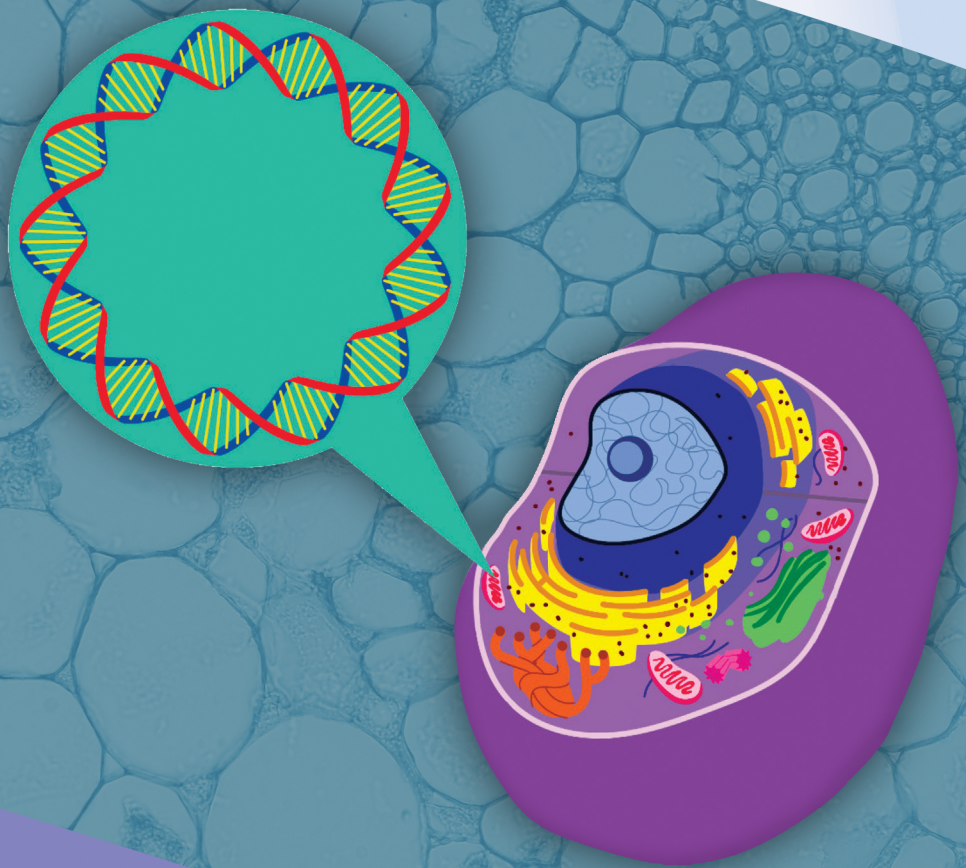
Mitochondrial DNA and the Immuno-inflammatory Response:

New Frontiers to Control Specific Microbial Diseases



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(Volume 3)

Mitochondrial DNA and the Immuno-inflammatory Response: New Frontiers to Control Specific Microbial Diseases

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Volume # 3

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FOREWORD

Microorganisms are the most abundant and diverse beings on Earth and are capable of occupying various ecological niches. Among them, there are pathogenic microorganisms that have the ability to cause infections or diseases when interacting with a host, who they need to thrive and survive. Once the pathogen sets itself up in a host, it manages to avoid the host's immune response and uses its resources to replicate before spreading to new ones.

Infectious diseases are among the main cause of morbidity and mortality worldwide and are a major challenge for the biomedical sciences. Recently, much progress has been made towards unraveling the mechanisms of microbial pathogenesis, including the immuno-inflammatory response elicited by the parasite-host relationship. It is worth mentioning that the mitochondrial DNA stands out, known for its role in oxidative phosphorylation and maternally inherited mitochondrial diseases. The release of mitochondrial DNA into the cellular cytoplasm and out to the extracellular milieu activates different pattern recognition receptors and innate immune responses leading to robust actions.

Mitochondrial DNA and the Immuno-inflammatory response: new frontiers to control specific microbial diseases aims to present state-of-the-art coverage on topics central to the understanding of the interactions between pathogenic microorganism (bacteria and virus) and hosts. The book is divided into six chapters written by professionals with expertise in the field of cell biology and immuno-inflammatory response. The chapters cover the complexity of mitochondrial metabolism; the mitochondrial dysfunction in leprosy; mitochondria and the host immune cell against *Mycobacterium tuberculosis;* disturbance of mitochondrial function in *Streptococcus pneumoniae* infection; inflammatory response in Zika virus infection and mitochondrial dysfunction; and the potential role of mtDNA as an important marker of hyper inflammation in the progress of COVID-19. This book represents a comprehensive and an indispensable tool for researchers in immunology and microbiology wishing to keep abreast with the latest developments in cellular immunology and mitochondrial DNA. In addition, it provides a reliable reference for undergraduate and graduate students in their pursuit of becoming competent future immunologists/microbiologists, as well as for health professionals in general.

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PREFACE

The first cases of the infection caused by the virus SARS-CoV-2 were reported in December, 2019, in China, hence the name of the disease: COVID-19 (Corona Virus Disease, year 2019). The world saw the emergence of a global pandemic in 2020, which still poses a threat to global health in 2021, despite the recent mass vaccination. In some countries like the USA, Brazil and India, the number of deaths is still worrisome. The most important lesson learnt from this disease is its destructive potential of triggering a sudden and uncontrolled inflammatory response to the virus, which can rapidly decimate populations worldwide. That was our biggest motivation for choosing the topic ***Mitochondrial DNA and the Immuno-inflammatory response: new frontiers to control specific microbial diseases*** as the second volume in the book series **"Frontiers in Inflammation"**.

Our objective is to present to the reader a book on the topic of "inflammation" in its broadest sense, including relatively recent scientific discoveries concerning the active participation of a cell organelle—mitochondria—and its respective constituents, mainly mitochondrial DNA (mtDNA), in the immuno-inflammatory responses. It is worth mentioning that, coincidently, this organelle was studied by James P. Allison, PhD, and Tasuku Honjo, PhD, who were awarded the Nobel Prize in Physiology or Medicine for their discovery regarding cancer therapy by inhibition of negative immune regulation. Thus, the mechanism for oxygen sensing (mitochondria) has fundamental importance in Physiology and Pathology, in areas such as the metabolism, immune response and ability to adapt to exercise. All in all, the role of mitochondria goes far beyond their contribution to cellular energy metabolism. Mitochondria are multifuncional organelles that actively participate in the immuno-inflammatory response in several pathologies. To develop this subject, we chose some pathologies which have already been studied under the light of this specific area. Therefore, this book will address: (1) two diseases (one bacterial and the other one viral) in which the exacerbation of the inflammatory response can lead to neuropathies— leprosy (one of the oldest diseases in the world) and Zika fever (a relatively new disease in Brazil)—and (2) three diseases (two bacterial and one viral) in which the exacerbation of the inflammatory response can lead to irreversible lung damage that can cause rapid death—tuberculosis, pneumonia and the most recent global pathology, COVID-19. In addition, the introductory chapter of this book deals with updates on mitochondria as multifunctional organelles, enabling Cell Biology to better interface with Physiology, Pathology and Immunology.

Our goal is to provide up-to-date content on the chosen topic, aiming at broadening horizons and awakening readers, especially infectologists and pathologists, about the importance of investigation and research on the subject of inflammation, a very fascinating and promising topic for new discoveries of therapeutic targets.

We hope that this content may be useful in universities, hospitals and scientific research centers, as well as for health professionals in general. It is worth mentioning that each author and co-author presents their experience in their area of expertise in Cell Biology and Infectious Diseases.

Brazil is one of the countries listed with a high incidence of leprosy, Zika fever, tuberculosis, bacterial pneumonia and COVID-19. We would like to express our sincere thanks to all authors who have contributed chapters to this book. We would also like to thank Bentham Science Publishers for the publication opportunity and for their support in disseminating knowledge.

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DEDICATION

This book is dedicated to healthcare professionals and all individuals who have dealt or are dealing with one or more of the illnesses covered in this book.

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CHAPTER 1

An Auspicious Bacterium: How Mitochondria can be Beneficial to the Innate Immunity through Aerobic Exercises

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Abstract: Mitochondria are highly relevant organelles with regard to their unique function in generating energy and contributing to metabolism within the cell. Furthermore, recent studies suggest that they might have an influence on the innate immune and inflammatory responses, thus affecting antiviral immunity (as example: Zika virus (ZIKV), hepatitis C virus (HCV), dengue virus and SARS-CoV-2 virus) and antibacterial immunity as well (*Streptococcus pneumoniae, Mycobacterium leprae* and *Mycobacterium tuberculosis*). Therefore, this chapter aims at bringing a relevant debate about the role of mitochondria and their multifunctional capacity. We intend to discuss the complexity of mitochondrial metabolism, especially during aerobic physical exercises, which causes the modulation of the gene expression of proteins that lead to mitochondrial proliferation and, thus, promote health. In addition, considering the injuries caused by hypoxia, this chapter also stresses the enormous potential of mitochondria to enable the survival of eukaryotic cells by allowing them to turn to aerobic respiration, as shown in previous scientific studies. In conclusion, this chapter points out the importance of mitochondrial biogenesis (both natural and stimulated

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biogenesis by aerobic exercise) and the benefits this organelle brings to the health, arguing that they go far beyond cellular respiration and oxidative phosphorylation.

Keywords: Aerobic exercises, Immunoinflammatory response, Mitochondrial biogenesis.

1. MITOCHONDRIAL BIOGENESIS

The way in which life emerged on Earth is the subject of study by many scientists throughout the history of science. Assumptions and theories have been elaborated to suggest how cellular metabolism developed [1]. Despite those scientific efforts, the origin of the eukaryotic cell remains unknown. Nevertheless, it is known that there are two main cell types: the prokaryote and the eukaryote cell. The prokaryote cell is a simpler example consisting of cytoplasm, genetic material, cell wall, plasma membrane, cilia, flagella, ribosome and plasmid. The eukaryotic cell has some components similar to the prokaryotic cell, while it has additional components such as mitochondria, Golgi apparatus, nucleus, lysosomes, secretion vesicles, endoplasmic reticulum and peroxisomes (which can be found in most eukaryotic cells). However, glyoxysomes, chloroplasts, chlorophylls and the cell wall are present only in the plant cell. Together, these peculiarities make the eukaryotic cell (Fig. **1**) a very complex system. In order to understand the complexity of these cells, it is necessary to have a closer look at relevant events in tissues, organs, systems and organisms.

The energetic role of mitochondria is intimately linked to the origin of the eukaryotic cell and their development in complex organisms. Comprehension of the evolutionary origin of mitochondria is essential for understanding any biological structure or process specially involved in birth, aging-related diseases and cell death. In this context, it is relevant to begin this review by introducing the meaning of "Biogenesis", mentioned by Attardi *et al*. [2] and Leaver *et al*. [3] to refer to the production of new mitochondria inside the cells. According to the review of Nisoli *et al*. [4], mitochondrial division occurs concurrently with the nuclear division. Despite the kinetics of mitochondrial division not coinciding with cell proliferation all the time, it is verified that, in muscle cells, mitochondria divide during both events: myogenesis and physical exercise. Furthermore, mitochondria can also duplicate after some special circumstances, such as under benzodiazepine treatment, inhibitors of oxidative phosphorylation, phorbol esters and calcium modulation [4].

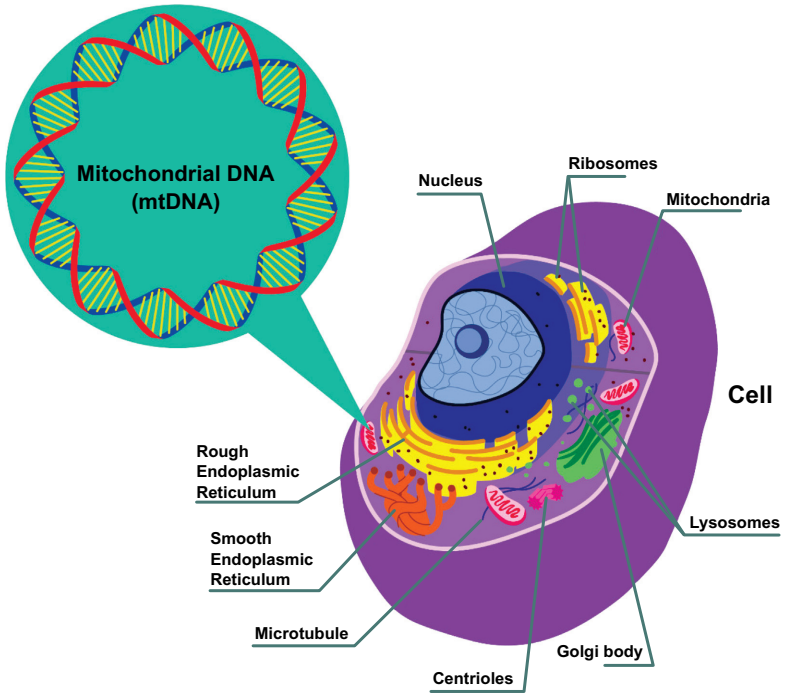

Fig. (1). Eukaryotic cell and its organelles. Note that mitochondria are the only intracellular organelle with DNA inside, besides the nucleus.

The scientist Lynn Margulis was the main proponent of the endosymbiotic theory of the mitochondria's evolution. She thoroughly changed the understanding of the evolution of nuclear cells by proposing that it was the result of symbiotic fusions of bacteria. Throughout her career, Margulis's research has not received due credit in the scientific community and her article entitled "On the Origin of Mitosing Cells" appeared in 1967, after being rejected by about fifteen journals [5]. Margulis defended the theory that cellular organelles, such as mitochondria and chloroplasts, had been independent bacteria, and this knowledge was ignored for another decade, only being accepted after robust genetic evidence [6, 7]. Anderson *et al*. [8] reported that complete genomic sequences for many mitochondria, as well as for some bacteria, were a consistent demonstration to explain the origin of mitochondria. In addition, they argued that phylogenetic reconstructions with genes encoding proteins active in metabolism and energy translation were the confirmation of the simplest version of the endosymbiosis hypothesis. These same authors warned that the hypotheses of hydrogen and syntrophy for the origin of the mitochondria, however, were not yet completely clear, but that future research in this direction would probably show that the evolution of hydrogenosomes could be related to that of mitochondria.

CHAPTER 2

Mitochondrial Dysfunction in Leprosy: Shedding Light on the Neurodegenerative Consequences

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Abstract: Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* or *Mycobacterium lepromatosis*. Dermal tissue macrophages and Schwann cells from peripheral nerves are the main host cells for the pathogen. The clinical manifestations of this disease depend basically on the host's immune response to *M. leprae*. However, genes relevant to both innate and adaptive immune responses also seem to contribute to leprosy acquisition and to determine its clinical forms. The crucial clinical problem in leprosy is represented by episodes of intense inflammation. They represent a major problem in the course of leprosy, as reactional episodes can be responsible for permanent damage to nerves, causing deformities. Among bacterial pathogens, infection of peripheral nerves is a unique property of *M. leprae*. The intensity of the inflammatory reaction in response to tissue damage caused by pathogens is strongly associated with mitochondria and their respective mitochondrial DNA, since this organelle and its constituents act as potent ligands for several innate immunity receptors. In this chapter, we will first describe the general context of leprosy and its various clinical forms, diagnosis and treatment, highlighting episodes of acute inflammatory response during this pathology and, finally, we will outline some cellular mechanisms that lead to neurodegenerative consequences in leprosy. The literature partially attributes these to cytokines and, mainly, to TNF-α, as well as to changes in mitochondrial dynamics, especially mitochondrial DNA, when mitochondrial dysfunction seems to be involved in the pathogenesis of neuritis in leprosy.

Keywords: Leprosy, Mitochondrial dysfunction, Neuritis.

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1. LEPROSY-AN OVERVIEW

Leprosy, or Hansen's disease (HD), is a chronic infectious disease caused by *Mycobacterium leprae* or *M. lepromatosis*, with neural, skin, and upper airway mucosal involvement. Its transmission mechanism is still unknown, but it is speculated that it probably occurs from person to person through the inhalation of bacilli present in upper airways secretions [1, 2]. This may occur in the prolonged contact between susceptible and genetically predisposed individuals and untreated multibacillary leprosy patients [3]. Thus, the nasal mucosa is the main route of entry or exit of *M. leprae* [1]. Humans are the main host and reservoir of *M. leprae*, but it has also rarely been found in chimpanzees, sooty mangabey monkeys, cynomolgus macaques and red squirrels. It is also present in about 15% of wild nine-banded armadillos in the southern United States. Armadillos develop multibacillary infection and are the only verified environmental reservoir of *M. leprae* [4].

Leprosy is the oldest documented disease in human history and, still today, over 200,000 new cases are still reported worldwide each year, despite the employment of multidrug therapy (MDT) by the Word Health Organization (WHO). The incidence of leprosy, as well as tuberculosis, malaria, Chagas disease and leishmaniasis, has become a parameter for differentiating social conditions between countries. However, the behavior of several endemic diseases in Brazil and other countries cannot be fully explained by the stages of economic development, which led to the role of genetic factors gaining prominence in scientific research along with the distribution of the disease in clusters, families or communities with a common genetic background. However, it is now known that, despite advances in treatment and prospects for leprosy patients since the introduction of MDT three decades ago, the global incidence of leprosy remains high in countries such as India and Brazil.

Research focusing on the link between human genetics and leprosy susceptibility is extremely useful for further elucidating how and why people develop this disease [1, 6]. Moreover, there are reports that vitamin D (VDR, a gen receptor on chromosome 12q12) may be associated with susceptibility to leprosy, as well as a variety of factors associated with innate and adaptive immunity [7 - 11]. Also, genetic regulation of the innate immune response in leprosy, as demonstrated by different polymorphisms of the NOD2 gene, has been associated not only with increased susceptibility to this illness but also with inflammatory manifestations episodes [12, 13].

Furthermore, Polycarpou & Lockwood and Mi *et al.* [12, 14] mentioned a fundamental role of the innate immune system in simultaneous dysregulation of

the inflammatory response during reactional episodes in leprosy, due to genetic variability in genetic polymorphisms associated with Toll-like receptors. In 2012, leprosy ranked sixth on the World Health Organization's neglected tropical diseases scale [15]. In 2015, it was still part of the list, assuming, the tenth position. Diseases such as AIDS, tuberculosis and malaria no longer appeared in the first positions in this list in 2015, having been replaced with other pathologies, such as: Buruli ulcer, cysticercosis, dengue, Guinea worm disease, echinococcosis, fascioliasis and sleep disease [16]. In other words, although there is a fluctuation in the order of occurrence of these pathologies worldwide, leprosy continues to occupy a position among the pathologies with the highest incidence and public health problem in some regions around the world, according to WHO [16].

More up-to-date data provided by WHO also reports that, in 2019 [17], a total of 202,185 new cases of leprosy were detected globally. Brazil, India and Indonesia are at the top of this list with more than 10,000 cases each, while 13 other countries—Bangladesh, Democratic Republic of Congo, Ethiopia, Madagascar, Mozambique, Myanmar, Nepal, Nigeria, Philippines, Somalia, North Sudan, Sri Lanka and United Republic of Tanzania—reported 1,000 to 10,000 cases each [5]. To sum up, leprosy is an ancient and deforming disease caused by *M. leprae*, which needs continued vigilance, especially when it comes to the detection and treatment of undiagnosed cases.

2. CLINICAL FORMS OF LEPROSY

Humans are the main reservoir of leprosy, while, in the Americas, the armadillo is also a significant one. Approximately 95% of the population is immune to *M. leprae* and does not become sick when infected by it, although there are no tools to detect subclinical infection. Among those who fall ill, what determines the evolution of the disease is the patient's own immune response and genetic susceptibility [1, 18, 19]. The spectral pathology of leprosy can be diagnosed using two coexisting classification systems. According to Ridley & Jopling [20], leprosy is characterized by five different types of clinical manifestations: tuberculoid (TT), borderline tuberculoid (BT), borderline (BB), borderline lepromatous (BL) and lepromatous (LL) (Fig. **1** and Table **1**). Indeterminate leprosy is considered the first manifestation of the disease, and, after a variable period of time, it evolves to cure or into one of the clinical forms mentioned above [2]. The classification proposed by WHO is based on the number of lesions and determines the treatment regimen [5, 17]. According to WHO, individuals with more than five lesions are classified as multibacillary (MB) patients, whereas individuals with less than five lesions are classified as paucibacillary (PB) patients (Table **1**).

CHAPTER 3

The Multifaceted Interface Between the Host Immune Cell and *Mycobacterium Tuberculosis* - Mitochondria at the Crux of the Matter

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Abstract: Tuberculosis (TB) is a contagious infectious disease that is a major cause of morbidity, being one of the top 10 causes of death worldwide, and the leading one from a single infectious agent. Also called "White Plague" in the past, TB is an airborne disease, propagated when multibacillary people spread *M. tuberculosis* by coughing or sneezing. The disease typically affects the lungs (pulmonary TB), but can also affect other sites (extrapulmonary TB). TB is curable and preventable: about 85% of the people who develop the disease may be successfully treated with a 6-month multidrug regimen. The treatment has the additional benefit of preventing onward transmission.

Macrophages are the first host cell to get in contact with *M. tuberculosis*. They also have important effector functions, regardless of whether the infection evolves to a chronic or latent form. However, *M. tuberculosis* evades host cell innate defense mechanisms, manipulates organelles and cell metabolism, as well as host cell death pathways. This complex interaction between the host cell and the bacillus determines the outcome of the infection. In this context, mitochondria and mitochondrial DNA (mtDNA) contribute to triggering cell death by necrosis. However, excessive necrosis may lead to tissue damage, which disrupts granulomas and benefits *M. tuberculosis* transmission. We intend to revisit the major aspects of this intricated and multifaceted interface between the host immune cell and *M. tuberculosis* and discuss how mitochondria are the crux of the matter.

Keywords: Inflammation, Mitochondria, Necrosis, Tuberculosis.

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1. TUBERCULOSIS-AN OVERVIEW

Tuberculosis is an ancient, contagious and chronic infectious disease caused by *Mycobacterium tuberculosis*, or Koch's bacillus. *M. tuberculosis* may infect human lungs when a pulmonary environment is favorable to its survival, as it takes advantage of environmental changes. The bacillary proliferation forms lung colonies, and, if not controlled, part of the bacilli may migrate to other areas of the respiratory tract. Consequently, TB can spread *via* bronchogenic, lymphatic and hematogenous pathways [1].

There is a gross estimate that, within 24 hours, an infected person may spread up to 3.5 million tuberculosis bacilli, in the form of microscopic droplets eliminated through coughing, sneezing or speaking. These tiny particles may remain in the air for roughly eight hours and deposit on various objects, clothing, and even dust. Occasionally, the smallest droplets can be inhaled by other individuals, and, if it is not taken by the mucociliary clearance in the airways, the bacilli can reach the alveoli, becoming potentially infective. In past decades, a common route of *M. bovis* infection, a bacillus similar to *M. tuberculosis* causing bovine tuberculosis, was the ingestion of contaminated milk and meat. Once inhaled or ingested, the bacillus remains inactive for about three days.

Afterwards, the bacillus starts its 18-hour self-renewing cycle. In this phase, the innate immune defense mechanism is fully activated [1]. The *M. tuberculosis* infection produces an initial inflammatory lesion, called Ghon's complex. Between the third and the eighth week of infection, the bacilli have already formed a lung colony capable of producing an inflammatory reaction that triggers the destructive process of the lung parenchyma. Additionally, the bacilli may propagate through lymphatic vessels to adjacent lymph nodes, establishing the primary complex of the infection. The initiation of the immune response creates a specific nodule or tubercle granuloma. Then, the infection may progress to chronic tuberculosis or, more rarely, to acute progressive tuberculosis. The infection may also remain dormant, which allows latent bacilli to resume their destructive action years after that initial event. Strikingly, humans present significant resistance against this emerging aggression (roughly 90% of the population), and the most frequent outcome is the regression of the pathological process and spontaneous cure, with consequent recovery, scarring, or calcification of tissue damage [1]. However, sterilizing immunity has not been described in the case of human tuberculosis.

It is worth mentioning that the factors that determine the course of tuberculosis are still not fully understood. In addition to the contagion by *M. tuberculosis*, both

genetic predisposition and individual immune response are reported to be probable causes that trigger TB [1].

The clinical features presented by tuberculosis are extremely complex, due to the multiplicity of symptoms that can pose a challenge to diagnosing it. In the initial phase, the infection is almost always silent and characterized by mild manifestations. It is also difficult to be detected by image techniques, such as X-ray. The evolution of the severe TB disease is described by the appearance of high fever (symptom that already mirrors the intense systemic inflammatory manifestation), accompanied by night sweats, persistent weight loss, chest pain, a cough, increased expectoration, tiredness and dyspnea. Later, hemoptysis characterizes a serious condition, especially when associated with other symptoms. The definitive tuberculosis diagnosis is made mainly through the laboratory analysis of sputum, either culture or smear, although pulmonary images, either X-ray or tomography, and tuberculin skin test are auxiliary methods employed [1].

Interestingly, tuberculosis and leprosy are caused by different etiological agents belonging to the same genus, wrongly leading to the idea that both mycobacteria established an antagonistic relationship due to their immunological competition. In other words, it is broadly debated whether tuberculosis inhibits the occurrence of leprosy, a phenomenon that would potentially explain a change in the European epidemiological profile (Grmek, 1983 APUD [1]).

Studies of human skeletons have shown that tuberculosis is an old disease, having been affecting humans for thousands of years. Its origin remained unknown until 24th of March, 1882, when the German doctor Robert Koch announced his discovery of the bacillus and its disease [2, 3].

Right after having identified the etiological agent of TB, Koch examined the tissues of some animal varieties and phlegm of contaminated individuals that had had tuberculosis infection. The confirmation of the presence of the bacillus allowed Koch to develop the culture procedures for isolation of the bacillus, and also for inoculating the material in a variety of animals, such as guinea pigs, hamsters, rabbits, monkeys, dogs, cats and chickens. In this interim, Koch attested the uniqueness of the pathologies to differentiate several diseases from tuberculosis [1].

According to Koch, tuberculosis is a pathology caused exclusively by the bacillus that received his name. It affects both humans and animals. Both eliminate the bacillus through sneezing and phlegm. Despite the contagious nature of TB, Koch was cautious in disqualifying the hereditary condition of the disease, suggesting the need for further studies on the phenomenon [1].

CHAPTER 4

Mitochondrial DNA and *Streptococcus pneumoniae* Infection – Induction of Immuno-inflammatory Response

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Abstract: *Streptococcus pneumoniae*, or pneumococcus, is one of the leading causes of morbidity and mortality associated with lower respiratory infections. Usually, it colonizes asymptomatically the human upper respiratory tract, but it can eventually migrate to other body sites to cause invasive and non-invasive diseases. The polysaccharide capsule (CPS) is the main pneumococcal virulence factor and it is used in the currently available vaccines against this pathogen. However, novel therapeutic and prevention approaches are urgently needed to target emergent non-vaccine serotypes, especially those associated with antimicrobial resistance. Besides CPS, pneumococcus has several other virulence factors that contribute to its pathogenesis, including surface proteins (*e.g.*, CbpA), the pore-forming toxin pneumolysin (PLY), as well as enzymes that produce hydrogen peroxide (H_2O_2). Here, we describe the pathogenesis of pneumococcal infections as well as host cell molecular signaling, focusing on major molecules responsible for host cell invasion and translocation, and disturbance of mitochondrial function, resulting in mitochondrial DNA (mtDNA) leakage, inflammation and tissue damage. Understanding molecular and immuno-inflammatory mechanisms underlying pathogenesis and pathogen-host cell interactions is crucial to developing novel approaches to prevent and treat pneumococcal diseases.

Keywords: Mitochondrial DNA, Pneumococcus, *Streptococcus pneumoniae*.

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1. INTRODUCTION

Streptococcus pneumoniae, also referred to as pneumococcus, is a gram-positive diplococcus-shaped human pathogen and a leading agent of infectious diseases worldwide. In 2016, *S. pneumoniae* was the most common cause of morbidity and mortality from lower respiratory infections globally, contributing to over 1,189,937 deaths [1]. Pneumococcus colonizes the upper respiratory tract of 20-80% of healthy children, and of 5-35% of healthy adults. Colonization is usually asymptomatic, and young children are considered the main reservoir of pneumococcus. On the other hand, asymptomatic colonization is also considered a prerequisite for the establishment of invasive pneumococcal disease (IPD). Risk groups for developing severe pneumococcal disease include young children (especially < 2 years old), elderly adults (> 65 years old), and immunocompromised individuals [2 - 5]. The polysaccharide capsule (CPS) is considered the main pneumococcal virulence factor, protecting the bacteria from opsonophagocytosis [6]. The pneumococcal CPS 2 is chemically and antigenically diverse, allowing for the classification of pneumococcal strains into around 100 different capsular types, or serotypes [7]. For all serotypes, CPS is responsible for stimulating the production of specific antibodies, and for this reason, currently available pneumococcal conjugate vaccines (PCV) are based on this target. However, current PCVs provide protection against only 10 (PCV10) or 13 (PCV13) capsular types among those identified so far. Reduction in the occurrence of serotypes targeted by PCVs has led to a great reduction in IPD cases and herd protection, but it has also contributed to the phenomenon of serotype replacement. Serotype replacement consists of the emergence of pneumococcal disease associated with non-vaccine serotypes, and can become a serious problem for the control of the disease.

Pneumococcus has also been listed as one of the current antibiotic-resistance threats, and an increase in antimicrobial resistance among pneumococci, including those belonging to non-vaccine serotypes, has raised concerns about the effectiveness of empiric antimicrobial therapy for pneumococcal disease [8, 9].

To overcome these issues related to the current CPS-based vaccines and the increasing antimicrobial resistance rates, new vaccines that can provide immunological protection regardless of serotype and/or novel treatment approaches that can cover antibiotic-resistant strains need to be developed.

2. PNEUMOCOCCAL PATHOGENESIS: VIRULENCE FACTORS, INFLAMMATION AND MITOCHONDRIAL DNA LEAKAGE

Streptococcus pneumoniae colonizes the upper respiratory tract, mainly the nasopharynx, from where it can spread to adjacent sites or more distant

organs/systems, including middle ear, lungs, bloodstream, spleen, heart and central nervous system (CNS), among others. Although traditionally considered an extracellular pathogen, it can also assume an intracellular lifestyle. This ability may help the microorganism evade some defense mechanisms and spread within the human host.

Pneumococcus has various virulence factors that contribute to its adherence to the airway epithelium, evasion of host immune defenses, tissue penetration, and invasion and survival within host cells. These factors include a CPS, the pore-forming toxin pneumolysin (PLY), several surface proteins (*e.g.*, choline binding proteins, metalloproteases, neuraminidases, pili, pneumococcal surface protein A *etc.*), as well as the pyruvate oxidase SpxB and the α-glycerophosphate oxidase GlpO, enzymes that produce hydrogen peroxide (H_2O_2). Many pneumococcal virulence factors directly or indirectly damage host tissues or induce host inflammatory responses, facilitating tissue penetration, but two of them are of great importance: the choline binding protein A (CbpA) and PLY [10, 11].

Choline binding proteins (Cbp) are a family of proteins that are noncovalently attached to the phosphorylcholine (ChoP) domain on the pneumococcal teichoic acid and has several functions. CbpA, also called pneumococcal surface protein C (PspC), is one of the major pneumococcal adhesins, responsible for binding pneumococci to different host cells; it has specific motifs for binding to the polymeric immunoglobulin receptor (pIgR) on nasopharyngeal and lung epithelial cells and the laminin receptor (LR) on endothelial cells, promoting adherence, endocytosis and translocation across epithelial and endothelial barriers [12].

PLY is a pore-forming toxin that is released during bacterial autolysis, although more recent evidence suggest that PLY may also be exposed on pneumococcal surface [13, 14]. PLY has a wide range of mechanisms of action, playing a pivotal role in the pathogenesis of pneumococcal infections. It induces extensive pro-inflammatory effects, directly lyses or induces apoptosis of different host cells, inhibits mucociliary clearance and separates tight junctions of the human respiratory epithelial cells [10].

In the lungs, CbpA binds to the polymeric immunoglobulin receptor (pIgR) on the alveolar epithelium, whereas PCho-bearing teichoic acid binds to the platelet activating factor receptor (PAFR), promoting firm adherence, followed by replication and initiation of host damage responses. Ultimately, it leads to the development of lobar pneumonia. Different cell wall pathogen-associated molecular patterns (PAMPs) and other molecules of *S. pneumoniae* induce signaling through the Tolllike receptor (TLR) pathways, such as peptidoglycan components by TLR2 and PLY by TLR4. As a result, the nuclear factor kappa-B

CHAPTER 5

Mitochondrial DNA Role in Zika Virus Infection

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Abstract: Zika virus (ZIKV) is a member of the *Flavivirus* family. ZIKV infection ranges from asymptomatic to a mild disease in adults. However, in 2015, ZIKV infection became a public health emergency in the Americas associated with neurological alterations such as Guillain-Barré syndrome (GBS) in adults and congenital zika syndrome (CZS). By blocking type I IFN interferon signaling pathways, ZIKV evades the immune system and infects cells expressing the T cell immunoglobulin mucin domain-1 (TIM-1) and TAM (Tyro3, AXL, and Mer) receptors, such as neural progenitor cells. Moreover, ZIKV seems to orchestrate a process of astrocytic hypoxia that leads to the production of reactive oxygen species (ROS), mitochondrial DNA (mtDNA) fragmentation, and apoptosis. In recent decades, the active participation of mitochondria in the immuno-inflammatory response has been reported in several pathologies. In this context, mtDNA seems to have an essential role in triggering the innate immune response by activating inflammasomes, activating the cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway, and also activating toll-like receptors that lead to IFN production and viral clearance. Here, we present an overview of some mechanisms of inflammatory response present in ZIKV infection, which contributes to mitochondrial dysfunction, mtDNA release, and tissue damage.

Keywords: Inflammation, Mitochondrial DNA, Neuroinflammation, Zika virus.

1. INTRODUCING ZIKA VIRUS (ZIKV)

Zika virus (ZIKV) belongs to the *Flaviviridae* family, *Flavivirus* genus, which includes Dengue virus, Yellow Fever, West Nile virus, St. Louis encephalitis, and Japanese encephalitis. ZIKV was isolated from sentinel rhesus monkeys in 1947

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in the Zika forest in Uganda to study the wild cycle of yellow fever. Subsequently, this pathogen was isolated in *Ae. Africanus* mosquito, but the first description of human infection occurred in 1954, in serum samples collected from West African inhabitants [1, 2].

ZIKV presents icosahedral symmetry and a genome consisting of a single positive-strand RNA encoding a single polyprotein (Fig. **1**). This polyprotein encodes three structural proteins (capsid, membrane, and envelope) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B e NS5). The structural membrane protein plays a vital role in the fusion process between the viral envelope and the host membrane, while the viral envelope protein is involved in receptor binding, membrane fusion, and viral assembly, in addition to being the main target of neutralizing antibodies [3]. Non-structural proteins are involved in essential processes such as evasion of the virus to the host's immune system and mechanism of viral replication [4]. Genotypic analysis shows that ZIKV has only one serotype, with two distinct strains, Asian (P6-740) and African (MR766) [5].

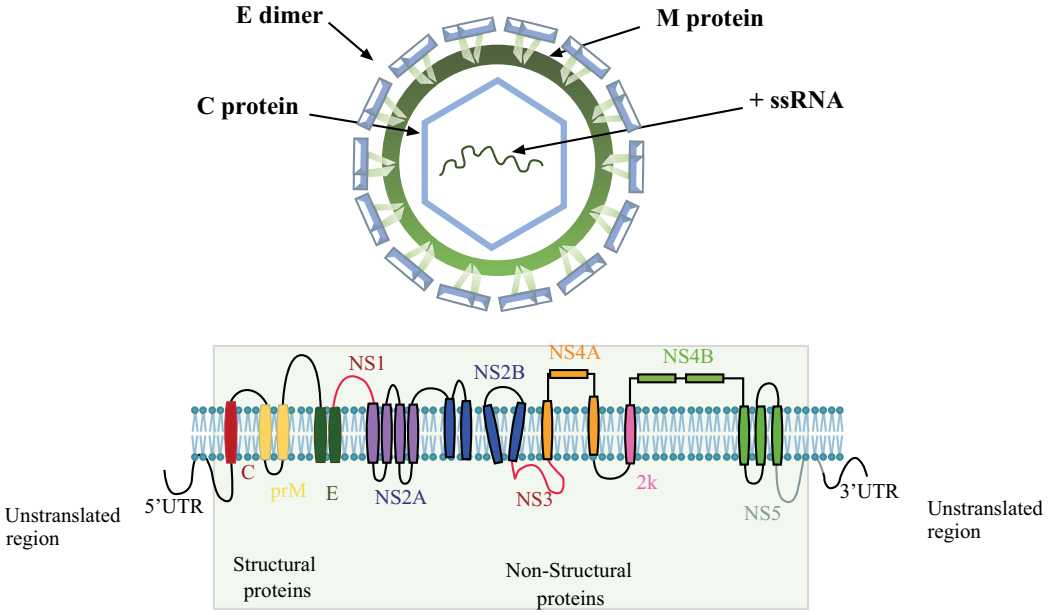

Fig. (1). Schematic Zika virus (ZIKV) structure and viral polyproteins. As described above, the main structural compartments of ZIKV are composed of an icosahedral capsid (C), an envelope protein (E dimer), membrane protein (M), and a genome consisting of single-strand positive-sense RNA (+ssRNA). At the bottom of the figure, structural (C, E, prM) and non-structural proteins (NS1-NS5) are presented.

The transmission occurs mainly through the bite of the *Aedes* mosquitoes genus; therefore, ZIKV is classified as an arbovirus (arthropod-borne virus). In addition,

another non-vector-mediated transmission has been described in the literature, such as blood transfusion [6], sexual transmission [7], and vertical transmission [8 - 10]. In addition, the viral genome was detected in different body fluids such as urine [11], saliva [12], breast milk [13], and vaginal fluid [14].

The extrinsic incubation stage is the period between the occurrence of the blood meal with the infected blood and becoming infectious. This period has an average of ten days and may vary from 3 to 12 days. After that period, the mosquito is able to transmit the virus to a susceptible host [15 - 17]. ZIKV can infect fibroblasts, keratinocytes, Langerhans cells, monocytes, and macrophages present at the inoculation site [18, 19]. Also, neural progenitor cells (NPC) [20], radial glial cells [21], astrocytes [22], microglia [23], and trophoblasts cells [24, 25] can also be infected by ZIKV. It is known that the virus is able to bind to TIM-1 and TAM receptor families (Tyro3 and AXL) and to DC-SIGN receptors that are expressed on the plasma membrane of host cells [26]. Radial glia, astrocytes, microglia, and endothelial cells showed high expression of AXL receptors [27]. Interestingly, studies had described that expression of AXL receptors could confer vulnerability to ZIKV infection [26, 28, 29].

ZIKV infection is characterized by asymptomatic to mild disease. Symptomatic cases occur in 27-50% of infected individuals [30]. The clinical symptoms are similar to other arboviruses such as Dengue and Chikungunya virus, characterized by a rash (located frequently on the neck, torso and limbs, palms and soles), mild to moderate fever, non-purulent conjunctivitis, and arthralgia, and there may be retro-ocular pain, myalgia between other non-specific symptoms. The symptomatic condition usually persists for 3-7 days, except for arthralgia, which may keep for some weeks [31].

The first description of ZIKV infection in humans was in 1954, and the infection did not appear to induce chronic symptoms or impact pregnant women [32]. However, in 2015, the first cases of ZIKV infection were confirmed in northeastern Brazil, and microcephaly in newborns was associated with infection during pregnancy [33]. The S139N mutation in the Asian strain has been described as being responsible for the increase in virulence, neurotropism, and apoptosis in human neural progenitor cells and is associated with the cases of microcephaly related [34].

The vertical transmission has significant impacts on pregnant women with severe clinical abnormalities that usually affect child development, defined as Congenital Zika Syndrome (CZS). The causes of the development of CZS in only a few infants exposed during the embryonic stage are not yet evident in the literature. However, the literature suggests that the trimester in which the pregnant woman is

CHAPTER 6

Mitochondrial Dysfunction and the Immuno-inflammatory Response Induced by SARS-CoV-2 Infection: the Role of Mitochondrial DNA

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Abstract: In 2019, a new coronavirus (SARS-CoV-2) was identified in China and had rapidly spread across the world. Its associated disease, coronavirus disease 2019 (COVID-19), has led to millions of deaths in 2020-2021. Studies have been demonstrating that SARS-CoV-2 induces a systemic hyperinflammatory state, which is associated with a decreased cytotoxic capacity and impaired Type I interferon (IFN) response. Moreover, iron dysfunction/hyperferritinemia in association with hyperinflammation leads to oxidative stress and apoptosis. Altogether, these cellular events contribute to COVID-19 severity. In viral infections, systemic and cellular alterations can promote mitochondrial dysfunction. In this regard, dysfunctional mitochondria can trigger the immune response, leading to the release of mitochondrial damage-associated molecular patterns, including mitochondrial DNA (mtDNA) and reactive oxygen species (mtROS). mtDNA is known to promote a beneficial antiviral response; however, sustained nocive stimuli, such as SARS-CoV-2, could turn this response into oxidative stress and exacerbated inflammation leading to tissue injury. In addition, mtDNA can be released into the extracellular space and induce a proinflammatory state in neighboring cells. Here, we highlight the potential role of mtDNA as an important marker of hyperinflammation in the progress of COVID-19. Furthermore, we briefly discuss the role of mtROS and its interactions with the mitochondrial antiviral signaling (MAVS), which can also contribute to COVID-19 immunopathogenesis.

Keywords: Coronavirus, COVID-19, Immune response, Inflammation, Mitochondrial DNA.

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1. INTRODUCING CORONAVIRUS INFECTIOUS DISEASE 2019 *(*COVID-19*)*

In December 2019, a substantial increase in pneumonia cases with an unknown cause was observed in Wuhan, China [1, 2]. Genome sequencing was carried out, and the severe acute respiratory coronavirus 2 (SARS-CoV-2, also known as 2019-nCoV) was identified as responsible for this new disease, which was further called coronavirus disease 2019 (COVID-19) [3]. After the dramatic increase in the number of cases in China, the virus promptly spread to other countries with a very high impact in Europe and the United States [4, 5]. Subsequently, by March 11th 2020, the World Health Organization declared COVID-19 a pandemic [6].

Belonging to the *Coronaviridae* family, coronaviruses are enveloped viruses with a single-stranded RNA of positive polarity as their genome. They can be found among humans and animals and are mainly responsible for respiratory and enteric diseases [7]. Before the 2002-2004 SARS-CoV outbreak in China, it was believed that coronaviruses could not cause severe illness in humans. Not only the SARS-CoV outbreak but also the Middle East respiratory syndrome (caused by MERS-CoV) epidemics 10 years later proved that these viruses are relevant pathogens of public health concern [8, 9]. Importantly, compared to SARS-CoV and MERS-CoV, SARS-CoV-2 has spread more rapidly due to globalization, exceeding the number of cases and deaths of previous epidemics [10].

The major route of SARS-CoV-2 transmission is through viral particles eliminated in the event of coughing and/or sneezing [5, 11]. Studies have shown that direct inoculation of viral particles through manipulation of contaminated surfaces may also occur since SARS-CoV-2 can remain viable in different surfaces such as plastic, glass, and stainless steel for various hours up to a few days [12 - 14]. Transmission through aerosol is also relevant, mainly in hospital settings, since various aerosol-generating proceedings such as bronchoscopy and orotracheal intubation are frequent [14, 15]. Lastly, studies have shown that SARS-CoV-2 can be present in the feces of infected patients, which highlights the potential for fecal-oral transmission [16].

COVID-19 symptoms frequently appear after an incubation period of ~5.2 days and can be classified as mild, moderate, severe, or critical illness based on the severity of symptoms [5]. In this context, the most common symptoms are fever, cough, and shortness of breath [17, 18]. Occasionally, non-respiratory symptoms such as palpitations, diarrhea, or headache can precede the respiratory symptoms [19]. In severe cases, patients may experience dyspnea and decreased oxygen saturation, while critical cases present with respiratory failure and septic shock [19].

In regards to COVID-19 severity, several factors can contribute to determining if an individual infected with SARS-CoV-2 will develop a self-limited disease with flu-like symptoms or a severe case requiring intensive care and mechanical ventilation. To mention a few, since the beginning of COVID-19 pandemic, studies have been showing that older age and comorbidities (such as diabetes, chronic obstructive pulmonary disease, cardiovascular disease, and cancer) are significantly associated with disease severity [20 - 23]. Moreover, an exacerbated immune response in association with coagulation disorders is also associated with poor outcomes [24 - 27]. Some studies also demonstrate that the frequency of severe cases is higher among men when compared to women, especially for elderly patients [24, 28]. As expected, high SARS-CoV-2 viral load (and inversely lower cycle threshold values) has also been observed in severe cases [29, 30]. Lastly, co-infections by various pathogens have been a cause of concern, which have empirically generated some treatment protocols including broad-spectrum antibiotics and antiparasitic drugs [31, 32]. Whether all these factors can synergistically contribute to COVID-19 severity and lethality is a question that must be answered.

2. THE IMMUNOPATHOGENESIS OF COVID-19

It is known that SARS-CoV-2 can induce a robust immune response. Thus, considering the urgent need to understand the mechanisms involved in COVID-19 immunopathogenesis, and subsequently contribute to the development of new treatment and vaccine strategies, several studies have been performed in the last two years to assess different aspects of the immuno-inflammatory response driven by SARS-CoV-2 infection.

Firstly, the exacerbated release of circulating cytokines and chemokines leading to a sustained state of hyperinflammation has been extensively reported [24 - 26, 33]. This immunological event, which is also known as “cytokine storm,” is promoted by the uncontrolled and sustained activation of T cells and macrophages, which leads to high serum levels of proinflammatory cytokines such as IL-2, IL-6, IL-8, TNF-α, IL-1β; and chemokines such as MCP-1/CCL-2 and IP-10/CXCL-10 [24, 33]. Elevations in circulating levels of anti-inflammatory cytokines such as IL-10 can also be observed, probably as an attempt to suppress the inflammatory process [34, 35]. Notably, this phenomenon of excessive cytokine release is accompanied by an increase in levels of acute-phase proteins [36]. We and others have demonstrated that hyperferritinemia is an important and early indicator of inflammation in hospitalized patients, easily accessible during clinical follow-up, and considered a relevant predictive factor for disease severity and death by COVID-19 [37 - 39]. In this context,

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DILVANI OLIVEIRA SANTOS

Prof. Dilvani Oliveira Santos is a senior professor at the Federal Fluminense University (UFF) in Niterói, RJ, Brazil. She was a visiting professor at the University of Ghent - Belgium (fellowship: Capes / 2009). She holds Post-Doctorate in Cell Immunology at Innogenetics - Ghent - Belgium ([fellowship: World Health Organization (WHO) / 1996] and [fellowship: CNPq / 1992-1994]). Recently, she filed a patent at the National Institute of Industrial Property (INPI, Brazil) 2019: “Pharmaceutical composition with seaweed drawn fractions and its use as a leishmanicide agent” registration number BR10201901811. She was a Guest Editor of the international scientific journal “Frontiers in Medicine” (2020/2022). Prof. Dilvani Santos has published several scientific articles and books (most of them on Leprosy and cutaneous Leishmaniasis). In addition, she is a writer of multilingual children's books as a teaching tool for health promotion in childhood.

PAULO RENATO ZUQUIM ANTAS

Dr. Paulo Renato Zuquim Antas has been mentoring and coaching a team that develops both clinical and basic research embracing the field of human infections and vaccinations. Dr. Antas's laboratory group has contributed to the fight against tuberculosis in individuals naturally exposed to *Mycobacterium tuberculosis* at Oswaldo Cruz Foundation (FIOCRUZ), RJ, Brazil. Most of his scientific time is currently dedicated to studying several aspects of the involvement of inflammatory cytokines, and the T cells in the immunity to mycobacteria. Part of his work is also focused on (1) identifying immunologic predispositions to tuberculosis, (2) identifying new biomarkers for mycobacterial diseases, and (3) uncovering the in vitro aspects that afford partial protection by the BCG vaccine. These have allowed him to be well-positioned in his permanent position as a senior researcher to work as an independent investigator in tuberculosis.