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THESIS

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By:

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Theme:

Exploration of intracytosolic levels of LDs during the interaction between red blood cells and macrophages infected with *Pseudomonas aeruginosa*

Under the supervision of Professor Mourad ARIBI

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List of abbreviations

C

CD: Cluster of differentiation
CO_2: Carbon dioxide
Conc: Concentration

D

DMEM: Dulbecco's Modified Eagle Medium

E

EPS: Exopolysaccharides

F

FBS: Fetal bovine serum

H

HLA-DR: Human Leukocyte Antigen – DR Isotype

I

IL: Interleukin

L

LDL: low-density lipoprotein
LDs: Lipid droplets

M

M1: Pro-inflammatory macrophages
M2: Anti-inflammatory macrophages
MDMs: Monocyte-derived macrophages
MerTK: efferocytosis receptor c-Mer tyrosine kinase
MHC: Major histocompatibility complex
MMPs: Matrix metalloproteinase
MOI: Multiplicity of infection

O

ORO: Oil red O

P

Pa: *Pseudomonas aeruginosa*
PBMCs: Peripheral blood mononuclear cells
PBS: phosphate buffered saline
pH: potential of hydrogen

Q

QS: quorum sensing

R

RBCs: Red blood cells
Rh: Rhesus
RNS: Reactive nitrogen species
ROS: Reactive oxygen species

T

T3SS: type III secretion system
T4SS: type IV secretion system
TBET: Trypan blue exclusion test
TGs: Triglycerides
TNF: tumor necrosis factor

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Introduction

Pseudomonas aeruginosa is a gram-negative bacterium known for its ability to cause a wide range of infections. It belongs to the *Pseudomonadaceae* family. It is an opportunistic pathogen that particularly affects individuals with compromised immune systems or underlying health conditions [1].

P. aeruginosa produces a variety of virulence factors that contribute to its pathogenicity. It is frequently found in aquatic environments, rhizosphere, hospitals and other healthcare settings and industrial settings. It exhibits intrinsic and acquired resistance to many antibiotics due to its impermeable outer membrane, efflux pumps, and ability to produce β-lactamases and other enzymes that degrade antibiotics [1].

Monocyte-derived macrophages (MDMs) are a subset of macrophages that originate from circulating monocytes [2]. The production of monocytes occurs in the bone marrow through a process called hematopoiesis. Subsequently, monocytes are released into the bloodstream where they circulate until they receive signals to migrate into tissues. In tissues, monocytes differentiation into macrophages under the influence of the local environment and neighbouring cells [3]. It expresses different cluster of differentiation such as: CD14, CD11b, CD68 and CD11c. Macrophages are classified into two major phenotypes: M1 (classically activated) and M2 (alternatively activated) macrophages.

This classification helps understand the diverse roles of macrophages in immunity, inflammation, tissue repair, and homeostasis. Lipid droplets (LDs), stored in macrophages, are closely associated with the stimulation of phagocytic activity of M1 macrophages, but the exact mechanisms remain unclear [4]. They also serve as intracellular energy stores by compartmentalizing lipids, mainly in the form of triglycerides (TGs) [5]. In addition to their role in phagocytosis, these LDs play a crucial role in modulating macrophage immunity by regulating the production of inflammatory cytokines and influencing the response to infections [6]. They act as intracellular signaling centers, thus participating in the regulation of immune and inflammatory responses.

Red blood cells (RBCs) exists in abundance as a host cell in the human body, outnumbering the following most abundant circulating blood cells by some hundred times at least[7]. Their huge number and routine circulation between distant locations in the body make them primarily qualified to transduce signals of danger or infection between different components and organs of the immune system[8]. They play a significant role in nucleic acid binding, complement regulation, metabolism, and vascular regulation. Internal components of RBCs, such as hemoglobin and heme, play a role in innate immunity, capable of generating antimicrobial reactive oxygen species (ROS) to defend against invading hemolytic microbes, as well as promoting pathologic inflammatory and autoimmune responses [7].

I. Literature review

I. 1. *Pseudomonas Aeruginosa*

I.1.1.Definition:

Pseudomonas Aeruginosa is a gram-negative bacterium known for its ability to cause a wide range of infections. It belongs to the *Pseudomonadaceae* family. It is an opportunistic pathogen that affects particularly individuals with compromised immune systems or underlying health conditions [1].

I.1.2.Taxonomy:

Domain	Bacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	*Pseudomonadales*
Family	*Pseudomonadaceae*
Genus	*Pseudomonas*
Species	*aeruginosa*

Table I.1.1: *Pseudomonas Aeruginosa* taxonomy

I.1.3.Virulence factors:

P. aeruginosa produces a variety of virulence factors that contribute to its pathogenicity, including: exopolysaccharides (EPS), exotoxins, type III secretion system (T3SS), type IV secretion system (T4SS), quorum sensing (QS) systems, Antibiotic resistance mechanisms, adhesion molecules and iron acquisition systems [2].

I.1.4.*Pseudomonas aeruginosa* habitat:

P. aeruginosa is frequently found in aquatic environments, rhizosphere, hospitals and other healthcare settings and industrial settings.

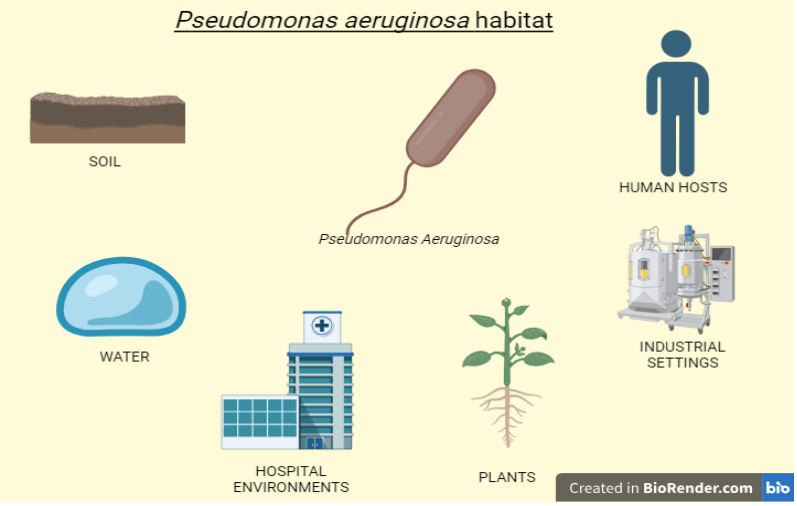

Figure I.1.1: *Pseudomonas aeruginosa* habitat.

I.1.5. Resistance and pathogenicity:

Pseudomonas aeruginosa is a versatile opportunistic pathogen capable of causing a wide range of infections, particularly in individuals with compromised immune systems, underlying health conditions, or medical devices[1].

Antibiotic Resistance: *Pseudomonas aeruginosa* exhibits intrinsic and acquired resistance to many antibiotics due to its impermeable outer membrane, efflux pumps, and ability to produce β-lactamases and other enzymes that degrade antibiotics[2].

Adaptation to Host Environment: *Pseudomonas aeruginosa* is adept at adapting to diverse host environments, including the respiratory tract, urinary tract, skin, and soft tissues[2].

Nosocomial Infections: *Pseudomonas aeruginosa* is a leading cause of nosocomial (hospital-acquired) infections, particularly in critically ill patients, those with burns, cystic fibrosis, or immunocompromised individuals[3].

I.1.6. Interaction with host:

The interaction of *pseudomonas aeruginosa* with the host includes different mechanisms. The main aspects of this interaction are as follow:

1. **Adhesion and colonization:**

Pili and Fimbriae: *Pseudomonas aeruginosa* uses pili and fimbriae to adhere to epithelial cells and other surfaces[4].

Flagella: The bacterium uses its flagella for motility, which helps it reach and colonize host tissues[4].

2. **Biofilm formation:**

Extracellular polymeric substance (EPS): *Pseudomonas aeruginosa* forms biofilms by producing EPS, which encases the bacterial community and protects it from the host

immune system and antibiotics. Biofilms are particularly problematic in chronic infections[4].

3. **Immune Evasion:**

Pseudomonas aeruginosa uses the T3SS to inject effector proteins into host cells and uses the alginate as a component to protect itself from phagocytosis and oxidative stress[4].

4. **Toxin production:**

Pseudomonas aeruginosa produces Exotoxin A to stop cells proteins synthesis, elastases and proteases to degrade host tissues and components, Pyocyanin to help generate reactive oxygen species.

5. **Invasion and dissemination:**

P.aeruginosa secretes phospholipases to degrade phospholipids of the host cell, and siderophores to sequester iron from the host.

6. **Host immune response:**

Innate immune response: The host immune system responds to *Pseudomonas aeruginosa* by attempting phagocytosis and producing cytokines[5].

Adaptive immune response: The adaptive immune response involves the activation of T cells and B cells, which produce antibodies targeting *Pseudomonas aeruginosa* antigens[6].

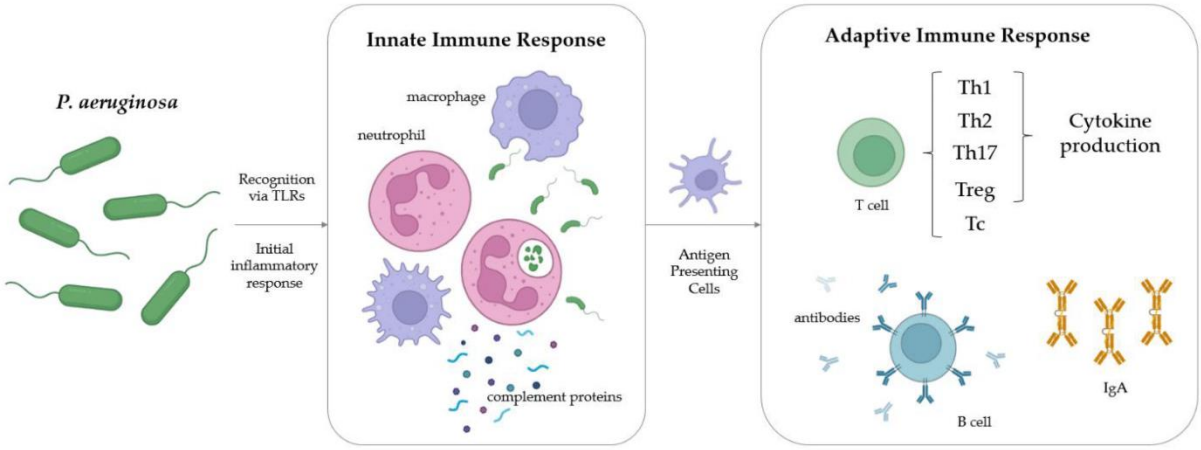

Figure I.1.2: A summary of the key aspects of the host immune response against *P. aeruginosa* infection[7].

I.2. Monocyte-derived macrophages

I.2.1Definition:

Monocyte-derived macrophages (MDMs) are a subset of macrophages that originate from circulating monocytes [8]. The production of monocytes occurs in the bone marrow through a phenomenon called hematopoiesis. Subsequently, monocytes are released into the bloodstream where they circulate until they receive signals to migrate into tissues. In tissues, monocytes differentiation into macrophages under the influence of the local environment and neighbouring cells [9].

I.2.2.Ontogeny:

Monocyte-derived macrophages ontogeny includes a few steps that goes:

Monocytes production: In the bone marrow, monocytes are produced from hematopoietic stem cells as part of the hematopoiesis process. Monocyte progenitors comes from the differentiation of hematopoietic stem cells into common myeloid progenitors, which then differentiate into monocyte progenitors[10].

I.2.3.MDMs identification markers:

CD14: CD14 is a well-known marker for monocytes and macrophages. It is a glycoprotein that is expressed on the surface of monocytes, macrophages, and dendritic cells [11].

CD11b (Integrin alpha M): CD11b is a component of the integrin receptor family and is expressed on the surface of monocytes, macrophages, neutrophils, and some dendritic cells [11].

CD68: CD68 is a glycoprotein that is highly expressed in macrophages, including MDMs [12].

CD163: CD163 is a scavenger receptor primarily expressed on monocytes and macrophages [13].

CD206 (Mannose Receptor): CD206 is a C-type lectin receptor expressed on macrophages, dendritic cells, and some endothelial cells[11].

CD11c: CD11c is a marker for dendritic cells, but it is also expressed on a subset of macrophages, including MDMs[11].

HLA-DR : Human leukocyte antigen-DR (HLA-DR) is a major histocompatibility complex class II molecule expressed on antigen-presenting cells, including monocytes and macrophages [11].

MerTK: Mer tyrosine kinase (MerTK) is a receptor tyrosine kinase that is predominantly expressed on macrophages, including MDMs [11].

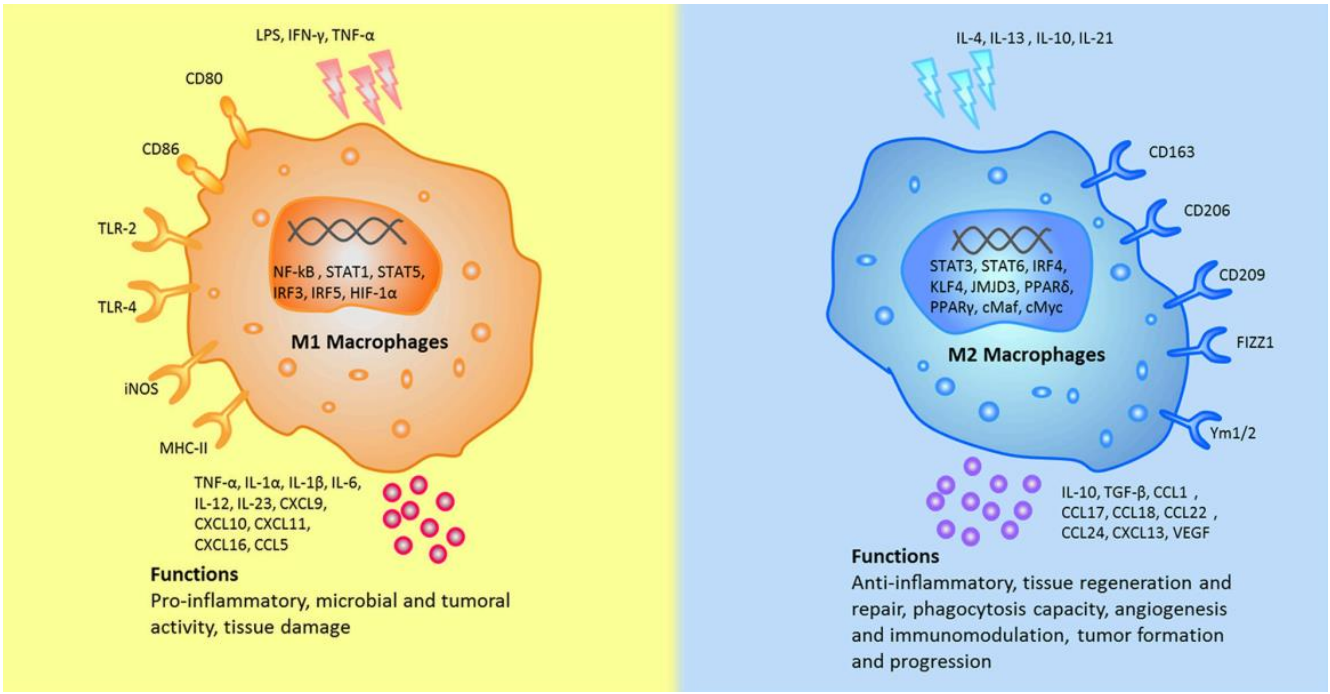

Figure I.2.1: Comparison of stimuli, surface markers, secreted cytokines, and biological functions between M1 and M2 macrophages [14].

I.2.4.Phenotypes:

M1 macrophages: Pro-inflammatory macrophages are classically activated macrophages that respond to microbial products and pro-inflammatory cytokines. M1 macrophages are characterized by the production of pro-inflammatory cytokines such as IL-1β, TNF-α, IL-6, and IL-12. These cytokines play a role in promoting inflammation and immune responses against pathogens [15].

M2 macrophages: Anti-inflammatory or regulatory macrophages are alternatively activated macrophages that respond to anti-inflammatory cytokines. Unlike M1 macrophages, M2 macrophages are recognized for their production and expression of anti-inflammatory cytokines such as IL-10 and TGF-β. Additionally, they secrete factors that promote tissue repair and remodelling [15].

M2 macrophages have three different functional sub-types m2a (associated with tissue repair), m2b (associated with immunoregulation) and m2c (associated with the resolution of inflammation and tissue remodelling)[16].

I.2.5. Functions:

MDMs play a crucial role in immune responses, tissue homeostasis, and disease processes. Here are some main functions of MDMs:

Function	Details
Phagocytosis	MDMs are a first degree phagocytic immunocytes capable of engulfing and digesting numerous particles, such as viruses, bacteria, fungi, as well as dead cells and cellular debris[17].
Antigen Presentation	MDMs are professional antigen-presenting cells that capture and present antigens to CD4+ T lymphocytes on their MHC II[18].
Tissue Repair and Remodelling	MDMs play a major role in cell repair and remodelling after an injury or an infection by the secretion of growth factor, matrix metalloproteinases (MMPs), and other molecules that promote angiogenesis, remodelling and wound healing[19].
Regulation of Inflammation	MDMs have an important role in inflammation regulation for they have both, pro-inflammatory and anti-inflammatory phenotypes, depending on the signals they receive from the micro-environment[20].
Host Defence	Through different mechanisms such as phagocytosis, production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and secretion of antimicrobial peptides, MDMs contribute on killing directly invading micro-organisms[18].
Immune Regulation	Through the expression chemokines and cytokines, MDMs regulate the activation, differentiation and function of different cells such as T cells, B cells, dendritic cells and NK cells[18].

Table I.2. Monocyte-derived macrophages functions

I.2.6.Lipid droplets functions:

Macrophage lipid droplets serve several important functions in cellular tissue homeostasis, immune responses, and metabolism. Here are some key functions of macrophage lipid droplets:

Function	Details
Lipid Storage and Energy Reserve	Lipid droplets in macrophages serve as storage organelles for neutral lipids, primarily triglycerides and cholesterol esters[21].
Cholesterol Homeostasis	Macrophage lipid droplets play a crucial role in maintaining cholesterol homeostasis by sequestering excess cholesterol and preventing its accumulation in the plasma membrane[21].
Foam Cell Formation	In the context of atherosclerosis, macrophages take up modified low-density lipoproteins (LDL) and accumulate cholesterol esters, leading to the formation of foam cells within atherosclerotic plaques[22].
Immune Regulation	Lipid droplets in macrophages can serve as platforms for the integration of metabolic and immune signalling pathways[15].
Antimicrobial Defence	Lipid droplets in macrophages have been shown to play a role in antimicrobial defence against intracellular pathogens[23].
Stress Response and Cell Survival	Under conditions of cellular stress, such as oxidative stress or nutrient deprivation, macrophage lipid droplets may undergo dynamic changes in size and composition as part of the cellular stress response[24].

Table I.2.2. Monocyte-derived macrophages lipid droplets functions

I.3. Erythrocytes

I.3.1.Definition:

Erythrocytes exist ample in the human body as the most abundant cell type. These cells account nearly 70 percent of the total cell count in an average adult [25]. Despite losing of their nucleic components and organelles during erythropoiesis, their huge number and routine passage between distant locations in the body allow them to transduce signals of danger or infection between different components and organs of the immune system [26].

I.3.2.Ontogeny:

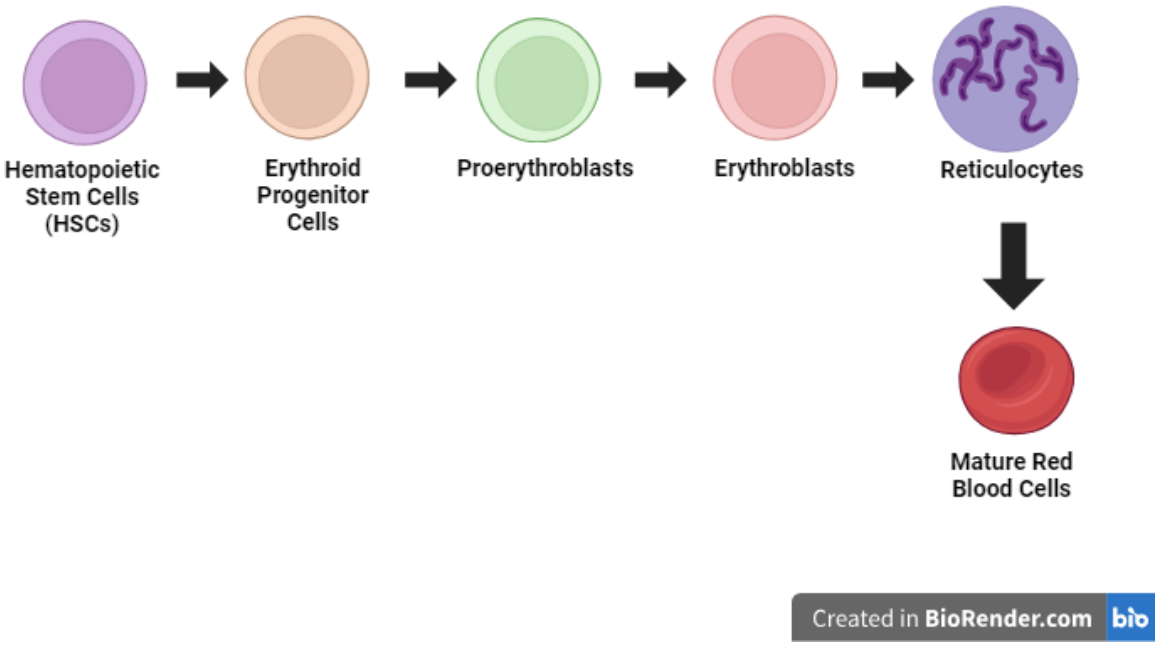

Figure I.3.1: Red blood cells ontogeny.

I.3.3.RBCs identification markers:

Red blood cells are usually identified based on their morphology (disc shape and absence of nucleus). However, the surface markers used to identify RBCs are limited due to the lack of organelles and membrane proteins. These are the markers commonly used to identify erythrocytes or characterize red blood cells:

Blood Group Antigens: Erythrocytes mainly known for the expression of blood group antigens on their surface, including ABO antigens and Rh antigens[27].

CD235a: Glycophorin a is involved in maintaining RBCs structural integrity and is commonly used as a marker for erythrocytes in flow cytometry[28].

CD71: It is expressed at low levels on reticulocytes and not mature erythrocytes, but we can consider it as an identification marker since it is used for identifying immature erythrocytes[29].

CD147: Also known as basigin, is a transmembrane glycoprotein that is not exclusively expressed on the surface of erythrocytes but can be used as an identification marker of RBCs in some studies[30].

I.3.4. Functions:

Oxygen Transport : RBCs are primarily known for transporting oxygen from the lungs to tissues throughout the body [31].

Carbon Dioxide Transport: Red blood cells play a crucial role in transporting CO_2 from tissues to lungs for exhalation [32].

Buffering pH: Erythrocytes help regulate the pH of the blood by acting as buffers.

Nitric Oxide Transport: Red blood cells are also involved in transporting nitric acid throughout the body, which is a signalling molecule that helps regulate blood flow and vascular tone[33].

Maintaining Cell Shape and Flexibility : Their biconcave disc shape that allows them to perform various functions without rupturing tissues [34].

Immune Modulation: Erythrocytes have recently been considered as immunocytes due to their crucial role in immunology. They help transduce signals between distant cells and assuring other immune functions[26].

I.4. Problematic and objectives

I.4.1 Problematic

Pseudomonas aeruginosa, a gram-negative opportunistic bacterium, is responsible for various human infections and is known for its ability to adapt to adverse environmental conditions. Macrophages, as tissue phagocytic cells, play an essential role in immune regulation by capturing and presenting antigens.

Lipid droplets (LDs), stored in macrophages, are closely associated with stimulating the phagocytic activity of M1 macrophages, but the exact mechanisms remain unclear. These LDs also serve as intracellular energy stores by compartmentalizing lipids, mainly in the form of triglycerides (TGs). These LDs, in addition to their role in phagocytosis, also play a crucial role in modulating macrophage immunity by regulating the production of inflammatory cytokines and influencing the response to infections. Acting as intracellular signaling centers, they participate in regulation immune and inflammatory responses.

On the other hand, red blood cells, the most abundant cells in humans, can play a role in immune complex elimination by promoting phagocytosis and antigen presentation through immune adhesion, contributing to the regulation of inflammatory responses. In this context, our objective is to evaluate the interaction between red blood cells and macrophages infected with *Pseudomonas aeruginosa*, focusing on LDs and TG levels.

I.4.2 Objectives

- Evaluate of the interaction between red blood cells and macrophages infected with *Pseudomonas aeruginosa*.
- Determine the levels of lipid droplets and triglyceride.

I.4.3 Purpose

Our aim is to explore the implication of lipid droplets in the polarization of macrophages and red blood cells during *Pseudomonas aeruginosa* infection.

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