ELUCIDATING THE ROLE OF DENDRITIC CELLS AND B CELLS IN IMIQUIMOD (IMQ)-INDUCED PSORIASIS-LIKE MOUSE MODEL

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by

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LIST OF SYMBOLS

°C	Degree celsius
%	Percent
µl	Microliter
cc	Cubic centimetre
G	Gauge
g	Gram
kDa	Kilodaltons
L	Litre
mg	Microgram
mg/kg	Microgram per kilogram
ml	Millilitre
mm	Millimetre
n	Sample size
nm	Ultraviolet range
pg	Picogram
pg/ml	Picogram per mililitre
rpm	Revolutions per minute
α	Alpha
β	Beta
γ	Gamma

LIST OF ABBREVIATIONS

AID	Activation-induced cytidine deaminase
AIRE	Autoimmune regulator
APCs	Antigen-presenting cells
BAFF	B cell activating factor
BALB/c	Bagg albino (inbred research mouse strain) genotype c
BCR	B cell receptor
Blys	B lymphocyte stimulator
Bregs	Regulatory B cells
cAMP	Cyclic adenosine monophosphate
CCL	C-C Motif Chemokine Ligand
CD	Cluster of differentiation
cDNA	Complementary deoxyribonucleic acid
CDPs	Common dendritic cell progenitors
CLP	Common lymphoid progenitors
CSF2	Colony-Stimulating Factor 2
CTLA	Cytotoxic T-lymphocyte antigen
CXCL	C-X-C Motif Chemokine Ligand
CXCR	C-X-C chemokine receptor
DAMPs	Damage-associated molecular patterns
DCs	Dendritic cells
DETCs	Dendritic epidermal T cells
DNA	Deoxyribonucleic acid
EGF	Epidermal growth factor
ELISA	Enzyme-linked immunosorbent assay
FGF	Fibroblast growth factor
Flt3	Fms-like tyrosine kinase 3
FSC	Forward scatter
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GC	Germinal centre
GC content	Guanine-Cytosine content
GM-CSF	Granulocyte-macrophage colony-stimulating factor

GMP	Granulocyte-macrophage progenitors
HLA-DRA	Major Histocompatibility Complex, Class II, DR α
HRP	Horse radish peroxidase
HSCs	Hematopoietic Stem Cells
IACUC	Institutional Animal Care and Use Committee
IFN	Interferons
IFPA	International Federation of Psoriasis Association
Ig	Immunoglobulins
IL	Interleukin
IMID	Immune-mediated inflammatory disease
IMQ	Imiquimod
iNOS	Inducible Nitric Oxide Synthase
KGF	Keratinocytes growth factor
MHC	Major histocompatibility complex
MIQE	Minimum Information for Publication of Quantitative Real-Time PCR Experiments
MLPs	Myeloid lineage progenitors
MZ	Marginal zone
NETs	Neutrophil extracellular traps
NFATC1	Nuclear factor of activated T cells; cytoplasmic 1
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK	Natural killer cells
PAMPs	Pathogen-associated molecular patterns
PASI	Psoriasis Area and Severity Index
PDE	Phosphodiesterase
PD-L1	Programmed death-ligand 1
PRR	Pattern recognition receptor
psA	Psoriatic arthritis
PUVA	Psoralen plus ultraviolet A
RA	Rheumatoid arthritis
RNA	Ribonucleic acid
RT-PCR	Real-time polymerase chain reaction
SD	Standard deviation
SLE	Systemic lupus erythematosus

sp	Species
SSC	Side scatter
TD	T-cell dependent
Tfh	T follicular helper cells
TI	T-cell independent
TLR	Toll-like receptor
TNF	Tumour necrosis factor
Tregs	Regulatory T cells
USM	Universiti Sains Malaysia
VEGF	Vascular endothelial growth factor
VLA	Very Late Antigen

LIST OF APPENDICES

MENJELASKAN PERANAN SEL DENDRITIK DAN SEL B DALAM IMIQUIMOD (IMQ)-MODEL TETIKUS SEPERTI PSORIASIS TERINDUKSI

ABSTRAK

Psoriasis adalah gangguan kulit inflamasi kronik yang dicirikan oleh hiperproliferasi keratinosit dan gangguan imun. Walaupun psoriasis adalah penyakit yang dimediasi sel T, bukti yang semakin meningkat menunjukkan peranan penting sel dendritik (DC) dan sel B dalam mendorong, mengekalkan dan mengawal keradangan psoriatik. Oleh itu, kajian ini bertujuan untuk menerangkan peranan DC dan sel B dalam model tetikus seakan psoriasis yang diinduksi oleh imiquimod (IMQ) melalui analisis berasaskan masa mengenai tindak balas imun dalam kulit, limpa dan darah. Tetikus BALB/c dibahagikan kepada kumpulan kawalan (n=6) dan IMQ-terinduksi (n=6), dengan sampel dikumpulkan pada hari ke-3, hari ke-5 dan hari ke-7. Keradangan seperti psoriasis diinduksi melalui aplikasi topikal IMQ, yang menyebabkan peningkatan ketebalan lipatan kulit, skor Indeks Kawasan dan Keparahan Psoriasis (PASI) yang diubah, dan splenomegali berbanding dengan kumpulan kawalan. Analisis histologi (pewarnaan hematoksilin dan eosin (H&E) dan pewarnaan trichrome Masson) mendedahkan ciri-ciri utama psoriasis, termasuk hiperplasia epidermis, hiperkeratosis, infiltrasi sel imun dan saluran darah yang jelas, serta peningkatan ketumpatan sel imun dalam limpa. Yang menarik, pulp putih limpa menunjukkan pembesaran pusat germinal (GC) yang ketara, menunjukkan peningkatan aktiviti limfoid. Sitometri aliran digunakan untuk menganalisis dinamik sel DC dan B merentasi sampel. Keputusan menunjukkan kecenderungan peningkatan dalam populasi sel DC $CD11c^{hi/+}MHCII^{+}$ merentasi semua sampel, menekankan peranan mereka dalam persembahan antigen dan pengaktifan imun. Seterusnya, sel B

B220^{+}CD38^{+} meningkat dalam limpa, manakala sel B CD19^{+}CD38^{+} adalah lebih tinggi dengan ketara dalam kulit tetapi menurun dalam darah, mencadangkan migrasi dan pengaktifan dinamik. Analisis ekspresi gen berikutnya (RT-PCR) bagi *CD11c*, *H2-Aa*, *BAFF*, *IL-10*, *IL-6* dan *CXCR5* menunjukkan peningkatan yang konsisten dalam kumpulan yang diinduksi IMQ, menyokong keadaan radang yang berterusan yang didorong oleh pengaktifan sel DC dan B. Analisis sitokin menggunakan ELISA menunjukkan peningkatan tahap serum BAFF, IL-10 dan IL-6 pada setiap titik masa, mengukuhkan lagi peranan mereka dalam keradangan kronik dan pengaktifan sel B. Secara keseluruhan, peningkatan penanda sel DC dan sel B pada peringkat selular dan molekul, bersama dengan kenaikan sitokin pro- dan anti-radang, mencerminkan tindak balas imun yang kuat dan dinamik. Penemuan ini mengesahkan kejayaan pembentukan IMQ model tetikus seperti psoriasis terinduksi dan menyokong objektif kajian dalam meneroka penglibatan dinamik DC serta sel B semasa perkembangan penyakit. Ia juga memberikan asas untuk penyelidikan terapeutik pada masa hadapan.

ELUCIDATING THE ROLE OF DENDRITIC CELLS AND B CELLS IN IMIQUIMOD (IMQ)-INDUCED PSORIASIS-LIKE MOUSE MODEL

ABSTRACT

Psoriasis is a chronic inflammatory skin disorder characterised by keratinocyte hyperproliferation and immune dysregulation. Although psoriasis is a T cell-mediated disease, increasing evidence suggests the important roles of dendritic cells (DCs) and B cells in initiating, sustaining and regulating psoriatic inflammation. Therefore, this study aims to elucidate the roles of DCs and B cells in an imiquimod (IMQ)-induced psoriasis-like mouse model through a time-based analysis of immune responses in the skin, spleen and blood. BALB/c mice were divided into control (n=6) and IMQ-induced (n=6) groups, with samples collected on day 3, day 5 and day 7. Psoriasis-like inflammation was induced *via* topical IMQ application, leading to increased skinfold thickness, modified Psoriasis Area and Severity Index (PASI) scores and splenomegaly compared to controls. Histological analysis (hematoxylin and eosin (H&E) and Masson's trichrome staining) revealed hallmark psoriasis features, including epidermal hyperplasia, hyperkeratosis, immune cell infiltration and visible blood vessel observation, as well as increased immune cell density in the spleen. Notably, the white pulp of the spleen exhibited significant germinal centre (GC) enlargement, indicating heightened lymphoid activity. Flow cytometry was used to analyse DC and B cell dynamics across samples. The results demonstrated an increasing trend in $CD11c^{hi/+}MHCII^{+}$ DC populations across all samples, accentuating their involvement in antigen presentation and immune activation. Concurrently, $B220^{+}CD38^{+}$ B cells increased in the spleen, while $CD19^{+}CD38^{+}$ B cells were significantly higher in the skin but decreased in the blood, suggesting distinct migration and activation dynamics. Subsequent gene expression analysis (RT-PCR) of

CD11c, *H2-Aa*, *BAFF*, *IL-10*, *IL-6* and *CXCR5* revealed consistent upregulation in the IMQ-induced group, supporting a sustained inflammatory state driven by DC and B cell activation. ELISA-based cytokine analysis showed elevated serum levels of BAFF, IL-10 and IL-6 at each time point, further reinforcing their role in chronic inflammation and B cell activation. Overall, the increment of DC and B cell markers at both cellular and molecular levels, accompanied by elevated pro- and anti-inflammatory cytokines, reflects a robust and evolving immune response. These findings affirm the successful establishment of the IMQ-induced psoriasis-like mouse model and support the study objectives in elucidating the dynamic involvement of DCs and B cells during disease progression as well as offering a foundation for future therapeutic research.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Psoriasis is a common chronic inflammatory skin disorder marked by abnormal keratinocyte proliferation and immune cell infiltration (Sieminska *et al*., 2024). According to the International Federation of Psoriasis Association (IFPA), it affects about 2-3% of the global population, with a prevalence of 2.3% in Malaysia (Psoriasis Association of Malaysia, 2020). Considering the widespread impact of psoriasis, it is essential to understand the underlying mechanisms, especially the intricate interplay between genetic factors, environmental triggers and immune system dysregulation that drives its pathogenesis. Immune system dysregulation involves an aberrant activation of innate and adaptive immune pathways, including the excessive production of pro-inflammatory cytokines such as IL-17 and IL-23, which perpetuate chronic inflammation and keratinocyte hyperproliferation (Kamiya *et al*., 2019; Dand *et al*., 2020; Rakhshan *et al*., 2020).

Psoriasis pathogenesis is complex, beginning with triggers such as antimicrobial peptides (AMPs) received by antigen-presenting cells (APCs) which then activate and recruit various immune cells that exacerbate the inflammatory response and drive the characteristic hyperproliferation of keratinocytes (Sieminska *et al*., 2024). Early research indicates that dendritic cells (DCs) trigger innate immune stimulation and cytokine secretion (Jariwala, 2007). Meanwhile, T cells, especially the T helper (Th)1 and Th17 subsets, play a crucial role in sustaining the inflammation seen in psoriatic lesions, a finding later supported by another recent study (Nussbaum *et al*., 2021; Antal *et al*., 2022). Identifying key cytokines produced by T cells, including TNF-α, IL-17 and IL-23, has led to targeted biological therapies like

etanercept and ustekinumab. Etanercept blocks TNF-α activity by preventing its binding to TNF receptors, while ustekinumab targets the p40 subunit shared by IL-12 and IL-23, inhibiting downstream inflammatory responses (Brodmerkel *et al*., 2019). This cytokine-specific blockade disrupts the inflammatory cascade, alleviating skin lesions and improving patient quality of life. Hence, these treatments have significantly reduced psoriasis severity and opened new opportunities for targeting immune components in the management of psoriasis (Sieminska *et al*., 2024).

Despite these advances, many patients experience relapse or only partial remission, thus requiring a more comprehensive understanding of psoriasis pathophysiology (Balak *et al*., 2022). Emerging evidence suggests that, beyond T cells, both DCs and B cells play crucial roles in initiating and perpetuating psoriatic inflammation (Torres-Ruiz and Shoenfeld, 2019; Branisteanu *et al*., 2022). DCs act as key antigen-presenting cells, driving the activation and recruitment of other immune cells and amplifying the inflammatory response, while B cells contribute through antibody production and cytokine secretion (Torres-Ruiz and Shoenfeld, 2019; Grän *et al*., 2020). Psoriasis has traditionally been considered a T-cell-mediated disease and the involvement of B cells is less commonly reported. Hence, investigating the role of B cells in psoriasis pathogenesis is a novel research area (Nguyen and Soulika, 2019; Noor *et al*., 2024). Psoriasis is considered an autoimmune disease, therefore B cells, with the help of APCs like DCs, likely play a role in its mechanisms. This shift in understanding emphasises the complex interactions among immune cells and the role of DCs and B cells in regulating immune responses (Kim *et al*., 2017; Wang and Bai, 2020a).

B cells, generally associated with humoral immunity and antibody production, are now recognised for their roles in autoimmune diseases, including psoriasis. Studies

have shown that B cells can produce pro-inflammatory cytokines, present antigens and modulate the activity of other immune cells, including T cells and DCs. For example, a previous study demonstrated that mice lacking B cells or with IL-10-deficient B cells exhibited severe inflammation when exposed to IMQ, indicating the protective role of IL-10 (Alrefai *et al*., 2016). Another clinical study found that B cells in psoriatic lesions express higher activation markers, such as CD20 and are involved in forming ectopic lymphoid structures, further implicating them in disease pathogenesis (Lu *et al*., 2016).

DCs are professional APCs that are essential for the activation of T cells and the initiation of adaptive immune responses. In psoriasis, DCs have been shown to accumulate in the skin and produce cytokines such as IL-23, which drives Th17 cell responses (Torres-Ruiz and Shoenfeld, 2019). Markers such as CD11c and MHCII are typically used to identify these activated DC populations in inflamed tissues (Torres-Ruiz and Shoenfeld, 2019). DCs have been suggested to interact with B cells in psoriatic skin to form a pathogenic axis to sustain chronic inflammation (Kim *et al.,* 2017). However, the precise nature of these interactions and their contributions to the overall disease process are yet to be fully elucidated.

The roles of B cells, aided by DCs, in psoriasis development are still under active investigation. B cells expressing CD19, CD38 or B220 have been implicated in inflammatory responses (Sieminska *et al.,* 2024), with some subsets potentially exerting regulatory effects via IL-10 (Heath *et al.*, 2019; Sieminska *et al*., 2024). These immune cells potentially play a critical role in exacerbating psoriasis and point toward new therapeutic targets, which will probably lead to further revising the current understanding of psoriasis pathogenesis. A comprehensive view of psoriasis could uncover a broader range of targets beyond soluble mediators, including cellular

components like B cells and DCs, potentially offering novel insights into the disease. Genes such as *B cell activating factor* (*BAFF)*, *IL-6* and *CXCR5* may also reflect disease-associated B cell survival, pro-inflammatory cytokine activity and immune cell trafficking, respectively (Sieminska *et al.*, 2024).

The imiquimod (IMQ)-induced psoriasis-like model replicates key features of human psoriasis, engaging both innate and adaptive immunity (Wang *et al.,* 2023; Walvekar *et al*., 2024). Compared to xenografts and transgenic models (Rodríguez-Martínez *et al.*, 2017a), IMQ-treated BALB/c mice provide a simpler, reproducible system for studying systemic immune connections in psoriasis. Previous studies using the IMQ model have highlighted inflammatory mediators, but only recently has attention turned to the contributions of B cells and DCs (Jabeen *et al.*, 2020; Huang *et al.*, 2023; Tran *et al*., 2024). Hence, this current study aims to clarify the roles of B cells and DCs in psoriasis using the IMQ-induced model by analysing key cytokines, markers and gene profiles to identify potential therapeutic targets. This approach also allows for comprehensive cellular populations between the skin (the primary site of inflammation), the blood (the systemic network) and the spleen (a secondary lymphoid organ) to provide valuable insights into both systemic and localised aspects of the disease. To capture distinct phases of disease development, day 3 represents the early onset, day 5 reflects the stage of immune cell infiltration and day 7 corresponds to the progression phase. Genes associated with these cell types, including *CD11c* and *H2-Aa* (linked to DC antigen presentation) (Brunner *et al.*, 2023), *BAFF* (critical for B cell survival) (Liu *et al*., 2021), *IL-10* and *IL-6* (cytokines influencing B cell and DC function) (Sakai *et al.,* 2023) and *CXCR5* (involved in B cell localisation) (Pan *et al.,* 2022), provide mechanistic insight into their contribution to disease pathology.

1.2 Problem statement

Psoriasis treatments vary by severity, with topical agents and phototherapy used for mild to moderate cases and systemic drugs, including biologics, reserved for severe cases (Huang *et al*., 2019; Le and Torres, 2022; Fargnoli e*t al.*, 2023). Biologics such as etanercept and ustekinumab target T cell-mediated pathways in which etanercept neutralises TNF-α, while ustekinumab blocks IL-12 and IL-23 to inhibit Th1 and Th17 responses (Li *et al*., 2022; Hassan *et al.*, 2023). Despite their efficacy, these treatments often fail to sustain remission, with recurrence, adverse effects, treatment resistance, high costs and variable responses posing major challenges (Tian and Lai, 2022). These limitations warrant the need for therapeutic strategies beyond T cell-focused approaches.

The traditional view of psoriasis as a T cell-mediated disease is supported by the abundance of T cells in lesions, elevated Th1/Th17 cytokines and the success of T cell-targeted therapies (Zhang *et al*., 2023). However, this focus has overlooked the roles of DCs and B cells, which are increasingly recognised for contributing to disease progression (Branisteanu *et al*., 2022). DCs initiate and modulate immune responses, while B cells, beyond antibody production, influence immune regulation (Heath *et al*., 2019; Sieminska *et al.*, 2024). Advances in autoimmune therapies have shown the potential of targeting these cells. Abatacept blocks CD80/CD86 co-stimulatory signals on DCs to reduce T cell activation in rheumatoid arthritis (Ahamada and Wu, 2023), while iscalimab inhibits CD40-CD40L interactions on DCs to suppress T cell responses in Sjögren's syndrome and transplantation (Nashan *et al*., 2018; Ristov *et al.*, 2018; El Jamal and Shibli, 2024). For B cells, belimumab inhibits BAFF to reduce autoantibody production in SLE and targeted JAK inhibitors using polylactic acid nanoparticles have been used to suppress B cell activity during flares (Álvarez *et al.*,

2023). These findings accentuate the importance of exploring immune components beyond T cells, as DCs and B cells may also play crucial roles in psoriasis pathogenesis and offer new therapeutic opportunities.

In conjunction with this, the IMQ-induced psoriasis-like mouse model is a widely used experimental system replicating many aspects of human psoriasis. Despite its extensive use, most studies utilising this model have focused on the roles of T cells and cytokines, thus often neglecting the potential contributions of B cells and DCs (Badanthadka and D'Souza, 2020; Costa *et al*., 2022). Additionally, these past studies typically did not assess how these cells change over time, which could reveal key insights into the progression of psoriasis and potential points for intervention.

Thus, this research addresses the gaps in understanding the roles of DCs and B cells in the evolving psoriasis pathogenesis using the IMQ-induced psoriasis-like mouse model. By employing a time-point-based experimental approach, the study investigates how the roles of these cells evolve throughout disease development. Systematic analysis of immune responses by DCs and B cells at multiple time points in three main sites such as skin (local inflammation), spleen (immune cell activation site) and blood (circulating immune responses), will offer a comprehensive understanding of the dynamic cellular mechanisms driving psoriasis. Notably, this study is the first to dissect both DC and B cell responses simultaneously across skin, spleen and blood in a time-based manner in providing a unique integrated view of psoriasis progression. In short, this approach could reveal new insights into the roles of B cells and DCs and potentially lead to innovative therapeutic strategies and a deeper understanding of the disease.

1.3 Research questions

1) How do psoriasis-like lesions progress in an IMQ-induced mouse model, as characterised by changes in PASI score, skinfold thickness and histological features of the skin and spleen at different time points?
2) What are the changes in DC and B cell populations in the skin, spleen and blood of the IMQ-induced psoriasis-like mouse model at different time points?
3) How does the gene expression of *CD11c*, *H2-Aa*, *BAFF*, *IL-10*, *IL-6* and *CXCR5* genes vary in the skin, spleen and blood of the IMQ-induced psoriasis-like mouse model at different time points?
4) What are the circulating levels of extracellular cytokines BAFF, IL-10 and IL-6 in the serum of the IMQ-induced psoriasis-like mouse model and how do these levels vary at different time points?

1.4 Research hypothesis

DCs and B cells play significant roles in psoriasis-like inflammation by contributing to initiating and progressing immune responses that drive disease pathogenesis.

1.5 Research objectives

1.5.1 General objective

To elucidate the role of DCs and B cells in an IMQ-induced psoriasis-like mouse model.

1.5.2 Specific objectives

1) To establish the psoriasis mouse model using IMQ-induced topical application.
2) To determine the expression of DCs and B cell populations in the skin, spleen and blood in the IMQ-induced psoriasis-like mouse model at different time points.
3) To evaluate the gene expression of *CD11c*, *H2-Aa*, *BAFF*, *IL-10*, *IL-6* and *CXCR5* in the skin, spleen and blood of the IMQ-induced psoriasis-like mouse model at different time points.
4) To assess the circulating levels of extracellular cytokines BAFF, IL-10 and IL-6 in the serum of the IMQ-induced psoriasis-like mouse model at different time points.

CHAPTER 2

LITERATURE REVIEW

2.1 Psoriasis

Psoriasis is a chronic skin condition in which inflammation is highly affected by genetic abnormality and driven by autoimmunity. It is classified as an immune-mediated inflammatory disease (IMID), also noted by a dermatologic-related condition (Kamiya *et al*., 2019; Rendon and Schäkel, 2019). Psoriasis affects approximately 2 to 3% of the global population and has a reported prevalence of 2.3% in Malaysia (Rendon and Schäkel, 2019). Beyond its cutaneous manifestations, psoriasis imposes a substantial burden on patients due to its visible and often stigmatising lesions, leading to psychological distress, depression, impaired quality of life and increased healthcare costs (Kamiya *et al.*, 2019; Rendon and Schäkel, 2019). This chronic disease usually appears with well-demarcated erythematous plaques with slivery and/or white scales (Dhabale and Nagpure, 2022). These abnormal appearances regularly appear on knees, elbows, body trunk and scalp. Since psoriasis has many forms, it can be categorised into two major groups which are non-pustular (guttate, vulgaris, erythrodermic, palmoplantar, inverse, scalp) and pustular which some photographic images can be seen in Figure 2.1 (Kimmel and Lebwohl, 2018).

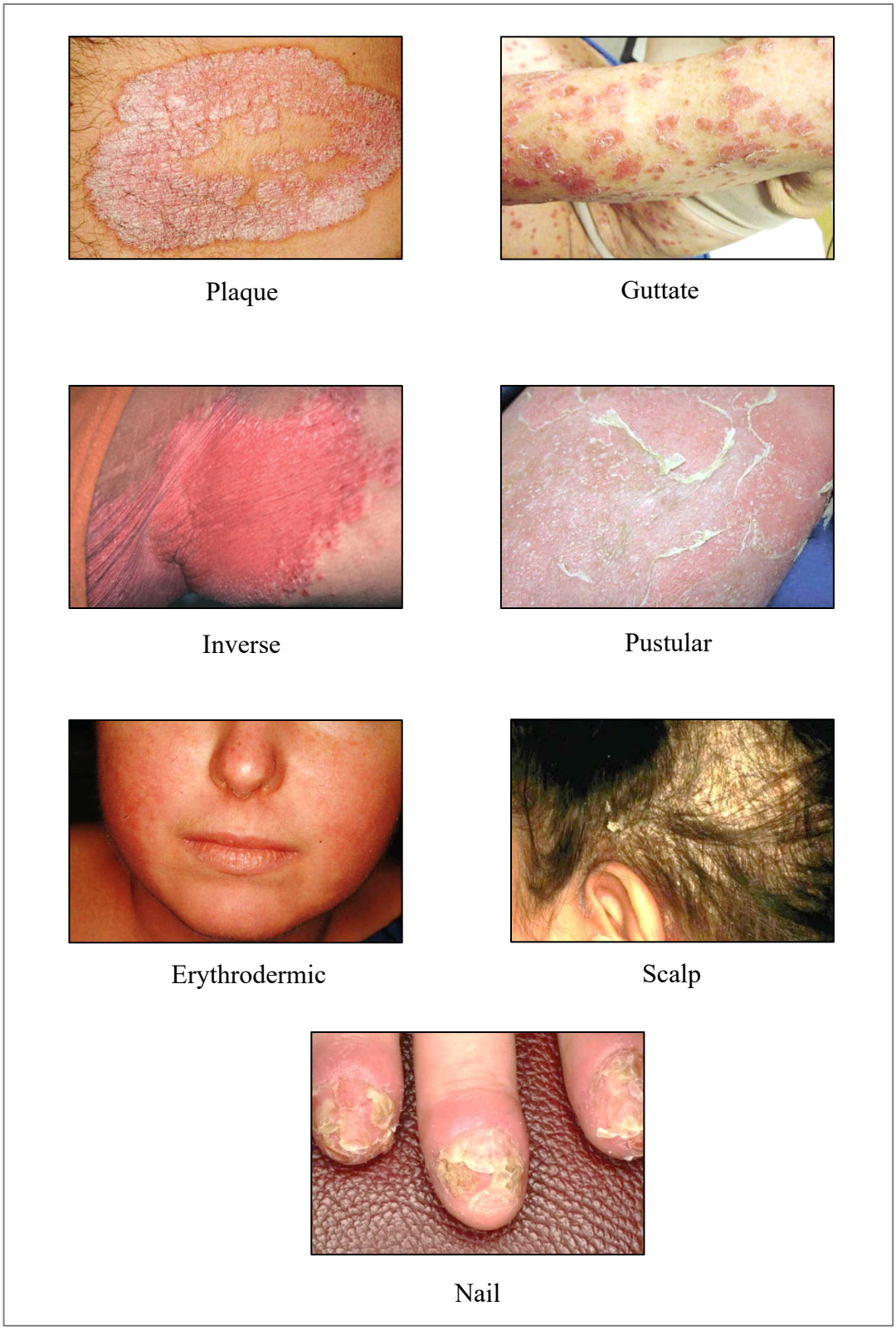

Figure 2.1 Common types of psoriasis consist of plaque, guttate, inverse, pustular, erythrodermic, scalp and nail. Photographs are adapted from Kimmel and Lebwohl, (2018).

2.1.1 Immunopathogenesis of psoriasis

Psoriasis pathogenesis is highly hypothesised that regardless of which cause, the early specific signal perceived by DCs creates the chain reactions of T cells' inflammatory downstream pathways, eventually leading to the expansion of keratinocytes. In short, psoriasis completes its development in four major phases: initiation, stimulated innate immune response, responsive adaptive immune response and epidermal expansion. The most common hypothesis suggests that nucleic acid complex remnant of AMPs such as LL-37 or self-DNA is presented and captured by Toll-like receptors (TLR)-8 (TLR-7 in mice) of DCs. In response, stimulated DCs release TNF-α, IFN-γ, IL-1, IL-12 and IL-23. These cytokines will co-stimulate naïve T cells for their clonal expansion and stimulation to differentiate into Th cells (Sameer and Nissar, 2021; Chhabra *et al*., 2022; Noor *et al*., 2022; Miura *et al*., 2023).

Stimulated DCs are said to further excrete inflammatory mediators IFN-α and IFN-β in activating each other to secrete more pro-inflammatory mediators such as IL-12 and TNF-α. The naïve T cells are also recruited in the psoriatic lesions (Wu *et al*., 2023a). Subsequently, in an adaptive immune response phase, these DCs activate these T cells *via* costimulatory signals to generate its differentiation into Th1, Th17 and Th22 cells. Th1 cells secrete IFN-γ, TNF-α and IL-2 which intensify the inflammatory conduit by triggering keratinocytes and DCs (Rendon and Schäkel, 2019). Meanwhile, Th22 cells secrete IL-22, which is responsible for the characteristics of a psoriatic skin lesion in releasing keratinocyte-derived T cell-recruiting chemokines. IL-22 gives rise to the altered tissue phenotype, including epidermal hyperplasia, acanthosis and parakeratosis (Kanda, 2021). Th17 cells are activated by IL-1 and primarily respond to IL-23, IL-12 and TNF-α to produce IL-17 upon migrating to the psoriatic dermis. IL-17 then binds to its receptor on keratinocytes, triggering the release of TNF and CCL20.

With TNF-α, IL-17 allows further neutrophil assemblage to produce ROS, α-defensin with antimicrobial potential, CXCL8, IL-6 and CCL20 (Chiricozzi *et al*., 2011; Kanda, 2021). Additionally, this cytokine combination stimulates keratinocytes to produce chemokines such as CXCL1, CXCL2, CXCL3, CXCL5 and IL-8, which further enhance the inflammatory response by attracting more immune cells to the site of inflammation (Yamanaka *et al*., 2021). Keratinocytes produce vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and angiopoietin to initiate neoangiogenesis (Chen *et al*., 2023). This process promotes the formation of new blood vessels, resulting in the characteristic red colouration of psoriasis lesions due to the dense vascular network beneath the skin (Rendon and Schäkel, 2019; Martins *et al*., 2020). The overall summarised known cellular and molecular pathogenesis of psoriasis is illustrated in Figure 2.2.

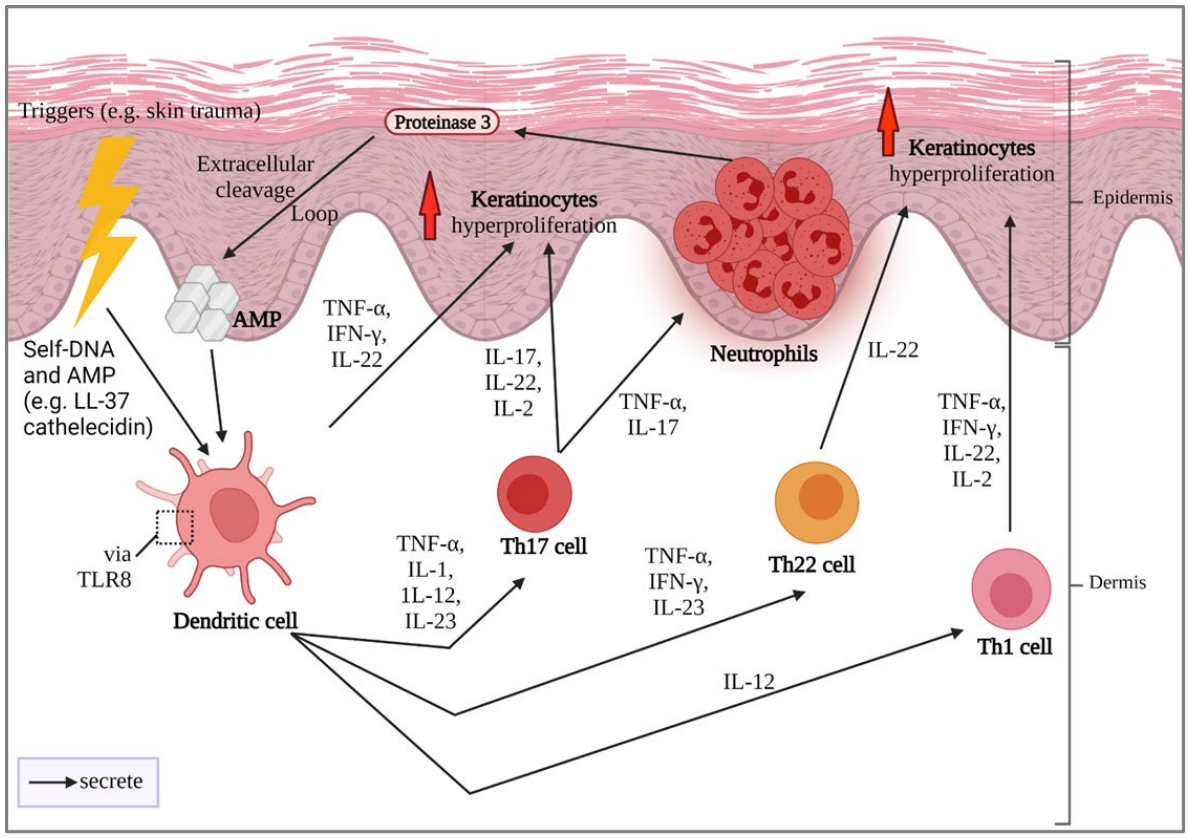

Figure 2.2 Summarised pathogenesis of psoriasis. Triggers such as skin trauma promote self-DNA release and antimicrobial peptides (AMPs) such as LL-37 cathelicidin to form complexes that activate dendritic cells (DCs) *via* TLR8. Activated DCs secrete proinflammatory cytokines (e.g., TNF-α, IL-1, IL-12, IL-23) to cause differentiation of T helper (Th)1, Th17 and Th22 cells. These Th cells amplify inflammation by producing IL-17, IL-22, TNF-α and IFN-γ cytokines to stimulate keratinocyte hyperproliferation and neutrophil infiltration. The resulting inflammatory feedback loop contributes to psoriasis lesion formation.

Inflammatory cells can continuously invade the psoriatic lesion due to the secretion of cytokines and chemokines in the host defence pathway. Persistent VEGF expression accelerates inflammatory cell accumulation by promoting alternative vascular pathways. During advanced epidermal hyperproliferation, keratinocytes and dermal fibroblasts produce pro-inflammatory and growth factors (TNF-α, IL-1β, TGF-β, KGF, EGF), contributing to tissue remodelling and extracellular matrix reorganisation (Liarte *et al*., 2020; Chen *et al.*, 2023). Moreover, the psoriatic plaque contains numerous macrophages that release IL-6, IL-12 and IL-23 and inducible nitric oxide synthase (iNOS) (Wu *et al*., 2023a). The plague also undergoes a respiratory burst, producing reactive oxygen species (ROS) and becoming granulated. This event causes the formation of neutrophil extracellular traps (NETs) suspected to be involved in the pathogenesis of psoriasis (Chiang *et al*., 2019).

Overall, a wide array of immune cells has been implicated in psoriasis, including DCs, T cells (Th1, Th17, Th22), macrophages, neutrophils, B cells and innate lymphoid cells. DCs initiate immune activation through antigen presentation and cytokine release; T cells drive inflammation *via* effector cytokines; macrophages and neutrophils amplify the response through cytokine secretion and NET formation (Sameer and Nissar, 2021; Kanda, 2021; Leiferman *et al.*, 2022). B cells, though less explored, may contribute by antigen presentation and cytokine production, potentially influencing these immune pathways at various stages, with support from APCs like DCs (Alrefai *et al.*, 2016; Rendon and Schäkel, 2019). Innate lymphoid cells support early immune activation (Ten Bergen *et al.,* 2020). While this reflects the overall immune landscape, the precise contributions of each cell subset remain unclear. Continued research is needed to delineate their roles in disease progression and inform the development of targeted therapeutic strategies (Kahlert *et al*., 2019; Wu *et al.*, 2023a).

2.2 Dendritic cells (DCs)

2.2.1 Development

DCs are primary APCs that link innate and adaptive immunity. The development of DCs exhibits differences between humans and mice, with multiple DC subsets emerging based on locality, phenotype and specific growth factors. Although the development of DCs varies between humans and mice, these differences do not compromise the relevance of findings from murine models. The mechanisms underlying DC differentiation and function in mice provide valuable insights that are often reproducible and applicable to human immunological contexts. This reproducibility is crucial for understanding the immune dysregulation associated with psoriasis. Thus, the distinct yet complementary nature of DC development in both species enhances the overall understanding of immune responses and facilitates the translation of research findings into potential therapeutic strategies.

DCs originate from common myeloid progenitors (CMP) and common lymphoid progenitors (CLP) in the bone marrow. These progenitors give rise to various types of DCs, including classical or conventional DCs, plasmacytoid DCs (pDCs) and follicular DCs, the latter of which are suggested to originate from lymphoid follicles within germinal centres (GCs) (Abd El-Aleem *et al*., 2022). Major types include conventional DCs (cDCs), myeloid DCs (mDCs) and follicular DCs (FDCs) (Cabeza-Cabrerizo *et al*., 2021). Although FDCs have similar names and morphology to other DCs, the different origin from mesenchymal cells means the cells should be excluded when considering DC development from the bone marrow. In humans, DCs develop from granulocyte-macrophage progenitors (GMPs), multi-lymphoid progenitors (MLPs) and common myeloid progenitors (CDPs), which differentiate into pDCs or

preclassical DCs (pre-cDCs). Pre-cDCs can further differentiate into cDC1 and cDC2 subpopulations (Cabeza-Cabrerizo *et al*., 2021; Marmonti *et al*., 2022).

Various cytokines and transcription factors influence the maturation of DCs. Fms-like tyrosine kinase 3 (Flt3) is pivotal for maintaining and establishing cDCs and pDCs, while the Flt3-L/Flt3 complex supports DC development in lymphoid and mucosal organs. CDPs differentiate into cDC1, cDC2 and pDCs under the influence of Flt3-L, with similar regulatory pathways observed in mice (Chrisikos *et al*., 2019). Granulocyte-macrophage colony-stimulating factor (GM-CSF), a glycoprotein known as colony-stimulating factor 2 (CSF2), regulates DC differentiation and is essential for their survival *in vitro*. This factor also contributes to myeloid cell development and maturation, reflecting similarities in DC development between species (Mashima *et al*., 2020).

DCs function as professional APCs, presenting antigens to $CD4^+$ T cells *via* MHC class II molecules. Immature DCs can navigate peptide-MHCII complexes intracellularly through the MHCII ubiquitin biochemical process which occurs on a conserved lysine within the β-chain of the cytoplasmic domain. Following pathogen invasion, TLRs induce DC maturation, downregulating their phagocytic and endocytic abilities while enhancing their expression of co-stimulatory molecules and cytokines, such as type 1 interferons (IFNs) (Cho and Roche, 2013; Kozono *et al.,* 2023). These processes optimise DCs for effective antigen presentation to T cells.

Mature DCs translocate and express MHCII molecules on their cell surface, facilitating T cell recognition. Notably, MHCII molecules are also present in B cells; however, DCs exhibit longer ubiquitin chain lengths than B cells. In addition to MHCII, DCs express other hematopoietic surface markers, including CD11c, Flt3 and CD45 (Cho *et al*., 2013; Kozono *et al*., 2023).

DCs orchestrate $CD4^+$ T cell activation during pathogen invasion by engaging costimulatory receptors and upregulating MHCII molecules. DCs capture naive T cells and present antigens, promoting their differentiation into effector T cells. DCs also produce tolerogenic cytokines that facilitate the differentiation of regulatory T cells (Tregs), fostering self-tolerance (Zhu and Zhu, 2020; Scheib *et al*., 2022). Given their critical role in modulating T cell responses, DCs represent a promising target for therapeutic interventions in autoimmune conditions such as psoriasis (Kim *et al*., 2021).

2.2.2 Activation

DCs communicate directly with other immune cells through cell surface protein recognition and cytokine secretion, particularly for activating innate immunity (Iwasaki and Medzhitov, 2015; Ye *et al*., 2020). Such communication is critical during the initial invasion of a pathogen that breaches the first-line defence of the immune system. Before the pathogen can replicate, DCs act as a secondary defense by detecting pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) on the pathogen's surface (Roh and Sohn, 2018). In addition to PAMPs, DCs can recognise exogenous signals like cytokines and endogenous signals, such as self-DNA, resulting from infection-induced changes (Domogalla *et al*., 2017). Immature DCs detect these signals through pattern recognition receptors (PRRs) like TLRs, which activate transcription factors crucial for their function. In diseases such as psoriasis, DCs utilise TLRs to detect psoriatic self-DNA and other activating triggers (Zepeda-Cervantes *et al*., 2020). Furthermore, DCs can engulf foreign bodies *via* phagocytosis or micropinocytosis, processing and presenting antigens on their surface through MHC molecules (Keselowsky *et al*., 2020).

Once activated, DCs migrate to secondary lymphoid organs, such as lymph nodes, through afferent lymphatic vessels in communicating with T cells. DCs uniquely present antigens to naïve T cells as the primary APCs, initiating antigen-specific immune responses (Hilligan and Ronchese, 2020). Concomitantly, DCs highly expressed co-receptors on its cell surface such as CD80, CD86 and CD40 to promote further T cell activation (Maverakis *et al*., 2015; Azeem *et al*., 2020).

2.2.3 Tolerance

DCs are essential for maintaining B cell tolerance through various mechanisms, including regulating B cell activation, influencing regulatory B cell (Breg) activity and facilitating central B cell tolerance. These functions help prevent autoantibody generation and autoimmune conditions by controlling abnormal B cell responses to self-antigens (Chekol Abebe *et al*., 2021). DCs regulate B cell activation and differentiation by presenting self-antigens that promote tolerance (Fucikova *et al.*, 2019). DCs present self-antigens to B cells without co-stimulatory signals or with inhibitory cues for B cell anergy or apoptosis, hence removing autoreactive B cells (Heath *et al.,* 2019). This helps to suppress autoantibody production and prevents autoimmune responses (Hasegawa *et al*., 2018; Heath *et al*., 2019).

Moreover, DCs promote the development and function of Bregs, specialised B cells with immunosuppressive properties (Fucikova *et al*., 2019). Bregs secrete anti-inflammatory cytokines like IL-10 and TGF-β to inhibit autoreactive T cells and other immune effectors (Boldison *et al.,* 2020). DCs assist in the differentiation of naïve B cells into Bregs by providing co-stimulatory signals and creating cytokine-rich environments, thus reducing autoimmune reactions and preserving immune tolerance (Fucikova *et al.,* 2019).

Additionally, in the bone marrow and secondary lymphoid organs, DCs are critical for central B cell tolerance, where immature B cells undergo selection processes to eliminate autoreactive clones. In these sites, DCs present self-antigens to immature B cells, prompting deletion or anergy of autoreactive clones. This process prevents the emergence of autoantibody-producing B cells and significantly contributes to B cell tolerance maintenance (Hasegawa *et al*., 2018; Fucikova *et al*., 2019; Chekol Abebe *et al*., 2021).

2.3 B cells

2.3.1 Development

B cells originate from hematopoietic stem cells (HSCs) in the bone marrow, where the cells undergo maturation. The common lymphocyte precursors derived from HSCs differentiate into pro-B cells (immature B cells). This maturation process involves three primary mechanisms establishing antibody diversity: V(D)J recombination, somatic hypermutation and class-switch recombination (King *et al*., 2021). Sequential expression of B cell receptors (BCRs) occurs, with BCRs composed of heavy (V_H, D_H and J_H) and light chains (V_L-J_L) linked by disulfide bonds (Solanki *et al*., 2022). These receptors undergo assembly and rearrangement, with the Ig heavy chains being generated before the light chains, forming the basis for the B cell lineage to produce antibodies capable of binding to a wide variety of antigens (Collins and Watson, 2018).

During the rearrangement of the Ig heavy chain, the D and J segments combine in common lymphoid progenitors (CLPs) and pre-pro B cells. The DJ segments are then joined with the upstream variable (V) region as the cells progress to the pre-B cell stage (Chi *et al*., 2020). The selection of either the κ or λ light chain ultimately results in the generation of functional Ig, such as IgM, from the μ heavy chain (Collins *et al.,* 2018; Chi *et al*., 2020). Immature B cells express the complete IgM BCR before exiting the bone marrow and transitioning into transitional B cells upon entering the secondary lymphoid organs such as the spleen (Collins *et al.,* 2018; Chi *et al*., 2020).

In the secondary lymphoid organs, immature B cells undergo transitional stages known as T1 and T2. Transitional T1 B cells, which still have limited survival potential, migrate to the spleen, where the cells transition into T2 B cells, which are more mature and capable of further differentiation. At this point, B cells follow one of two developmental paths depending on whether the cells receive T cell help.

T cell-independent (TI) B cells, such as Marginal Zone (MZ) B cells, arise from the T2 stage. These MZ B cells are predominantly located in the marginal zone of the spleen, respond rapidly to blood-borne antigens and are short-lived, primarily expressing IgM. MZ B cells can quickly mount immune responses without needing T cell help, making them effective in early immune responses (Baumgarth, 2021).

Meanwhile, T cell-dependent (TD) B cells develop into follicular B cells, which reside in the B cell follicles of lymphoid organs. These cells require help from T cells to activate, which occurs entirely through TCR-MHCII interactions. During this process, follicular B cells present processed antigens to T cells, receiving signals that promote their activation. Once activated, follicular B cells can either become short-lived plasma cells, which produce antibodies rapidly or enter the GCs for further maturation (Zhang *et al*., 2018). Within the GCs, follicular B cells undergo clonal expansion, somatic hypermutation and affinity maturation, enhancing their antigen specificity. Then, the cells either differentiate into memory B cells capable of rapid response upon re-exposure to the antigen or plasmablasts which secrete high-affinity antibodies (Hendricks *et al*., 2018). This pathway, driven by T cell help, is essential for generating a durable and precise immune response (Hendricks *et al*., 2018; Stebegg *et al.,* 2018).

2.3.2 Activation

The mechanism by which immature B cells become activated involves several processes mediated by BCR interactions with their associated antigens, primarily occurring in secondary lymphoid organs such as the spleen and lymph nodes. Generally, B cells can be activated without T cell stimulation, leading to the classification of this response as TI, which occurs *via* two major types: Type 1 and Type 2.

In TI Type 1 activation, B cells receive signals from BCRs and TLRs. Specifically, transitional B cells interact with foreign antigens *via* TLR-7 and/or TLR-9 (murine TLR8), as well as through complement and CD40, acting as 'innate' cells that capture antigen information (Kremlitzka *et al*., 2016; Allman *et al*., 2019). In contrast, TI Type 2 activation is characterised by the ability of multivalent antigens to be recognised by BCRs, which leads to cross-linking on the BCR surface and initiates the activation process (Fukao *et al.,* 2021). Once stimulated, transitional B cells differentiate into MZ B cells and subsequently act as short-lived plasmablasts to secrete high levels of IgM for a rapid response to foreign antigens. This mechanism occurs within several days, providing a timely response before full B cell activation *via* $CD4^+$ T cell help is established (Zhou *et al*., 2020b).

In contrast to TI activation, B cells also preferentially interact with $CD4^+$ T cells in a process known as TD activation. This pathway is the most common where activated $CD4^+$ T cells stimulate B cell activation through the interaction of CD40 ligand on T cells with CD40 on B cell surfaces, along with CXCR5 signalling, regardless of prior B cell exposure to other epitopes of the same antigen (Wangriatisak *et al*., 2022). Consequently, B cells within GCs undergo proliferation, somatic hypermutation and immunoglobulin class-switch recombination. Initially, these activated B cells express low-affinity IgM. Following activation within the GC, the cells may express other

immunoglobulins, such as IgA, IgE and IgG. In some cases, activated B cells express IgM without switching to other isotypes. These class-switching processes involve the excision of unwanted isotypes, which are influenced by cytokine signalling from T cells and other cells.

Specialised T follicular helper (Tfh) cells secrete cytokines that enhance B cell differentiation within the TD pathway. This process enables activated B cells to enter the B cell follicle, destined to produce specific antibody subtypes and undergo massive proliferation in the GC. After completing these processes, B cells exit the GC to differentiate into either memory B cells or plasmablasts (short-lived precursors to long-lived plasma cells) (Bonasia *et al*., 2021; Wangriatisak *et al.,* 2022).

2.3.2(a) Germinal centres (GCs)

During an immune response, the germinal centre (GC) is a transient structure within secondary lymphoid organs such as the spleen and lymph nodes. It plays a crucial role in the maturation and differentiation of B cells to produce high-affinity antibodies. Immature B cells that encounter antigens are assembled in the outer cortex of the lymph nodes or the white pulp of the spleen. These B cells then transition into transitional B cells and receive T-dependent (TD) stimulation, as the formation of GCs requires help from $CD4^+$ T cells (Young and Brink, 2021).

In the dark zone, activated B cells, called centroblasts, undergo rapid clonal expansion, resulting in a substantial increase in cell numbers. This expansion amplifies the immune response against a specific antigen, generating numerous identical daughter cells with the same BCR specificity (Olatunde *et al*., 2021; Young and Brink, 2021). Concurrently, somatic hypermutation occurs, driven by activation-induced cytidine deaminase (AID). AID introduces point mutations in the variable regions of

immunoglobulin genes, particularly in the rearranged BCR genes. This process generates a diverse pool of BCRs with varying affinities for the antigen. As B cells undergo these mutations, activation markers such as CD38 are upregulated, signalling their differentiation (Olatunde *et al*., 2021).

Following somatic hypermutation, B cells enter the process of affinity maturation. B cells expressing BCRs with higher affinity for the antigen are preferentially selected for survival through a positive selection process. These higher-affinity B cells are better equipped to bind to antigens presented by follicular dendritic cells (FDCs) in the light zone, leading to their continued proliferation and differentiation (Olatunde *et al*., 2021; Pan *et al.,* 2022). In contrast, B cells with lower-affinity BCRs are more likely to undergo apoptosis, ensuring that only the most effective B cells contribute to the immune response. This selective process enhances the overall effectiveness of the antibody response, ultimately resulting in the production of high-affinity antibodies in the later stages of B cell maturation (Olatunde *et al*., 2021; Young and Brink, 2021; Pan *et al.,* 2022; Inoue and Kurosaki, 2024).

Once completed, B cells move into the light zone, competing for antigens. Higher-affinity B cells, identified by CD19 and B220 expression, are more likely to engage with antigens and survive. The light zone is crucial for interactions with Tfh cells and FDCs (Mintz and Cyster, 2020). B cells with high-affinity BCRs bind to antigen-antibody complexes displayed by FDCs, which provide survival signals. Tfh cells, expressing CXCR5, migrate to B cell follicles and GCs, providing co-stimulatory signals such as CD40L and IL-21, which activate B cells and support plasma cell generation (Olatunde *et al*., 2021; Pan *et al.,* 2022).

B cells also express CXCR5 to aid their localisation within the GC, facilitating interactions with Tfh cells. IL-6 and IL-21 promote B cell responses and Tfh cell