N° d'ordre : _____

UNIVERSITY OF TLEMCEN - ABOU-BEKR BELKAÏD

FACULTY SNV-STU - DEPARTMENT OF BIOLOGY

LABORATORY OF APPLIED MOLECULAR BIOLOGY AND IMMUNOLOGY

- BIOMOLIM-

MEMORY

Submitted for the degree of

Master in Biological Sciences

Immunology Specialty

By: ABDERRAHMAN Walid

Defended on

14/06/2026

Topic:

Influence of red blood cells inducing hypoxic stress on lipid droplet formation in human monocytes.

_____under the supervision of professor **Mourad ARIBI**_____

Jury

Pr. Mohammed CE Ismet SMAHI	Professeur	Université de Tlemcen, Algérie	Président
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Dr. Zineb HADJIDJ	MCB	Université de Tlemcen, Algérie	Examinatrice

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Résumé

Résumé :

Introduction : Les globules rouges (GR) sont désormais reconnus comme des cellules immunomodulatrices actives capables d'influencer les réponses immunitaires innées. Les monocytes, en tant qu'effecteurs centraux de l'immunité innée, accumulent des gouttelettes lipidiques (GL) intracellulaires lors de leur activation organites établis comme des plateformes inflammatoires inductibles. La communication fonctionnelle entre les érythrocytes déficients en hémoglobine (GR^{-}/Hb^{-}) et le métabolisme lipidique des monocytes demeure peu caractérisée. Cette étude avait pour objectif d'évaluer *ex vivo* l'impact des GR autologues déficients en hémoglobine sur le métabolisme lipidique des monocytes notamment le cholestérol cellulaire total (tccCHOL), les triglycérides intracellulaires (iTAG) et l'accumulation des gouttelettes lipidiques.

Matériels et méthodes : Du sang veineux périphérique a été prélevé chez des patients présentant une déficience en hémoglobine. Les monocytes ont été isolés par centrifugation sur gradient de densité, puis purifiés par adhérence sélective sur plastique. Les GR autologues ont été récupérés du même gradient et remis en suspension dans du DMEM complet. Quatre conditions expérimentales ont été établies. Le tccCHOL et l'iTAG ont été dosés par méthodes enzymatiques colorimétiques et normalisés aux protéines totales. Les gouttelettes lipidiques ont été visualisées par coloration à l'Oil Red O et quantifiées par fluorescence totale corrigée (CTCF) via Fiji/ImageJ et CellProfiler.

Résultats : La teneur en cholestérol cellulaire total est restée stable dans les quatre conditions, sans différence statistiquement significative entre les groupes. En revanche, les niveaux de triglycérides intracellulaires ont présenté un profil nettement différent. L'accumulation des gouttelettes lipidiques, évaluée par CTCF (Oil Red O), a augmenté significativement en réponse à la stimulation par LPS et/ou Hb, avec un effet maximal observé en double stimulation LPS+Hb+.

Conclusion : Cette étude montre que les GR déficients en Hb modulent le métabolisme lipidique des monocytes en favorisant l'accumulation de gouttelettes lipidiques, particulièrement en double stimulation LPS+Hb+, positionnant ces organites comme acteurs clés de l'inflammation innée dans un contexte de déficience en hémoglobine.

Mots clés : Globules rouges, Monocytes, Gouttelettes lipidiques, Triglycérides, Déficience en hémoglobine, Immunité innée, Cholestérol.

Abstract

Abstract:

Introduction: Red blood cells (RBCs) are now recognized as active immunomodulatory cells capable of influencing innate immune responses. Monocytes, as central effectors of innate immunity, accumulate intracellular lipid droplets (LDs) upon activation — organelles established as inducible inflammatory platforms. The functional communication between hemoglobin-deficient erythrocytes (RBC^{-}/Hb^{-}) and monocyte lipid metabolism remains poorly characterized. This study aimed to assess ex vivo the impact of autologous hemoglobin-deficient RBCs on monocyte lipid metabolism, including total cellular cholesterol (tccCHOL), intracellular triglycerides (iTAG), and lipid droplet accumulation.

Materials and Methods: Peripheral venous blood was collected from hemoglobin-deficient patients. Monocytes were isolated by density gradient centrifugation and purified by selective plastic adherence. Autologous RBCs were recovered from the same gradient and resuspended in complete DMEM. Four experimental conditions were established. tccCHOL and iTAG were quantified by enzymatic colorimetric methods and normalized to total proteins. Lipid droplets were visualized by Oil Red O staining and quantified by corrected total cell fluorescence (CTCF) using Fiji/ImageJ and CellProfiler.

Results: Total cellular cholesterol remained stable across all four conditions, with no statistically significant difference between groups. In contrast, intracellular triglyceride levels showed a markedly different profile. Lipid droplet accumulation, assessed by CTCF (Oil Red O), increased significantly in response to LPS and/or Hb stimulation, with a maximal effect observed under dual LPS+Hb+ stimulation.

Conclusion: This study demonstrates that Hb-deficient RBCs modulate monocyte lipid metabolism by promoting lipid droplet accumulation, particularly under dual LPS+Hb+ stimulation, positioning these organelles as key players in innate inflammation in the context of hemoglobin deficiency.

Keywords: Red blood cells, Monocytes, Lipid droplets, Triglycerides, Hemoglobin deficiency, Innate immunity, Cholesterol.

الملخص

الملخص :

المقدمة: : تُعرف خلايا الدم الحمراء الآن بأنها خلايا مناعية نشطة قادرة على التأثير في الاستجابات المناعية الفطرية. وتُعدّ الخلايا الوحيدة، باعتبارها الفاعل الرئيسي في المناعة الفطرية، مسؤولة عن تراكم قطرات الدهون داخل الخلايا عند تنشيطها، وهي عضيات تُعتبر منصات التهابية قابلة للتحفيز. ولا يزال التواصل الوظيفي بين كريات الدم الحمراء ناقصة الهيموجلوبين واستقلاب الدهون في الخلايا الوحيدة غير مفهوم بشكل كافٍ. هدفت هذه الدراسة إلى تقييم تأثير كريات الدم الحمراء الذاتية ناقصة الهيموجلوبين على استقلاب الدهون في الخلايا الوحيدة خارج الجسم الحي، بما في ذلك الكوليسترول الخلوي الكلي، والدهون الثلاثية داخل الخلايا، وتراكم قطرات الدهون .

المواد والطرق: جُمعت عينات دم وريدي طرفي من مرضى يعانون من نقص الهيموجلوبين. عُزلت الخلايا الوحيدة باستخدام الطرد المركزي بتدرج الكثافة، ونُقيت عن طريق الالتصاق الانتقائي بالبلاستيك. تم استخلاص كريات الدم الحمراء الذاتية من نفس التدرج وإعادة تعليقها في وسط DMEM كامل. تم تحديد أربع حالات تجريبية. تم قياس الكوليسترول الخلوي الكلي (tccCHOL) والدهون الثلاثية داخل الخلايا (iTAG) باستخدام طرق قياس الألوان الإنزيمية، وتمت معايرتهما بالنسبة إلى البروتينات الكلية. تم تصوير قطرات الدهون باستخدام صبغة الزيت الأحمرO ، وتم قياسها كميًا باستخدام تقنية التألق الخلوي الكلي المصحح (CTCF) باستخدام برنامجي Fiji/ImageJ وCellProfiler .

النتائج: ظل الكوليسترول الخلوي الكلي مستقرًا في جميع الحالات الأربع، دون وجود فرق ذي دلالة إحصائية بين المجموعات. في المقابل، أظهرت مستويات الدهون الثلاثية داخل الخلايا نمطًا مختلفًا بشكل ملحوظ. ازداد تراكم قطرات الدهون، الذي تم تقييمه باستخدام تقنية) CTCF الزيت الأحمر(O ، بشكل ملحوظ استجابةً لتحفيز LPS و/أوHb ، مع ملاحظة أقصى تأثير عند التحفيز المزدوج بـ LPS وHb.

الخلاصة: تُظهر هذه الدراسة أن كريات الدم الحمراء المُفتقرة للهيموجلوبين تُعدّل استقلاب الدهون في الخلايا الوحيدة عن طريق تعزيز تراكم قطرات الدهون، لا سيما في ظل التحفيز المزدوج بالليبوساكاريد والهيموجلوبين، مما يجعل هذه العضيات عناصر أساسية في الالتهاب الفطري في سياق نقص الهيموجلوبين.

الكلمات المفتاحية، كريات الدم الحمراء، الخلايا الوحيدة، قطرات الدهون، الدهون الثلاثية، نقص الهيموجلوبين، المناعة الفطرية، الكوليسترول.

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Au terme de ce travail, il nous est agréable d'adresser nos plus sincères remerciements à toutes les personnes qui ont contribué, de près ou de loin, à l'élaboration de ce mémoire de fin d'études.

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Enfin, nous adressons nos remerciements les plus chaleureux à nos familles, pour leur amour inconditionnel, leur patience et leur soutien indéfectible qui ont été notre force tout au long de ce parcours.

Merci à tous.

Dédicace

❥

Je dédie ce modeste travail, fruit de longues années d'efforts et de persévérance,

À mes très chers parents,

Ma mère, source inépuisable d'amour et de tendresse, dont les prières m'ont accompagné à chaque étape de ma vie. Mon père, mon premier modèle, qui m'a appris la valeur du travail, de l'honneur et de la dignité.

À mes chers frères,

Pour leur présence, leur complicité et les moments de joie partagés qui rendent la vie plus belle.

À mon encadreur, Monsieur le Professeur Aribi Mourad,

En témoignage de ma reconnaissance pour sa guidance et son dévouement.

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Monsieur Hichem Zerrouk, Madame Sedjai Dounia et Madame Nour El Houda Bendjeffel,

Pour leur soutien précieux, leur bienveillance et leur générosité.

Je vous dédie ce travail avec tout mon amour et ma gratitude.

❥

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Liste of abbreviations

Liste of abbreviations :

RBC : Red Blood Cell

Hb : Hemoglobin

LD : Lipid Droplet

EV : Extracellular Vesicle

RBC-EV : Red Blood Cell-Derived Extracellular Vesicle

CR1 : Complement Receptor 1

DARC : Duffy Antigen Receptor for Chemokines

TLR : Toll-Like Receptor

TLR9 : Toll-Like Receptor 9

CD163 : Cluster of Differentiation 163

HO-1 : Heme Oxygenase-1

HSC : Hematopoietic Stem Cell

CMP : Common Myeloid Progenitor

GMP : Granulocyte-Macrophage Progenitor

MDP : Macrophage-Dendritic Cell Progenitor

CCR2 : C-C Chemokine Receptor Type 2

DGAT1 : Diacylglycerol Acyltransferase 1

TAG : Triacylglycerol

PGE2 : Prostaglandin E2

ABCA1 : ATP-Binding Cassette Transporter A1

ABCG1 : ATP-Binding Cassette Transporter G1

LXR : Liver X Receptor

SREBP : Sterol Regulatory Element-Binding Protein

25-HC : 25-Hydroxycholesterol

IFN : Interferon

TNF-α : Tumor Necrosis Factor Alpha

IL : Interleukin

ROS : Reactive Oxygen Species

SARS-CoV-2 : Severe Acute Respiratory Syndrome Coronavirus 2

COX-2 : Cyclooxygenase-2

CTCF : Corrected Total Cell Fluorescence

Liste of abbreviations

DMEM : Dulbecco's Modified Eagle's Medium

EDTA : Ethylenediaminetetraacetic Acid

GPO-POD : Glycerol Phosphate Oxidase-Peroxidase

HIF-1α : Hypoxia-Inducible Factor 1-alpha

iNOS : Inducible Nitric Oxide Synthase

iTAG : Intracellular Triacylglycerol

LPS : Lipopolysaccharide

MO : Monocyte

NF-κB : Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells

Nrf2 : Nuclear Factor Erythroid 2-Related Factor 2

PBS : Phosphate-Buffered Saline

PBMC : Peripheral Blood Mononuclear Cell

PPARγ : Peroxisome Proliferator-Activated Receptor Gamma

PS : Phosphatidylserine

SIRPα : Signal Regulatory Protein Alpha

TBET : Trypan Blue Exclusion Test

tccCHOL : Total Cellular Cholesterol Content

Introduction

Introduction:

Red blood cells (RBCs), traditionally viewed as passive oxygen carriers, are increasingly recognized as active regulators of immune homeostasis. Beyond their essential role in gas exchange, erythrocytes participate in the clearance of immune complexes, circulating nucleic acids, and inflammatory mediators, thereby shaping innate and adaptive immune responses (Stowell et al., 2018; Stephenson et al., 2022). Mature human RBCs express complement receptor 1 (CR1/CD35), enabling the sequestration and removal of complement-opsonized immune complexes, while recent evidence has revealed their capacity to sense extracellular DNA through Toll-like receptor 9 (TLR9), highlighting an unexpected role in immune surveillance and inflammatory regulation (Cornacoff et al., 1995; Lam et al., 2021). These discoveries have fundamentally altered the classical perception of erythrocytes, positioning them as dynamic components of the immune network rather than inert bystanders.

Importantly, alterations in erythrocyte physiology profoundly influence their immunomodulatory properties. Defects in hemoglobin content are associated with oxidative imbalance, membrane remodeling, altered vesiculation, and the release of bioactive metabolites capable of affecting neighboring immune cells. However, despite growing evidence linking dysfunctional erythrocytes to chronic inflammation and immune dysregulation, the mechanisms through which hemoglobin-deficient RBCs shape innate immune cell function remain poorly understood.

Monocytes represent an ideal target for such erythroid-derived signals. As circulating sentinels of the innate immune system, monocytes rapidly respond to environmental perturbations by adapting their inflammatory, metabolic, and antimicrobial programs. Beyond their established roles in cytokine production, phagocytosis, and antigen presentation, monocytes exhibit remarkable metabolic plasticity that governs the magnitude and quality of immune responses (Kapellos et al., 2020; Bagger et al., 2021). Recent advances in immunometabolism have demonstrated that cellular metabolism is not merely a consequence of activation but a central determinant of immune cell fate and function.

Introduction

Among the metabolic organelles orchestrating these responses, lipid droplets (LDs) have emerged as critical regulators of innate immunity. Once considered inert lipid storage structures, LDs are now recognized as highly dynamic organelles integrating lipid metabolism, inflammatory signaling, and host defense mechanisms (Bozza and Viola, 2010). Activation of pattern-recognition receptors, particularly TLR2 and TLR4, rapidly induces LD biogenesis through coordinated activation of lipogenic pathways involving PPARγ, SREBP-1, and DGAT1, resulting in the accumulation of neutral lipids within activated monocytes and macrophages (Melo and Weller, 2024). These organelles serve as specialized platforms for the synthesis of bioactive lipid mediators and participate directly in the regulation of inflammatory responses during infection and tissue injury.

Beyond their role in acute inflammation, lipid droplets have recently gained attention as hallmarks of trained immunity, a form of innate immune memory characterized by long-lasting functional reprogramming following an initial stimulus (Netea et al., 2020). Trained monocytes undergo profound metabolic rewiring involving enhanced lipid biosynthesis, cholesterol metabolism, and epigenetic remodeling, resulting in amplified responses upon secondary challenge. In particular, increased intracellular cholesterol accumulation and alterations in cholesterol homeostasis have emerged as central drivers of trained immunity, linking lipid metabolism to sustained inflammatory potential (Bekkering et al., 2018; Sohrabi et al., 2024). The quantification of total cellular cholesterol (tccCHOL) therefore represents a valuable metabolic signature reflecting the establishment of trained immune programs.

Whether erythrocyte-derived signals contribute to this metabolic reprogramming remains largely unexplored. Given that hemoglobin-deficient RBCs display profound alterations in redox status, membrane composition, and lipid metabolism, we hypothesized that they may influence monocyte immunometabolic adaptation by promoting lipid droplet formation and cholesterol accumulation. Such mechanisms could constitute an unrecognized pathway through which dysfunctional erythrocytes shape innate immune memory and inflammatory responses.

Introduction

In this study, we investigated the impact of hemoglobin-deficient RBCs on lipid droplet biogenesis and total cellular cholesterol accumulation in human monocytes. By exploring these metabolic hallmarks of trained immunity, we sought to determine whether altered erythrocytes can actively reprogram monocyte function and contribute to the establishment of a persistent innate immune phenotype.

Objectives and Aims of the Study:

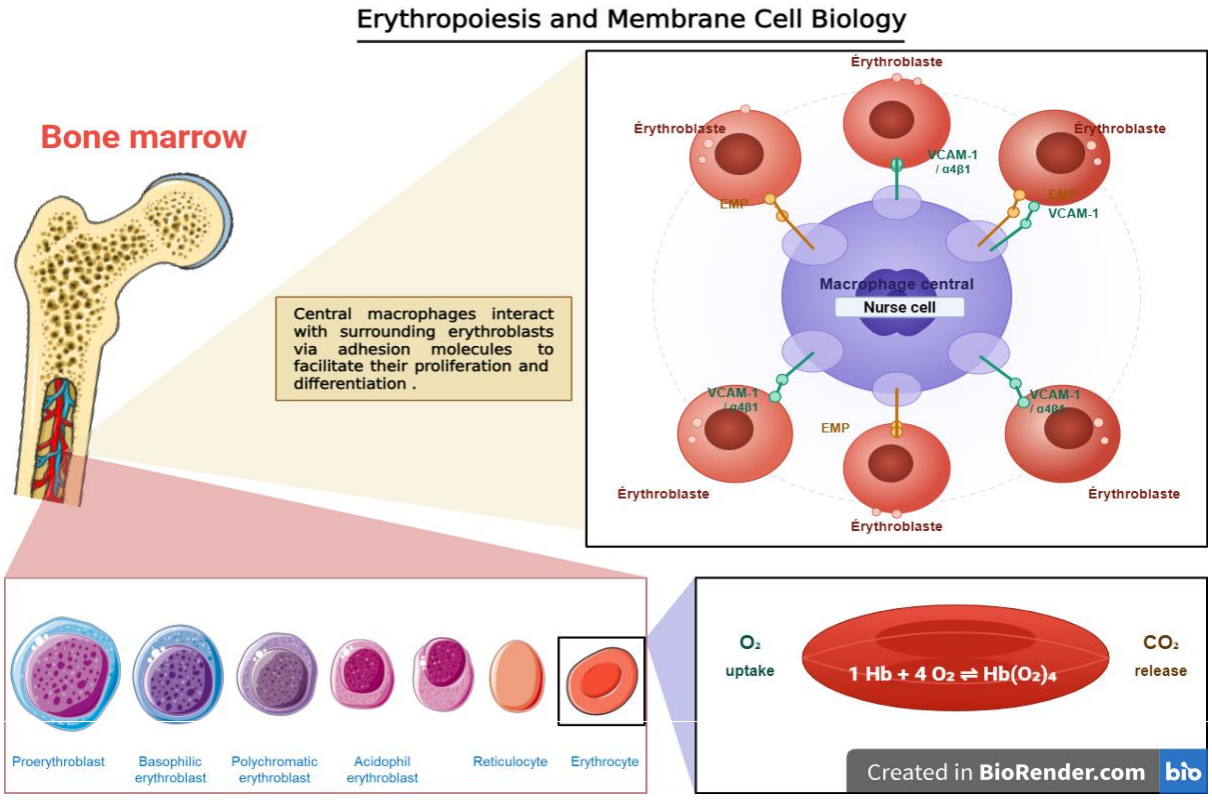
Despite considerable advances in our understanding of erythrocyte immunomodulation, monocyte biology, and LD-mediated inflammation, the functional crosstalk between circulating RBCs and monocytes and its impact on LD dynamics remains incompletely characterized. The present study was therefore designed to investigate the modulation of monocyte activation in the context of erythrocyte-monocyte interactions; characterize the kinetics and magnitude of LD biogenesis in monocyte under defined inflammatory stimuli; and assess the downstream immunological consequences of LD formation. By integrating lipid imaging, this work aims to delineate a previously underexplored axis of innate immune regulation with potential relevance to infectious, inflammatory, and metabolic disease

I. LITERATURE REVIEW:

1. RED BLOOD CELLS: CELLULAR BIOLOGY, SENESCENCE, AND IMMUNE FUNCTIONS

1.1. Erythropoiesis and Membrane Cell Biology:

Developmentally, the origins of the erythrocyte lineage can be traced back to the embryonic yolk sac, where primitive erythrocytes are generated prior to the definitive emergence of hematopoietic stem cells (Dzierzak et Bigas 2018). Mature red blood cells are generated through erythropoiesis, a finely regulated process occurring within specialized bone marrow niches known as erythroblastic islands (Moras et al. 2017). Within these islands, central macrophages interact closely with surrounding erythroblasts via adhesion molecules—such as VCAM-1, α4β1 integrin, and erythroblast macrophage protein (EMP)— (Fang et al. 2025). Terminal maturation requires the dramatic expulsion of the erythroblast nucleus, generating a pyrenocyte that exposes phosphatidylserine for prompt engulfment by the central macrophage (De Back et al. 2014; Giri et Tamgadge 2025). This vital deformability is conferred by a highly specialized membrane skeleton, composed of a spectrin-actin filamentous network tethered to a fluid lipid bilayer via integral proteins like band 3 (Huang 2026).



LITERATURE REVIEW

1.2. RBC Senescence and Erythrophagocytosis:

During their approximately 120-day lifespan, circulating erythrocytes accumulate oxidative and mechanical damage, leading to progressive biophysical and biochemical membrane alterations (Huang 2026) .

Senescent erythrocytes undergo significant remodeling, characterized by desialylation of surface glycoproteins and the oxidative-induced clustering of band 3, creating neoantigens that bind naturally occurring autologous immunoglobulins (IgG) (De Back et al. 2014).

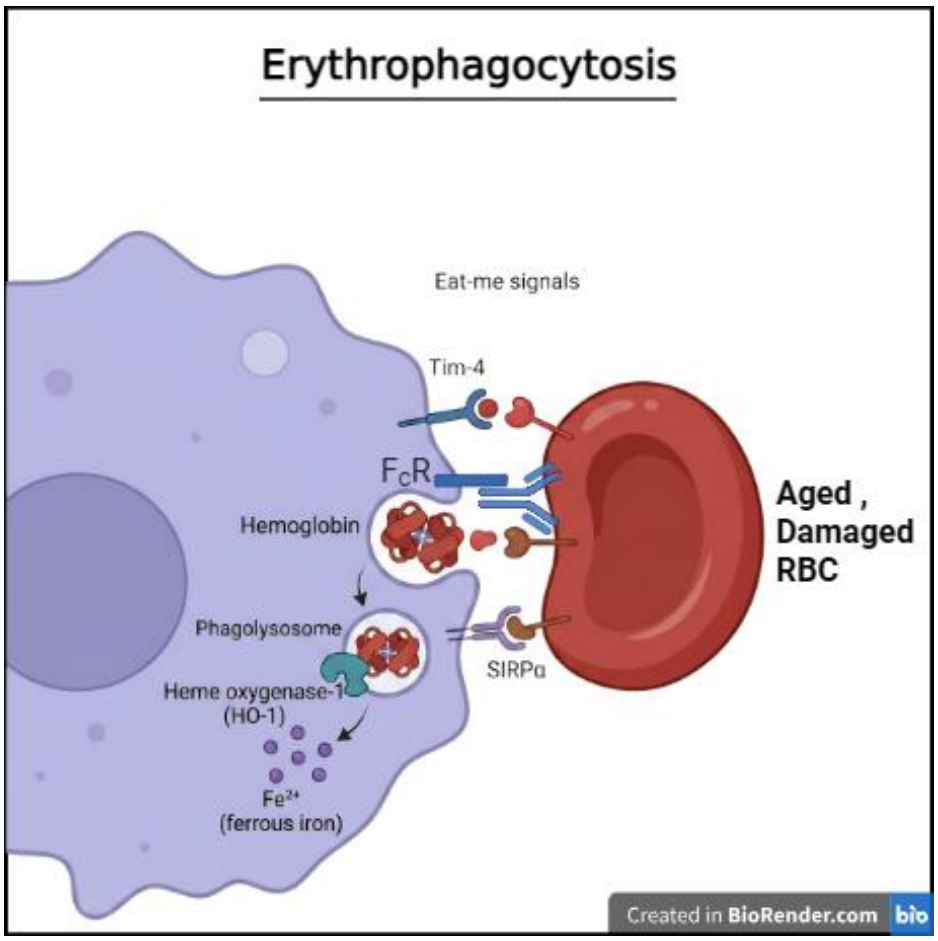
Concurrently, cellular aging and injury promote the loss of membrane lipid asymmetry, resulting in the externalization of phosphatidylserine (PS), which acts as a highly conserved "eat-me" signal (Joshi et al. 2025).

Furthermore, conformational changes in the CD47 molecule induced by oxidative stress switch its typical "don't eat me" inhibitory signal into a pro-phagocytic signal (De Back et al. 2014).

In the splenic red pulp, the maturation of specialized iron-recycling macrophages responsible for RBC clearance is critically driven by the cytokine IL-33 and erythrocyte-derived hemin (D. Xu et Huang 2020).

These macrophages recognize "eat-me" signals via receptors such as Tim-4, Fc receptors, and signal regulatory protein alpha (SIRPα), enzymatically degrading internalized hemoglobin via heme oxygenase-1 (HO-1) to recycle essential ferrous iron (Lévesque et al. 2021).

Pathological conditions, such as malaria and COVID-19, induce severe biochemical alterations in erythrocytes, decreasing their deformability and accelerating their phagocytic clearance by macrophages (Bester et al. 2022).



1.2. Immunomodulatory Roles of Erythrocytes:

Far from being inert oxygen transporters, mature human erythrocytes actively participate in the innate immune system by interacting directly with various inflammatory mediators and immune cells (Karsten et Herbert 2020).

Erythrocytes prominently express the Duffy Antigen Receptor for Chemokines (DARC), which binds an array of inflammatory chemokines—including IL-8 and MCP-1—serving as a dynamic reservoir that regulates systemic chemokine gradients (Karsten et Herbert 2020) .

In addition to DARC, RBCs express complement receptor 1 (CR1), allowing them to bind complement-opsonized immune complexes in the circulation for safe transport to liver and spleen macrophages in a process called immune-adherence clearance (De Back et al. 2014).

Erythrocytes also function as critical immune sentinels capable of detecting cell-free mitochondrial DNA and bacterial CpG DNA through their surface expression of the nucleic acid-sensing Toll-like receptor 9 (TLR9) (Lam et al. 2021).

During hyperinflammatory states like sepsis or severe COVID-19, the overwhelming binding of nucleic acids to RBC-TLR9 provokes the release of pro-inflammatory cytokines, exacerbating innate immune activation (Dobkin et Mangalmurti 2022).

Similarly, during severe systemic inflammatory response syndrome (SIRS), massive release of cytokines alters the overall immune landscape, underscoring the role of secretome components and circulating cells in modulating profound inflammatory kinetics (Martí-Chillón et al. 2025).

1.4. RBC-Derived Extracellular Vesicles: Biogenesis, Signaling, and Therapeutic Potential:

Throughout their lifespan, erythrocytes continuously release nanoscale lipid bilayer structures known as red blood cell-derived extracellular vesicles (RBC-EVs), a process that sharply accelerates during cellular aging, oxidative stress, and systemic inflammation (L. Xu et al. 2021).

RBC vesiculation serves as a critical homeostatic mechanism to shed damaged membrane components and oxidized hemoglobin, thereby delaying premature erythrocyte removal from the circulation (Huang 2026).

In the context of cardiovascular diseases, extracellular vesicles serve as critical intercellular messengers that transfer bioactive proteins and microRNAs to target cells, regulating cellular phenotypes and disease progression (Fu et al. 2020).

Specifically, RBC-EVs derived from patients with type 2 diabetes transfer functional arginase-1 to endothelial cells, inducing severe vascular dysfunction by generating oxidative stress and compromising nitric oxide bioavailability (Collado et al. 2025).

Once internalized by innate immune cells like monocytes and macrophages, RBC-EVs can trigger robust pro-inflammatory responses, stimulating the secretion of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (L. Xu et al. 2021).

Furthermore, during pathological conditions such as sickle cell disease, the externalization of phosphatidylserine on RBC-EVs confers potent procoagulant

properties that amplify the coagulation cascade and promote thrombo-inflammation (Joshi et al. 2025).

Due to their vast abundance and specific molecular cargo, circulating RBC-EVs and their dynamics during systemic inflammatory syndromes hold significant promise as accessible, non-invasive liquid biopsy biomarkers (Martí-Chillón et al. 2025).

Finally, capitalizing on their lack of nuclear DNA and excellent biocompatibility, researchers are exploring engineered RBC-EVs as a highly efficient, targeted nanodelivery platform for therapeutic interventions (L. Xu et al. 2021).

2. HEMOGLOBIN: STRUCTURAL FUNCTION, HEMOLYTIC TOXICITY, AND MACROPHAGE RESPONSE

2.1. Structural Biology and Physiological Function:

Hemoglobin (Hb) is a highly conserved tetrameric metalloprotein comprising two alpha and two beta globin subunits (Gell 2018). Each globin chain covalently binds an iron-protoporphyrin IX (heme) prosthetic group within a hydrophobic pocket that protects the iron from irreversible oxidation (Gell 2018). The primary physiological function of Hb is the reversible binding and transport of oxygen from the lungs to peripheral tissues (Helms et al. 2018). Oxygen binding triggers a cooperative allosteric conformational shift from a low-affinity tense (T) state to a high-affinity relaxed (R) state (Gell 2018). This oxygen-carrying capacity is finely tuned by heterotropic regulators, including protons (the Bohr effect), carbon dioxide, and 2,3-diphosphoglycerate (Gell 2018). Finally, sequestering Hb strictly within the erythrocyte membrane is essential to prevent its potent nitric oxide (NO) scavenging activity, thereby preserving vascular tone and homeostasis (Helms et al. 2018) .

2.2. Heme Toxicity and Monocyte/Macrophage Polarization:

Once internalized by the macrophage, heme is enzymatically degraded by heme oxygenase-1 (HO-1), yielding cytoprotective metabolites including biliverdin, carbon monoxide, and ferrous iron (Vijayan et al. 2018).

Heme serves as the most potent physiological inducer of HO-1, triggering its robust transcription via the Nrf2 signalling pathway to restore cellular redox homeostasis (Immenschuh et al. 2017) . The profound accumulation of intracellular heme and subsequent HO-1 upregulation drives a dynamic phenotypic shift in the

monocyte/macrophage population (Schaer et Buehler 2013). This distinct metabolic reprogramming polarizes macrophages into a highly specialized, non-foamy phenotype referred to as M-hem or M-Hb (Schaer et Buehler 2013).

M-hem macrophages are uniquely characterized by suppressed expression of pro-inflammatory cytokines, high antioxidant capacity, and highly efficient iron recycling capabilities (Vijayan et al. 2018). Ultimately, this HO-1-dependent polarization serves as a critical adaptive, anti-inflammatory mechanism that limits oxidative tissue injury and accelerates the resolution of inflammation during haemolytic stress.

3. MONOCYTES: IDENTITY, DEVELOPMENTAL ORIGINS, AND INNATE IMMUNE EFFECTOR FUNCTIONS

3.1. Defining Monocytes and Their Place in Innate Immunity:

Monocytes represent a heterogeneous population of circulating mononuclear phagocytes that constitute approximately 10% of human leukocytes and serve as critical effectors in the innate immune system (Cormican et Griffin 2020). These cells are characterized by remarkable plasticity, allowing them to sense environmental changes and differentiate into macrophages or dendritic cells upon migration into tissues (Orozco et al. 2021).

Circulating monocytes are conventionally classified into three distinct subsets based on the expression of surface markers CD14 and CD16: classical (CD14++CD16-), intermediate (CD14++CD16+), and non-classical (CD14+CD16++) (Williams et al. 2023).

In murine models, these populations correspond to Ly6C-high inflammatory monocytes, which are rapidly recruited to sites of infection, and Ly6C-low patrolling monocytes, which monitor the luminal surface of endothelial cells (Shi et Pamer 2011). This heterogeneity extends beyond surface markers to transcriptomic profiles, suggesting that monocyte subsets may exist as a continuum of differentiation rather than discrete populations (Williams et al. 2023).

While classical monocytes primarily act as precursors for tissue-resident macrophages during homeostasis, they can also give rise to monocyte-derived dendritic cells (moDCs) under specific inflammatory conditions (Rigamonti et al. 2023).

Furthermore, recent phenotypic analyses have identified novel subpopulations, such as large CD14+ monocytes, which exhibit distinct adhesion molecule expression and inflammatory responses (Merah-Mourah et al. 2020).

The dynamic balance between these subsets is essential for maintaining immune equilibrium and orchestrating effective responses against pathogens (Meskini et al. 2024).

Consequently, monocytes function not only as replenishers of the tissue macrophage niche but also as direct mediators of inflammation and host defence (Trzebanski et al. 2024a).

3.2. Hematopoietic Origins and Developmental Pathways of Monocytes:

Monocytes originate from hematopoietic stem cells (HSCs) in the bone marrow, where they undergo a hierarchical differentiation process regulated by specific transcription factors and cytokines (Yusoff et al. 2025).

Traditionally, monopoiesis was described as a linear pathway progressing from common myeloid progenitors (CMPs) to granulocyte-macrophage progenitors (GMPs) and subsequently to macrophage-dendritic cell progenitors (MDPs) (Orozco et al. 2021).

However, recent lineage tracing studies using single-cell RNA sequencing have challenged this dogma by revealing a dual origin for classical monocytes (Pimenov et al. 2024).

It is now established that classical monocytes can arise independently from either GMPs, generating neutrophil-like monocytes, or from MDPs, which produce dendritic cell-like monocytes (Trzebanski et al. 2024).

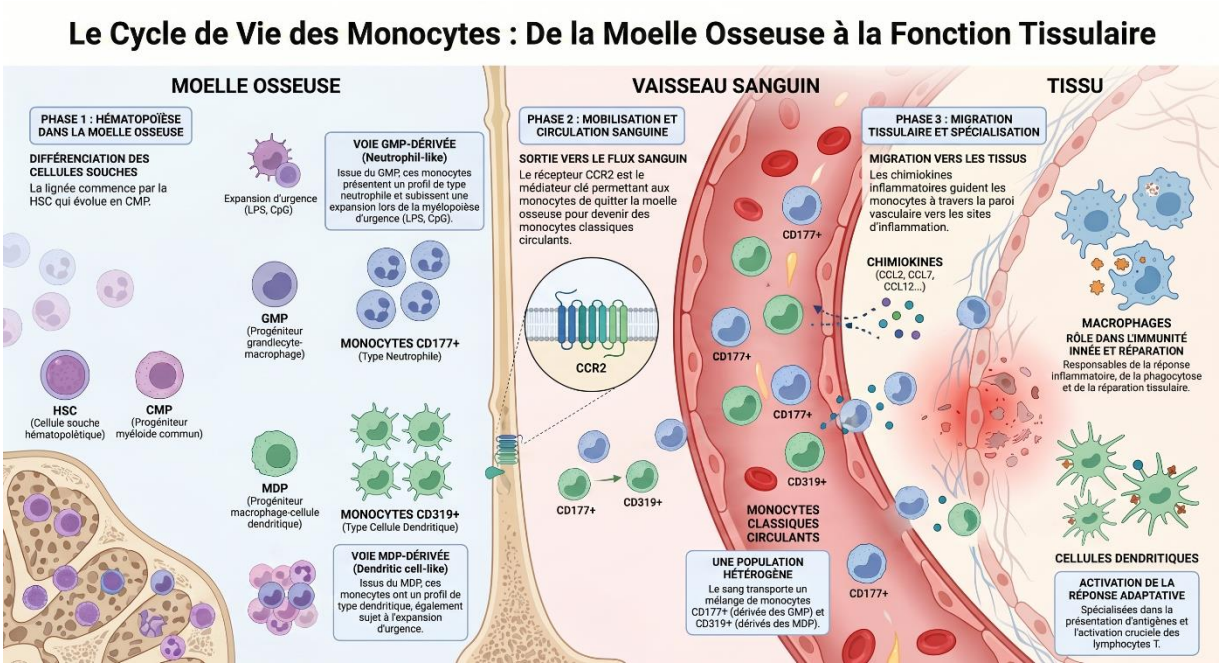
These distinct developmental trajectories are conserved in the steady state, where GMP-derived monocytes significantly outnumber their MDP-derived counterparts in the circulation (Trzebanski et al. 2024).

Under conditions of emergency myelopoiesis, inflammatory signals such as LPS or CpG can differentially promote the expansion of these distinct monocyte lineages from the bone marrow (Orozco et al. 2021).

The identification of specific surface markers, such as CD177 for GMP-derived cells and CD319 for MDP-derived cells, now allows for the discrimination of these subsets in peripheral blood.

Furthermore, the mobilization of these cells from the bone marrow into the bloodstream is critically dependent on CCR2 signalling, particularly during infection (Shi et Pamer 2011).

This refined understanding of monocyte ontogeny suggests that the functional fate of a monocyte within tissues is pre-determined by its hematopoietic origin (Trzebanski et al. 2024).



3.3. Monocytes as Key Mediators of Innate Immune Defence and Tissue Homeostasis:

As pivotal architects of the innate immune response, monocytes are rapidly recruited to inflamed tissues where they exert direct antimicrobial functions and orchestrate tissue repair (Shi et Pamer 2011).

Upon tissue entry, they can differentiate into inflammatory macrophages or monocyte-derived dendritic cells, a fate decision influenced by local environmental cues and specific transcription factors like IRF5 (Orozco et al. 2021).

In the context of bacterial infections, such as Listeria monocytogenes, recruited monocytes differentiate into TNF- and iNOS-producing dendritic cells (Tip-DCs) which are essential for pathogen clearance (Shi et Pamer 2011).

Recent evidence highlights that monocyte function is dictated by ontogeny; GMP-derived monocytes exhibit superior phagocytic activity and neutrophil-like behaviors such as extracellular trap formation (Trzebanski et al. 2024).

Conversely, MDP-derived monocytes are functionally distinct, showing a propensity for antigen presentation and differentiation into specific tissue-resident macrophages, such as those in the lung interstitium (Pimenov et al. 2024).

Monocytes also play a crucial role in the resolution of inflammation, although their persistence in tissues can contribute to pathology, such as fibrosis or atherosclerosis (Rigamonti et al. 2023). Through the activation of pattern recognition receptors like TLRs, monocytes initiate signaling cascades, including the NF-κB pathway, to release pro-inflammatory cytokines (Chi et al. 2025). Additionally, monocytes can undergo epigenetic reprogramming after initial stimulation, a phenomenon known as trained immunity, which enhances their response to secondary challenges (Williams et al. 2023). Thus, the versatility of monocytes allows them to function as both immediate effectors of immunity and precursors for long-term tissue maintenance (Meskini et al. 2024).

IV. LIPID METABOLISM IN MONOCYTES AND MACROPHAGES: CHOLESTEROL, TRIGLYCERIDES, AND LIPID DROPLET DYNAMICS

4.1. Lipid Droplet Biogenesis and Its Role in Macrophage Activation:

Lipid droplets (LDs) have emerged as highly dynamic organelles that serve as central hubs integrating cellular metabolic reprogramming with innate immune responses in monocytes and macrophages (Zhang et al. 2021).

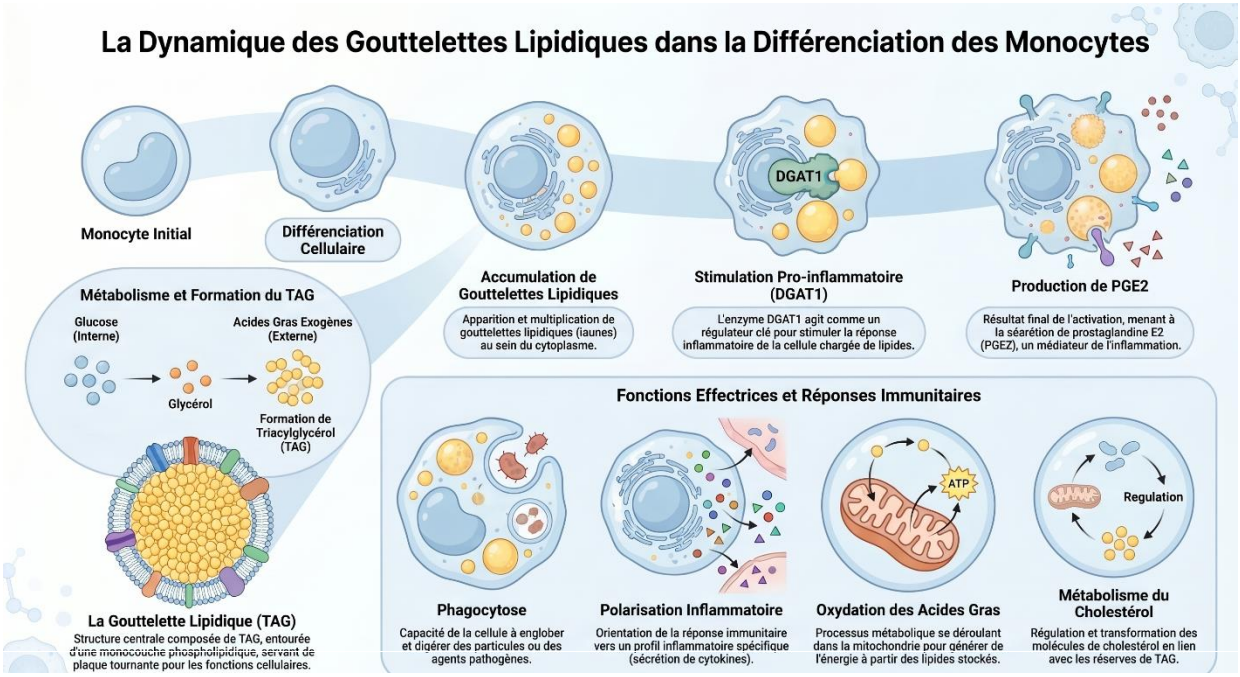
The accumulation and dynamic regulation of LDs are fundamentally required for the differentiation of monocytes into functional macrophages, driving their subsequent phagocytic and inflammatory capacities (Singh et Sen 2021).

Upon pro-inflammatory stimulation, macrophages undergo a metabolic commitment toward triacylglycerol (TAG) synthesis, a process strictly dependent on DGAT1 that is

indispensable for optimal inflammatory activation and prostaglandin E2 production (Castoldi et al. 2020).

Interestingly, this classical activation-induced lipid droplet formation relies primarily on the esterification of exogenous fatty acids with glucose-derived glycerol, rather than on de novo fatty acid synthesis (Rosas-Ballina et al. 2020).

Consequently, these dynamic shifts in lipid synthesis, fatty acid oxidation, and cholesterol metabolism profoundly dictate macrophage polarization, phagocytosis, and overall bioenergetics (Yan et Horng 2020).



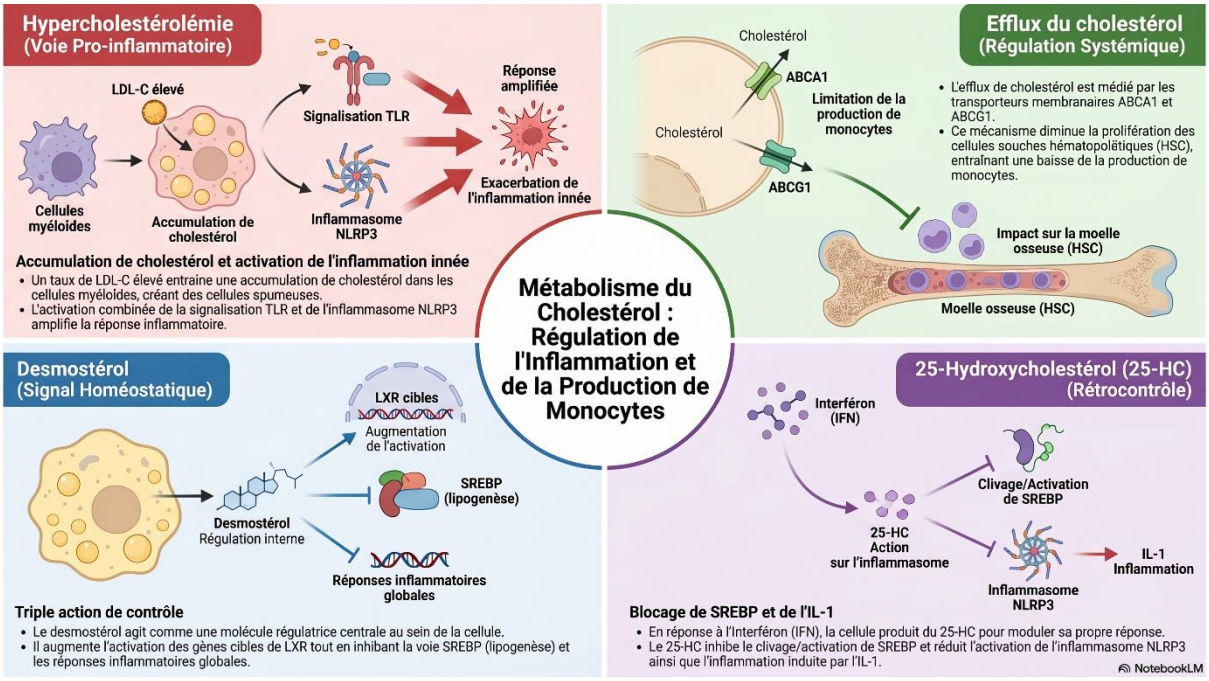
4.2. Cholesterol Metabolism and Its Regulatory Impact on Innate Immune Signaling:

Concurrently, hypercholesterolemia drives cholesterol accumulation within myeloid cells, triggering Toll-like receptor (TLR) signaling and inflammasome activation, which further exacerbates innate immune inflammation (Tall et Yvan-Charvet 2015).

Conversely, cholesterol efflux pathways regulated by ABCA1 and ABCG1 not only mitigate foam cell formation but also restrain hematopoietic stem cell proliferation, thereby limiting excessive monocyte production in the bone marrow (Murphy et al. 2014).

The regulated accumulation of desmosterol, an intermediate in cholesterol biosynthesis, acts as a critical homeostatic signal in foam cells to activate LXR target genes while suppressing SREBP-mediated lipid synthesis and inflammatory responses (Spann et al. 2012).

Furthermore, the interferon-induced cholesterol metabolite 25-hydroxycholesterol (25-HC) exerts a negative feedback mechanism by antagonizing SREBP processing, thereby suppressing interleukin-1-driven inflammation and inflammasome overactivation (Reboldi et al. 2014).

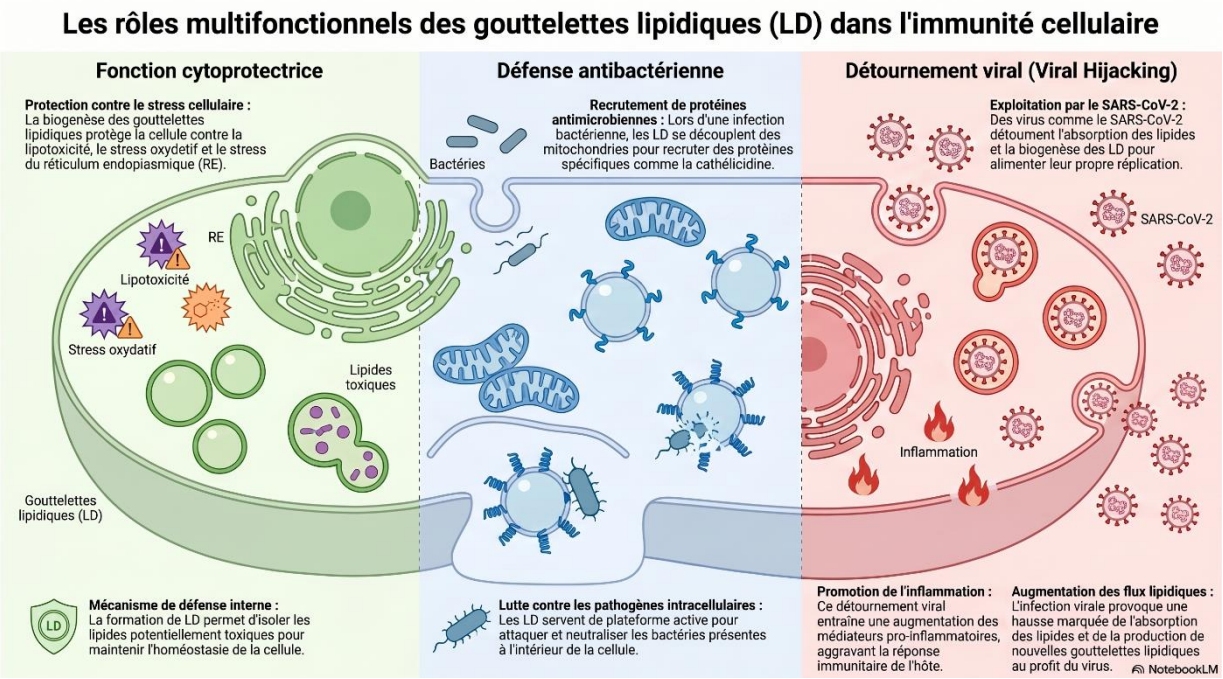


4.3. Cytoprotective and Antimicrobial Functions of Lipid Droplets in Disease Contexts:

Beyond basic homeostasis, the biogenesis of LDs acts as a critical cytoprotective mechanism against lipotoxic, oxidative, and endoplasmic reticulum stress across various human pathologies (Zadoorian et al. 2023).

In the context of infection, mammalian LDs physically uncouple from mitochondria and accumulate antimicrobial proteins, such as cathelicidin, to orchestrate a direct, protein-mediated intracellular defense against bacterial pathogens (Bosch et al. 2020).

However, this metabolic dependency is readily hijacked by viruses, as demonstrated by SARS-CoV-2, which upregulates lipid uptake and LD biogenesis to fuel viral replication and amplify the production of pro-inflammatory mediators (Dias et al. 2020).



II. Matériel et Methodes:

Confidentiel

III. Results and Interpretation:

Confidentiel

IV. Discussion:

Confidentiel

V. Conclusions and Perspectives:

Confidentiel

VI. References

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