidermal hyperplasia and any one of them alone is not sufficient to produce the IL-23 effect **(Rizzo et al., 2011).** IL-23 also induces mixed inflammatory cells to infiltrate the dermis and it enhances TNF-α production by macrophages **(Coimbra et al., 2012).**

7. *Interleukin 17:*

IL-17 family is composed of 6 isoforms (IL17A - IL17F) of those 6 IL17A and IL17F are the main isoforms expressed by T lymphocytes in psoriasis. IL 17A & F have the ability to work as monomers; each one binds alone to the receptor or work as a heterodimer with each other IL-17A/F **(Ariza et al., 2012; Girolomoni et al., 2012; Kupetsky et al., 2013).** IL 17A is about 20 times stronger than IL-17F in activating downstream genes whereas the IL-17A/IL-17F heterodimer have intermediate activity. IL-17 exerts its biological action through binding to family of receptors IL-17R (A-E) **(Girolomoni et al., 2012).**

IL-17 is produced by Th17 cells, Tc17 cells, γδ T cells, neutrophils and mast cells. It is interesting to know that mast cells and neutrophils are present in higher densities than Th17 cells in psoriatic plaques **(Ariza et al., 2012; Girolomoni et al., 2012).**

IL-17 works with IL-22 to initiate immune response against extracellular invaders by inducing antimicrobial peptides and chemokines expression resulting in neutrophil recruitment. IL-17 has a role in tissue inflammatory response on the other hand IL-22 is responsible for tissue regeneration **(Girolomoni et al., 2012).**

Evidences supporting the role of IL-17 in psoriasis development include increased blood level of IL-17 in psoriatic patients correlated with disease severity. IL-17 mRNA was detected in psoriatic plaques. Furthermore, in experimental models blocking of IL-17A improved psoriasis-like pathology along with the fact that the response to anti TNF-α therapy is associated with reduction in IL-17 signaling and down regulation of IL-17 regulated genes among responders only **(Coimbra et al., 2012; Girolomoni et al., 2012).**

IL-17 (**Figure 8**) produces and maintains psoriatic plaques through different mechanisms **(Girolomoni et al., 2012)**. IL-17 recruits different types of cells to the psoriatic lesions through induction of keratinocyte chemokine (CCL20) expression. it binds the chemokine receptor (CCR6). Thus $CCR6^{+}$ cells like Th-17 and mDCs is being recruited to psoriatic plaques creating positive feedback loop and maintaining the presence of both cells in psoriatic lesions. Worth mentioning responders to anti TNF-α therapy show downregulation of CCL20. Furthermore, IL-17A stimulates the expression of CXCL1, CXCL3, CXCL5, CXCL6 and CXCL8 chemokines activating CCR1 and CXCL2. The latter further activates CCR2. Both CCR1 and CCR2 are involved in neutrophil attraction to form subcorneal micro-abscesses in psoriatic epidermis **(Girolomoni et al., 2012).**

Moreover, IL-17A increases IL-6 secretion by fibroblasts and mDCs which, in turn, drives more T cells to the Th17 phenotype (auto reactive loop). IL-17A also stimulates production of TNF-α and IL-1 by mDCs and macrophages **(Coimbra et al., 2012; Girolomoni et al., 2012)**.

Another role of IL-17A in psoriasis development is achieved via induction of antimicrobial peptides expression as β-defensin 2, S100A7, S100A8 and S100A9 which act as stimulus for innate immune response **(Coimbra et al., 2012; Girolomoni et al., 2012)**.

Recently psoriasis has been considered as a systemic condition with higher rate of developing cardiovascular accident, diabetes and obesity. IL-17A, presents at higher concentrations in the blood of psoriatic patients compared to normal control, has been linked to atherogenesis **(Girolomoni et al., 2012).**

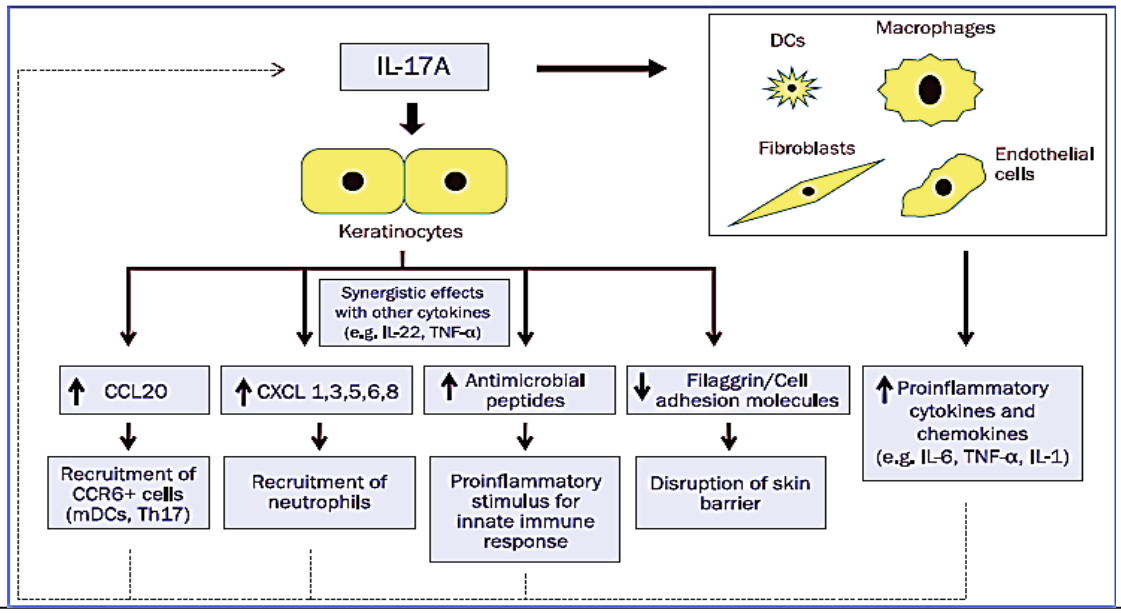


Figure 8: Roles of interleukin (IL)-17A in psoriasis. IL-17A stimulates the production of pro-inflammatory chemokines and cytokines, These mediators promote infiltration of multiple cell types to psoriatic lesions, including myeloid dendritic cells (mDCs), Th17 cells and neutrophils. Dotted lines indicate that some of these actions may act as a positive feedback loop which sustains the chronic inflammatory response in the skin. DCs, dendritic cells; TNF, tumor necrosis factor **Adopted from Girolomoni et al., 2012**

8. *Interleukin 22*

Different cells produce IL-22 includes Th22, Th17, Th1, DCs and macrophages **(Coimbra et al., 2012; Fujita, 2013).**

In the skin IL-22 targets the keratinocytes for amplification as well as maintenance of inflammation **(Coimbra et al., 2012).** IL-22 mediates the crosstalk between epidermal cells and leukocytes rather than regulating immune cells. Furthermore, IL-22 acts with IL17 in regulating the antimicrobial peptide production including the induction of S100A7, S100A8, S100A9, β-defensin-2, and β-defensin-3 gene expression. IL-22 also induces epidermal hyperplasia (acanthosis), and inhibits keratinocyte terminal differentiation (hypogranulosis). Moreover, induces pro-inflammatory responses, such as the production of multiple cytokines, various chemokines, and acute-phase proteins from different cell types **(Baliwag et al., 2015; Coimbra et al., 2012; Fujita, 2013; Tohyama et al., 2012)**. To sum up IL-22 works on keratinocytes rather than immune cells regulating three functions: (i) production of antimicrobial peptides, (ii) keratinocyte differentiation and (iii) keratinocyte migration **(Sabat et al., 2007)**.

IL-22 is one of the most important cytokines in the pathogenesis of psoriasis **(Tohyama et al., 2012).** This is evident via many facts; the up-regulated IL-22 expression in psoriatic lesions clearly decreases after anti-psoriatic therapy. Even more, IL22R expression is enhanced in psoriatic skin compared to normal skin. Serum IL-22 levels are also higher in psoriasis patients than in healthy controls, and these levels significantly correlate with disease severity. Furthermore, the significant reduction of serum IL-22 after treatment is achieved **(Coimbra et al., 2012; Fujita, 2013).**

The production of IL-22 is influenced by IL-6 and IL-23 *in vitro*. However, *in vivo* IL-6 seems to have no role. It seems that the IL-23 induced keratinocyte hyperplasia is mediated through IL-22. It was proved that IL-22 blockade abolished significantly the IL-23 induced keratinocyte hyperplasia **(Ariza et al., 2012; Coimbra et al., 2012).** Furthermore, Tohyama and coworkers proved that IFN-α enhances the effect of IL 22 on keratinocytes via increasing IL-22 Receptor expression **(Tohyama et al., 2013)**.

9. *Interleukin 19,interleukin 20, interleukin 24:*

They are of IL-10 family. Activated monocytes secrets both IL-19 and IL-20 while IL-24 is secreted by Th2 cells, monocytes and melanocytes **(Zheng et al., 2007).** Their receptors are expressed mainly on epithelial cells and signals through STAT3 promoting tissue remodeling, wound healing, and antimicrobial peptide expression (**Baliwag et al., 2015).** TNF-α and IL1β induce the production of IL-19 and IL-20 from myeloid cells. As IL22, they promote the crosstalk between keratinocytes and activated myeloid cells **(Zheng et al., 2007).**

h- Chemokines:

Chemokines are small cytokines (8-11 kDa in size), small chemotactic substances guide the movement of leukocytes towards inflammation sites. **(Lee and Hwang, 2012).** As chemo-attractant factors they stimulate directional movement of all types of leukocytes through regulated group of chemokine receptors expressed on the surface of T cell subsets including Th1, Th2, Th17, Treg, and memory T cells. This enable T cells to differentially respond to specific chemokines. Along with this chemotactic ability, chemokines enhance the leukocytes' rolling and adhesion to endothelial cells

by enhancing the affinity and avidity of leukocytes' surface β1 and β2 integrins **(Lee and Hwang, 2012)**.

In the skin, keratinocytes are capable of expression of multiple chemokines that attract certain leukocytes, such as T cells or dendritic cells (DCs), toward the epidermis **(Lee and Hwang, 2012).**

1. *CCR 6:*

CCR6 has an important role in the chemotaxis of Th17. CCL20 is the recognized CCR6's ligand, however human β-defensin 2 (hβD2) attracts leukocytes via CCR6 as well. In psoriatic patients, peripheral Th17 cells expressing CCR6 are increased both in skin and in blood. IL-23 from DCs maintains dermal $CCR6^+$ Th17 cells **(Lee and Hwang, 2012).**

Langerhans cells also express CCR6 and are attracted to CCL20. CCR6 is supposed to have a role in macrophage maturation although it is not expressed on fully mature macrophage **(Lee and Hwang, 2012).**

2. *CCL 20:*

As mentioned before it is an exclusive ligand for CCR6. It is expressed normally by keratinocytes and dermal endothelial cells. This expression is increased in pro-inflammatory conditions as in the presence of TNF-α, IL-17, IL-1α and IFN-γ. In psoriasis, CCL20 is excessively expressed in epidermis and endothelial cells. CCL20 recruits circulating CCR6-expressing cells e.g. Th17 cells, γδT cells - both produce Th17 cytokines - and LCs into inflamed epidermis **(Lee and Hwang, 2012).**

3. *β-defensin 2:*

human β defensin 2 (hβD2) signals through CCR6 also. It is highly expressed in the epidermis of psoriatic skin. β defensin gene high copy numbers are associated with susceptibility to psoriasis. Increased hβD2 production in psoriasis may act in synergy with CCL20 to attract the needed populations of $CCR6^+$ expressing cells, including Th17 cells **(Lee and Hwang, 2012)**.

4. *CCR10:*

In psoriasis, CCR10-expressing T cells accounts for more than 90% of T cell population and migrate into epidermis under the effect of keratinocyte derived CCL27, the CCR10 ligand whose expression is upregulated by TNF- α **(Lee and Hwang, 2012)**.

5. *CXCR3:*

CXCR3 is upregulated on Th 1 cells which migrate from dermis to epidermis under the influence of its ligands; CXCL9, CXCL10, and/or CXCL11 derived from keratinocytes. It is also involved in trans-endothelial migration of T cells **(Lee and Hwang, 2012).**

6. *CCR5 & CCL5:*

CCL5 (RANTES) is one of the ligands for CCR5. It is highly expressed in psoriatic lesions. Calcipotriol, an active vitamin D3 analogue and a therapeutic for psoriasis, inhibits CCL5 keratinocyte production. Moreover, epidermal T cells and dermal macrophages' CCR5 expression was higher in lesional compared to non lesional skin. $CCR5^{+}$ T cells move to epidermis through interaction with CCL3 and keratinocyte -derived CCL5. **(Lee and Hwang, 2012).**

To sum up many chemokines and their receptors are accused in psoriasis development and progression. Targeting single chemokine ligand or receptor is challenging due to redundancy of the chemokine system. However, in mouse models of psoriasis, therapeutics targeting the CCR6 pathway showed efficacy. Furthermore, the mice lacking CCR6 resist induction of psoriasiform lesions on IL-23 injection **(Lee and Hwang, 2012).**

i- Keratinocyte:

Activated Keratinocytes are involved in two processes during psoriasis development 1st involves the hyperplasia and increased cell division with altered differentiation 2nd involves secretion of cytokines (**Coimbra et al., 2012).**

Keratinocytes hyperplasia and altered differentiation is a hallmark feature of psoriasis pathology which occurs under effect of multiple cytokines. IL 19, IL 20, IL 22, IL 24 and others enhance epidermal hyperplasia and altering its differentiation as well as activating keratinocyte for secretion of different mediators **(Baliwag et al.,**

2015; Zheng et al., 2007). Early in psoriasis, keratinocyte activation is under the effect of Th1 cytokines e.g. IFN-γ and IL22. Later on Th 17 cytokines take the upper hand e.g. IL-6, IL-17 and IL-22. Macrophages and dendritic cells maintain such activation via secreting TNF-α, IL-6, IL- 18, IL-19 and IL-20. Other mediators involved in keratinocyte activation are produced by keratinocyte itself as TGF-α and nerve growth factor (NGF) and by dermal stromal cells mediators e.g. keratinocyte growth factor (KGF), insulin-like growth factor 1(IGF-1), and fibroblast growth factor 10 **(Coimbra et al., 2012; Sabat et al., 2007)**.

Keratinocytes produce various mediators that enhance more immune cell migration, induce angiogenesis and activate dermal stromal cells. Of their products, IL-6, IL-8, TGF-α, TGF-β, and amphiregulin act as neutrophil attractants and growth factors for endothelial cells. Moreover, TGF-α and amphiregulin stimulate keratinocytes hyperproliferation and are, along with TGF-β, ligands for IL-1 and epidermal growth factor receptor, which are expressed in psoriasis **(Coimbra et al., 2012)**.

j- Mannose Binding Lectin:

Mannose Binding Lectin (MBL) is very important player of innate immune system (uniprotkb: P11226). It is a Ca^{+2} dependent collectins family member (**Dommett et al., 2006; Takahashi, 2011**). Collectins are pattern recognition molecules structurally composed of carbohydrate recognition domain and collagen- like structures **(Jack et al., 2001)**.

Innate immune system is responsible for the primary defense against harm to the body. First the invading pathogens were thought to be the only targets of innate immune system later on other targets have been identified including abnormal self-epitopes e.g. cells and tissues damaged through apoptosis, trauma, inflammation, malignancy or other kinds of tissue injury **(Takahashi, 2011).**

Successful innate immune protection includes both recognition and effector mechanisms. The recognition of injurious agents is the responsibility of Pattern recognition molecules. Pathogen associated molecular patterns (PAMPs) are conserved structures in a particular class of microorganisms, which are not present

within the host. These structures are not easily discarded as they are essential for the viability of the microorganism **(Bergman, 2011)**.

Pattern recognition molecules/receptors recognize and bind to these specific patterns (PAMPs) in the harmful agents such as the recognition of lipopolysaccharide moiety by TLR4 or the recognition of carbohydrate moiety by MBL. These molecules are found as cell membrane proteins on innate cells, or as embedded proteins in the extracellular matrices, or as soluble proteins circulating in the blood e.g. MBL **(Takahashi, 2011)**.

MBL was primarily acknowledged as a pattern-recognition molecule able to distinguish microbial non-self from self, then it become appreciated that MBL can also bind to altered self-molecules like apoptotic and necrotic cells **(Downing et al., 2005; Heitzeneder et al., 2012)**.

MBL synthesis and secretion:

MBL, in human, is synthesized mainly in liver (95%) and the remaining amount is synthesized in kidney, thymus, tonsil, small intestine and vagina. It was also detected in the lungs of healthy individuals and on the smooth muscle of the airway following infection **(Jack et al., 2001; Takahashi, 2011)**. MBL is detected in skin after burn and in brain after trauma. Following UVB exposure, MBL is detected in the skin and mostly recruited into the damaged sub-epithelial tissue from local capillaries. MBL aids in clearing apoptotic keratinocytes **(Takahashi, 2011).** This wide distribution of MBL indicates its role as a surveillance system in innate immune system **(Takahashi, 2011)**.

MBL serum level is affected by genetic and non-genetic factors e.g. age, sex, hormones and infection. MBL is an acute phase protein whose serum levels may increase 2–3 folds in response to infection or other inflammatory stimuli **(Heitzeneder et al., 2012).**

MBL Chemical Structure

MBL is formed of subunits of trimers (**Figure 9**) i.e. each subunit contains 3 peptide chains (trimer) (**Dommett et al., 2006; Takahashi, 2011**). The peptide chain composed of 21 residues, cysteine rich, N terminus domain for the disulfide binding

between chains, followed by glycine rich collagen like domain composed of 19 repeats of Glycine, X and Y where X and Y may be any amino acid but frequently proline and hydroxylproline. These repeats get interrupted at one site forming a hinge, then α helical coiled-coil hydrophobic part representing the neck of 34 residue, and finally the carbohydrate domain with globular configuration at the C terminus (112 residue) **(Dommett et al., 2006; Takahashi, 2011)**. Then subunits get oligomerized in higher order structures to be functional e.g. tetramers and hexamers. Thus the ability of MBL to bind to the carbohydrate moieties on pathogens depends on the oligomerization into higher order structure **(Dommett et al., 2006; Takahashi, 2011; Worthley et al., 2005)**. The hinge in the collagenous region give MBL protein its bouquet shaped orientation (**Figure 9**) **(Dommett et al., 2006)**.

The carbohydrate recognition domain binds sugars e.g. mannose, N-acetyl-D-glucosamine, N-acetyl-mannosamine, fructose and glucose **(Worthley et al., 2005**). These carbohydrates not only present on the surface of many pathogens e.g. bacteria and viruses but also abnormal self-tissues, which have endogenous neo-epitopes that become exposed on apoptotic cells, cell debris, injured and damaged tissues **(Takahashi, 2011).** MBL also binds phospholipids, nucleic acids and non-glycosylated proteins (**Dommett et al., 2006**).

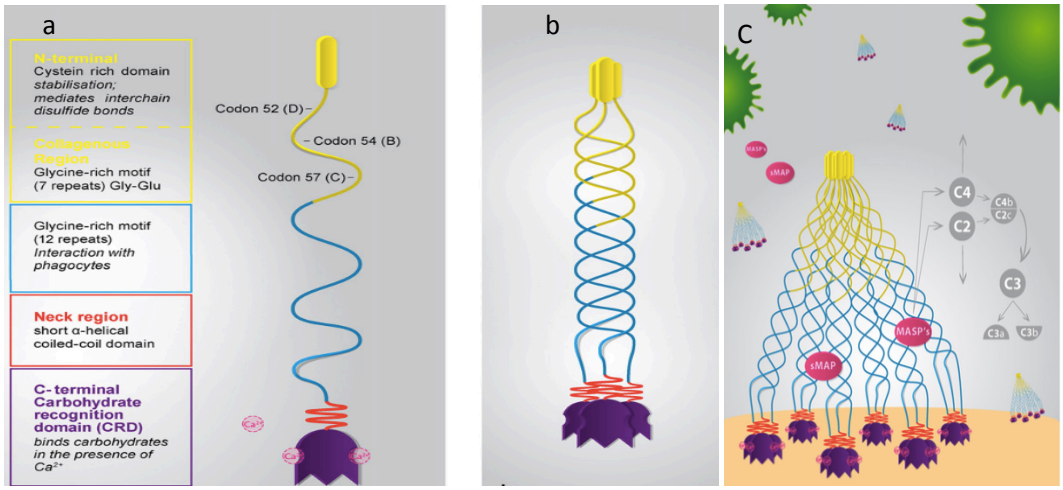


Figure 9: MBL structure.

a: peptide chain
b: MBL trimer subunit of 3 peptide chain
c: bouquet like orientaion of tetramers or more. **adopted from Heitzeneder et al., 2012**

MBL Gene

MBL is encoded in human by *mbl 2* gene, (**Figure 10**) on chromosome10q 21–24 (**Dommett et al., 2006; Turan et al., 2014**). *mbl* 1 is a pseudogene in human, however it is functional in rats **(Turan et al., 2014)**. It has 4 exons; exon 1 encodes for the cysteine rich N terminal domain, the signal peptide and part of collagenous region, exon 2 encodes the rest of the collagenous region, exon 3 for the neck region, exon 4 encodes the carbohydrate binding domain (**Dommett et al., 2006**).

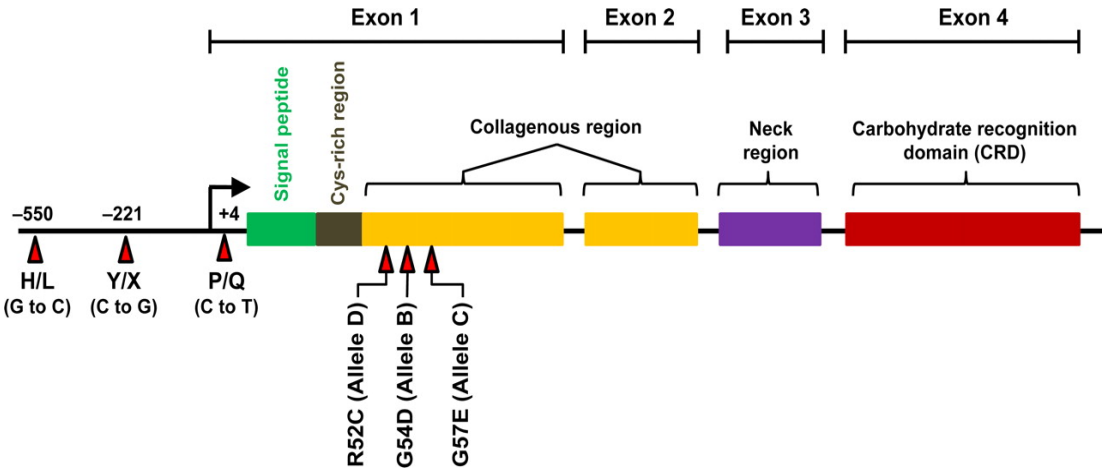


Figure 10: *mbl* 2 gene. **Adopted from Ram et. al., 2010**

Different polymorphisms have been identified of *mbl2* gene **(Figure 10)**. Three SNPs present in exon 1; at codon 52 (D allel), codon 54(B allel), and codon 57 (C allel). A refers to the wild type allel. B allele is the commonest of the exon 1 polymorphisms. C allele is common in sub Saharan African population (**Dommett et al., 2006**; **Heitzeneder et al., 2012**).

Other *mbl* gene polymorphisms occur at promoter site. They include the haplotypes H/L (-619), Y/X (−290 for) and P/Q (−66) **(Heitzeneder et al., 2012).** The exon 1 SNPs B and C are associated with faster degradation of their multimers and exist mainly as lower order. Functionally, they have a lower mannose binding capacity and do not activate complement. This glycine-rich motif defect is not present in the D allele, thus the functional defect is not so obvious. Moreover, the D variant only happens in association with the high producing promoter variant H **(Heitzeneder et al., 2012**).

In general, MBL B, C and D variants can function as an opsonin, but uniformly have a severely reduced capacity of complement activation. The deficient MBL might range from100 ng/ml up to 1 µg/ml depending on the measurement approach **(Dommett et al., 2006**; **Heitzeneder et al., 2012; Worthley et al., 2005)**.

Susceptibility to various conditions have been linked to *mbl* gene polymorphism e.g. brucellosis **(Bayram et al., 2012)**, leprosy **(De Messias-Reason et al., 2007)** and immune diseases as Psoriasis **(Turan et al., 2014).**

MBL Functions

Functional MBL exerts several functions including opsonization, induction of pro-inflammatory responses early in infection, and lectin dependent complement activation (**Dommett et al., 2006; Takahashi, 2011**).

1. *Complement activation*:

Upon binding the carbohydrate moiety of the pathogen, MBL activates one of the MBL-associated serine Proteases (MASPs), which are of four types MASP 1, 2, 3 and MAp 19 a truncated MASP2 resulting in complement activation. MBL/MASP interaction occurs at the collagen like domain **(Jack et al., 2001; Dommett et al., 2006**). MBL- MASP2 complex (**Figure 11**) cleaves C4 & C2 forming C4bC2a which is a C3 convertase (**Dommett et al., 2006**). MBL–MASP1 complexes also can activate C3 directly (**Dommett et al., 2006**; **Flyvbjerg, 2010).**

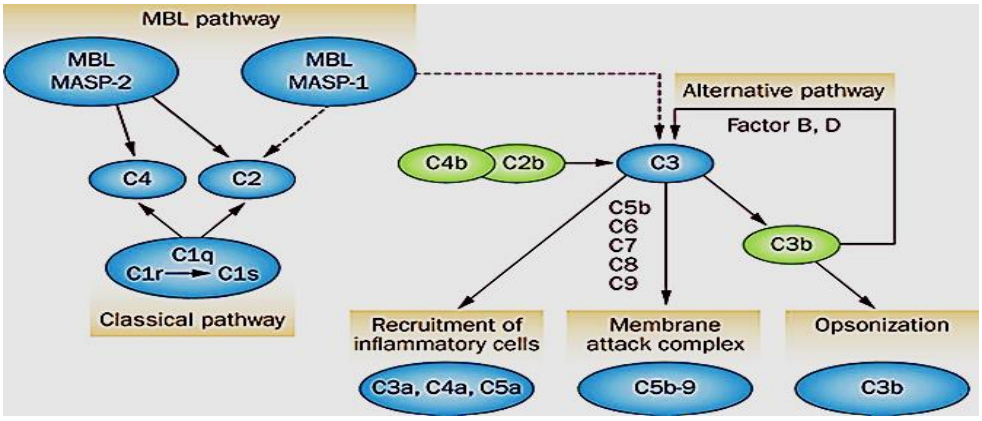


Figure 11: Complement activation pathways. Antibodies bind to C1 complex activates classical pathway. The Lectin pathway is activated via MBL's recognition of carbohydrates Spontaneous C3 activation starts the alternative pathway. All pathways meets at C3 cleavage with anaphylatoxin secretion & inflammatory cells recruitment, opsonization, and the membrane attack complex formation with pathogen lysis. MBL, mannose-binding lectin; MASP, mannose-binding-lectin-associated serine proteases. **Adopted from Flyvbjerg, 2010**.

Upon activation, complement works on opsonization of the pathogen, chemotaxis and phagocyte activation. Complement also directs pathogen lysis via the membrane attack complex formation **(Heitzeneder et al., 2012).**

2. *The promotion of (complement-independent) opsonophagocytosis:*

An important function common to collectin group is to up-regulate the expression of cell surface phagocytic receptors. For example MBL increase the expression of Scavenger Receptor A on Kupffer Cells (liver macrophage) enhancing their ability to phagocytize lipid A, an active moiety of LPS, *Staphylococcus aureus, and Escherichia coli* **(Ono et al., 2006)**.

Thus, MBL can opsonize the microbes directly through its carbohydrate-binding domain and with phagocytes through its collagen like domain (**Tsutsumi et al., 2005).** This is along with its indirect opsonizing ability via lectin pathway of complement activation (**Figure 12**) **(Ono et al., 2006; Macdonald et al., 2010)**.

3. *The removal of apoptosis products:*

MBL interacts (**Figure 12**) with CD 91 and calreticulin receptors on the surface of macrophages facilitating apoptotic and necrotic cell phagocytosis. MBL recognizes exposed terminal sugars of cytoskeletal proteins on their surfaces. CD 91 receptor is highly expressed on the surface of human macrophages and is involved in the recognition and phagocytosis of more than 30 different ligands. This occurs via the collagenous region of MBL binding to the calreticulin/CD-91 complex on phagocytes, resulting in the ingestion of the apoptotic cell **(Duus et al., 2010; Heitzeneder et al., 2012)**.

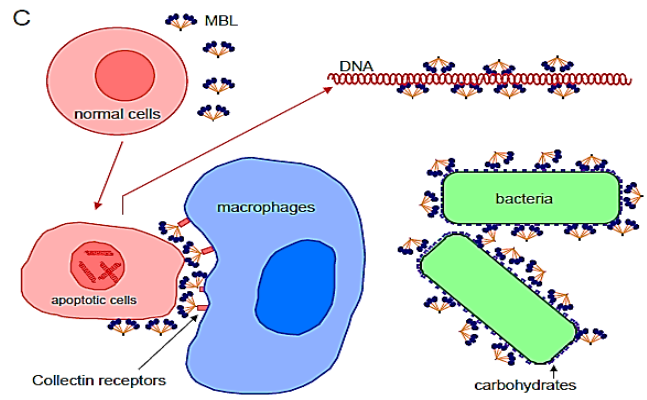


Figure 12: Functions of MBL.

The trimeric MBL subunits interact with receptors via the collagenous-tail domains. MBL recognizes and binds to microorganism surface mannose and N-acetylglucosamine sugars activating lectin pathway. Moreover, MBL binds to both apoptotic cells and phagocytes, promoting the uptake of apoptotic cells. **Adopted from Tsutsumi et al., 2006.**

4. The modulation of inflammation:

Mannose binding lectin was primarily identified as complement system activator on binding the pathogen carbohydrate moieties **(Jack et al., 2001)**. With more elaborate research its function as immune response regulator and its anti-inflammatory property protecting the body against excessive – unneeded- immune responses that may cause excess tissue damage **(Wang et al., 2011a; Downing et al., 2003; Downing et al., 2005; Wang et al., 2011b).**

MBL affects cytokine secretion in concentration dependent manner; at low levels MBL increases TNF α, IL 6 and IL 1 liberation from monocytes in vitro and decreases their release at high concentrations; **(Jack et al., 2001).**

MBL not only interacts with apoptotic cells but also to different cells expressing normal self-patterns. MBL ability to interact with immune cells was discovered first then the nature of receptors recognize it was identified. MBL can bind to monocyte derived dendritic cells (moDcs) especially the immature ones **(Downing et al., 2003).** Thereafter, Downing and coworkers proved the binding of MBL to other immune cells e.g. monocytes, B lymphocytes and natural killer cells. Such interaction was inhibited in serum suggesting that it happens in the extravascular spaces at sites of inflammation and has anti-inflammatory and tolerogenic effect. So they suggested that MBL existence act as a disease modifier **(Downing et al., 2005).** Then Shimizu and coworkers proved that MBL and other members of collectins family bind to TLR4, CD14 and MD-2 receptors **(Shimizu et al, 2009).**

MBL interaction with immune cells modulates inflammatory response. MBL recognizes and binds to the peptidoglycan portion of the Gram positive bacterial cell wall and modulates the macrophage response to it **(Nadesalingam et al., 2005)**. MBL inhibits peptidoglycan induced TNF-α secretion and enhances IL-8 – a chemokine - secretion. MBL null mice died of septic shock in response to the *S. aureus* infection. The induced excess tissue damage was attributed to the elevated levels of TNF-α and IL-6 in their blood (cytokine storm) **(Shi et al., 2004)**. MBL down-regulates macrophage-mediated inflammation (reduced TNF-α and IL-6) while enhancing phagocytosis (enhanced IL-8) in response to gram positive bacterial wall peptidoglycan **(Nadesalingam et al., 2005).** MBL clusters the lipoteichoic acid of *S.*

aureus and presents it to TLR2/6 heterodimer within phagosomes inside the macrophage enhancing TLR 2 signaling pathway **(Ip et al., 2008).** MBL binds to and inhibits signaling of TLR3, TLR9 & TLR4 **(Liu et al., 2014; Tang et al., 2015; Wang et al., 2011a**)

Another aspect of MBL role in inflammatory response has been studied in skin response to injury. The MBL null mice have abnormal response to burn (thermal injury) with reduced sloughing of eschar (dead skin) and interestingly abnormal epidermal acanthosis compared to wild type mice **(Møller-Kristensen et al., 2007; Takahashi, 2011).**

MBL Deficiency

MBL deficiency is considered one of the commonest immune deficiencies. Affected population suffers increased susceptibility to infection e.g. Cutaneous infections (**Dommett et al., 2006**). The prevalence rate of MBL mediated immune deficiency has been varied between 5% – 30% of the population **(Takahashi, 2011).**

Deficiencies of the MBL-MASP pathway have been associated with recurrent and serious infections, autoimmunity, atopic disease, recurrent miscarriage and cardiovascular pathology **(Worthley et al., 2005)**.

MBL replacement therapy

MBL replacement therapy (**Table 1**) has been tested both in human and in animal model of diseases. MBL was replaced either in a purified form out of human plasma or a recombinant human form. Both forms have been verified to be safe and tolerable for human use without anti-MBL antibodies production **(Petersen et al., 2006; Valdimarsson et al., 2004; Bang et al., 2008)**.

In human, MBL replacement therapy was given for MBL deficient pediatric cancer patients with chemotherapy induced neutropenia. This replacement was safe (**Frakking et al., 2009)** and successful in restoring circulating MBL level along with partial recovery to opsanophagocytosis function which was explained by partial loss of MBL complement activation function during purification, the lack of phagocytes after chemotherapy and presence of other genetic alterations affected another points in

the alternate pathway of complement activation **(Brouwer et al., 2009; Garred et al., 2002).**

In mice, MBL substitution rescued the MBL knockout (KO) mice burnt and infected with *Pseudomonas aeruginosa* from death **(Møller-Kristensen et al., 2006)**. MBL reconstitution also improved the survival of MBL KO mice infected with *Staphylococcus aureus* from 0 % survival in MBL KO to 45% after MBL substitution **(Shi et al., 2004)**. rhMBL substitution also rescued Ebola virus infected mice **(Michelow et al., 2011).**

Table 1: MBL replacement Therapy

	Model	**MBL Type**	**Dose**	**Method of administration**
Wang et al., 2011 a	THP1 cell line (Human monocytic cell line)	pdMBL*	15 µg/ml	Incubation
Wang et al., 2011 b	Peripheral blood monocyte derived dentritic cells	pdMBL	0, 1, 10 or 20 µg/ml	Incubation
Liu et al., 2014	Peripheral blood monocytes & monocyte derived dendritic cells	pdMBL	10 µg/ml	Incubation
Tang et al., 2015	Peripheral blood monocytes	pdMBL	10 µg/ml	Incubation
Zhou et al., 2010	Human primary embryonic kidney cell line (293T) & Huh-7.5 cell	pdMBL rhMBL** (R&D)	0.1, 0.3, 1, 3, or 10 µg/ml	Incubation

	Model	MBL Type	Dose	Method of administration
	lines			
Li et al., 2012	Neutrophil $2*10^6$ cell/ml	rhMBL (R&D)	2.5, 5, 10 µg/ml	Incubation
Shi et al., 2004	C57bl Mice	rhMBL NatImmune	75 µg/ mice/ d for 3 days in 200 µl saline	Intraperitoneal
Michelow et al., 2011	C57bl Mice	rhMBL NatImmune	*75 µg/mouse/12h for 10 d = 4.3 mg/kg *350 µg/mouse/12h for 10 d = 20 mg/kg	Intraperitoneal
Møller-Kristensen et al., 2006	C57bl Mice	rhMBL NatImmune	75 µg/ mouse in 200 µl saline at 12 and 2 h before and 24 h after the burn injury.	Intraperitoneal
Frakking et al., 2009	Human	pdMBL	0.2 mg/kg/3d 0.3 mg/kg/4d	Infusion
Bang et al., 2008	Human	pdMBL	Healthy: 6mg/week for 3 weeks Septicemic: 0.2mg/kg/ week or twice weekly	Infusion
Valdimarsson et al., 2004	Human	pdMBL	6mg/ week for 3 weeks	Infusion
Petersen et al., 2006	Human	rhMBL NatImmune	0.01, 0.05, 0.1, and 0.5 mg/Kg	Infusion

	Model	MBL Type	Dose	Method of administration
			once 0.1, 0.3 mg/Kg/3d	
Garred et al., 2002	Human	pdMBL	6mg/3d	Infusion

* pdMBL; Plasma derived MBL. ** rhMBL; recombinant human MBL.

So, MBL role has been upgraded from being just a pattern recognition molecule to a master innate immune response regulator interacting with other key player of the system e.g. TLRs **(Downing et al., 2005).**

At the inflammatory loci strong immune stimulation is most needed one to several days post infection. MBL though an acute phase reactant, presents at high concentration at inflammatory loci, it suppresses the monocytoid cell function. This is a desirable homeostatic mechanism to achieve a sterilizing but not self-damaging immune response. MBL interacts with autologous immune cells through receptors as TLRs enhancing phagocytosis and at the same time preventing excessive cytokine release and tissue damaging; tuning the immune response to injury avoiding the excessive tissue damage as for example induced by LPS; LPS tolerance **(Wang et al., 2011a).**

It is important to take in consideration that MBL through its role in distinguishing self from non-self and thereby elimination of invading pathogens, and its role in the clearance of abnormal self-components, particularly apoptotic cells guard against autoimmunity. On the contrary, deficient MBL could cause a defect in clearing dying cells and immune complexes, resulting in accumulation of hazardous self-components which increase the risk of dysregulated immune responses **(Downing et al., 2005).**

k- Toll like receptors:

Toll like Receptors (TLRs) is another important component of innate immune response. A family of pattern recognition receptors (PRPs) recognize distinct, conserved microbial parts and aid cells to recognize self from non-self in immune activation **(Hari et al., 2010; Pandey and Agrawal, 2006; Parker et al., 2007).** Both

exogenous and endogenous patterns are recognized by TLRs **(Connolly and O'Neill, 2012; Pandey and Agrawal, 2006; Parker et al., 2007).** Lipopolysaccharide (LPS), flagellin, lipopeptides, viral double-stranded RNA (dsRNA) and bacterial DNA are pathogen associated exogenous patterns. Endogenous patterns include heat-shock proteins, beta defensins, hyaluronic acid, fibronectin, fibrinogen, high mobility group box1 (HMGB1), heparin sulphate. According to their site in the cell TLRs are classified into:

I- Cell surface TLRs. & II- Intracellular TLRs found on intracellular organelles membranes as endososmes.

(Connolly and O'Neill, 2012; Pandey and Agrawal, 2006; Parker et al., 2007)

TLR 4 is an example of cell surface TLRs. It acts as homodimer and binds the LPS moiety in bacterial cell wall and viral proteins **(Connolly and O'Neill, 2012)**. Recently direct binding of MBL with TLR4 was proved **(Wang et al., 2011 a).** Other cell surface located TLRs are TLR2, TLR5, TLR6, TLR10. TLR2 heterodimerizes with TLR1 or TLR6 recognizing lipoteichoic acids, lipoproteins and lipoarabinomannan moieties **(Connolly and O'Neill, 2012; Pandey and Agrawal, 2006; Parker et al., 2007).** Intracellular TLRs are found embedded in membranes of vesicles as endosomes, lysosomes endoplasmic reticulum and endolysosomes. **(Connolly and O'Neill, 2012)**

Of intracellular TLRs (**Figure 13**) are TLR3, TLR7 & TLR8. TLR3 recognizes double stranded RNA. TLR 7 & 8 recognize single stranded RNA and imidazoquinoline derivatives e.g. imiquimod which is used as a drug against viral skin infections and skin precancerous and cancerous lesions **(Hari et al., 2010)**.

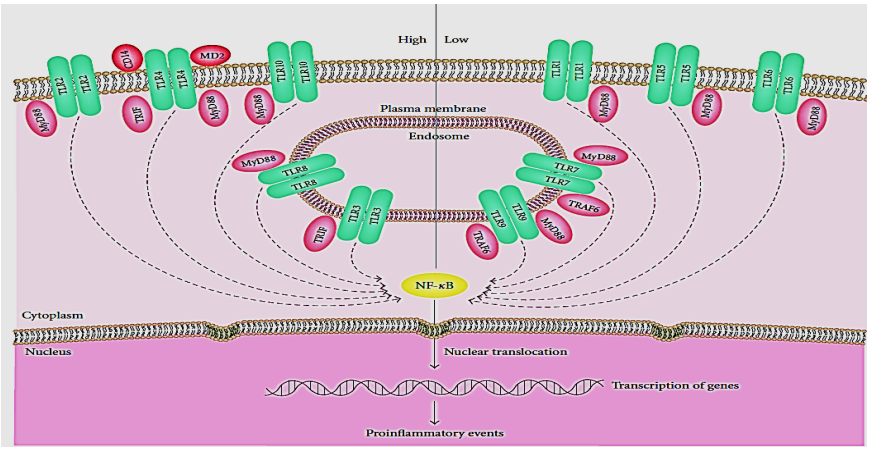


Figure 13: Site and Signaling pathway of TLRs inside the cell.
TLR1, 2, 4, 5, 6, &10 embedded in cell membrane and TLR3, 7, 8, & 9 on endosomal wall. **Adopted from Hari et al., 2010.**

Toll like Receptors signaling pathways:

TLRs are expressed on different sets of cells immune and non-immune cells (**Figure14**). In the skin different cells express TLRs, in the epidermis keratinocytes, melanocytes and Langerhans cells express TLRs**.** In the dermis, many cells express them including macrophages, dendritic cells, lymphocytes and others **(Hari et al., 2010).**

When TLRs binds to the activating moiety, they start one of two intracellular signaling pathways (**Akira &Takeda, 2004; Shinya et al., 2012)**:

I. MyD88 dependent (*myeloid differentiation factor 88*) signal ending in NF-κB release from the inhibitory effect of inhibitor of κB (IκB) proteins with induction of IL 6, IL 1, TNF α, and IL 8 gene expression **(Pandey and Agrawal, 2006; Parker et al., 2007)**.

II. TRIF mediated pathway (*Toll IL-1 receptor (TIR) domain containing adaptor inducing IFN-β*) This non MyD88 dependent pathway leads to both the induction of NF-κB and type 1 IFN (*Interferon*) liberation **(Parker et al., 2007; Pandey and Agrawal, 2006)**.

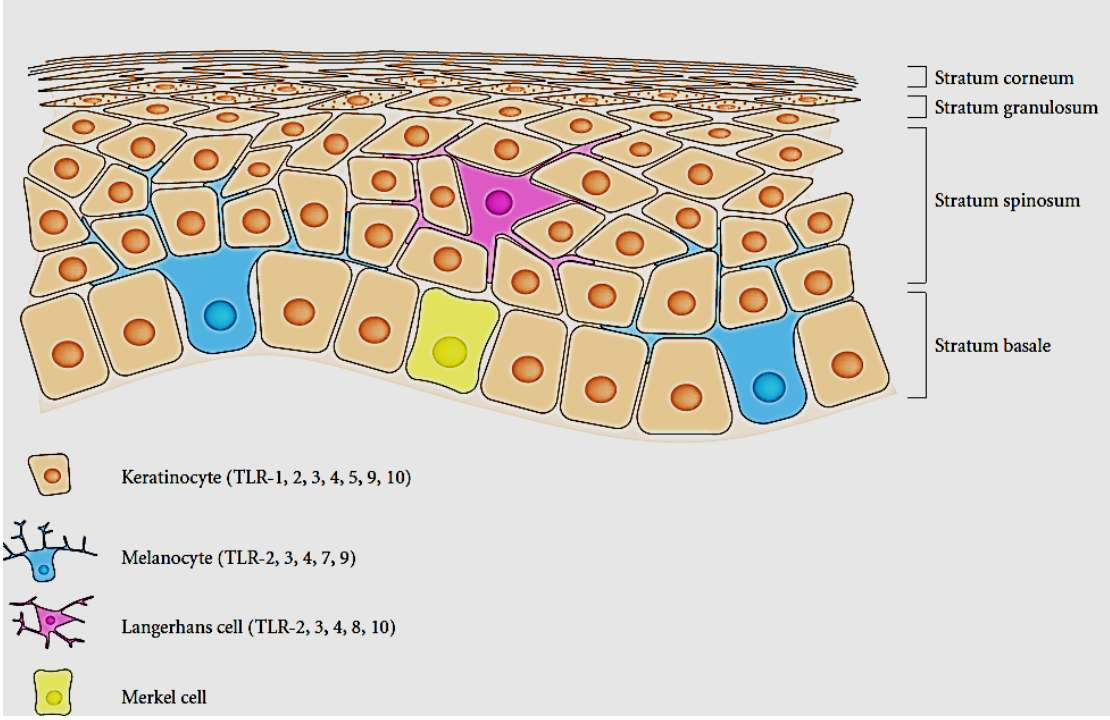


Figure 14: Distribution of TLRs in the skin.

Skin cross section showing keratinocytes and other epidermal cells express different TLRs as melanocytes, Langerhans cells, and Merkel cells. **Adopted from Hari et al., 2010**

Inhibitors of TLR signaling pathway have been identified as IRF-4 (Interferon regulatory factor) **(Parker et al., 2007).** Recently, MBL has been identified as another inhibitor of LPS induced TLR4 activation of NF-κB **(Wang et al., 2011a).**

Toll like Receptors functions:

Upon activation via TLRs, immune cells initiate phagocytosis, killing of pathogens, various chemokine & cytokine production, antigen presentation to T cells and leukocyte activation, thereby initiating adaptive immune response **(Hari et al., 2010).**

1. Control of innate immune response:

So the primary functions of TLRs is the recognition of PAMPs and the induction of innate immune system; antimicrobial peptides and proteins secretion along with activation of macrophage with production of various cytokines (IL-1β, IL-6, type I INFs and TNF-α) and chemokines **(Pasare &Medzhitov, 2004)**.

2. Control of adaptive immune responses:

Another function of TLRs is the control of T cell activation. As already known for T cell activation the TCR (T cell Receptor) binds the antigens of microbes presented in the MHC molecules of APCs, however this binding to be functioning need the presence of co-stimulatory CD 80/CD 86 molecules on the surface of APCs. The induction of these co-stimulatory molecules on APCs i.e. immature DCs is the responsibility of TLRs. So the engagement of TLRs on DCs in vivo is required to induce T cell priming. This function of TLRs is very important in preventing the improper T cell recognition of self-epitopes and subsequently the development of autoimmune disease **(Pasare &Medzhitov, 2004)**.

Toll like Receptors also release the T cells from the suppressor effect of T regulatory cells via the secretion of IL-6, however this release is balanced to ensure proper response to invading pathogens without excessive T cell activation and tissue damage **(Pasare &Medzhitov, 2004)**.

It is important to notice that despite TLRs main function is to induce protective immune responses, under some circumstances, activation of them may end up with autoimmune disease **(Pasare &Medzhitov, 2004)**.

Toll like receptors and Psoriasis:

TLRs are expressed in both immune and non-immune cells. Monocytes and dendritic cells are examples of immune cells highly responsive to TLRs and their activating moieties **(Hari et al., 2010; Parker et al., 2007; Pandey and Agrawal, 2006)**. When TLRs are stimulated in monocytes, various types of cells are recruited e.g. dendritic cells, T lymphocytes and natural killers. TLRs also contribute in intercellular communication so that optimal cell response is ensured **(Hari et al., 2010).**

Cells in tissues with barrier functions (in contact with outer environment) e.g. skin, genitourinary tract, GIT and respiratory system express TLRs, keratinocytes and melanocytes of the skin are examples of non-immune cells expression TLRs **(Hari et al., 2010; Pandey and Agrawal, 2006; Parker et al., 2007)**.

Toll like receptors have been implicated in psoriasis development **(Jiang et al., 2013).** TLR 9 is involved in the activation of pDCs by the LL37/self DNA complex ending in type I INF release and subsequent myeloid DCs maturation and auto-reactive T cells activation with continuous feedback loop (**Hari et al., 2010)**.

TLR9 is upregulated in psoriatic skin **(Morizane et al., 2012)**. Both TLR5 and TLR9 are regulated by the transforming growth factor-α (TGF-α) an important growth factor highly expressed in psoriasis. TGF-α, a keratinocyte growth and stimulating factor, binds to EGF receptor (EGF-R) on keratinocytes surface activating them. TGF-α increases TLR5 and TLR9 mRNA expression and subsequently increases proinflammatory cytokine, IL-8, and the antimicrobial peptide, hβD-2 production **(Miller et al., 2005)**. Both LL-37 & TLR9 are over expressed in psoriasis, LL-37 increases expression of TLR9 and enhances its binding to CpG and genomic DNA **(Morizane et al., 2012)**.

TLR7 and 8 has been implicated in psoriasis pathogenesis as their agonist, Imiquimod, exacerbates psoriasis and induces psoriasiform lesions in mice **(Van der**

Fits et al., 2009). TLR7 & 9 are expressed on plasmacytoid DCs (pDCs) whereas TLR8 is expressed on myeloid DCs **(Hari et al., 2010; Jiang et al., 2013)**. Jiang and coworkers used antagonists to TLR7, 8&9 as therapy for psoriasis like lesions induced by IL23 injections in mice. They reported inhibition in Th1 and Th17 responses **(Jiang et al., 2013)**. Antagonists of TLR7, 8 & 9 have been used in preclinical models for treatment of autoimmune diseases as arthritis, lupus and uveitis **(Jiang et al., 2013**). Thus, TLR antagonism is a promising target for psoriasis treatment **(Jiang et al., 2013).**

TLR3 signaling has been recently associated with enhanced keratinocyte expression of the common p40 subunit of both IL23 & IL12 which link TLR3 with wound healing and probably psoriasis **(Ramnath et al., 2015)**.

TLR4 and TLR2 have been linked to psoriasis development. TLR4 expression in the skin was higher in psoriasis than normal skin **(Garcia-Rodriguez et al., 2013; Seung et al., 2007).** In another study Baker and coworker showed that in psoriatic lesional epidermis, TLR2 was highly expressed on upper epidermal keratinocyte more than on the basal layer keratinocytes **(Baker et al., 2003; Garcia-Rodriguez et al., 2013).** Moreover, Heat shock proteins e.g. HSP60 heavily expressed by keratinocytes of psoriasis, trigger TLR4 on APCs leading to maturation and secretion of TNF-α and IL-12. Furthermore, the keratinocyte derived fibronectin act on TLR4 pathway of APCs (Langerhans cells) causing their maturation, TNF α and IL 12 liberation, along with antigen presentation to autoreactive T cells **(Gaspari, 2006; Hari et al., 2010).** The upregulated AMPs in psoriasis, S100A8 and S100A9, are endogenous activators of TLR4 **(Garcia-Rodriguez et al., 2013**). Furthermore, antikeratin 16 monoclonal antibodies increase the expression levels of TLR 2, TLR4 and NF-κB nascent polypeptide-associated complex. Anti-keratin 16 antibodies are highly detected in the serum of psoriasis patients **(Hari et al., 2010)**.

On the systemic level, TLR4 & to a lesser extent TLR2 gene expression were higher in psoriatic patients' peripheral blood monocytes than in control. This could infer a role of TLR4 & TLR2 in the pathological systemic inflammatory status in psoriasis patients which leads to and / or aggravates other associated chronic inflammatory diseases e.g. cardiovascular accidents, diabetes mellitus **(Garcia-Rodriguez et al., 2013**).

Topical and systemic retinoids have anti-inflammatory activity through inhibition of TLR2 signaling which may be part of the mode of actions of retinoids in psoriasis treatment **(Hari et al., 2010; Tenaud et al., 2007)**. Retinoids have also been proved to have anti-inflammatory & immunomodulatory activity through their inhibitory effects on TLR4/ NFκB pathway blocking various cytokine secretion including IL1β, IL 6, IL12 and TNF-α **(Kim et al., 2013; Gu et al., 2010).**

It is interesting to know that monomethyl fumarate (fumaric acid ester), an immunotherapeutic for psoriasis, greatly inhibits LPS signaling via TLR4 in dendritic cells, inhibits NF-κB activation, lowers IL 12 & IL 10 production, and modulates monocytes-derived DC polarization i.e. fumaric acid ester 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).**

To sum up TLRs are critical component of innate immune system and are involved with dendritic cells and other APCs in priming the adaptive immune response. TLRs, as part of immune system, are involved in psoriasis development and their antagonists proven to have anti-psoriatic effect.

Aim of the work

In this study we aimed at establishment of psoriasis model in C57Bl mice background using Imiquimod daily topical application. We tested the best schedule for induction and maintenance of disease. We aimed at exploring the best methods for assessment of disease induction and progression using clinical, dermoscopic, histopathological and molecular techniques. Establishment of this model paves the way for testing different potential therapeutics for psoriasis along with exploring the disease pathogenesis itself.

Following the establishment of this psoriasis model, we proceeded to use it for testing the efficacy of MBL, an immune response modifier, in treating this psoriasiform induced inflammation. The effectiveness of MBL was evaluated on 4 levels; clinically dermoscopically, histopathologically and eventually the molecular pathways involved in the response were explored.

This work introduces a new potential therapeutic for psoriasis. This potential therapeutic offers blocking of an early step in the disease pathogenesis without the side effects that complicate other therapeutics including lymphoma development, reactivation of TB infection or being contraindicated in HIV or HCV patients.

Materials And Methods

Psoriasis animal model:

Eight to 11 week female C57BL mice were obtained from Theodore Bilharz Research Institute, Cairo. Animals were maintained and handled in accordance with NIH guidelines for the care and use of laboratory animals. Induction of psoriasis was executed as previously reported with few modifications **(Chamcheu et al., 2016; Van der Fits et al., 2009)**. In brief, the back of each mouse was shaved (area 2 X 2 cm^2 approx.) and the remaining hair was removed using a depilatory cream (Veet®; Reckitt Benckiser, UK). Forty eight hours later animals were evaluated clinically with the aid of DermLite DL3® dermoscope (3Gen, Inc., USA). Animals were sampled (skin & spleen) for further analysis for histopathologic evaluation and gene expression analysis serving as normal (negative control group). Each mouse received a daily topical dose of 62.5 mg (1/4 sachet) of Imiquimod (IMQ) cream (5%) (Aldara; 3M Pharmaceuticals) on the depilated back skin for 5 consecutive days, equivalent to a daily dose of 3.125 mg of the active compound. Following induction animals were divided into 2 groups one received the MBL treatment (n = 6) and the other received phosphate buffer saline (PBS) injection as control (n = 7). Both groups continued to receive same IMQ dose for the rest of treatment period (**Figure 15)**.

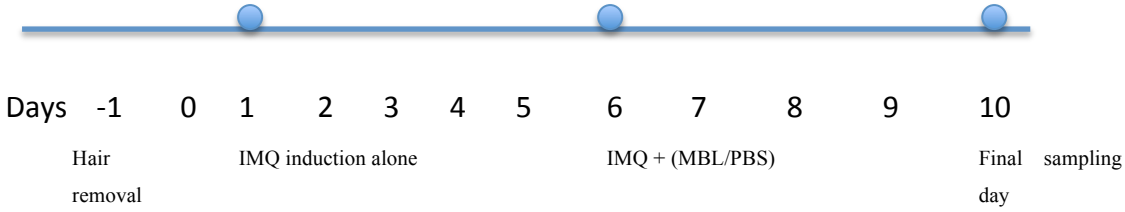


Figure 15: Schedule Of Psoriasis induction & treatment.

MBL treatment:

Recombinant mouse MBL2 was purchased from R&D Systems (Minneapolis, MN), and was reconstituted according manufacturer recommendation at 100 µg/ml of sterile PBS and kept at – 80 ^{0}C until injection. On the day of injection 20 µg (200 µl) were further diluted in an 800 µl PBS and each mice received 150 µl intralesional injection in the back using Hamilton® syringe (Sigma-Aldrich, USA), for 4

consecutive days. So the mice received daily dose of 3 µg of recombinant mouse MBL. Control mice received same volume of sterile PBS (150 µl) daily. On the fifth day of treatment skin & spleen samples were collected.

Clinical Evaluation:

Mice were evaluated clinically at 4 time points; a) Before induction on day 1. b) Mid induction phase on day 3. c) Before start of treatment on day 6 and finally, d) at the end of treatment at day 10. Clinical evaluation included weighing of mice, measuring thickness of back skin using electronic digital caliper (Titan, USA), assessment of extent of induction using erythema scaling and thickness score previously reported **(Chamcheu et al., 2016; Van der Fits et al., 2009)**. Erythema, scaling and thickness were given a score 0 - 4 (0; none, 1; mild, 2; moderate, 3; marked, 4; severe). A red panel was used for objective assessment of erythema.

Dermoscopic Evaluation:

For more precise evaluation of the psoriasis induction and the response to treatment, DermLite DL3® dermoscope was used. Dermoscope is a hand held device with magnification range 10 – 40 x for better visualization of surface skin and deeper down to dermal vessels and pigment. It was used to assess the mice at the same interval used for clinical evaluation, **Figure 16**. To our knowledge this is the first time dermoscopy is used in experimental dermatology for psoriasis induction and follow up of therapeutic response. According to the known dermoscopic criteria of psoriasis **(Lallas et al., 2012; Micali et al., 2011)**, the induction of psoriasis was ascertained by detection of dotted (glomerular) vessels distributed homogenously on the lesion, erythematous background and silvery white scales. The lesions were examined with non polarized light for evaluation of scaling followed by polarized light and finally with alcohol interface for better visualization of vascular pattern. We developed a scoring system to assess the response to therapy as illustrated in **Table 2**. The Scaling extent is given a score 0-3 with 0 = no changes, **Figure 17**, 1 = mild, **Figure 18**, 2 = moderate, **Figure 19**, and 3 = severe, **Figure 20**. Also the erythema and blood vessels is given similar score from 0-3 **Figures 21- 24**. The total score for each mouse is from 0-6.

Table 2: Scoring table for dermoscopic evaluation of therapeutic response:

	None (0)	Mild (1)	Moderate (2)	Severe (3)	Total score
Back ground erythema & Vessels					
Scales					

Figure 16: DermLite DL3 Dermoscope

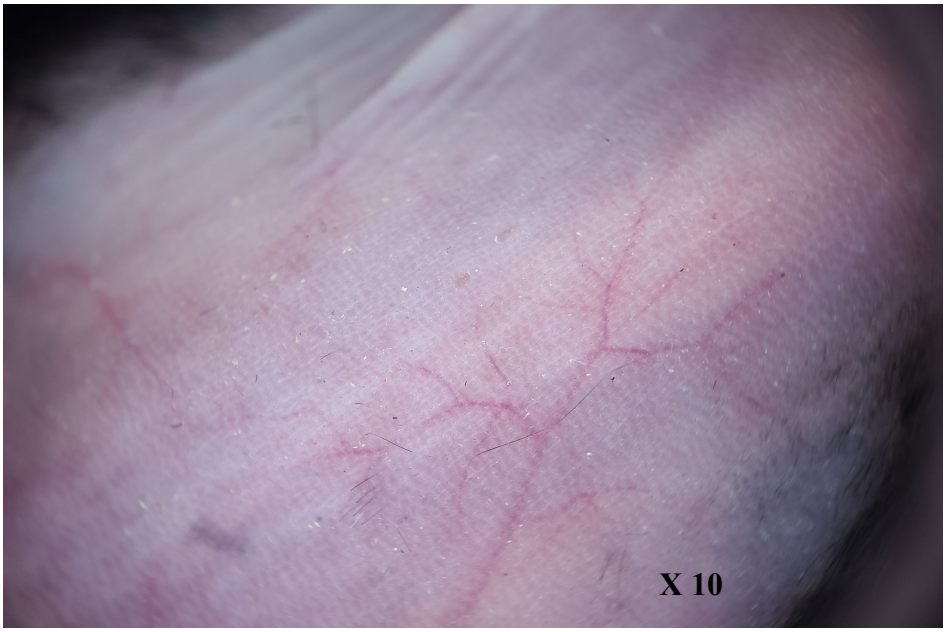


Figure 17: Scale grade 0

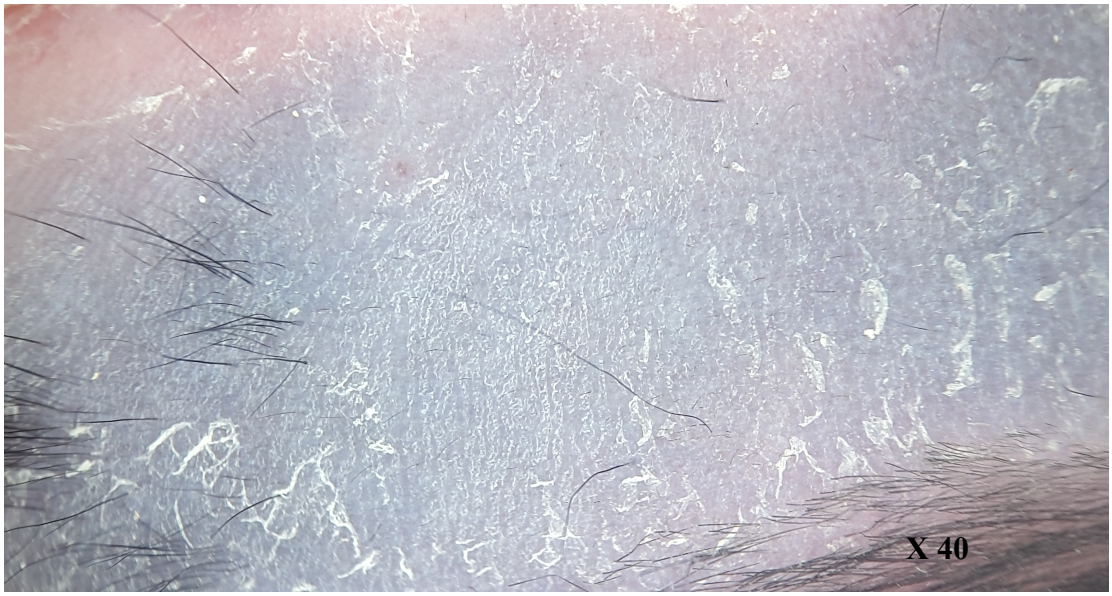


Figure 18: Scale grade 1

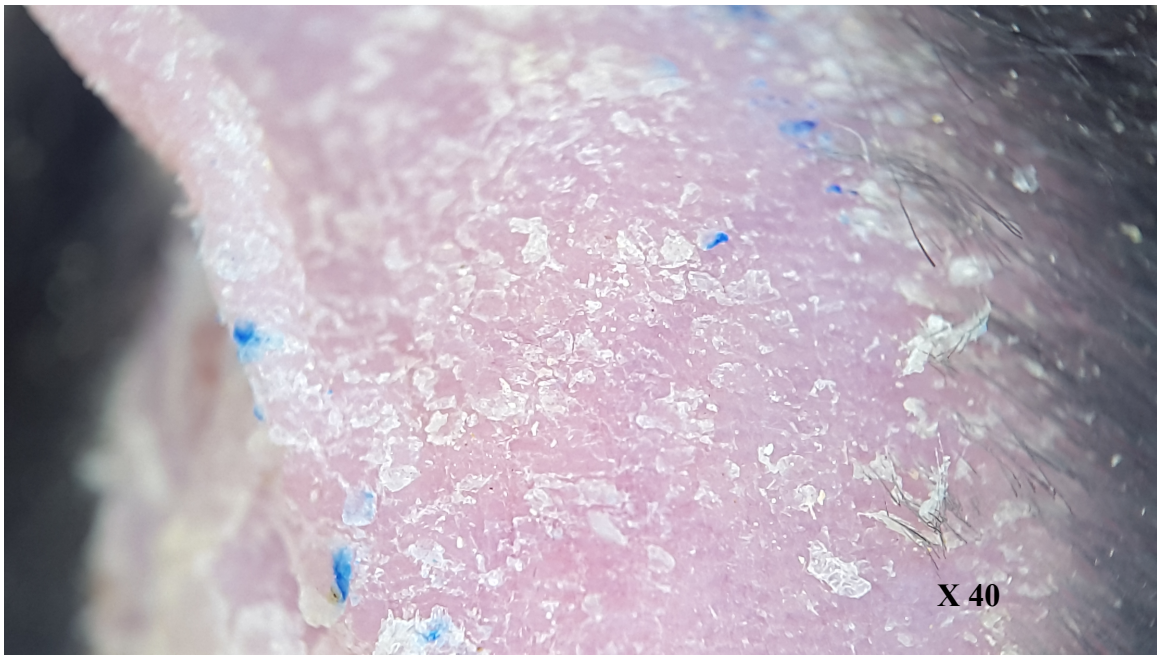


Figure 19: Scale grade 2

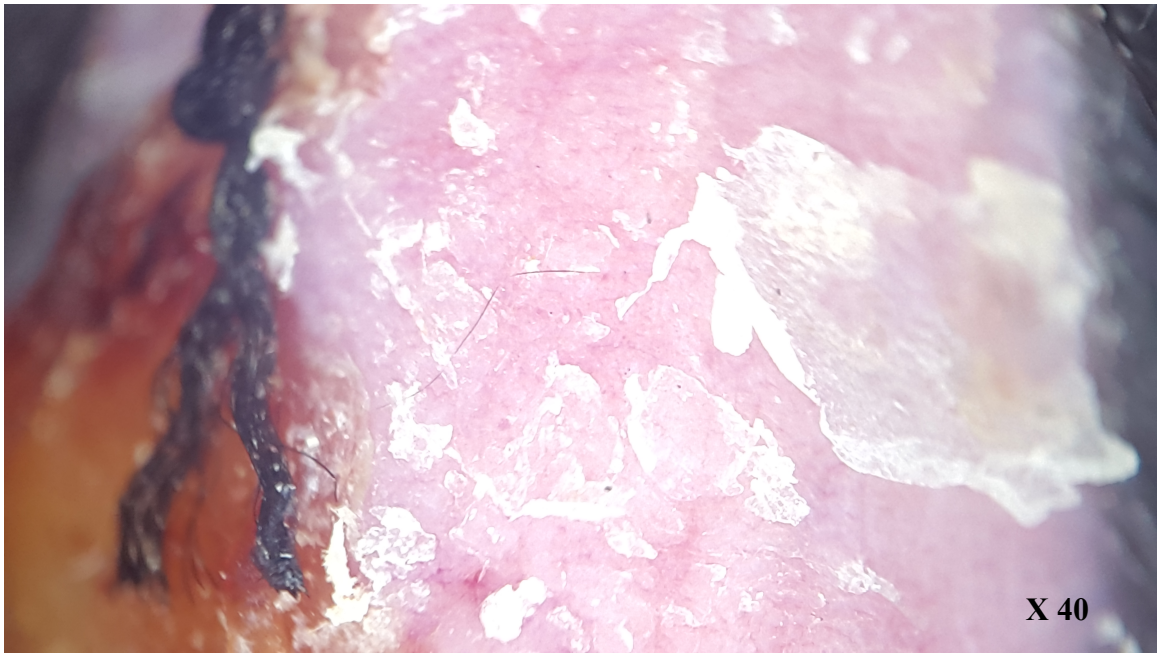


Figure 20: Scale grade 3

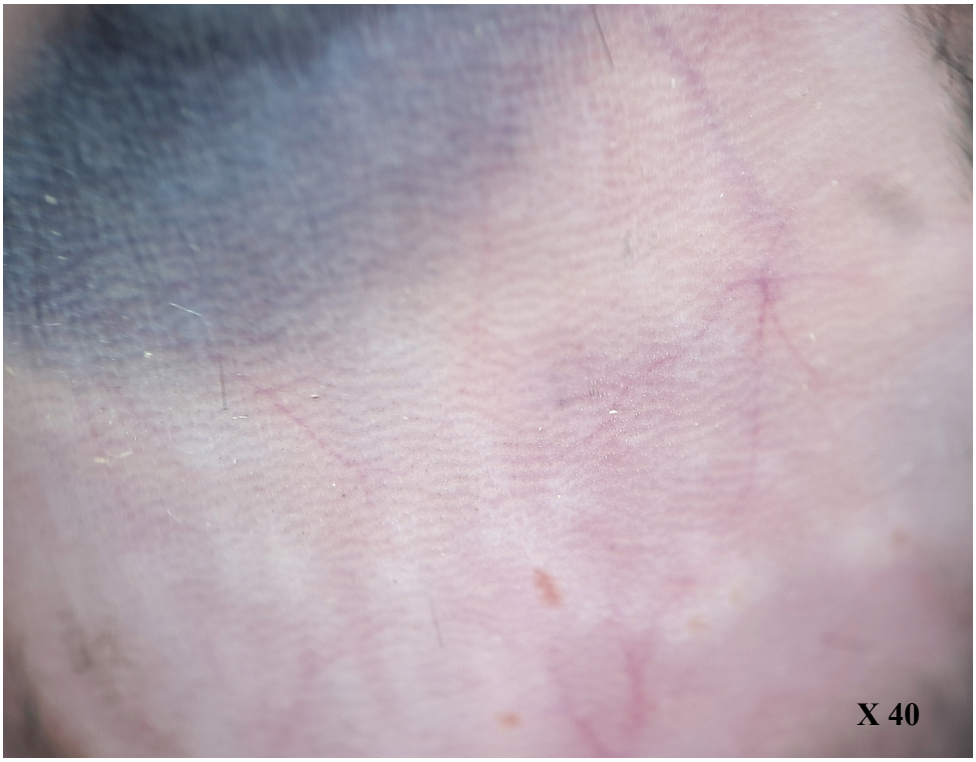


Figure 21: Erythema& vessel grade 0

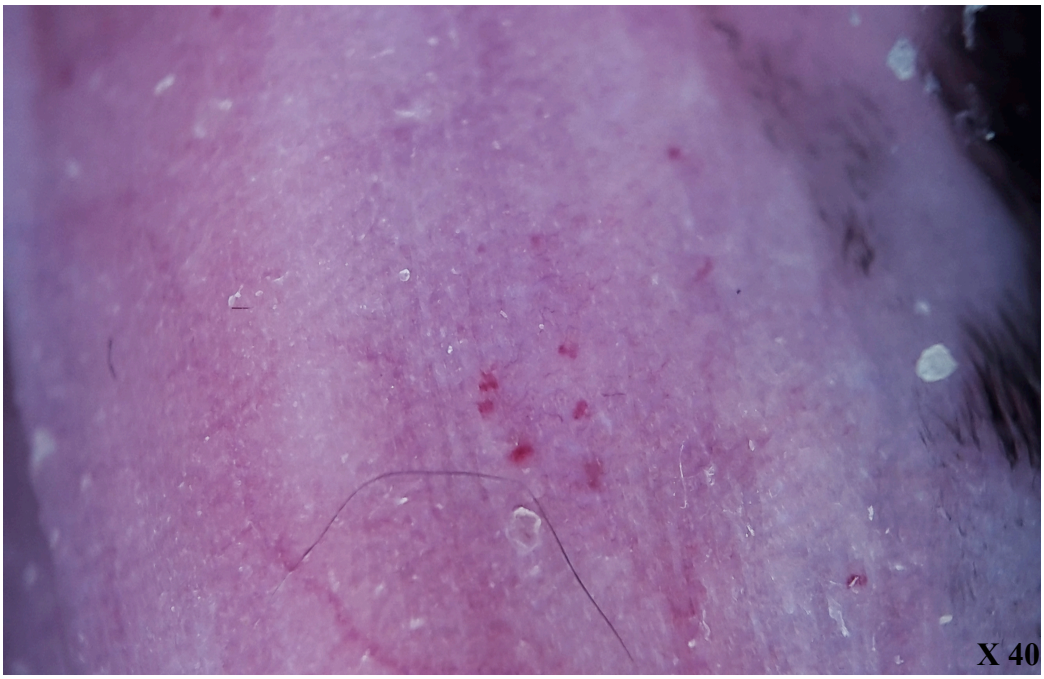


Figure 22: Erythema& vessel grade **1**

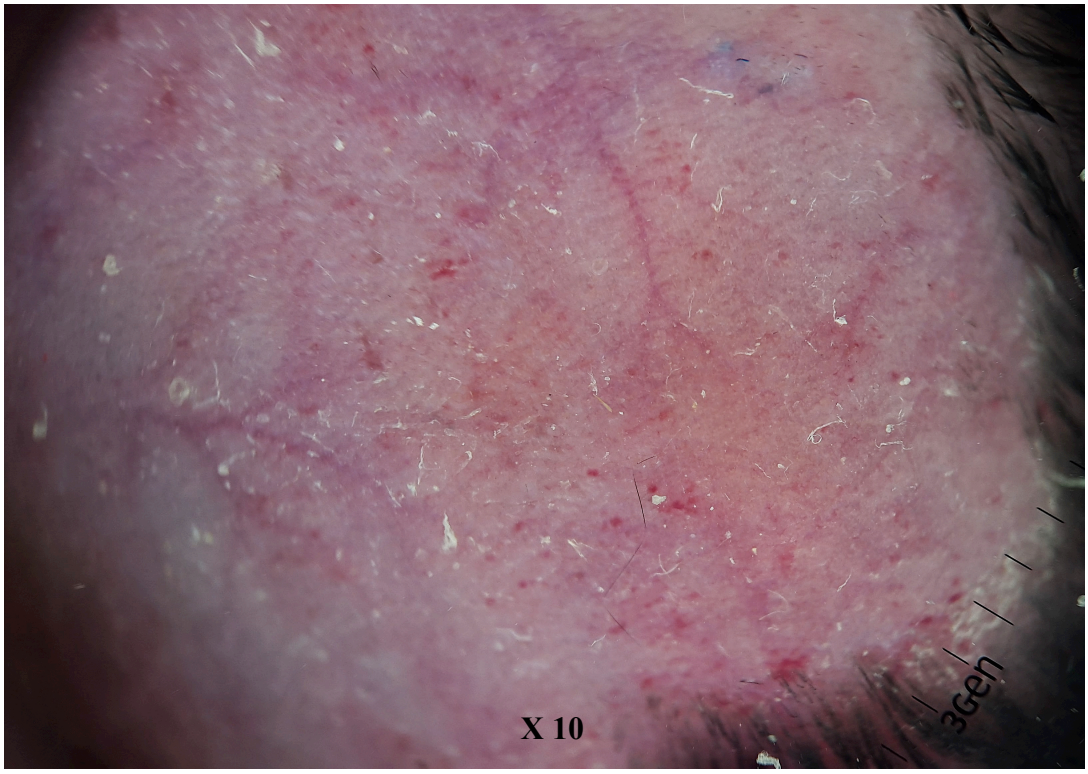


Figure 23: Erythema & vessel grade 2

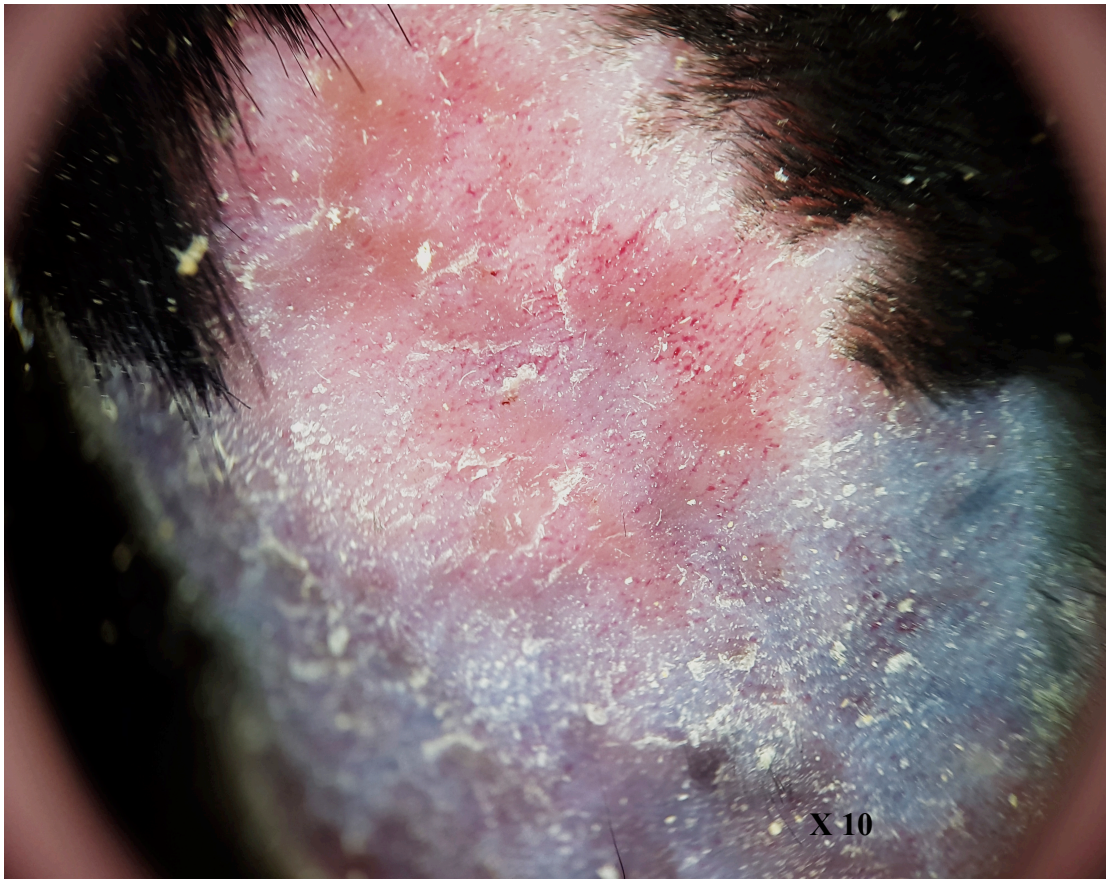


Figure 24: Erythema & vessel grade 3

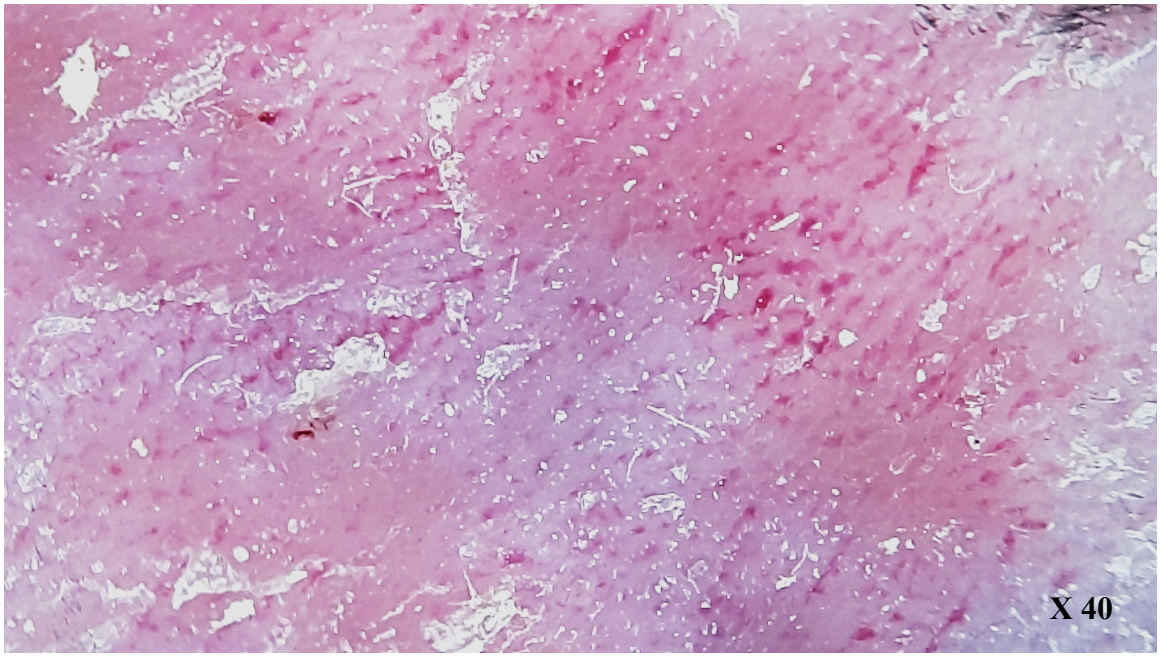


Figure 24, continued: Erythema & vessel grade 3

Histological Evaluation & Epidermal Thickness Assessment:

For histological examination, 5 ml punch biopsy from the back skin was immersed in 10% formalin. Samples were then embedded in paraffin wax, and sections were processed for H&E staining. The slides were examined under LEICA DM 1000 microscope (LEICA Microsystems, Wetzlar, Germany) with 10X & 40X magnification. Images under 10X magnification were further assessed using Image J **(Kim et al., 2015)**. To determine the average epidermal thickness 10-15 shots for each slide were taken then 6-8 readings /shot for the epidermal thickness (a straight perpendicular line is drawn from the basal layer to the upper stratum corneum) to assess the average epidermal thickness for each mouse.

Gene Expression analysis:

Skin samples were stored in RNAlater® (AMBION, Inc., USA) overnight then removed from it and kept at – 80 ^{o}C till total RNA was extracted using RNeasy Mini kit (Qiagen, Valencia, CA). Skin samples were first homogenized in 700 µl TRizol then 140 µl of chloroform was added. Centrifugation for 15 min at 12,000 x g at 4°C was followed for phase separation followed by column extraction according to manufacturer protocol. RNA (0.5µg) was reverse transcribed using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc, Waltham,

Massachusetts, USA). Quantitative real-time PCR was carried out using StepOnePlus PCR system (Invitrogen), and hypoxanthine-guanine phosphoribosyltransferase (Mm00446968_m1) served as endogenous control to calculate the relative amount of gene expression. Relative quantity of mRNA was calculated as respect to the mean of normal group, which is set to 1. TaqMan® ready made Gene expression assays for mouse IL6 (Mm00446190_m1) and IL12a (Mm00434169_m1) were measured.

Statistical analysis:

Quantitative data were statistically represented in terms minimum, maximum, median, mean and standard division (SD). Comparison between difference groups in the presents study was done using Independent samples T-Test for comparing two parametric groups, and using Mann-Whitney Test for comparing two nonparametric groups, and One-way ANOVA Test was used when comparison between more than two parametric groups with (LSD) or (Dunnett t Test) as multiple comparison tests, and Kruskall-Wallis Test was used when comparison between more than two nonparametric groups, and using Paired Samples T-Test for comparing two paired parametric groups, and using Wilcoxon Signed Ranks Test for comparing two paired nonparametric groups.

Qualitative data were statistically represented in terms number and percent. Comparison between difference groups in the presents study was done using Chi-Square Test.

A probability value (p value) less than or equal to (0.05) was considered significant and CI 95%. All statistical calculations were done using computer program SPSS (Statistical Package for Social Science) statistical program version (16.0). Graphs were done using Microsoft Excel program version 2010.

Results

I- Establishment of Psoriasis Mouse Model:

Imiquimod (Aldara ®, 5% Cream, 3M, USA) was applied daily for 6 days on the shaved back of mice. This successfully induced psoriasis-like lesions.

a. Imiquimod induces psoriasis-like lesions in mouse skin clinically:

The skin of the back of the mice was shaved and assessed 1 day before Imiquimod application and at 3 & 6 days after start of Imiquimod application. Skin started to show signs of inflammation in the form of erythema, scaling and increased thickness. The scoring of each parameter and the total score differed significantly between mice at day 0 and on day 6 as illustrated in **Table 3 and Figure 25.** These Psoriasis-like or psoriasiform lesions were consistent with Psoriasis pathology seen in human.

Table 3: Clinical score of mice after Imiquimod application:

Parameters	Groups	N*	Min.	Max.	Median	Mean ± S.D.	P value
Erythema	Normal	27	0.00	1.00	0.00	0.11 ± 0.32	0.001
	Imq*	24	2.00	4.00	3.50	3.33 ± 0.76	
Scaling	Normal	27	0.00	1.00	0.00	0.19 ± 0.40	0.001
	Imq*	24	2.00	4.00	3.00	3.38 ± 0.65	
Thickness	Normal	27	0.00	2.00	0.00	0.19 ± 0.48	0.001
	Imq*	24	1.00	4.00	4.00	3.38 ± 1.01	
Total	Normal	27	0.00	3.00	0.00	0.48 ± 0.80	0.001
	Imq*	24	7.00	12.00	10.00	10.08 ± 1.72	

*Imq: 6 days Imiquimod induction

**N: Number of mice

Skin fold thickness increased significantly with development of psoraisiform lesions (P= 0.001), **Table 4.**

Table 4: Skin fold thickness after Imiquimod application:

Parameters	Groups	N**	Min.	Max.	Median	Mean ± S.D.	P value
Skin fold thickness (mm)	Normal	20	0.43	0.80	0.63	0.63 ± 0.09	0.001
	Imq*	24	0.70	1.80	1.04	1.14 ± 0.33	

*Imq: 6 days Imiquimod induction

**N: Number of mice

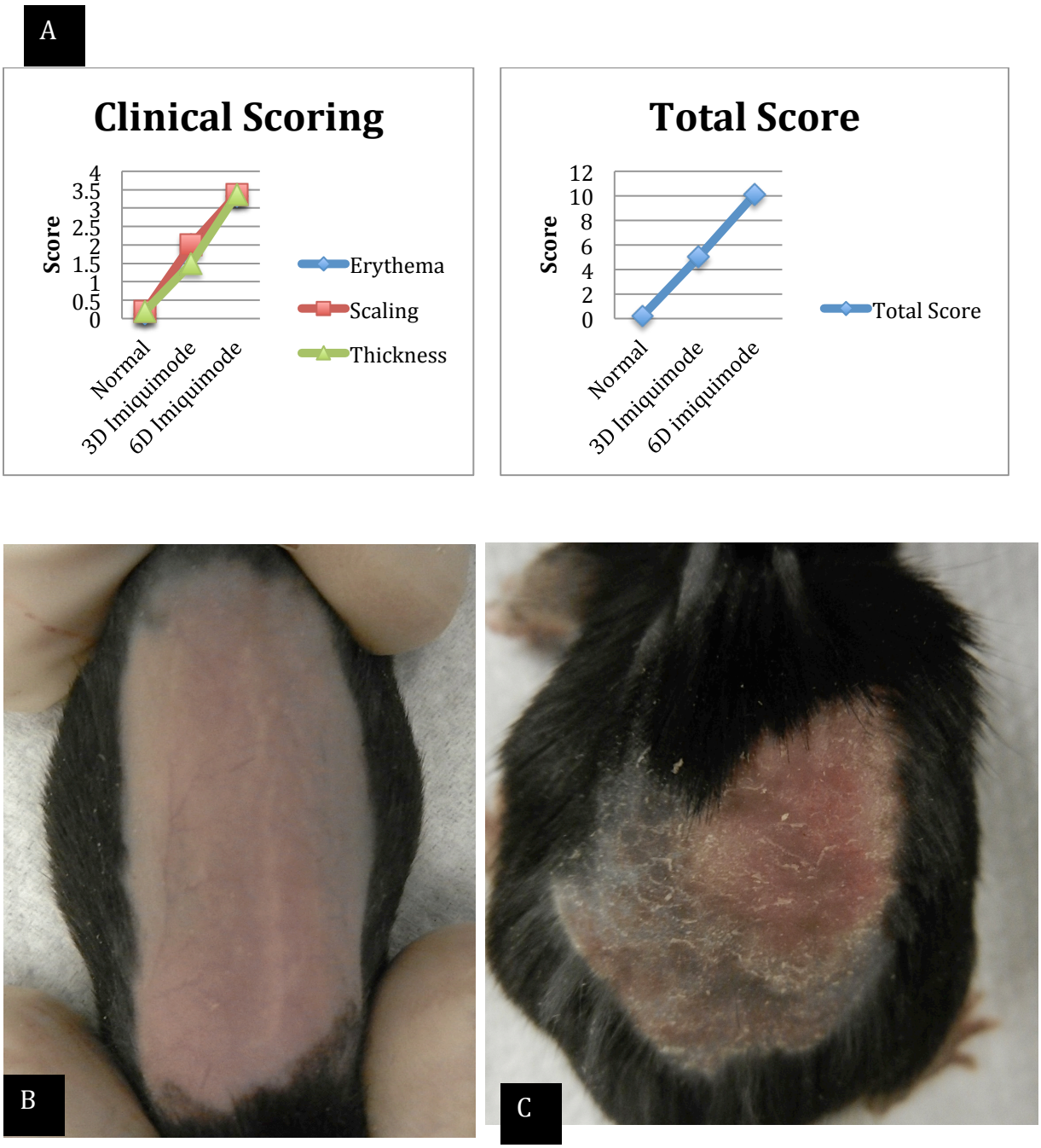


Figure 25: Successful induction of Psoriasis in mouse model. A) Graphic representation of progression of psoriasiform lesions from normal to 3 days and 6 days after Imiquimod application where erythema, scaling thickness has increased significantly ($P = 0.001$). B) Normal shaved back skin of mouse with 0 score on erythema scaling, thickness and total score. C) Psoriasis development with 4 score on erythema, scaling thickness and total score of 12.

b. Imiquimod induces psoriasis-like lesion in mouse skin dermoscopically:

Psoriasis induction was evaluated by dermoscopy. The characteristic dermoscopic features of psoriasis previously reported were evaluated and included in a scoring system to evaluate the induction and response to therapy. Two parameters were assessed; the dotted vessel and the background erythema component and the scaling component. The changes were given a score from 0 to 3 according to the severity of change. Scaling has increased significantly when compared to normal skin. The skin was thick, red with dotted blood vessels. Imiquimod-induced skin has significantly differed from normal skin regarding each of the two components and the total score (*P = 0.001*) **Table 5 and Figure 26.** Normal dermoscopic appearance was very thin transparent skin with long linear blood vessels easily seen on 10X without scales. The skin is very thin, it reflects the color of underneath tissues and when elevated to form a fold it is of skin colored, **(Figure 26)**.

Table 5: Dermoscopy Score in normal and Imiquimod induced psoriasiform lesions.

Parameter	Groups	N	Min.	Max.	Mean ± S.D.	P value
Erythema & Blood vessel	Normal	27	0.00	1.00	0.07 ± 0.27	0.001
	Imq*	16	1.00	3.00	2.50 ± 0.82	
Scaling	Normal	27	0.00	1.00	0.15 ± 0.36	0.001
	Imq*	16	1.00	3.00	2.56 ± 0.63	
Total	Normal	27	0.00	2.00	0.22 ± 0.51	
	Imq*	16	3.00	6.00	5.06 ± 1.29	0.001

*Imq: 6 days Imiquimod induction

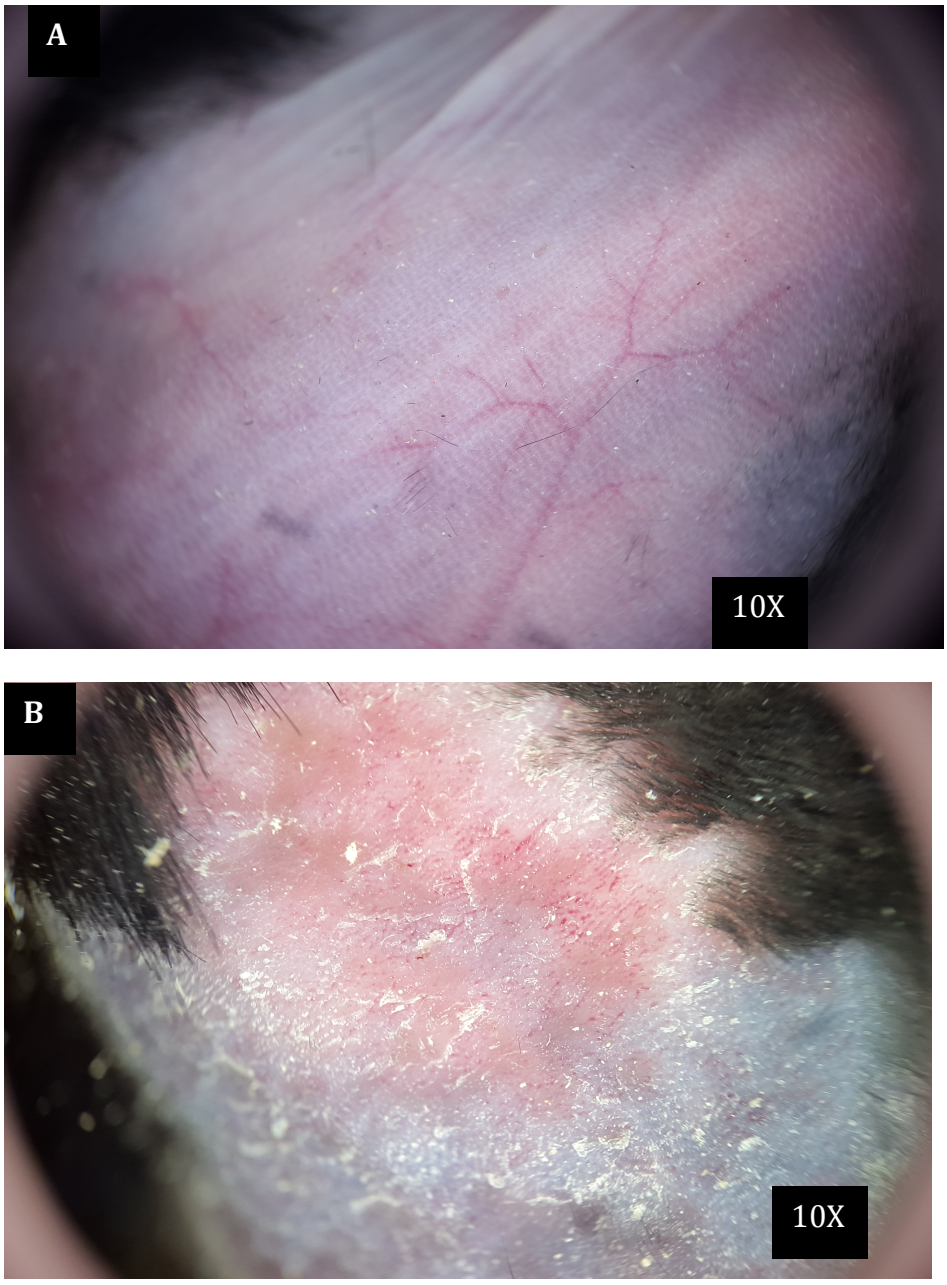


Figure 26: Dermoscopy of Imiquimod induced psoriasiform lesions. A) Normal mouse with thin transparent skin, linear branching vessels and skin colored fold. B) Induced skin with scaling and dotted vessel distributed on the lesion 10X.

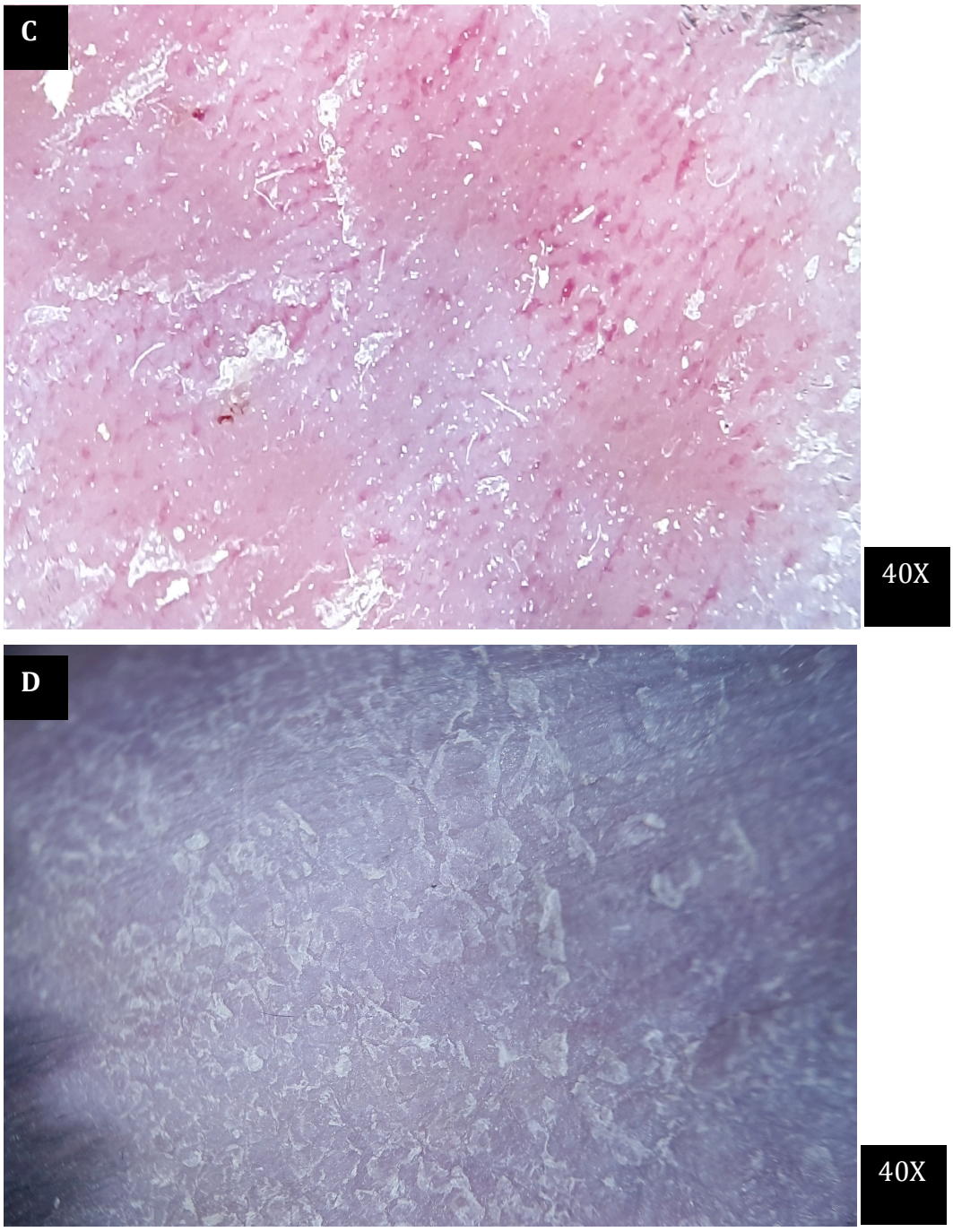


Figure 26, continued: Dermoscopy of Imiquimod induced psoriasiform lesions. C) Induced skin with white silvery scale and dotted vessels 40X. D) White silvery scale using non-polarized light mode.

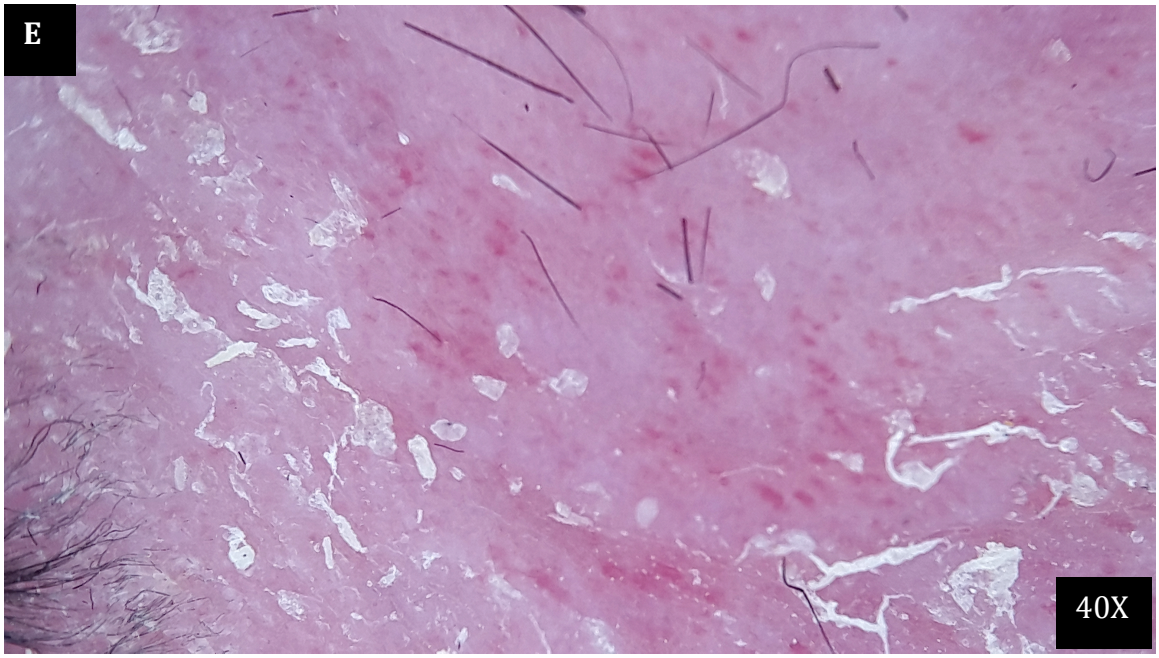


Figure 26, continued: Dermoscopy of Imiquimode induced psoriasiform lesions. E) Dotted vessels using polarized light mode.

c. Imiquimod induces psoriasiform histopathological changes in mouse skin.

Imiquimod treated skin showed acanthosis, hyperkeratosis and parakeratosis. In the dermis there is perivascular inflammatory infiltrate. The epidermal thickness has significantly increased (*P = 0.001*) from (17.7 µm ± 5.7µm) in normal skin to (83.4 µm ± 1.9 µm) in induced skin, **Table 6 & figure 27.**

Table 6: Imiquimod significantly increased epidermal thickness:

Groups	N	Min.	Max.	Median	Mean ± S.D.	P value
Normal	13	11.34	27.50	15.39	17.77 ± 5.74	0.001
Imq*	5	80.49	85.59	84.12	83.49 ± 1.92	

*Imq: 6 days Imiquimod induction

**N: Number of mice

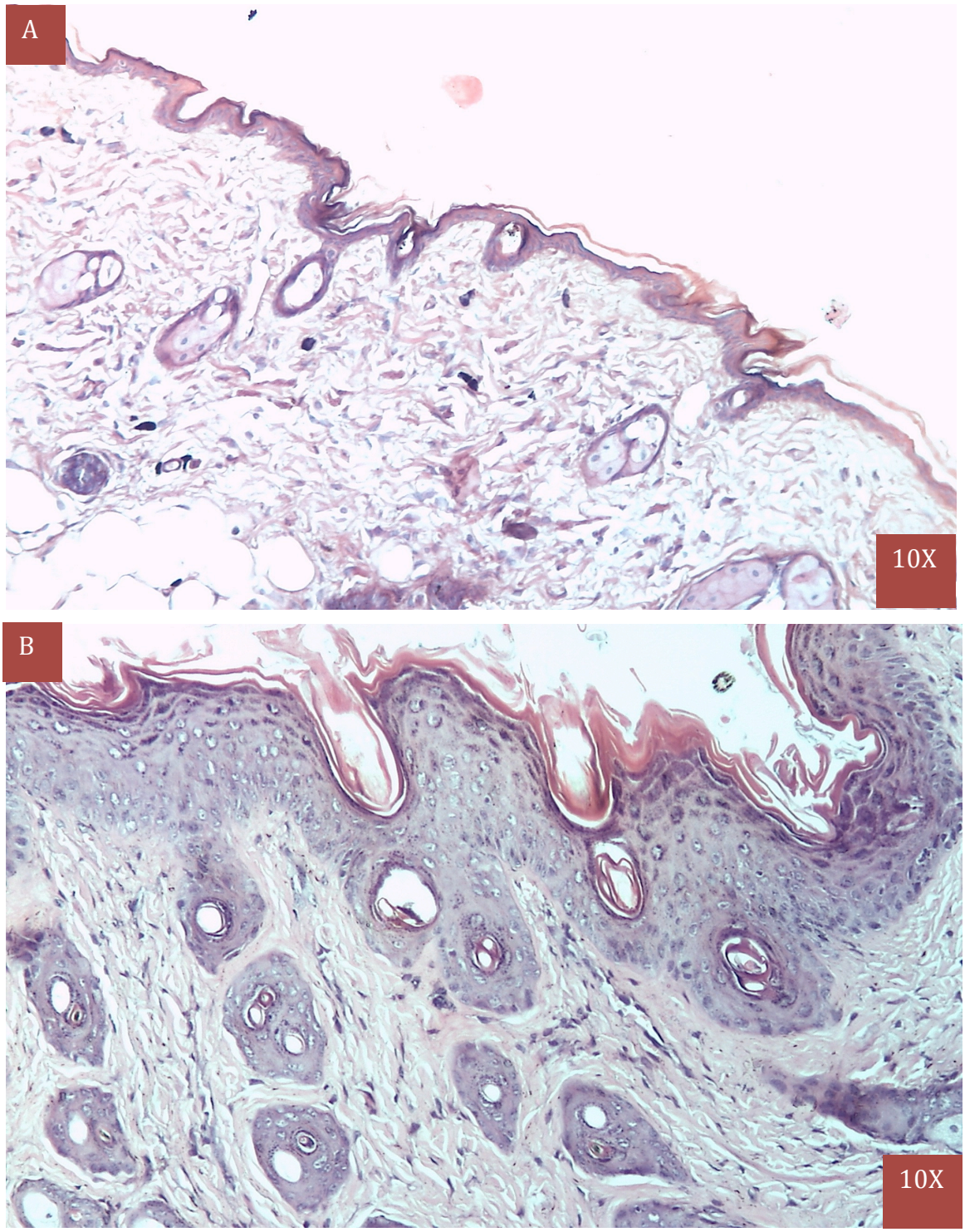
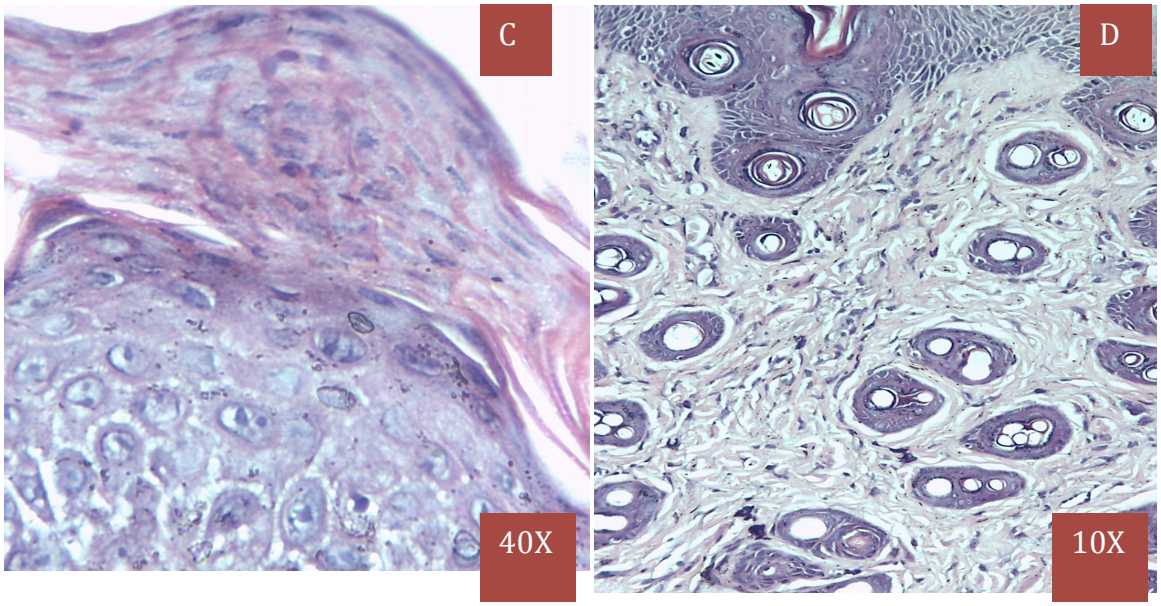


Figure 27: Psoriasiform changes in Imiquimod induced skin. A) Normal back skin of mice with thin epidermis, no rete ridges or inflammatory infiltrate. B) Imiquimod induced psoriasiform changes with acanthosis, hyperkeratosis& dermal inflammatory infiltrate.



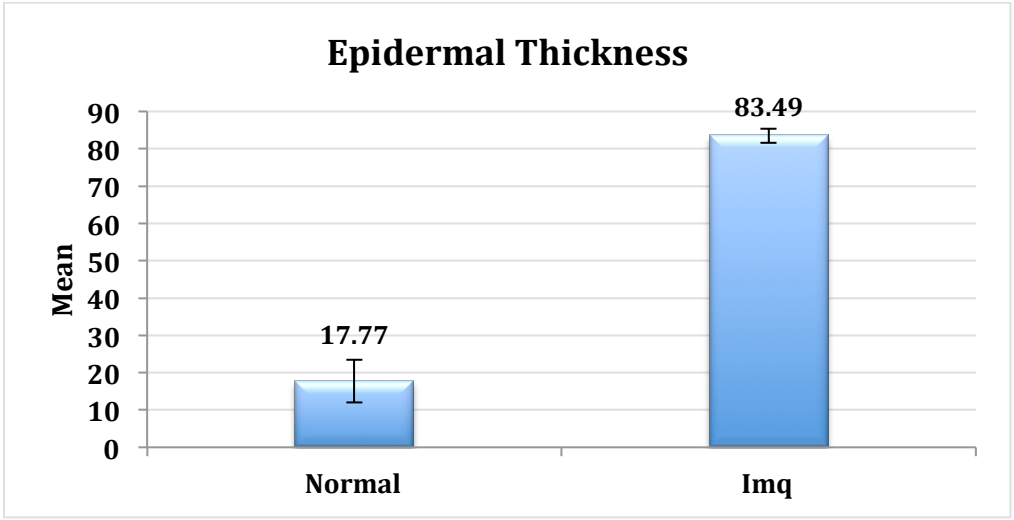


Figure27, continued: Psoriasiform changes in Imiquimod induced skin. C) Parakeratosis in stratum corneum. D) Dermal lymphocytic inflammatory infiltrate. E) Imiquimod increased epidermal thickness. The epidermal thickness of 10X images analyzed using Image J showed significant increase in epidermal thickness (*P= 0.001*)

II. Treatment of Psoriasiform lesions using MBL

MBL is an immune response modifier plays an essential role in fine tuning inflammatory responses in the extravascular spaces at site of inflammation.

a. MBL treated group started to gain weight:

MBL treated mice started to gain weight while PBS control mice continued to lose weight (*P=0.01*) **Table 7, Figure 28.**

Table 7: Percentage of weight change in MBL & PBS groups

Parameters		N**	Median	Mean ± S.D.	P value[a]
Weight	MBL	6	11.27	9.63 ± 4.54	0.010
	PBS	7	-7.04	-5.63 ± 9.02	

[a] P value between MBL and PBS using None-Parametric test (Mann-Whitney Test).

**N: Number of mice

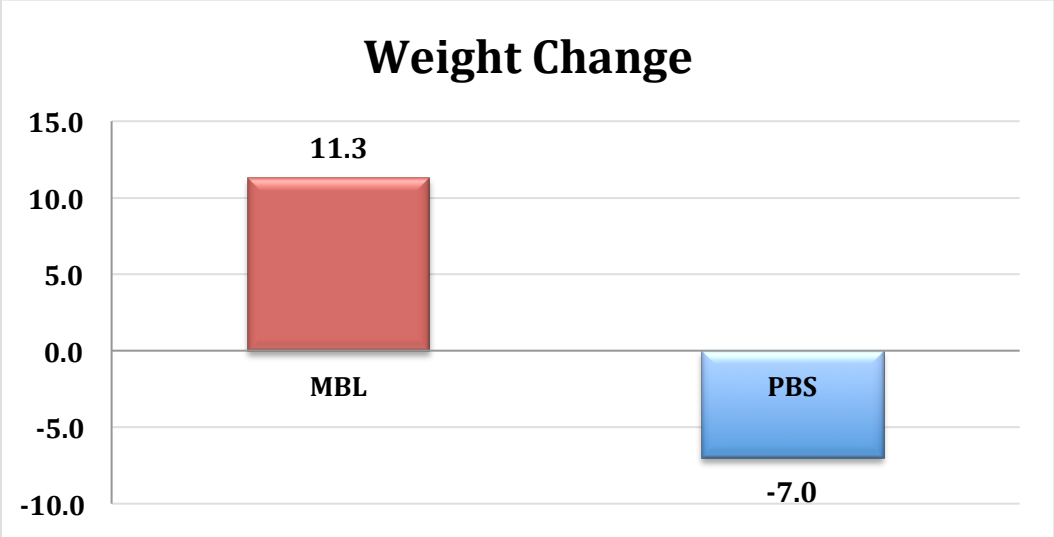


Figure 28: Weight change on MBL treatment. MBL group gained weight while PBS control group continued to loose weight.

b. MBL clinically reverse the imiquimod induced psoriasisform inflammation:

Intralesional injection of MBL successfully treated the psoriasiform skin lesions induced by imiquimod application. The skin of MBL treated mice showed significant reduction in all signs of psoriasiform lesions (erythema, scaling and thickness). On the other hand the control group (mice received only PBS) didn't show any significant improvement in the psoriasiform lesions compared to their clinical scoring before, **Table 8 & Figures 29, 30 &31.**

Table 8: MBL treats the Imiquimod induced psoriasifrom lesions

Parameters	Groups	N**	Median	Mean ± S.D.	P value[a]	P value[b]
Erythema	MBL	6	0.00	0.17 ± 0.41	0.001	0.001
	PBS	7	3.00	3.00 ± 0.58	0.219	
	Imq*	24	3.50	3.33 ± 0.76		
Scaling	MBL	6	1.00	1.00 ± 0.89	0.001	0.002
	PBS	7	4.00	3.71 ± 0.49	0.211	
	Imq*	24	3.00	3.38 ± 0.65		
Thickness	MBL	6	1.00	1.17 ± 1.47	0.002	0.036
	PBS	7	4.00	3.14 ± 1.46	0.909	
	Imq*	24	4.00	3.38 ± 1.01		
Total	MBL	6	2.00	2.33 ± 1.51	0.001	0.002
	PBS	7	11.00	9.86 ± 2.12	0.847	
	Imq*	24	10.00	10.08 ± 1.72		

[a] P value compared with Imq group using None-Parametric test (Mann-Whitney Test).

[b] P value between MBL group and PBS group using None-Parametric test (Mann-Whitney Test).

*Imq: 6 days imiquimod induction

**N: Number of mice.

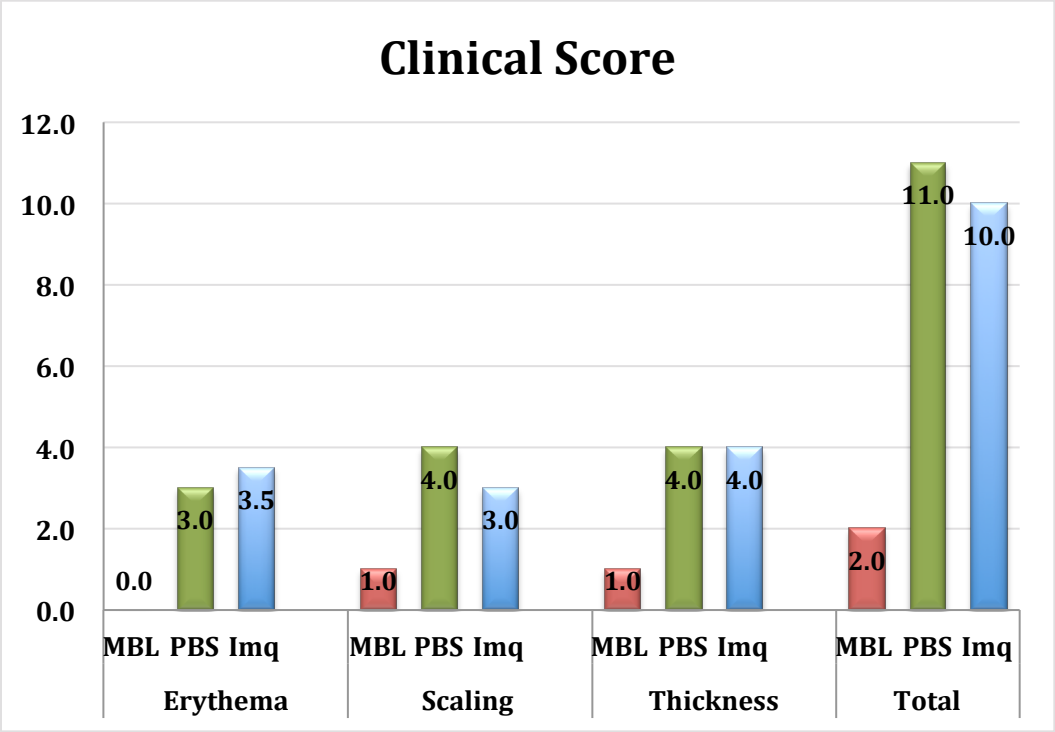
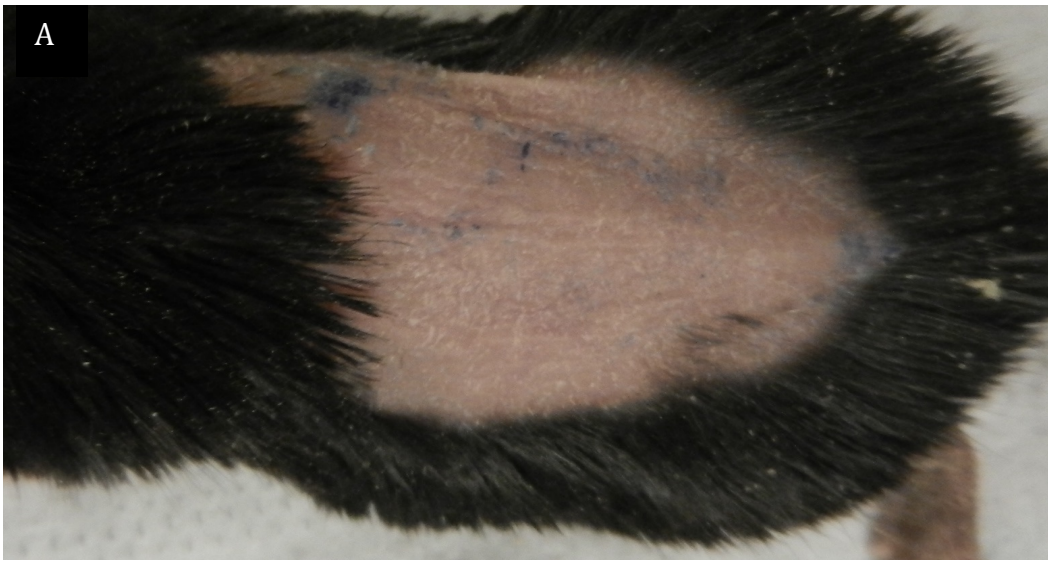


Figure 29: MBL improves psoriasiform lesional clinical score. MBL group showed significantly reduced clinical score in erythema, scaling, thickness, and total score compared to 6 days Imiquimod induction (Imq) scores ($P < 0.002$). PBS control group didn't show significant variation from Imq group ($P > 0.05$). MBL group had significantly lower clinical score compared to PBS control group ($P < 0.05$)



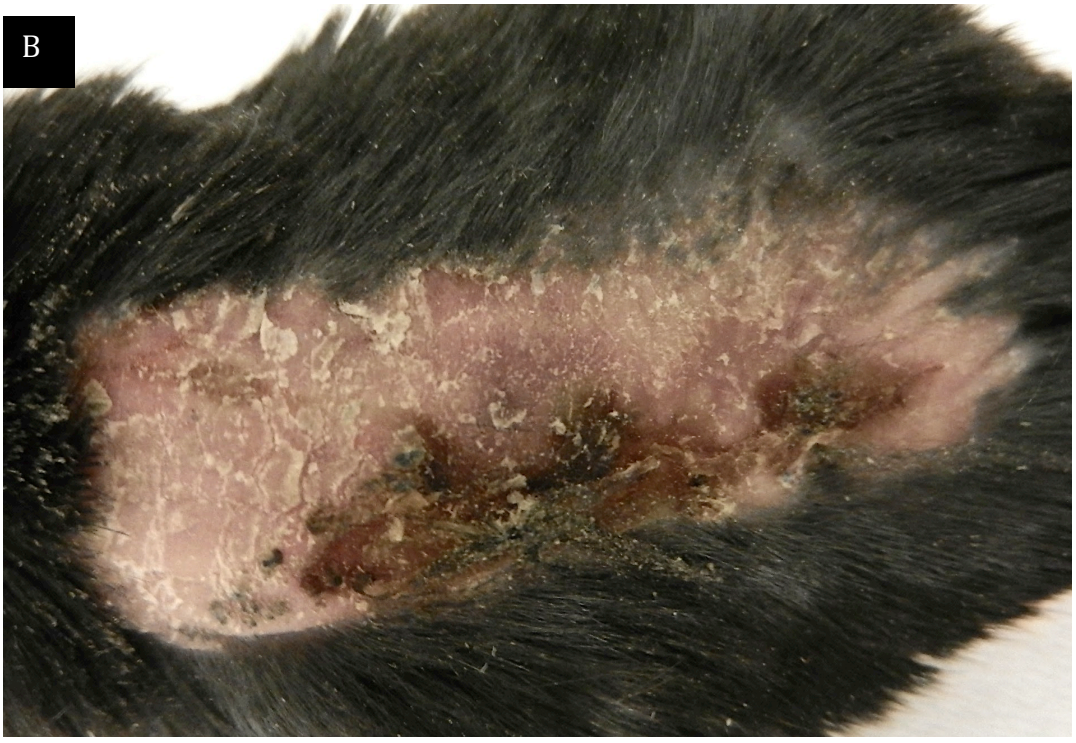


Figure 30: MBL injections improve Imiquimod induced psoriasis. A) MBL treated mouse with almost normal skin with mild scaling without erythema or increased thickness. B) PBS control group with marked scaling, erythema and thickening of the skin.

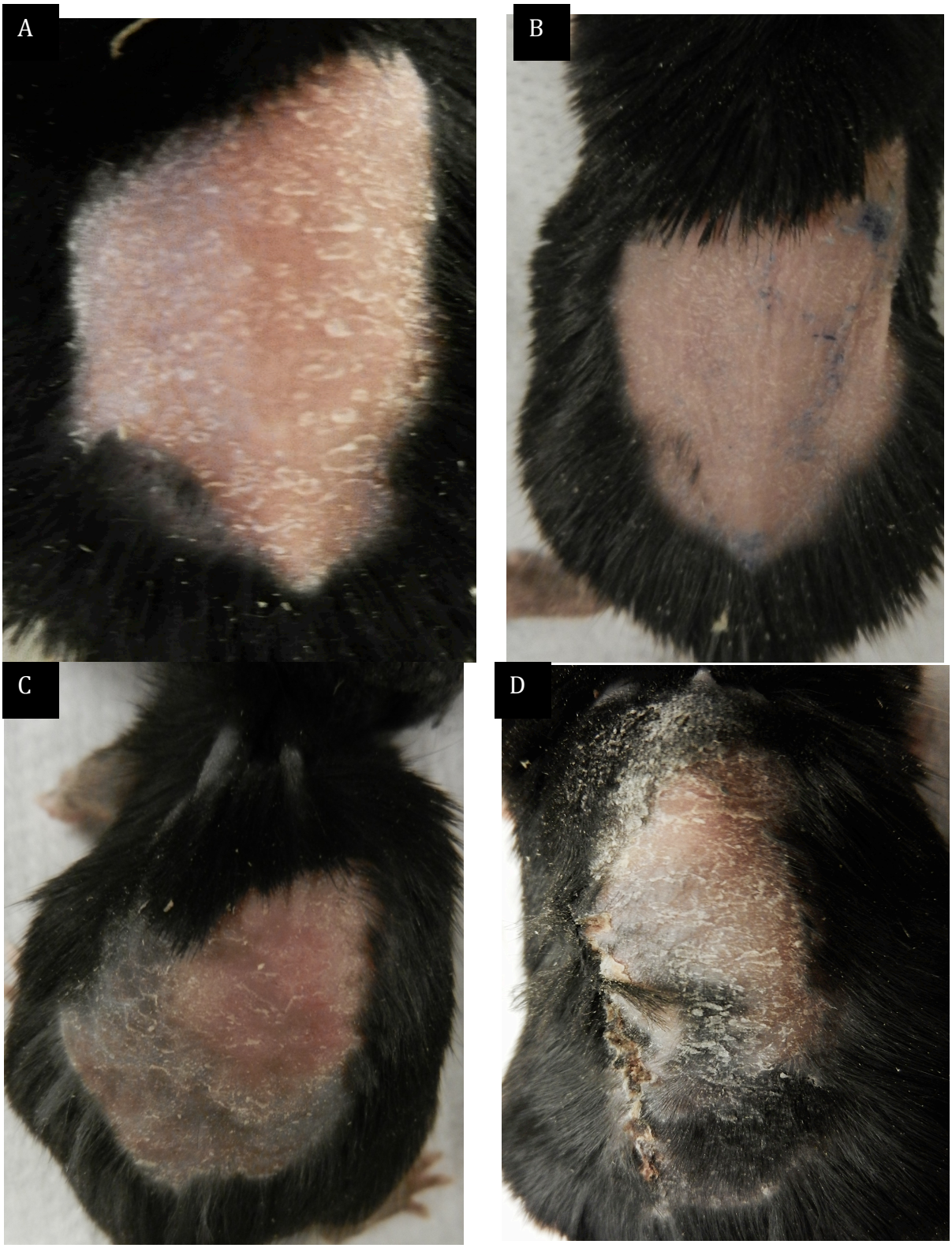


Figure 31: MBL treatment improves psoriasiform lesions. A) Imiquomod induced mouse before MBL treatment. B) Reversal of psoriasiform lesions after MBL treatment. C) Imiquimod induced mouse before PBS. D) Same mouse after PBS injection.

The skin fold thickness was significantly lower in MBL treated mice compared to PBS mice (*P = 0.045*), **Table 9**. While MBL treatment reduced the skin fold thickness significantly (*P = 0.005*) PBS received mice didn't show significant reduction in their skin fold thickness compared to 6 day imiquimod induction (*P = 0.943*), **Table 9, Figure 32.**

Table 9: Skin fold thickness in MBL treated & PBS control group

Parameters	Groups	N**	Median	Mean ± S.D.	P value[a]	P value[b]
Skin fold thickness	MBL	6	0.73	0.75 ± 0.17	0.005	0.045
	PBS	7	1.04	1.11 ± 0.36	0.943	
	Imq*	24	1.04	1.14 ± 0.33		

[a] P value compared with Imq group using None-Parametric test (Mann-Whitney Test).

[b] P value between MBL group and PBS group using None-Parametric test (Mann-Whitney Test).

*Imq: 6 days imiquimod induction

**N: Number of mice

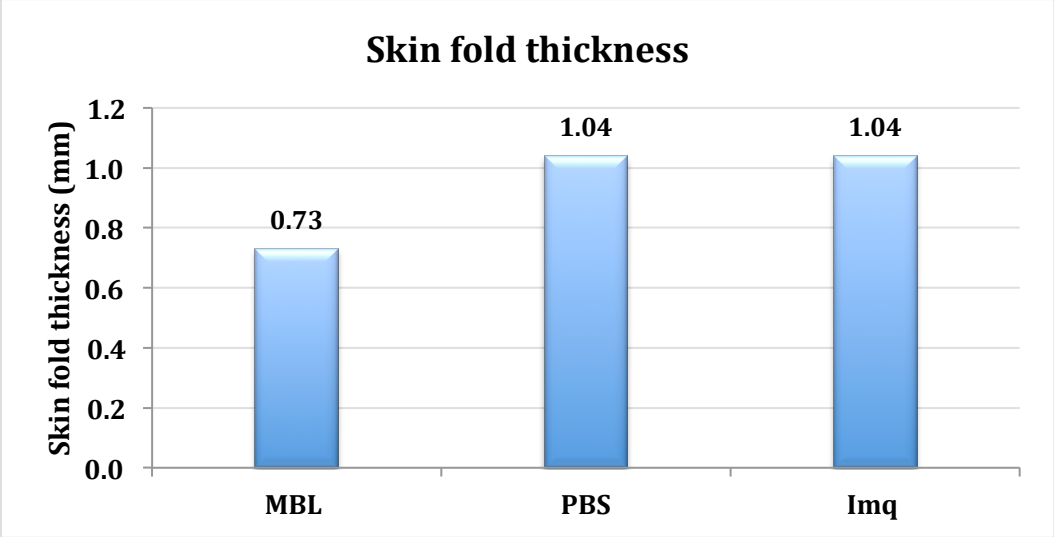


Figure 32: MBL reduced skin fold thickness. MBL treatment significantly reduced skin fold thickness (*P=0.005*) from 6 days imiquimod induction (Imq) thickness while PBS group didn't show significant reduction in skin fold thickness (P=0.943). MBL treated mice have significantly lower skin fold thickness than PBS control group (*P=0.045*).

c. MBL reversed the Imiquimod induced psoriasis on dermoscopic examination:

The improvement of Psoriasiform inflammation was also evident when examined by dermoscope with significant reduction (*P= 0.001*) of scaling, dotted vessels and background erythema score in MBL treated group while the PBS control group didn't vary significantly from the start of PBS injections, **Table 10 & Figures 33 & 34.**

Table 10: MBL treatment reduces dermoscopy scores.

Parameters	Groups	N**	Median	Mean ± S.D.	P value[a]	P value[b]
Erythema & Blood vessel	MBL	6	0.00	0.00 ± 0.00	0.001	0.001
	PBS	7	2.00	2.29 ± 0.76	0.399	
	Imq*	16	3.00	2.50 ± 0.82		
Scaling	MBL	6	1.00	1.00 ± 0.63	0.001	0.001
	PBS	7	3.00	3.00 ± 0.00	0.067	
	Imq*	16	3.00	2.56 ± 0.63		
Total	MBL	6	1.00	1.00 ± 0.63	0.001	0.002
	PBS	7	5.00	5.29 ± 0.76	0.942	
	Imq*	16	6.00	5.06 ± 1.29		

[a] P value compared with Imq group using None-Parametric test (Mann-Whitney Test).

[b] P value between MBL group and PBS group using None-Parametric test (Mann-Whitney Test).

*Imq: 6 days imiquimod induced mice

**N: Number of mice

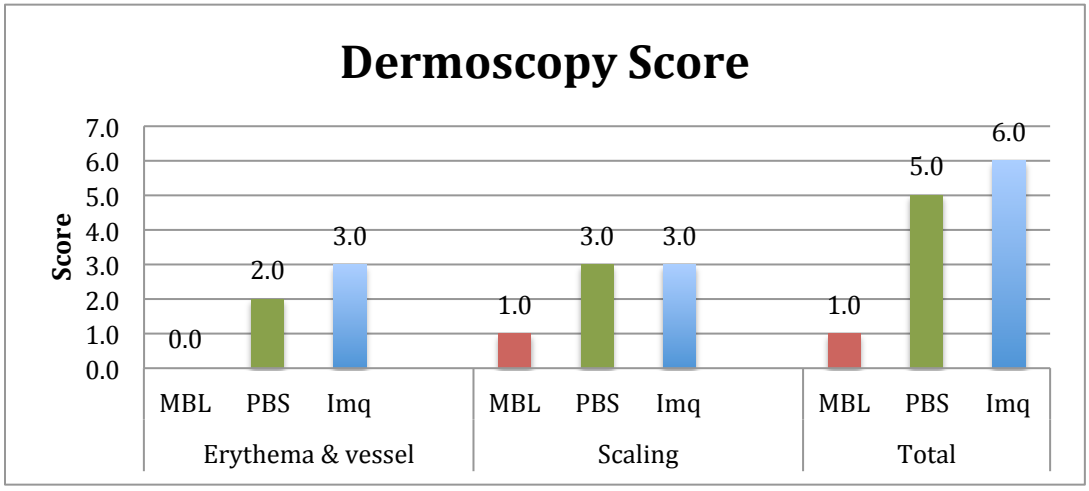


Figure 33: MBL reduced dermoscopic Scoring. MBL treatment significantly reduced dermoscopic changes (Erythema & vessel, Scaling and total score) compared to 6 days Imiquimod induction score (*P= 0.001*) while PBS group didn't show significant reduction on dermoscopic evaluation (Erythema *P= 0.399*, Scaling *P= 0.067* & total *P=0.942*).

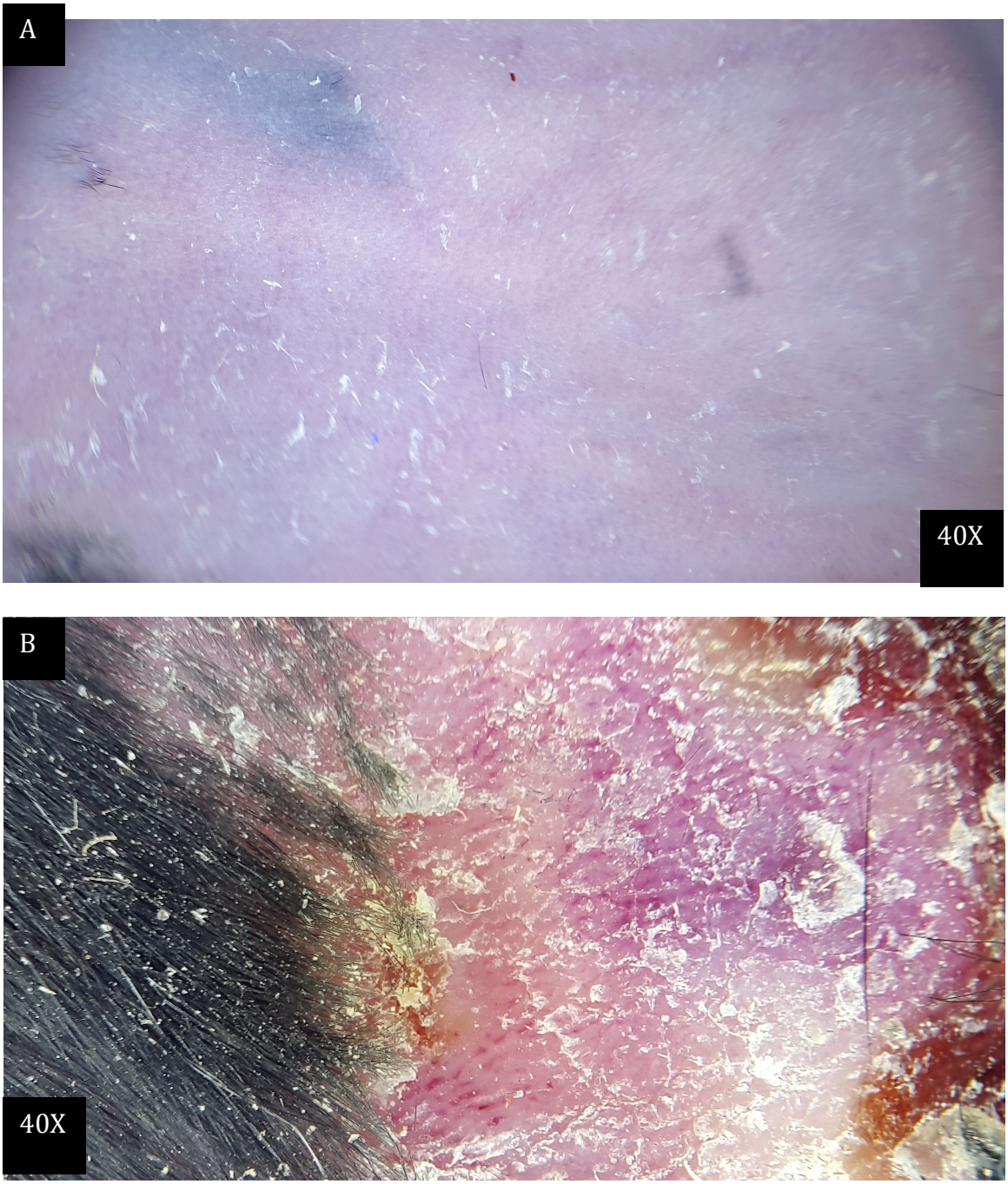


Figure 34: Dermoscopy of MBL treated mice VS PBS treated group. A) MBL treated mouse with no erythema or dotted vessels and with very mild silvery scales B) PBS mouse with marked background erythema, dotted vessels and thick silvery scales.

d. MBL improved psoriasiform pathologic changes of the skin:

Microscopic examination of H&E stained sections of MBL treated skin showed significant reduction in epidermal thickness (*P = 0.001*) compared to epidermal thickness of 6 days Imiquimod induction. PBS group, on the other hand didn't differ significantly from samples taken at 6 days of imiquimod induction (Imq) (*P = 0.064*), **Table 11, Figure 35.** Epidermal thickness was significantly reduced in MBL treated mice compared to PBS control group, **Table 11, Figure 35 & 36**

Table 11: MBL treatment reduced epidermal thickness

Groups	N**	Median	Mean ± S.D.	P value[a]	P value[b]
MBL	6	41.76	42.45 ± 4.82	0.001	0.001
PBS	6	73.12	75.79 ± 6.85	0.064	
Imq*	5	84.12	83.49 ± 1.92		

[a] P value between each group and the Imq group using Multiple Comparison test (Dunnett t Test).

[b] P value between MBL group and PBS group using Parametric test (Independent Samples T-Test).

* Imq: 6 days of Imiquimod induction

** N: Number of mice

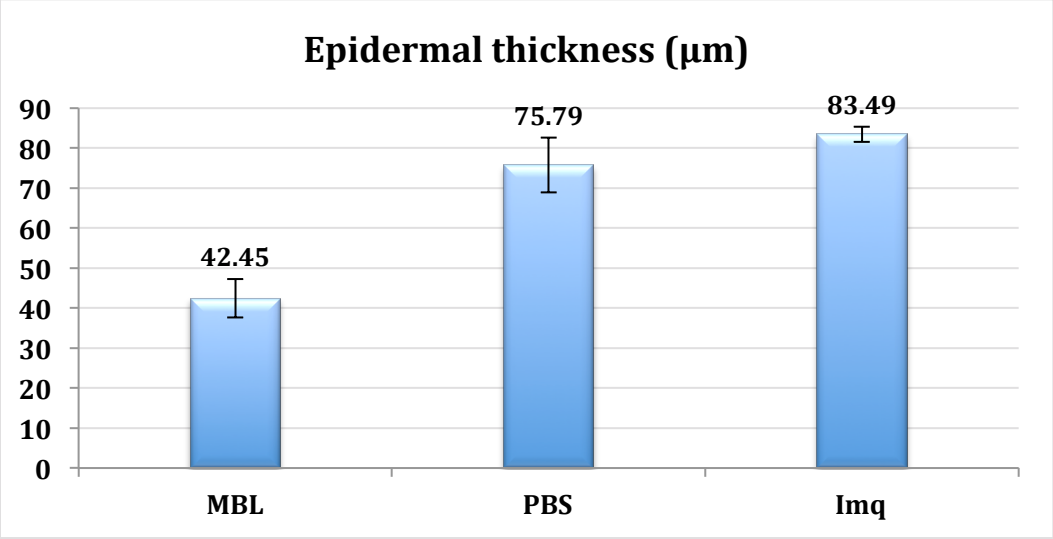


Figure 35: MBL treatment reduces epidermal thickness. MBL treatment significantly reduced epidermal thickness (*P = 0.001*) measured by 10X microscope with Image J analysis. On the other hand PBS didn't show significant reduction in epidermal thickness (*P = 0.064*) compared with 6 days Imiquimod mice.

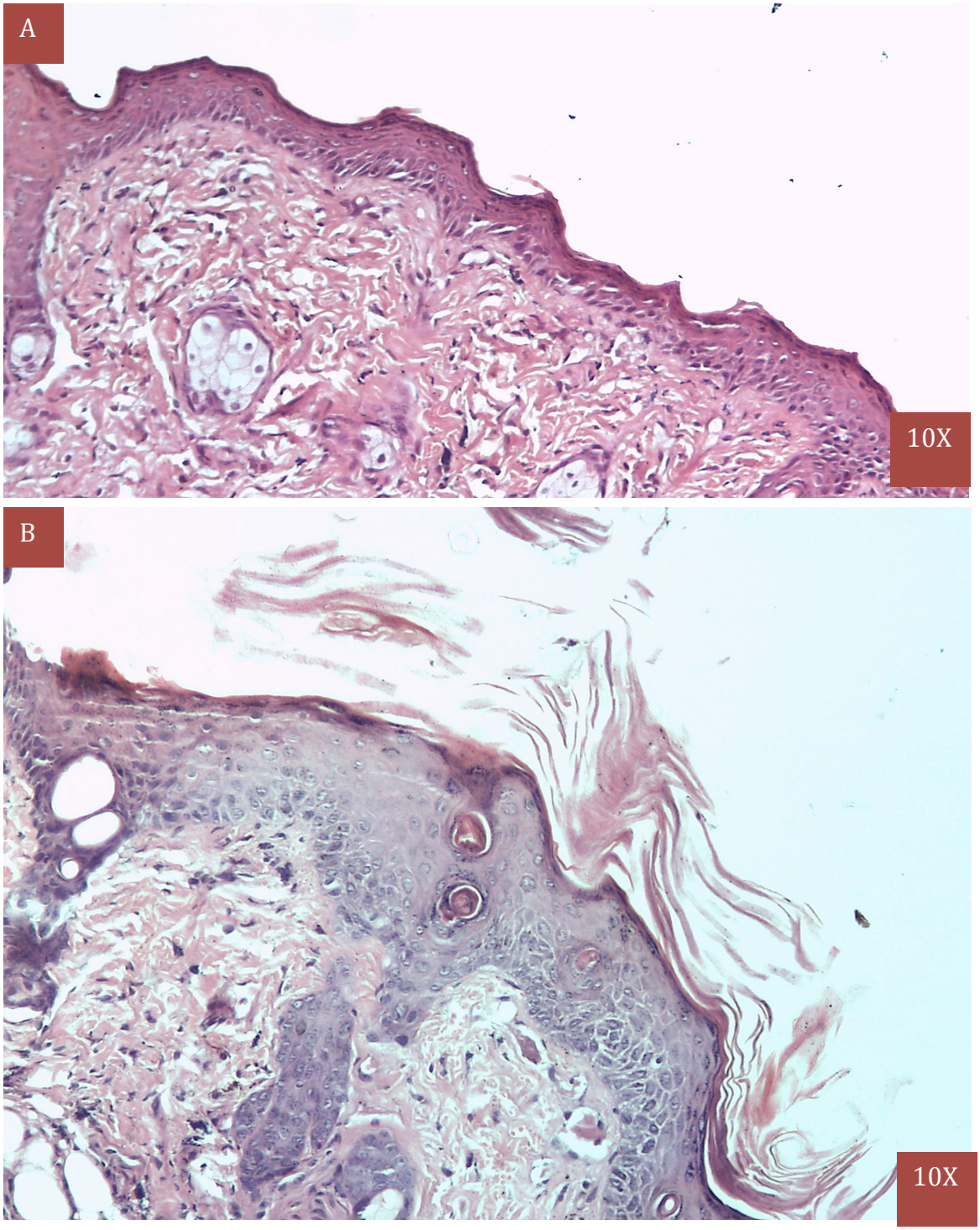


Figure 36: Epidermal thickness in MBL & PBS groups. A) MBL treated skin with significant reduction in epidermal thickness. B) PBS control group with non-significant change in epidermal thickness compared to 6 days Imiquimod mice.

e. **MBL affected distant areas of skin:**

Sites directly injected with MBL showed slightly better clinical, dermoscopic and pathologic scoring than distant areas of skin, however this difference was not statistically significant except in total clinical score, **Table 12, 13, 14 & 15 Figure 37.**

Table 12: MBL injected sites had similar clinical score as distant areas:

Parameters	Groups	N*	Median	Mean ± S.D.	P value[a]
Erythema	Injected area	6	0.00	0.17 ± 0.41	0.211
	Distant Area	6	0.50	0.67 ± 0.82	
Scaling	Injected area	6	1.00	1.00 ± 0.89	0.238
	Distant Area	6	2.00	1.67 ± 1.03	
Thickness	Injected area	6	1.00	1.17 ± 1.47	0.434
	Distant Area	6	1.00	1.83 ± 1.72	
Total	Injected area	6	2.00	2.33 ± 1.51	0.036
	Distant Area	6	4.00	4.17 ± 0.75	

[a] P value between injected area and distant area in Clinical Evaluation MBL using None-Parametric test (Mann-Whitney Test).

*N: number of mice

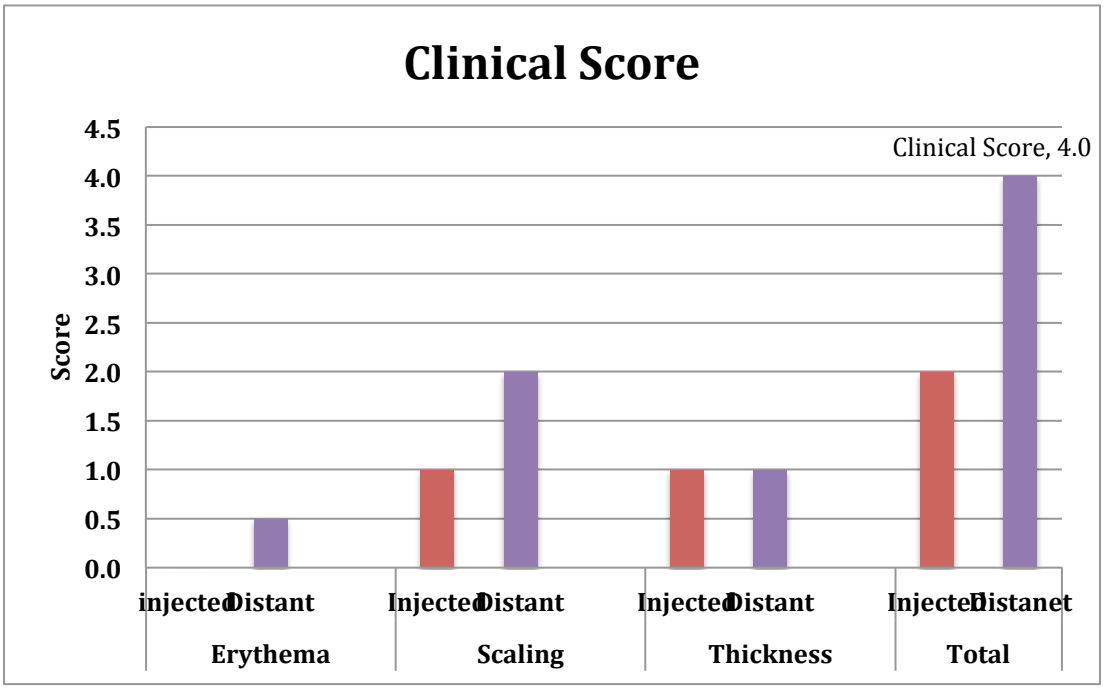


Figure 37: Clinical score for Distant and injected sites in MBL group. Distant area don't show significant difference than the improvement seen in injected sites except in the total score (*P = 0.036*)

Table 13: skin fold thickness similar in MBL injected sites & distant sites:

Parameters	Groups	N*	Median	Mean ± S.D.	P value[a]
Thickness (mm)	Injected area	6	0.73	0.75 ± 0.17	0.516
	Distant Area	6	0.75	0.82 ± 0.19	

[a] P value between injected area and distant area in skin fold thickness using None-Parametric test (Mann-Whitney Test).

*N: Number of mice.

Table 14: Dermoscopic scoring similar in MBL injected sites & distant sites:

Parameters	Groups	N	Median	Mean ± S.D.	P value[a]
Erythema & Blood vessel	Injected area	6	0.00	0.17 ± 0.41	0.138
	Distant Area	6	0.00	0.67 ± 0.82	
Scaling	Injected area	6	1.00	1.00 ± 0.89	0.171
	Distant Area	6	1.50	1.67 ± 1.03	
Total	Injected area	6	1.00	1.17 ± 1.47	0.067
	Distant Area	6	2.00	1.83 ± 1.72	

[a] P value between injected area and distant area in Dermoscopy using None-Parametric test (Mann-Whitney Test).

Table 15: MBL injected sites had similar histopathologic epidermal thickness as distant areas:

Groups	N*	Median	Mean ± S.D.	P value[a]
Injected area	6	41.76	42.45 ± 4.82	0.288
Distant Area	6	41.15	37.58 ± 9.50	

[a] P value between Injected area and distant area in epidermal thickness using Parametric test (Independent Samples T-Test).

*N: Number of mice

f. MBL mice had normal splenic Histology:

Mice continued to receive Imiquimod and PBS showed abnormal splenic pathology in the form of expanded white pulp, cellular hyperplasia & macrophage infiltration indicting an immune reactivation process of the spleen. These changes were not observed in MBL treated mice whose spleen showed normal histology**, Figure 38.**

The splenic weight in MBL treated group and PBS control group was significantly higher than normal spleen however there wasn't significant difference between the two groups, **Table 16, Figure 39.**

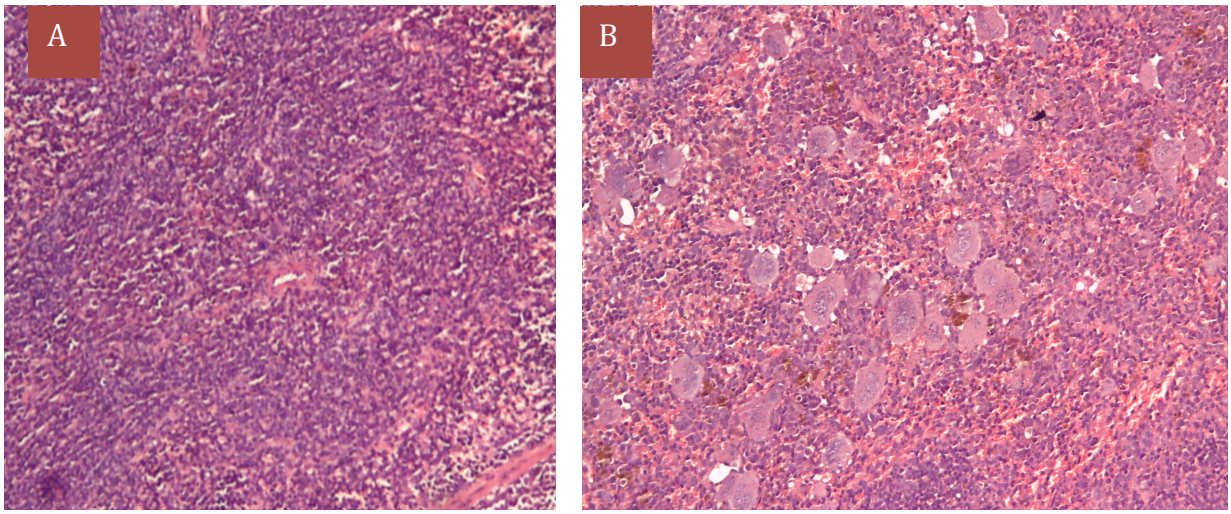


Figure 38: Splenic Histopathology. A) MBL treated mice spleen showing normal white pulp. B) PBS control spleen showing cellular hyperplasia &infiltration with macrophages.

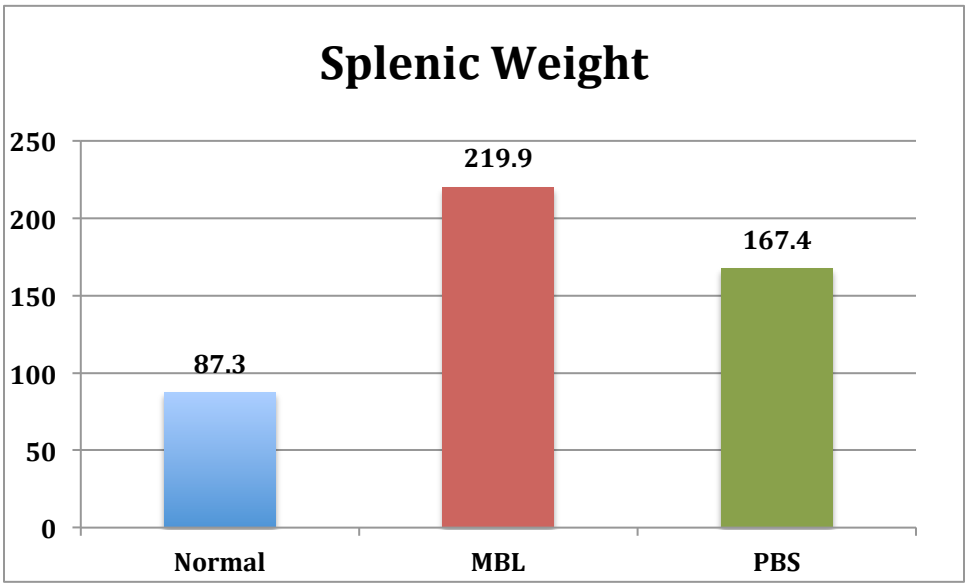


Figure 39: Splenic weight. MBL & PBS groups had significantly higher splenic weight than normal spleen (*P= 0.013, P= 0.001* respectively). Spleen weight is not significantly different between both groups (*P = 0.221*)

Table 16: Splenic weight is significantly higher than normal in MBL & PBS groups

Parameters	Groups	N*	Median	Mean ± S.D.	P value[a]	P value[c]
Splenic weight	Normal	9	87.30	86.30 ± 38.35		
	MBL	3	219.90	205.60 ± 34.08	0.013	0.221
	PBS	8	167.40	183.33 ± 54.31	0.001	

[a] P value between each group and Normal group using None-Parametric test (Mann-Whitney Test).

[c] P value between MBL group and PBS group using None-Parametric test (Mann-Whitney Test).

*N: Number of mice

g. MBL treatment altered cytokine expression in the treated skin:

MBL is immune response modifier secreted at sites of inflammation to fine tune the immune response to avoid excess tissue damage. On high concentration, MBL inhibits both TLR4 & TLR9 signaling subsequently NFκB dependent secretion of many cytokines including IL6 & IL12. IL-6 plays important role in Th17 differentiation, a key player in the pathogenesis of psoriasis. It also induces IL-8 secretion and enhances neutrophil chemotaxis to skin. Psoriatic patients have higher serum and skin IL-6. Its high level in the serum is an indicator of activity and reduction of its level is associated with response to methotrexate and UVB therapy in psoriasis **(Saggini et al., 2014)**

MBL treatment reduced IL-6 to expression level similar to its level in normal skin on the other hand PBS group showed significantly higher expression profile ($P = 0.05$) than both of them, **Table 17, Figure 40.**

Table 17: IL-6 Expression Profile:

Groups	N*	Median	Mean ± S.D.
Normal	4	0.83	0.91 ± 0.38[c]
MBL Group	4	0.91	0.97 ± 0.62[c]
PBS Group	5	3.39	3.20 ± 1.44[ab]

[a] Indicates that there is significant difference with Normal group at 0.05 significant level using None-Parametric test (Mann-Whitney Test).

[b] Indicates that there is significant difference with MBL group at 0.05 significant level using None-Parametric test (Mann-Whitney Test).

[c] Indicates that there is significant difference with PBS group at 0.05 significant level using None-Parametric test (Mann-Whitney Test).

*N: Number of mice.

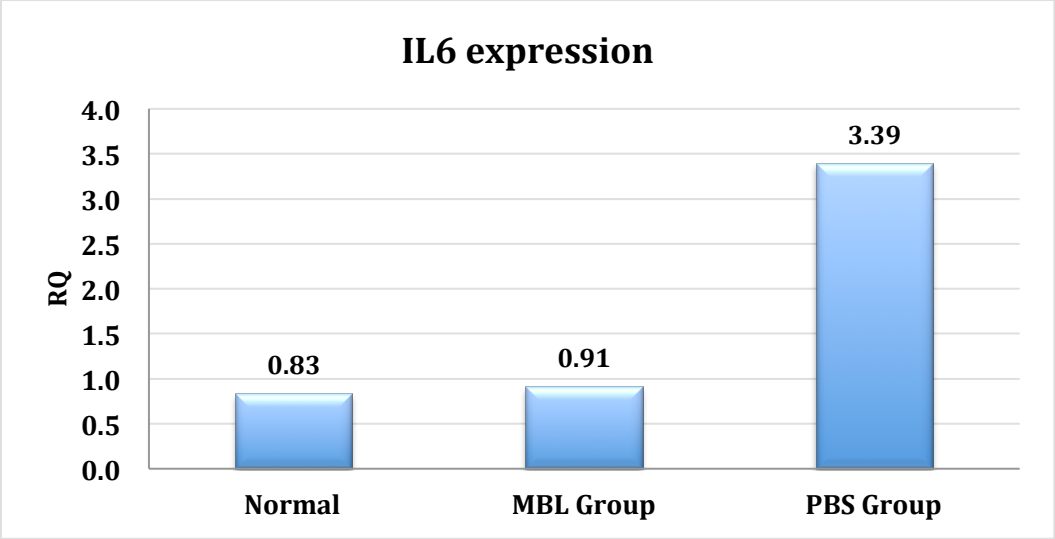


Figure 40: MBL reduced IL6 levels to Normal. MBL treated mice has expression level of IL6 like normal mice while PBS control group have significantly higher IL6 expression level ($P < 0.05$)

IL12 expression was similar in normal and PBS control group ($P=1$). MBL group had lower expression level than normal and PBS group, however it was statistically insignificant, **Table 18, Figure 41.**

Table 18: IL12 Expression in MBL & PBS:

Groups	N*	Median	Mean ± S.D.	P value[a]	P value[b]	P value[c]
Normal	3		1.05 ± 0.41	0.083	1	
MBL	2		0.26 ± 0.05			1
PBS	2		1.34 ± 1.60			

[a] P value between Normal and MBL groups
[b] P value between Normal and PBS groups
[c] P value between PBS and MBL groups
*N: Number of mice

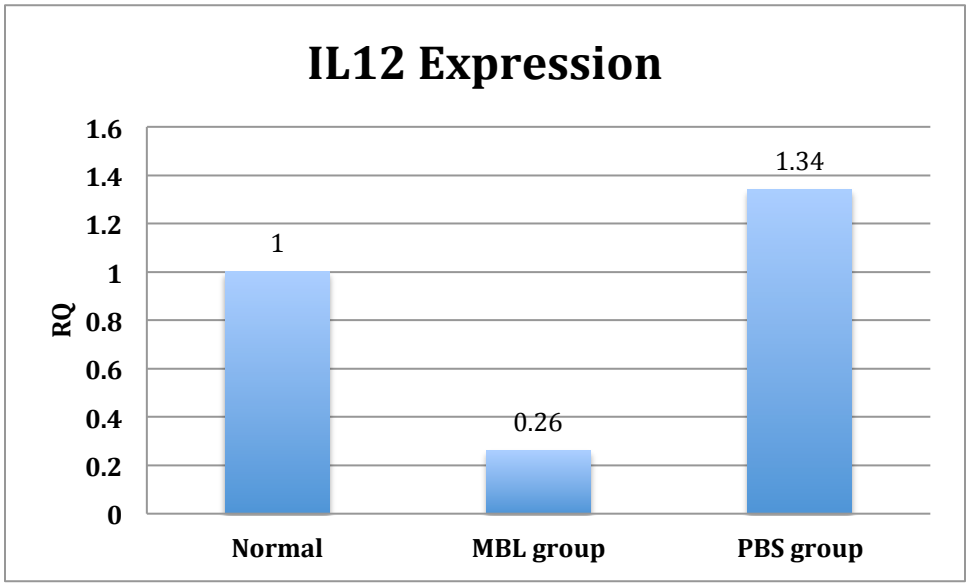


Figure 41: IL12 expression. MBL treated mice had lower expression level of IL12 than normal and PBS group however statistically insignificant.

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.

REFERENCES:

Akira S, Takeda K. (2004). Toll-like receptor signalling. Nat Rev Immunol. 4: 499-511.

Albanesi C, Scarponi C, Bosisio D, et al. (2010). Immune functions and recruitment of plasmacytoid dendritic cells in psoriasis Autoimmunity. 43: 215-9.

Ariza ME, Williams MV, Wong HK. (2013). Targeting IL-17 in psoriasis: from cutaneous immunobiology to clinical application. Clin. Immunol. 146: 131-9.

Baker BS, Ovigne JM, Powles AV, et al. (2003). Normal keratinocytes express Toll-like receptors (TLRs) 1, 2 and 5: modulation of TLR expression in chronic plaque psoriasis. Br J Dermatol. 148: 670-9. Quoted from Garcia-Rodriguez et al., 2013.

Baliwag, J., Barnes, D. H., & Johnston, A. (2015). Cytokines in psoriasis. Cytokine, 73(2), 342-350.

Bang, P., Laursen, I., Thornberg, K., Schierbeck, J., Nielsen, B., Valdimarsson, H., ... & Christiansen, M. (2008). The pharmacokinetic profile of plasma-derived mannan-binding lectin in healthy adult volunteers and patients with Staphylococcus aureus septicaemia. Scandinavian journal of infectious diseases, 40(1), 44-48.

Bayram N, Ozkinay F, Onay H, et al. (2012). Mannose-binding lectin gene codon 54 polymorphism susceptible to brucellosis in Turkish children. Turk J Pediatr. 54: 234-8.

Bergman IM. (2011). Toll-like receptors (TLRs) and mannan-binding lectin (MBL): on constant alert in a hostile environment. Ups J Med Sci. 116: 90-9.

Brotas AM, Cunha JM, Lago EH, et al. (2012). Tumor necrosis factor-alpha and the cytokine network in psoriasis. An Bras Dermatol. 87:673-81

Brouwer N, Frakking FN, van de Wetering MD, et al. (2009). Mannose-binding lectin (MBL) substitution: recovery of opsonic function in vivo lags behind MBL serum levels. J Immunol. 18: 3496-504.

Burden, A. D. and Kirby, B. 2016. Psoriasis and Related Disorders. Rook's Textbook of Dermatology, Ninth Edition. 1–64.

Büchau AS, Gallo RL. (2007). Innate immunity and antimicrobial defense systems in psoriasis. Clin Dermatol. 25: 616-24.

Callahan, J. A., Hammer, G. E., Agelides, A., Duong, B. H., Oshima, S., North, J., ... & Barrera, J. (2013). Cutting edge: ABIN-1 protects against psoriasis by restricting MyD88 signals in dendritic cells. The Journal of Immunology, 191(2), 535-539.

Chamcheu, J. C., Adhami, V. M., Esnault, S., Sechi, M., Siddiqui, I. A., Satyshur, K. A., ... & Wood, G. S. (2017). Dual Inhibition of PI3K/Akt and mTOR by the Dietary Antioxidant, Delphinidin, Ameliorates Psoriatic Features In Vitro and in an Imiquimod-Induced Psoriasis-Like Disease in Mice. Antioxidants & redox signaling, 26(2), 49-69.

Cai Y, Fleming C, Yan J. (2013). Dermal γδ T cells - A new player in the pathogenesis of psoriasis. Int Immunopharmacol. 16: 388-91.

Coimbra S, Figueiredo A, Castro E, et al. (2012). The roles of cells and cytokines in the pathogenesis of psoriasis. Int J Dermatol. 51: 389-95.

Connolly DJ, O'Neill LA. (2012). New developments in Toll-like receptor targeted therapeutics. Curr Opin Pharmacol. 12: 510-8.

De Messias-Reason IJ, Boldt AB, Moraes Braga AC, et al. (2007). The association between mannan-binding lectin gene polymorphism and clinical leprosy: new insight into an old paradigm. J Infect Dis. 196: 1379-85.

Denadai R. (2013). The role of plasmacytoid dendritic cells and interferon-alpha in the immunopathogenesis of psoriasis. Indian J Dermatol. 58: 247- 247

Dommett RM, Klein N, Turner MW. (2006). Mannose-binding lectin in innate immunity: Past, present and future. Tissue Antigens. 68: 193 - 209.

Downing I, Koch C, Kilpatrick DC. (2003). Immature dendritic cells possess a sugar-sensitive receptor for human mannan-binding lectin. Immunology. 109: 360-4.

Downing I, MacDonald SL, Turner ML, et al. (2005). Detection of an autologous ligand for mannan-binding lectin on human B lymphocytes. Scand J Immunol. 62: 507-14.

Duus K, Thielens NM, Lacroix M, et al. (2010). CD91 interacts with mannan-binding lectin (MBL) through the MBL-associated serine protease-binding site. FEBS J. 277: 4956-64.

Fantuzzi G, Reed DA, Dinarello CA. (1999). IL-12-induced IFN-gamma is dependent on caspase-1 processing of the IL-18 precursor. J Clin Invest. 104: 761-7.

Farkas A, Kemény L. (2012). Monocyte-derived interferon-alpha primed dendritic cells in the pathogenesis of psoriasis: new pieces in the puzzle. Int Immunopharmacol. 13: 215-8.

Fitch, E., Harper, E., Skorcheva, I., Kurtz, S. E., & Blauvelt, A. (2007). Pathophysiology of psoriasis: recent advances on IL-23 and Th17 cytokines. Current rheumatology reports, 9(6), 461-467.

Flyvbjerg A. (2010). Diabetic angiopathy, the complement system and the tumor necrosis factor superfamily. Nat Rev Endocrinol. 6: 94-101.

Frakking FN, Brouwer N, van de Wetering MD, et al. (2009). Safety and pharmacokinetics of plasma-derived mannose-binding lectin (MBL) substitution in children with chemotherapy-induced neutropaenia. Eur J Cancer. 45:505-12. Quoted from Heitzeneder et al., 2012.

Fredriksson T, Pettersson U. (1978). Severe psoriasis--oral therapy with a new retinoid. Dermatologica. 157:238-44. Quoted from Warren et al., 2009.

Fujita H. (2013). The role of IL-22 and Th22 cells in human skin diseases. J Dermatol Sci. 72: 3-8.

Garcia-Rodriguez S, Arias-Santiago S, Perandrés-López R, et al. (2013). Increased gene expression of Toll-like receptor 4 on peripheral blood mononuclear cells in patients with psoriasis. J Eur Acad Dermatol Venereol. 27:242-50.

Garred, P., Pressler, T., Lanng, S., Madsen, H. O., Moser, C., Laursen, I., ... & Koch, C. (2002). Mannose-binding lectin (MBL) therapy in an MBL-deficient patient with severe cystic fibrosis lung disease. Pediatric pulmonology, 33(3), 201-207.

Gaspari AA. (2006). Innate and adaptive immunity and the pathophysiology of psoriasis. J Am Acad Dermatol. 54: S67-80.

Girolomoni G, Mrowietz U, Paul C. (2012). Psoriasis: rationale for targeting interleukin-17. Br J Dermatol. 167: 717-24.

Griffiths C.E.M. & Barker J.N.W.N. (2010). Psoriasis. In: Rook's Textbook of Dermatology. (Burns T, Breathnach S, Cox N, Griffiths C, eds), 8th ed. Oxford, UK: Wiley-Blackwell, 871-930.

Gu, B., Miao, J., Fa, Y., Lu, J., & Zou, S. (2010). Retinoic acid attenuates lipopolysaccharide-induced inflammatory responses by suppressing TLR4/NF-κB expression in rat mammary tissue. International immunopharmacology, 10(7), 799-805.

Gudjonsson, J. E., Johnston, A., Dyson, M., Valdimarsson, H., & Elder, J. T. (2007). Mouse models of psoriasis. The Journal of investigative dermatology, 127(6), 1292.

Hari A, Flach TL, Shi Y, et al. (2010). Toll-like receptors: role in dermatological disease. Mediators Inflamm. 2010: 1-16.

Hanna, E., Abadi, R., & Abbas, O. (2016). Imiquimod in dermatology: an overview. International journal of dermatology, 55(8), 831-844.

Hébert HL, Ali FR, Bowes J, et al. (2012). Genetic susceptibility to psoriasis and psoriatic arthritis: implications for therapy. Br J Dermatol. 166: 474-82.

Heitzeneder S, Seidel M, Förster-Waldl E, et al. (2012). Mannan-binding lectin deficiency-Good news, bad news, doesn't matter? Clin Immunol.143: 22-38.

Hollox EJ, Huffmeier U, Zeeuwen PL, et al. (2008). Psoriasis is associated with increased beta-defensin genomic copy number. Nat Genet. 40: 23-5.

Ip WK, Takahashi K, Moore KJ, et al. (2008). Mannose-binding lectin enhances Toll-like receptors 2 and 6 signaling from the phagosome. J Exp Med. 205: 169-81.

Ivanov II, McKenzie BS, Zhou L, et al. (2006). The orphan nuclear receptor ROR gammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell. 126: 1121 - 33.

Jack DL, Klein NJ, Turner MW. (2001). Mannose-binding lectin: Targeting the microbial world for complement attack and opsonophagocytosis. Immunol Rev. 180: 86-99.

Jiang W, Zhu FG, Bhagat L, et al. (2013). Toll-Like Receptor 7, 8, and 9 Antagonist Inhibits Th1 and Th17 Responses and Inflammasome Activation in a Model of IL-23-Induced Psoriasis. J Invest Dermatol. 133: 1777-84.

Jiaravuthisan MM, Sasseville D, Vender RB, et al. (2007). Psoriasis of the nail: anatomy, pathology, clinical presentation, and a review of the literature on therapy**.** J Am Acad Dermatol. 57: 1-27.

Kagami S, Rizzo HL, Lee JJ, et al. (2010). Circulating Th17, Th22, and Th1 cells are increased in psoriasis. J Invest Dermatol. 130: 1373-83.

Kang, D., Li, B., Luo, L., Jiang, W., Lu, Q., Rong, M., & Lai, R. (2016). Curcumin shows excellent therapeutic effect on psoriasis in mouse model. Biochimie, 123, 73-80.

Kim, S. Y., Koo, J. E., Song, M. R., & Lee, J. Y. (2013). Retinol suppresses the activation of Toll-like receptors in MyD88-and STAT1-independent manners. Inflammation, 36(2), 426-433.

Kim, J., Nadella, P., Kim, D. J., Brodmerkel, C., da Rosa, J. C., Krueger, J. G., & Suárez-Fariñas, M. (2015). Histological stratification of thick and thin plaque psoriasis explores molecular phenotypes with clinical implications. PloS one, 10(7), e0132454.

Kupetsky EA, Mathers AR, Ferris LK. (2013). Anti-cytokine therapy in the treatment of psoriasis. Cytokine. 61: 704-12.

Laggner U, Di Meglio P, Perera GK, et al. (2011). Identification of a novel proinflammatory human skin-homing Vγ9Vδ2 T cell subset with a potential role in psoriasis. J Immunol. 187: 2783-93.

Lallas, A., Kyrgidis, A., Tzellos, T. G., Apalla, Z., Karakyriou, E., Karatolias, A., ... & Zalaudek, I. (2012). Accuracy of dermoscopic criteria for the diagnosis of psoriasis, dermatitis, lichen planus and pityriasis rosea. British Journal of Dermatology, 166(6), 1198-1205.

Lai, C. Y., Yeh, D. W., Lu, C. H., Liu, Y. L., Huang, L. R., Kao, C. Y., ... & Xiang, R. (2015). Identification of Thiostrepton as a Novel Inhibitor for Psoriasis-like Inflammation Induced by TLR7–9. The Journal of Immunology, 195(8), 3912-3921.

Lande R, Gregorio J, Facchinetti V, et al. (2007). Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. Nature. 449: 564-9.

Lee C, Hwang S. (2012). Pathophysiology of chemokines and chemokine receptors in dermatological science: A focus on psoriasis and cutaneous T-cell lymphoma. Dermatologica Sinica. 30: 128-135.

Lever, W. F. & Elder, D. E. (2009). Lever's histopathology of the skin (10th ed.). Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins.

Li, D., Dong, B., Tong, Z., Wang, Q., Liu, W., Wang, Y., ... & Duan, Y. (2012). MBL-Mediated Opsonophagocytosis of Candida albicans by Human Neutrophils Is Coupled with Intracellular Dectin-1-Triggered ROS Production. PLoS ONE, 7(12).

Liu, H., Zhou, J., Ma, D., Lu, X., Ming, S., Shan, G., ... & Zuo, D. (2014). Mannan binding lectin attenuates double-stranded RNA-mediated TLR3 activation and innate immunity. FEBS letters, 588(6), 866.

Lokitz M, Zhang W, Bashir M. (2005). Ultraviolet-B Recruits Mannose-Binding Lectin into Skin from Non-Cutaneous Sources. J Invest Dermatol. 125:166-73.

MacDonald SL, Downing I, Atkinson AP, et al. (2010). Dendritic cells previously exposed to mannan-binding lectin enhance cytokine production in allogeneic mononuclear cell cultures. Hum Immunol. 71: 1077-83.

Mahil, S. K., Capon, F., & Barker, J. N. (2016). Update on psoriasis immunopathogenesis and targeted immunotherapy. In Seminars in immunopathology. 38: 11-27.

Mathur AN, Chang HC, Zisoulis DG, et al. (2007). Stat3 and Stat4 direct development of IL-17-secreting Th cells. J Immunol. 178: 4901 - 7.

Micali, G., Lacarrubba, F., Massimino, D., & Schwartz, R. A. (2011). Dermatoscopy: alternative uses in daily clinical practice. Journal of the American Academy of Dermatology, 64(6), 1135-1146.

Michelow, I. C., Lear, C., Scully, C., Prugar, L. I., Longley, C. B., Yantosca, L. M., ... & Spear, G. T. (2011). High-dose mannose-binding lectin therapy for Ebola virus infection. Journal of Infectious Diseases, 203(2), 175-179.

Miller LS, Sørensen OE, Liu PT, et al. (2005). TGF-alpha regulates TLR expression and function on epidermal keratinocytes. J Immunol. 174: 6137-43.

Mitra A, Fallen RS, Lima HC. (2013). Cytokine-based therapy in psoriasis. Clin Rev Allergy Immunol. 44: 173-82.

Mizutani H, Ohmoto Y, Mizutani T, et al. (1997). Role of increased production of monocytes TNF-alpha, IL-1beta and IL-6 in psoriasis: relation to focal infection, disease activity and responses to treatments. J Dermatol Sci. 14: 145-53. Quoted from Turan et al., 2014.

Morizane, S., Yamasaki, K., Mühleisen, B., Kotol, P. F., Murakami, M., Aoyama, Y., ... & Gallo, R. L. (2012). Cathelicidin antimicrobial peptide LL-37 in psoriasis enables keratinocyte reactivity against TLR9 ligands. Journal of Investigative Dermatology, 132(1), 135-143.

Møller-Kristensen M, Hamblin MR, Thiel S, et al. (2007). Burn injury reveals altered phenotype in mannan-binding lectin-deficient mice. J Invest Dermatol. 127: 1524-31.

Møller-Kristensen, M., Ip, W. E., Shi, L., Gowda, L. D., Hamblin, M. R., Thiel, S., ... & Takahashi, K. (2006). Deficiency of mannose-binding lectin greatly increases susceptibility to postburn infection with Pseudomonas aeruginosa. The Journal of Immunology, 176(3), 1769-1775.

Nadesalingam J, Dodds AW, Reid KB, et al. (2005). Mannose-binding lectin recognizes peptidoglycan via the N-acetyl glucosamine moiety, and inhibits ligand-induced proinflammatory effect and promotes chemokine production by macrophages. J Immunol. 175: 1785 - 94.

Nestle FO, Conrad C, Tun-Kyi A, et al. (2005). Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production. J Exp Med. 202: 135 - 43.

Nestle FO, Kaplan DH, Barker J. (2009). Psoriasis. N Engl J Med. 361: 496-509.

Nestle FO, Turka L a, Nickoloff BJ. (1994). Characterization of dermal dendritic cells in psoriasis. Auto stimulation of T lymphocytes and induction of Th1 type cytokines. J Clin Invest. 94: 202-9.

Nofal, H (2014). Mannose binding Lectin gene polymorphism in psoriatic patients (Master's thesis). Retrieved from Faculty of Medicine library, Zagazig university.

Nograles KE, Zaba LC, Shemer A, et al. (2009). IL-22-producing "T22" T cells account for upregulated IL-22 in atopic dermatitis despite reduced IL-17-producing TH17 T cells. J Allergy Clin Immunol. 123: 1244-52.

Nomura I, Goleva E, Howell MD, et al. (2003). Cytokine milieu of atopic dermatitis, as compared to psoriasis, skin prevents induction of innate immune response genes. J Immunol. 171: 3262-9.

Ono K, Nishitani C, Mitsuzawa H, et al. (2006). Mannose-binding lectin augments the uptake of lipid A, *Staphylococcus aureus*, and *Escherichia coli* by Kupffer cells

through increased cell surface expression of scavenger receptor A. J Immunol. 177: 5517-23.

Ozawa A, Ohkido M, Haruki Y, et al. (1999). Treatments of generalized pustular psoriasis: a multicenter study in Japan. J Dermatol. 26: 141-9. Quoted from Sigurdardottir et al., 2013.

Pandey S, Agrawal DK. (2006). Immunobiology of Toll-like receptors: emerging trends. Immunol Cell Biol. 84: 333-41.

Parker LC, Prince LR, Sabroe I. (2007). Translational mini-review series on toll-like receptors: Networks regulated by toll-like receptors mediate innate and adaptive immunity. Clin Exp Immunol. 147: 199-207.

Pasare C, Medzhitov R. (2004). Toll-like receptors and acquired immunity. Semin Immunol. 16: 23-26.

Peric M, Koglin S, Dombrowski Y, et al. (2009). Vitamin D analogs differentially control antimicrobial peptide/"alarmin" expression in psoriasis. PLoS One. 4: 1-10.

Petersen, K. A., Matthiesen, F., Agger, T., Kongerslev, L., Thiel, S., Cornelissen, K., & Axelsen, M. (2006). Phase I safety, tolerability, and pharmacokinetic study of recombinant human mannan-binding lectin. Journal of clinical immunology, 26(5), 465-475.

Pollock R, Chandran V, Barrett J, et al. (2011). Differential major histocompatibility complex class I chain-related A allele associations with skin and joint manifestations of psoriatic disease. Tissue Antigens. 77: 554-61.

Prignano F, Ricceri F, Beccatti M, et al. (2012). Circulating dendritic cell subsets in psoriatic patients before and after biologic therapy. J Dermatol. 39: 274-5.

Ram, S., Lewis, L. A., & Rice, P. A. (2010). Infections of people with complement deficiencies and patients who have undergone splenectomy. Clinical microbiology reviews, 23(4), 740-780.

Ramnath, D., Tunny, K., Hohenhaus, D. M., Pitts, C. M., Bergot, A. S., Hogarth, P. M., ... & Sweet, M. J. (2015). TLR3 drives IRF6-dependent IL-23p19 expression and p19/EBI3 heterodimer formation in keratinocytes. Immunology and cell biology, 93(9), 771-779.

Reinholz M, Ruzicka T, Schauber J. (2012). Cathelicidin LL-37: an antimicrobial peptide with a role in inflammatory skin disease. Ann Dermatol. 24: 126-35.

Rizzo HL, Kagami S, Phillips KG, et al. (2011). IL-23-mediated psoriasis-like epidermal hyperplasia is dependent on IL-17A. J Immunol. 186: 1495-502.

Sabat, R., Philipp, S., Höflich, C., Kreutzer, S., Wallace, E., Asadullah, K., ... & Wolk, K. (2007). Immunopathogenesis of psoriasis. Experimental dermatology, 16(10), 779-798.

Saggini, A., Chimenti, S., & Chiricozzi, A. (2014). IL-6 as a druggable target in psoriasis: focus on pustular variants. Journal of immunology research, 2014.

Seung NR, Park EJ, Kim CW, et al. (2007). Comparison of expression of heat-shock protein 60, Toll-like receptors 2 and 4, and T-cell receptor gamma delta in plaque and guttate psoriasis. J Cutan Pathol. 34: 903-11. Quoted from Garcia-Rodriguez et al., 2013.

Shi, L., Takahashi, K., Dundee, J., Shahroor-Karni, S., Thiel, S., Jensenius, J. C., ... & Ezekowitz, R. A. B. (2004). Mannose-binding lectin-deficient mice are susceptible to infection with Staphylococcus aureus. The Journal of experimental medicine, 199(10), 1379-1390.

Shimizu T, Nishitani C, Mitsuzawa H, et al. (2009). Mannose binding lectin and lung collectins interact with Toll-like receptor 4 and MD-2 by different mechanisms. Biochim Biophys Acta. 1790:1705-10.

Shinya K, Ito M, Makino A, et al. (2012). The TLR4-TRIF pathway protects against H5N1 influenza virus infection. J Virol. 86: 19-24.

Sigurdardottir SL, Thorleifsdottir RH, Valdimarsson H, et al. (2013). The role of the palatine tonsils in the pathogenesis and treatment of psoriasis. Br J Dermatol. 168: 237-42.

Steinman RM, Idoyaga J. (2010). Features of the dendritic cell lineage. Immunol Rev. 234: 5-17.

Sticherling M. (2005). Mechanisms of psoriasis. Drug Discovery Today: Disease Mechanisms. 2: 275-281.

Strange A, Capon F, Spencer CC, et al. (2010). A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1. Nat Genet.42: 985-90.

Stuart PE, Hüffmeier U, Nair RP, et al. (2012). Association of β-defensin copy number and psoriasis in three cohorts of European origin. J Invest Dermatol. 132: 2407-13.

Swindell, W. R., Johnston, A., Carbajal, S., Han, G., Wohn, C., Lu, J., ... & Wang, X. J. (2011). Genome-wide expression profiling of five mouse models identifies similarities and differences with human psoriasis. PloS one, 6(4), e18266.

Tang, Y., Ma, D., Ming, S., Zhang, L., Zhou, J., Shan, G., ... & Zuo, D. (2015). Mannan-binding lectin reduces CpG DNA-induced inflammatory cytokine production by human monocytes. Microbiology and immunology, 59(4), 231.

Takahashi K. (2011). Mannose-binding lectin and the balance between immune protection and complication. Expert Rev Anti Infect Ther. 9: 1179-90.

Tenaud I, Khammari A, Dreno B. (2007). In vitro modulation of TLR-2, CD1d and IL-10 by adapalene on normal human skin and acne inflammatory lesions. Exp Dermatol. 16: 500-6. Quoted from Hari et al., 2010

Tohyama M, Yang L, Hanakawa Y, et al. (2012). IFN-α enhances IL-22 receptor expression in keratinocytes: a possible role in the development of psoriasis. J Invest Dermatol. 132: 1933-5.

Tsutsumi A, Takahashi R, Sumida T. (2005). Mannose binding lectin: genetics and autoimmune disease. Autoimmun Rev. 4: 364-72.

Turan H, Karkucak M, Yakut T, et al. (2014). Does MBL2 codon 54 polymorphism play a role in the pathogenesis of psoriasis? Int J Dermatol. 53: 34-8.

Ueno H, Schmitt N, Klechevsky E, et al. (2010). Harnessing human dendritic cell subsets for medicine. Immunol Rev. 234. 199-212.

Valdimarsson, H., Vikingsdottir, T., Bang, P., Saevarsdottir, S., Gudjonsson, J. E., Oskarsson, O., ... & Koch, C. (2004). Human Plasma-Derived Mannose-Binding Lectin: A Phase I Safety and Pharmacokinetic Study. Scandinavian journal of immunology, 59(1), 97-102.

Van der Fits, L., Mourits, S., Voerman, J. S., Kant, M., Boon, L., Laman, J. D., ... & Lubberts, E. (2009). Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. The Journal of Immunology, 182(9), 5836-5845.

Wang M, Chen Y, Zhang Y, et al. (2011) a. Mannan-binding lectin directly interacts with Toll-like receptor 4 and suppresses lipopolysaccharide-induced inflammatory cytokine secretion from THP-1 cells. Cell Mol Immunol. 8: 265 - 75.

Wang, M., Zhang, Y., Chen, Y., Zhang, L., Lu, X., & Chen, Z. (2011) b. Mannan-binding lectin regulates dendritic cell maturation and cytokine production induced by lipopolysaccharide. BMC immunology, 12(1), 1.

World Health Organization. (2016). Global report on psoriasis. Switzerland: WHO Press

Worthley DL, Bardy PG, Mullighan CG. (2005). Mannose-binding lectin: biology and clinical implications. Intern Med J. 35: 548-55.

Yano S, Banno T, Walsh R, et al. (2008). Transcriptional responses of human epidermal keratinocytes to cytokine interleukin-1.J Cell Physiol. 214: 1-13.

Yao Y, Richman L, Morehouse C, et al. (2008). Type I interferon: potential therapeutic target for psoriasis? PLoS One. 3: 1-14.

Zaba LC, Fuentes-Duculan J, Eungdamrong NJ, et al. (2009). Psoriasis is characterized by accumulation of immunostimulatory and Th1/Th17 cell-polarizing myeloid dendritic cells. J Invest Dermatol. 129: 79-88.

Zanvit, P., Konkel, J. E., Jiao, X., Kasagi, S., Zhang, D., Wu, R., ... & Abbatiello, B. (2015). Antibiotics in neonatal life increase murine susceptibility to experimental psoriasis. Nature communications, 6.

Zheng Y, Caro I, Ouyang W. (2007). Role of cytokine therapy in the treatment of psoriasis. Drug Discov. Today Ther. Strateg. 4: 25–31.

Zhou, Y., Lu, K., Pfefferle, S., Bertram, S., Glowacka, I., Drosten, C., ... & Simmons, G. (2010). A single asparagine-linked glycosylation site of the severe acute respiratory syndrome coronavirus spike glycoprotein facilitates inhibition by mannose-binding lectin through multiple mechanisms. Journal of virology, 84(17), 8753-8764.

Appendix

Appendix 1

Animal Psoriasis Sheet

Animal Serial number: ☐ C57Bl ☐ Balb c

Specific Mark:

Group: ☐ Control ☐ Treatment

Induction schedule:

Method: Amount:

	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Hair removal		Imq induction & Vaseline control								Imq + treatment & Imq + control					
Date																
Notes																

Samples collected:

Normal D ( ) Full Induction D ( ) TTT

☐ RNA extraction ☐ RNA extraction ☐ RNA extraction
☐ Formalin (w= g) ☐ Formalin (w= g) ☐ Formalin (w= g)
☐ Protein ☐ Protein ☐ Protein

Intervention:

Type: ☐ Treatment (MBL - Curcumin) ☐ Control (PBS- Vaseline)

Method/ Amount:
☐ Intra lesional ☐ Topical ☐ Oral/ systemic
Schedule:

	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Hair removal		Imq induction & Vaseline control								Imq + treatment & Imq + control					
Date																
Amount																

Tests done for the animal:
☐ RNA extraction ☐Histology ☐Immunohistochemistry ☐Eliza

Appendix 2:

Psoriasis Clinical & Dermoscopic Evaluation sheet:

Animal Serial number: ☐ Type: ☐ C57Bl ☐ Balb c

Specific Mark: Weight:

Group: ☐ Control ☐ Treatment

Date:

Day in the Course: ☐ Normal ☐ Mid induction ☐Full induction ( ) d
☐ Mid treatment ☐ Last treatment day

Clinical evaluation (Psoriasis Severity Index):

Photos: ☐ Y ☐ N

Redness (0-4)	Thickness (0-4)	Scaling (0-4)	Total (0-12)

Dermoscope:

	Pattern/color	Distribution	Score
Vessel			
Back ground erythema			
Scale			

RNA Extraction: Date: ( )

OD_{260}: Ratio: Concentration:

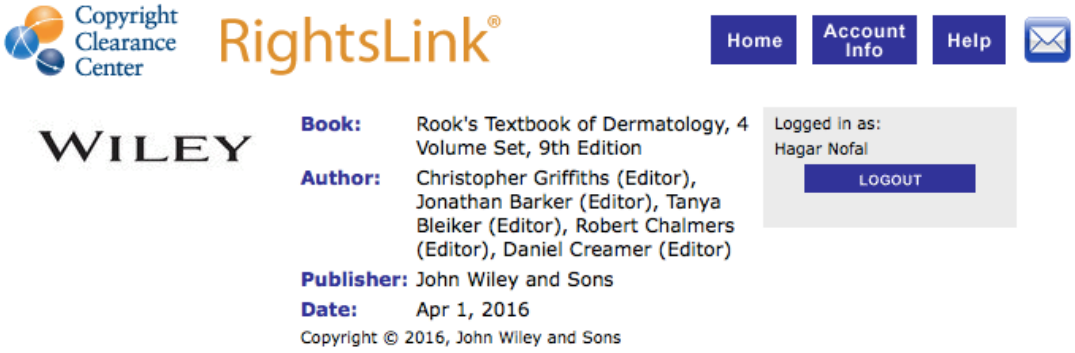
cDNA: Date: ( )

Real Time PCR: Date: ( )

Gene	Expression Level

Formalin: Date ( ) **IHC:** Date ( ) **Eliza:**

Appendix 3: Copyright permission for Figure1:



Order Completed

Thank you for your order.

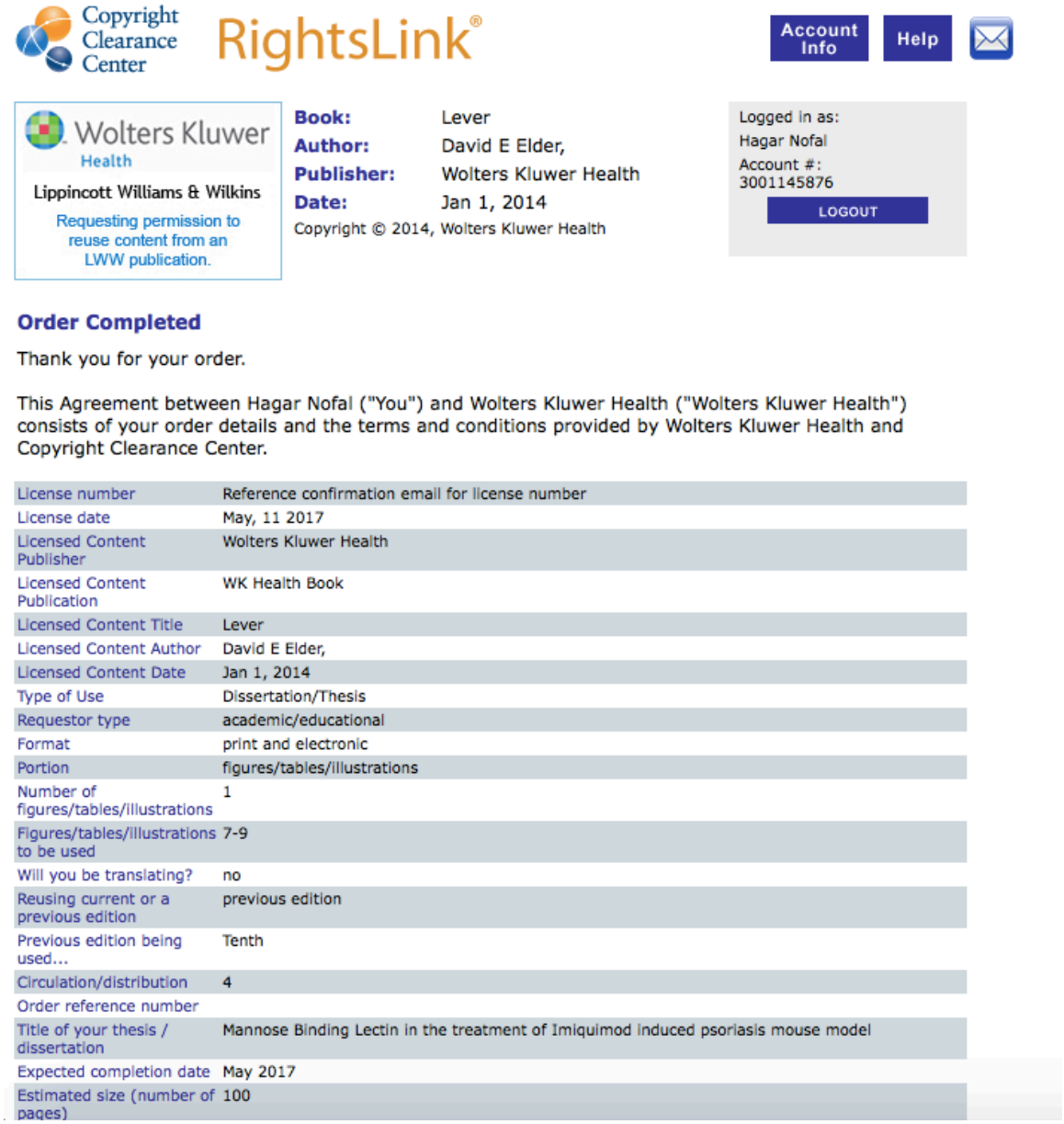
This Agreement between Hagar Nofal ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

Your confirmation email will contain your order number for future reference.

Printable details.

License Number	4099091157665
License date	Apr 30, 2017
Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	Wiley Books
Licensed Content Title	Rook's Textbook of Dermatology, 4 Volume Set, 9th Edition
Licensed Content Author	Christopher Griffiths (Editor), Jonathan Barker (Editor), Tanya Bleiker (Editor), Robert Chalmers (Editor), Daniel Creamer (Editor)
Licensed Content Date	Apr 1, 2016
Licensed Content Pages	4696
Type of use	Dissertation/Thesis
Requestor type	University/Academic
Format	Print and electronic
Portion	Figure/table
Number of figures/tables	1
Original Wiley figure/table number(s)	35.5
Will you be translating?	No
Title of your thesis /	Mannose Binding Lectin in the treatment of Imiquimod induced psoriasis mouse model

Appendix 4: Copyright Permission for Figure 2:



Copyright Clearance Center RightsLink®

Account Info | Help

Wolters Kluwer Health
Lippincott Williams & Wilkins
Requesting permission to reuse content from an LWW publication.

Book: Lever
Author: David E Elder,
Publisher: Wolters Kluwer Health
Date: Jan 1, 2014
Copyright © 2014, Wolters Kluwer Health

Logged in as:
Hagar Nofal
Account #: 3001145876
LOGOUT

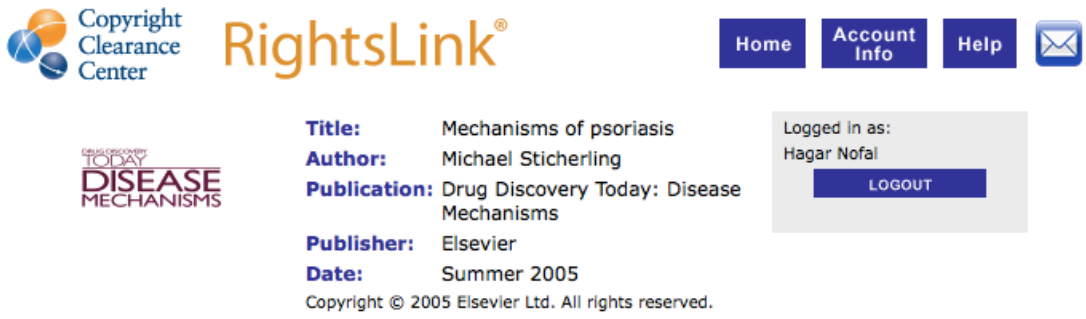
Order Completed

Thank you for your order.

This Agreement between Hagar Nofal ("You") and Wolters Kluwer Health ("Wolters Kluwer Health") consists of your order details and the terms and conditions provided by Wolters Kluwer Health and Copyright Clearance Center.

License number	Reference confirmation email for license number
License date	May, 11 2017
Licensed Content Publisher	Wolters Kluwer Health
Licensed Content Publication	WK Health Book
Licensed Content Title	Lever
Licensed Content Author	David E Elder,
Licensed Content Date	Jan 1, 2014
Type of Use	Dissertation/Thesis
Requestor type	academic/educational
Format	print and electronic
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Figures/tables/illustrations to be used	7-9
Will you be translating?	no
Reusing current or a previous edition	previous edition
Previous edition being used...	Tenth
Circulation/distribution	4
Order reference number	
Title of your thesis / dissertation	Mannose Binding Lectin in the treatment of Imiquimod induced psoriasis mouse model
Expected completion date	May 2017
Estimated size (number of pages)	100

Appendix 5: Copyright permission for Figure 3:



Order Completed

Thank you for your order.

This Agreement between Hagar Nofal ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

Your confirmation email will contain your order number for future reference.

Printable details.

License Number	4099081307816
License date	Apr 30, 2017
Licensed Content Publisher	Elsevier
Licensed Content Publication	Drug Discovery Today: Disease Mechanisms
Licensed Content Title	Mechanisms of psoriasis
Licensed Content Author	Michael Sticherling
Licensed Content Date	Summer 2005
Licensed Content Volume	2
Licensed Content Issue	2
Licensed Content Pages	7
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Format	both print and electronic
Are you the author of this Elsevier article?	No
Will you be translating?	No
Order reference number	

Appendix 6: Copyright permission for Figure 4:

Copyright Clearance Center RightsLink®

Home | Account Info | Help

Title: Monocyte-derived interferon-alpha primed dendritic cells in the pathogenesis of psoriasis: New pieces in the puzzle
Author: Árpád Farkas,Lajos Kemény
Publication: International Immunopharmacology
Publisher: Elsevier
Date: June 2012

Logged in as:
Hagar Nofal
LOGOUT

Order Completed

Thank you for your order.

This Agreement between Hagar Nofal ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

Your confirmation email will contain your order number for future reference.

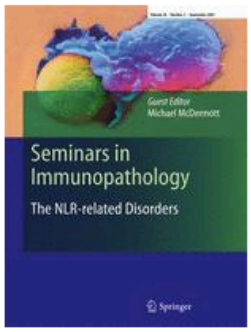
Printable details.

License Number	4099100904919
License date	Apr 30, 2017
Licensed Content Publisher	Elsevier
Licensed Content Publication	International Immunopharmacology
Licensed Content Title	Monocyte-derived interferon-alpha primed dendritic cells in the pathogenesis of psoriasis: New pieces in the puzzle
Licensed Content Author	Árpád Farkas,Lajos Kemény
Licensed Content Date	June 2012
Licensed Content Volume	13
Licensed Content Issue	2
Licensed Content Pages	4
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Format	both print and electronic
Are you the author of this Elsevier article?	No
Will you be translating?	No

Appendix 7: Copyright permission For Figure 5:

Copyright Clearance Center RightsLink®

Home | Create Account | Help



Title: Update on psoriasis immunopathogenesis and targeted immunotherapy
Author: Satveer K. Mahil
Publication: Springer Seminars in Immunopathology
Publisher: Springer
Date: Jan 1, 2015
Copyright © 2015, The Author(s)

LOGIN

If you're a copyright.com user, you can login to RightsLink using your copyright.com credentials. Already **a RightsLink user** or want to learn more?

Permissions Request

This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Springer and BioMed Central offer a reprint service for those who require professionally produced copies of articles published under Creative Commons Attribution (CC BY) licenses. To obtain a quotation, please email reprints@springeropen.com with the article details, quantity(ies) and delivery destination. Minimum order 25 copies.

Appendix 8: Copyright permission for Figure 6:

Copyright Clearance Center RightsLink®

Home | Account Info | Help

Title: Psoriasis of the nail: Anatomy, pathology, clinical presentation, and a review of the literature on therapy

Author: Michael M. Jiaravuthisan,Denis Sasseville,Ronald B. Vender,Francis Murphy,Channy Y. Muhn

Publication: Journal of the American Academy of Dermatology

Publisher: Elsevier

Date: July 2007

Copyright © 2007 American Academy of Dermatology, Inc. Published by Mosby, Inc. All rights reserved.

Logged in as:
Hagar Nofal
Account #:
3001145876
LOGOUT

Order Completed

Thank you for your order.

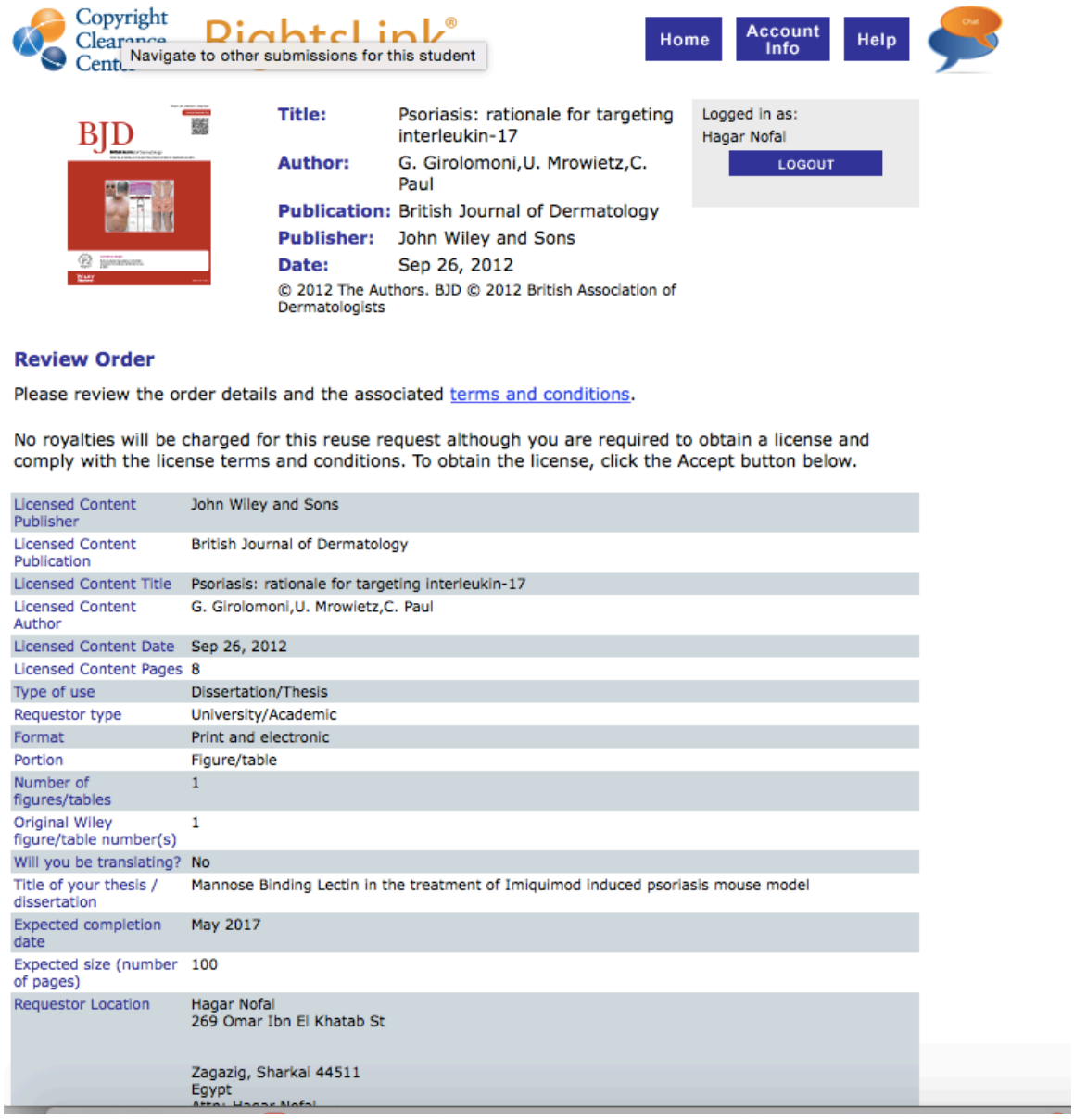
This Agreement between Hagar Nofal ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

Your confirmation email will contain your order number for future reference.

Printable details.

License Number	4100640495337
License date	May 02, 2017
Licensed Content Publisher	Elsevier
Licensed Content Publication	Journal of the American Academy of Dermatology
Licensed Content Title	Psoriasis of the nail: Anatomy, pathology, clinical presentation, and a review of the literature on therapy
Licensed Content Author	Michael M. Jiaravuthisan,Denis Sasseville,Ronald B. Vender,Francis Murphy,Channy Y. Muhn
Licensed Content Date	July 2007
Licensed Content Volume	57
Licensed Content Issue	1
Licensed Content Pages	27
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1

Appendix 9: Copyright permission for Figure 7:



Copyright Clearance Center RightsLink®

Navigate to other submissions for this student

Home | Account Info | Help

Title: Psoriasis: rationale for targeting interleukin-17

Author: G. Girolomoni,U. Mrowietz,C. Paul

Publication: British Journal of Dermatology

Publisher: John Wiley and Sons

Date: Sep 26, 2012

Logged in as:
Hagar Nofal
LOGOUT

Review Order

Please review the order details and the associated terms and conditions.

No royalties will be charged for this reuse request although you are required to obtain a license and comply with the license terms and conditions. To obtain the license, click the Accept button below.

Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	British Journal of Dermatology
Licensed Content Title	Psoriasis: rationale for targeting interleukin-17
Licensed Content Author	G. Girolomoni,U. Mrowietz,C. Paul
Licensed Content Date	Sep 26, 2012
Licensed Content Pages	8
Type of use	Dissertation/Thesis
Requestor type	University/Academic
Format	Print and electronic
Portion	Figure/table
Number of figures/tables	1
Original Wiley figure/table number(s)	1
Will you be translating?	No
Title of your thesis / dissertation	Mannose Binding Lectin in the treatment of Imiquimod induced psoriasis mouse model
Expected completion date	May 2017
Expected size (number of pages)	100
Requestor Location	Hagar Nofal 269 Omar Ibn El Khatab St Zagazig, Sharkal 44511 Egypt

Appendix 10: Copyright permission for Figure 8:

Copyright Clearance Center RightsLink®

Home | Account Info | Help

Title: Mannan-binding lectin deficiency — Good news, bad news, doesn't matter?

Author: Sabine Heitzeneder,Markus Seidel,Elisabeth Förster-Waldl,Andreas Heitger

Publication: Clinical Immunology

Publisher: Elsevier

Date: April 2012

Copyright © 2011 Elsevier Inc. All rights reserved.

Logged in as:
Hagar Nofal
LOGOUT

Order Completed

Thank you for your order.

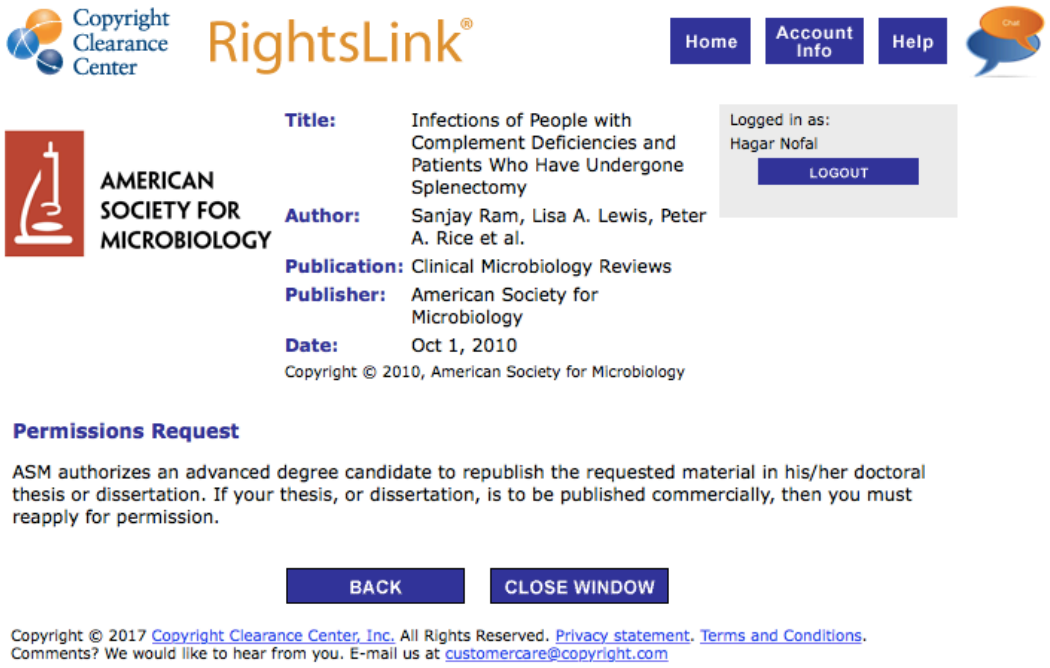
This Agreement between Hagar Nofal ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

Your confirmation email will contain your order number for future reference.

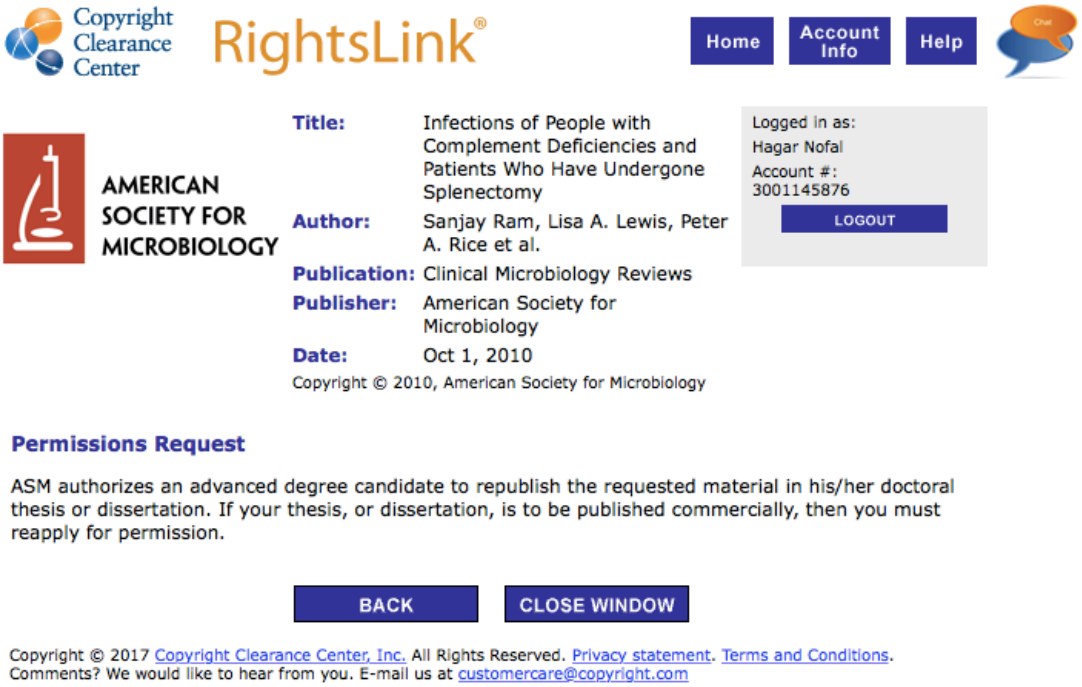
Printable details.

License Number	4099120763926
License date	Apr 30, 2017
Licensed Content Publisher	Elsevier
Licensed Content Publication	Clinical Immunology
Licensed Content Title	Mannan-binding lectin deficiency — Good news, bad news, doesn't matter?
Licensed Content Author	Sabine Heitzeneder,Markus Seidel,Elisabeth Förster-Waldl,Andreas Heitger
Licensed Content Date	April 2012
Licensed Content Volume	143
Licensed Content Issue	1
Licensed Content Pages	17
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Format	both print and electronic
Are you the author of this Elsevier article?	No
Will you be translating?	No
Order reference number	

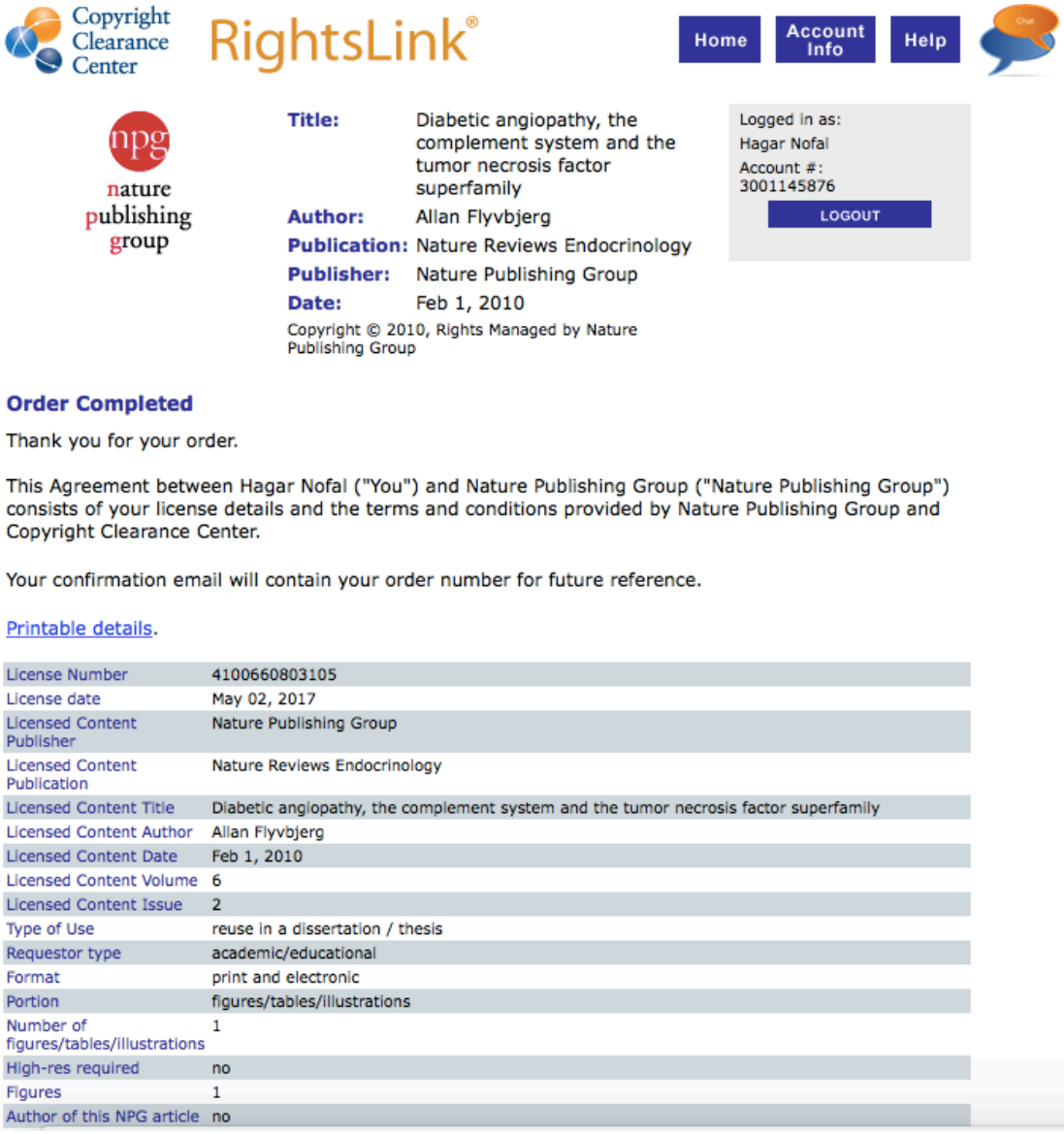
Appendix 11: Copyright permission for Figure 9



Appendix 12: Copyright permission for Figure 10:



Appendix 13: Copyright permission for Figure 11:



Copyright Clearance Center RightsLink®

Home | Account Info | Help

nature publishing group

Title: Diabetic angiopathy, the complement system and the tumor necrosis factor superfamily
Author: Allan Flyvbjerg
Publication: Nature Reviews Endocrinology
Publisher: Nature Publishing Group
Date: Feb 1, 2010

Copyright © 2010, Rights Managed by Nature Publishing Group

Logged in as:
Hagar Nofal
Account #:
3001145876
LOGOUT

Order Completed

Thank you for your order.

This Agreement between Hagar Nofal ("You") and Nature Publishing Group ("Nature Publishing Group") consists of your license details and the terms and conditions provided by Nature Publishing Group and Copyright Clearance Center.

Your confirmation email will contain your order number for future reference.

Printable details.

License Number	4100660803105
License date	May 02, 2017
Licensed Content Publisher	Nature Publishing Group
Licensed Content Publication	Nature Reviews Endocrinology
Licensed Content Title	Diabetic angiopathy, the complement system and the tumor necrosis factor superfamily
Licensed Content Author	Allan Flyvbjerg
Licensed Content Date	Feb 1, 2010
Licensed Content Volume	6
Licensed Content Issue	2
Type of Use	reuse in a dissertation / thesis
Requestor type	academic/educational
Format	print and electronic
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
High-res required	no
Figures	1
Author of this NPG article	no

Appendix 14: Copyright permission for Figure 12:

Copyright Clearance Center RightsLink®

Home | Account Info | Help

Title: Mannose binding lectin: Genetics and autoimmune disease
Author: Akito Tsutsumi,Reiko Takahashi,Takayuki Sumida
Publication: Autoimmunity Reviews
Publisher: Elsevier
Date: July 2005
Copyright © 2005, Elsevier

Logged in as:
Hagar Nofal
LOGOUT

Order Completed

Thank you for your order.

This Agreement between Hagar Nofal ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

Your confirmation email will contain your order number for future reference.

Printable details.

License Number	4099130876633
License date	Apr 30, 2017
Licensed Content Publisher	Elsevier
Licensed Content Publication	Autoimmunity Reviews
Licensed Content Title	Mannose binding lectin: Genetics and autoimmune disease
Licensed Content Author	Akito Tsutsumi,Reiko Takahashi,Takayuki Sumida
Licensed Content Date	July 2005
Licensed Content Volume	4
Licensed Content Issue	6
Licensed Content Pages	9
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Format	both print and electronic
Are you the author of this Elsevier article?	No
Will you be translating?	No
Order reference number	

Appendix 15: Copyright permission for Figure 13 &14:

Review Article

Toll-Like Receptors: Role in Dermatological Disease

Aswin Hari,[1,2] Tracy L. Flach,[1,2] Yan Shi,[1,2] and P. Régine Mydlarski[1,3]

[1]Immunology Research Group, University of Calgary, Calgary, AB, Canada T2N 4N1
[2]Department of Microbiology & Infectious Diseases, University of Calgary, Calgary, AB, Canada T2N 4N1
[3]Department of Medicine, University of Calgary, Calgary, AB, Canada T2N 4N1

Received 29 January 2010; Revised 27 April 2010; Accepted 1 July 2010

Academic Editor: Natalija Novak