Chapter 1

Introduction

1.1 Background

Innate immune response happens when the human body encounters pathogen and tissue damage, that acts as the body's first line of defense. The response immediately activates the body's defense mechanism by recognizing pathogen-associated molecular patterns (PAMPs) like bacterial lipopolysaccharide (LPS), viral RNA, or fungal β-glucans or damage-associated molecular patterns (DAMPs) like adenosine triphosphate (ATP), uric acid, or mitochondrial DNA (mtDNA) release (Land, 2018). Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-L-like receptors (RLRs), and inflammasomes are type of pattern recognition receptors (PRRs) each bind and detect different types of ligand involved in this pathway (Patergnani et al., 2021). A cascade of immune responses unfolds where PRRs promote the recruitment of cytokines to destroy the wound or heal the pathogens and cause inflammation response (Smith, 2018).

Without innate immunity, infection and cell/tissue damage would overwhelm the body before adaptive immunity could even start its cycle where the early stage of infection is the critical moment for increasing the survival rate. In some cases where the environment is under an extremely stressful condition, the downstream of an infection case could act as a double-edged sword since inflammatory response is evidently causing endothelial dysfunction which could lead to severe symptoms (De Boer et al., 2020). Generally, inflammatory responses promote immunosuppression to repair damaged tissue, but in the case of an impairment or dysregulation could lead to sepsis or chronic inflammation. This is highly found in previous cases of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV-2) during the pandemic era, where lots of patients were experiencing severe symptoms due to an impaired mitochondrial function activating toll-like receptor 9 (TLR-9) which trigger inflammation leading to endothelial cell dysfunction (Costa et al., 2022).

TLR-9 is primarily located in endosomal compartments, recognizing PAMPs such as unmethylated Cytosine-phosphate-Guanine (CpG) DNA motifs, a molecular signature abundantly found in bacterial and viral genomes. They also sense DAMPs, such as mtDNA released during cellular stress, apoptosis, or tissue injury (Rodriguez-Nuevo & Zorzano, 2019). This dual recognition capability positions TLR-9 as a critical sensor bridging infectious threats (e.g., sepsis, viral infections) and sterile inflammation (e.g., trauma, ischemia-reperfusion injury). Upon ligand binding, TLR-9 activates downstream signaling via the Myeloid Differentiation Primary Response 88-dependent pathway (MyD88-dependent pathway), triggering Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) and Interferon Regulatory Factor 7 (IRF7) to drive pro-inflammatory cytokines (e.g., TNF-α, IL-6, type I interferons) or immunosuppressive mediators (e.g., IL-10, IDO), depending on cellular context (Zheng et al., 2020). Dysregulation of TLR-9 signaling is implicated in various pathological conditions, including bronchopulmonary dysplasia (BPD), acute respiratory distress syndrome (ARDS), and systemic inflammatory response syndrome (SIRS) (Doğan et al., 2024).

Mesenchymal stem cells (MSCs) are recognized for their ability to modulate inflammation, reduce cell death, and promote tissue repair in various disease contexts (Regmi et al., 2019). Emerging studies suggest MSCs interaction with innate immune receptors such as Toll-like receptor 9 (TLR-9), which detects double-stranded DNA (dsDNA) and activates downstream MyD88/NF-κB signaling. Through this pathway, MSCs can influence cytokine production and immune responses. In models of lung injury and viral infection, MSCs have been shown to modulate TLR-9–related signaling to suppress inflammation and restore tissue homeostasis (Kim et al., 2024; Costa et al., 2022). Building on these findings, this study aims to investigate, through *in silico* analysis, whether dsDNA exposure affects the immunomodulatory behavior of MSCs under infection.

1.2 Objective

1. To investigate the effect of dsDNA on MSCs immunomodulation via TLR-9 signaling through in silico analysis of transcriptomic data

2. To identify genes and pathways associated with TLR-9–mediated immunomodulation in MSCs using in silico transcriptomic and enrichment analysis

1.3 Hypothesis

H_0 : dsDNA treatment has no effect on the expression of pro-inflammatory mediators on the downstream pathway of TLR9 activation in MSCs

H_1 : dsDNA treatment selectively downregulates pro-inflammatory effector molecules and adhesion factors, while simultaneously upregulating anti-inflammatory mediators downstream of TLR9 activation in MSCs.