Polymorphism of TLR-2 Gene and Risk factors Associated with Prevalence of *M.tuberculosis* in Participants Diagnosed of Pulmonary Tuberculosis in Ogun State, Nigeria.

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Being a M.Sc. Thesis Submitted to the Department of Biological Sciences, Faculty of Natural and Applied Sciences, Lead City University Ibadan, Oyo State, Nigeria

In Partial Fulfilment of the Requirement for the Award of Master Degree (MSc) Degree in Molecular Biology and Genomics

2025

Certification

This is to certify that **Rodiah Opeyemi Abubakre** with Matriculation Number **LCU/PG/003249** carried out this research work titled "Polymorphism of TLR-2 Gene and Risk factors Associated with Prevalence of *M.tuberculosis* in Participants Diagnosed of Pulmonary Tuberculosis in Ogun State, Nigeria"**.** under my supervision in the Department of Biological Sciences, Faculty of Natural and Applied Sciences, Lead City University, Ibadan, Oyo State, for the award of Master Degree (MSc) in Molecular Biology and Genomics and this has not been previously submitted.

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Dedication

I dedicate this project to Allah (SWT) for His mercies over my life and humanities at large.

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Acknowledgement

I want to acknowledge Lead City University and members of E- library for their support and knowledgeable impact. I am equally grateful to Dr. T.Y Raheem my ever dynamic supervisor for bringing out the best in me. I am also grateful to my Head of department, Dr. Felicia Adesina, Dr. Nike Bakare (Postgraduate coordinator),Dr. Enoch Ogene, Prof. Adeogun, Prof. Rosemary Audu, Dr. Tayo Sindiku, Dr. Bukola Bamikefa, Dr. Tajudeen Bamidele just to mention a few. Also to my parents Alhaji Bakare Rasheed. A, Alhaja Bakre Shakirah.A, for their unflinching love and support, My Families and siblings, AbdulRosheed. A, Roheemoh. A, Royhanah. A, Rosheedah. Mr Gbadamosi. K, and Mrs Bakare.I for their immeasurable support I am sincerely grateful to Dr. Roland Funwei of Babcock university teaching hospital(BUTH) for his help during the analysis, of the department for their encouragement and support. My daunting appreciation goes to my colleagues for sticking together till the end.

"Even though the above mentioned institutions and persons have assisted in the process of this research work, I alone stand responsible for the errors, if any found in this work"

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Table of Content

Content **Page**

Title Page i

Certification ii

Dedication iii

Acknowledgement iv

Table of Contents v

List of Tables x

List of Figures xi

Abstract xii

Chapter One: Introduction **1**

1.1 Background of the Study 1

1.2 Statement of the Problem 5

1.3 Justification of Study 5

1.4 Aim and Objectives of Study 6

1.4.1 Specific Objectives of the Study 6

1.5 Research Questions 6

1.6 Significance of the Study 7

1.7 Scope of Study 8

1.8 Limitation of Study 8

1.9 Operational Definition of Terms 8

Endnotes **10**

Chapter Two Literature Review **13**

2.1 Tuberculosis (TB) 13

2.1.1 Microbiology 14

2.1.2 Epidemiology 14

2.2 Natural History of TB 17

Lead City University Ibadan DO NOT COPY

2.2.1 Initial Infection 17

2.2.2 Latent Infection 17

2.2.3 Active Disease 18

2.3 Clinical Features 18

2.3.1 Pulmonary Disease 18

2.3.2 Extrapulmonary Tuberculosis 19

2.4 Diagnostic Considerations 19

2.4.1 Drug Susceptibility Testing 22

2.5 Treatment and Care 24

2.5.1 Drug-Susceptible Tuberculosis 25

2.5.2 Drug-Resistant Tuberculosis 26

2.6 Mycobacterium Tuberculosis Genome 28

2.6.1 Key Features 28

2.6.2 Evolutionary Origins 28

2.6.3 Global Lineages 29

2.6.4 Genomic Changes in Drug Resistance 31

2.6.5 Within-Host Diversity 32

2.7 Host- Mycobacterium Tuberculosis Interaction 33

2.7.1 Mycobacterium Tuberculosis Virulence 33

2.7.2 Host Response Against Mycobacterium Tuberculosis 34

2.7.2.1 Early Response 35

2.7.2.2 Granuloma Formation 38

2.8 Pattern Recognition Receptors (PRRs) in Mycobacterial Immunity 38

2.9 Toll-Like Receptors (TLRs) 39

Lead City University Ibadan DO NOT COPY

2.9.1 Discovery of TLRs 41
2.9.2 Structure and Characterization of TLRs 41
2.9.3 Members of TLR Family 43
2.9.4 Expression of TLRs 45
2.9.5 TLR Signaling Pathways 45
2.9.6 Myd88-Dependent Pathway 47
2.9.7 TRIF-Dependent Pathway 50
2.9.8 Function of TLRs 51
2.9.9 Consequences of The Released Cytokines 52
2.9.10 Importance of TLRs 52
2.9.11 Negative Feedback Regulators of TLRs 53
2.10 Toll Like Receptor 2 (TLR2) 54
2.10.1 Dimerization and Additional Co-Receptors of TLR2 57
2.10.1.1 Homodimerization 58
2.10.1.2 TLR2 Heterodimers 59
2.10.2 Other Co-Receptors Supporting TLR2 61
2.10.3 Optogenetics, An Innovative Approach to Study TLR Dimerization and Signaling 63
2.10.4 TLR2 Mediates Both Pro- and Anti-Inflammatory Responses 64
2.10.5 The Role of TLR2 in Infectious and Neurodegenerative Diseases 67
2.10.5.1 Sepsis 67
2.10.5.2 COVID-19 68
2.10.5.3 Neuroinflammation 69
2.10.5.4 Alzheimer's Disease 70
2.10.5.5 Parkinson's Disease (PD) 70

Lead City University Ibadan DO NOT COPY

2.10.6 Future Perspectives 73

Endnotes **75**

Chapter Three Methodology 85

3.1 Study Site 85

3.2 Study Participants 85

3.3 Sample Size 85

3.4 Study Design 87

Sampling Technique 87

3.5 Sample Collection and Transportation 87

3.6 Laboratory Analysis 88

3.6.1: Determination of Tb Status by Microscopy 88

3.6.1.1 Principle 88

3.6.1.2 Requirements 88

3.6.1.3 Composition of Strong Carbol Fuchsin 88

3.6.1.4 Procedure 88

3.6.2: Determination of M.Tb By Genexpert 89

3.6.3: Determination of M.Tb Drug Susceptibility By Genexpert 89

3.6.4 Participants'genomic Analysis 90

3.6.4.1 Procedure 90

3.6.5 Genotyping of Toll Like Receptors (TLRs) 91

3.7 Ethical Considerations 91

3.7.1 Approval from Health Research Ethics Committee 91

3.7.2 Confidentiality of The Participant's Data 92

3.8 Data Management and Analysis 92

3.9 Budget 92

Lead City University Ibadan DO NOT COPY

3.10 Work-Plan and Budget Presentation 93

Endnotes **94**

Chapter Four Results **95**

Results 95

Endnotes 117

Chapter Five Conclusion

5.1 Summary of Findings 118

5.2 Conclusion 118

5.3 Recommendations 119

5.4 Contribution to Knowledge 120

5.5 Suggested Areas Further Research 120

Bibliography 121

Appendices 130

Bio-Data 132

The University Compliance Certification 136

Lead City University Ibadan DO NOT COPY

List of Tables

Table **Title** **Page**

Table 1	TLR2-dependent pro- and anti-inflammatory responses.	66
Table 4	Work-plan and budget presentation	99
Table 4.1	Shows the socio-demographic characteristics of the Participants. In relation to age	99
Table 4.2	Shows the Comparison of the sensitivity and specificity of Microscopy result and GeneXpert screening of TB	101
Table 4.3	Distribution of Band Patterns of TLR2 Polymorphisms by Gender	103

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List of Figures

Figure	Tittle	Page

Figure 2.1	Worldwide Estimated TB (All Forms) Incidence in 2015.	16
Figure 2.2	Phylogenetic Analysis of the Mycobacterium Tuberculosis Complex (MTBC) Based On Genomic Data, Anchored by Mycobacterium Canettii	30
Figure 2.3	Innate Immune Response Against Mycobacterium Tuberculosis as it is Encountered by Alveolar Macrophages.	37
Figure 2.4	Structure of Tlrs.	42
Figure 2.5	Members of Tlrs Family and Their Location.	45
Figure 2.6	TLR Signaling Pathway.	47
Figure 2.7	TLR2 Myd88-Dependent Signalling Cascade. Dimerisation Is Initiated by Ligand Binding (Diacylated Lipopeptides (Lps) Or Triacylated Lps	57
Figure 2.8	TLR2 Homo- And Heterodimers and Additional Co-Receptors. Upon Ligand Binding, Dimerization Is Initiated, Often Requiring Additional Co-Receptors Such as Cluster of Differentiation 14 (CD14), CD44 Receptor, Integrin Receptors, Or CD36.	62
Figure 2.9	Concise Summary of TLR2's Role in Many Pertinent Inflammatory Disorders.	72
Figure 4.1	Prevalence of MTB Among TB Patients in Relation to Their Gender.	102
Figure 4.2	Labeled Electrophoresis Gel Showing Allelic Interpretation of Samples After Restriction Enzyme Digestion.	109
Figure 4.3	Distribution of Band Patterns of TLR2 Polymorphisms by Gender	110

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Abstract

Tuberculosis (TB) has remained a major cause of global mortality and is the single leading infectious killer only second to coronavirus disease 2019. Biological sex is an important determinant of health. Literature on the gender epidemiology of tuberculosis demonstrates gender differences in prevalence/notification rate, disease manifestation. The present study assessed toll like receptor 2 (TLR2), prevalence of *Mycobacterium Tuberculosis (M.TB)* and associated risk factors between male and female tuberculosis positive patients in Ogun state Nigeria.A total of 130 participants comprising of 83 M.TB positive individuals and 47 MTB negative individuals were recruited into the present study. One spot sputum sample was collected in sterile sputum container and 5mLs of venous blood was obtained from the anterior cubital vein using 5ml disposable, sterile syringe and dispensed into EDTA anticoagulated sample bottle for genomic analysis. Determination of TB status was done using both microscopy and geneXpert. DNA extraction was done using NIMR kit and TLRs genotyping was done using PCR. The result of the analysis shows a 100% specificity of the result of microscopy detection of TB compared to the result of GeneXpert detection of TB and also a 100% sensitivity of the result of microscopy detection of TB compared to the result of GeneXpert detection of TB. The prevalence of M.TB was higher among male subjects (58%) compared to female subjects (42%). The CC allele was more prevalent among male subjects (12) compared to females (7). The TT allele was also more prevalent among male subjects (5) compared to females (2). While the TC allele was slightly more prevalent among male subjects (24) compared to females (22) This study accomplished its goals, offering significant insights into the socio-demographic, genetic, and diagnostic factors of tuberculosis. The results highlight the essential interaction of behavioural, genetic, and environmental factors in determining tuberculosis susceptibility and outcomes.

Keywords: Mycobacterium Tuberculosis(M.TB), Toll-like receptors(TLRs), Diagnosis, Disease.

Word Count: 300

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Chapter One

Introduction

1.1 Background of the Study

Mycobacterium tuberculosis, commonly referred to as tubercle bacilli, is a pathogenic bacterium within the genus Mycobacterium and the primary causative agent of the majority of tuberculosis cases.[1] Initially identified in 1882 by Robert Koch, M. tuberculosis possesses a distinctive waxy covering on its cell surface, predominantly composed of mycolic acid, rendering the cell resistant to Gramme staining; hence, acid-fast detection methods are employed for its laboratory identification.[2]

Tuberculosis (TB) continues to be a significant contributor to global mortality and remains the foremost infectious killer, surpassed only by coronavirus illness 2019.[3.4] The tuberculosis epidemic impacts all global regions, although 30 nations disproportionately bear the burden, representing nearly 86% of the total expected incidence of tuberculosis infections in 2020.[5] The risk of tuberculosis differs not just globally among nations but also among subpopulations characterised by socio-demographic factors such as gender, age, race/ethnicity, immigration status, economic level, and employment within a specific country.[6] Existing discrepancies hinder the World Health Organization's (WHO) End Tuberculosis Strategy objectives and the advancement towards global tuberculosis eradication. In the absence of targeted treatments for high-risk populations, current gaps in tuberculosis are likely to be exacerbated; therefore, identifying these high-risk groups is the initial step towards implementing a pro-equity policy. Biological sex significantly influences health, as variations in genetic, hormonal, and epigenetic control affect the prevalence, manifestation, and management of diseases.[7] Nonetheless, genetics alone fails to account for the universally observed gender discrepancies, as these distinctions also stem from the social construction of gender.[8] Imposed gender roles, expectations, and norms can lead to disparities

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in access to material resources (such as prestige, power, and nourishment), health-related behaviours (for instance, smoking and drinking), and exposure to psychosocial stresses (including discrimination and violence).[8]

Contemporary study on the gender epidemiology of tuberculosis reveals disparities in prevalence and notification rates, illness manifestation, progression, case-fatality rates, treatment response, and along the continuum of tuberculosis pathogenesis and care.[9,10] Health inequities delineated by a singular characteristic, such as gender, overlook the complex realities of individuals' lives, where various identities and processes intersect to shape population health.[11] Prior research indicates that gender interacts with sociodemographic variables, such as age, to influence the risk of tuberculosis.[12] The existence of additional risk factors, such as human immunodeficiency virus (HIV), may further alter these consequences, as HIV is linked to an elevated risk of new Mycobacterium TB infection, reactivation of latent tuberculosis infection, and reinfection.[13]

In recent decades, much knowledge has been acquired regarding human innate and adaptive immunity and its principal components, including pattern recognition receptors (PRRs). These germline-encoded receptors swiftly recognise distinct molecular structures on pathogen surfaces, termed pathogen-associated molecular patterns (PAMPs) or endogenous damage-associated molecular patterns (DAMPs), thereby connecting non-specific immunity to particular immunity.[14, 15] As of now, two separate categories of pattern recognition receptors (PRRs) have been identified: membrane-bound receptors, which encompass Toll-like receptors (TLRs) and C-type lectin receptors (CLRs), and cytoplasmic proteins, including nucleotide-binding oligomerisation domain (NOD)-like receptors (NLRs), retinoic acid-inducible gene (RIG)-I-like receptors, absent in melanoma 2 (AIM2)-like receptors (ALRs), and DNA or RNA sensors, such as cyclic GMP-AMP synthase (cGAS).[16-18] The efficacy of the immune response in both the early and late stages of infection is considered crucial for

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determining the illness outcome. The initial determination may arise from the interactions between Mycobacterium tuberculosis and the diverse pattern recognition receptors (PRRs) present in innate immune system cells as well as in non-immune cells, including lung epithelial cells.[19, 20] While numerous studies have identified single nucleotide polymorphisms (SNPs) linked to varying susceptibility to tuberculosis in the receptors of the Toll-like receptor (TLR) and Nod-like receptor (NLR) families, the most significant associations between genetic polymorphisms and tuberculosis susceptibility typically do not manifest at the level of pattern recognition receptors (PRRs).[21-23] Moreover, the silencing of a single gene encoding pattern recognition receptors (PRRs) from the TLR or NLR families typically does not manifest significant abnormalities in murine models of Mycobacterium TB infection.[24] This observation indicates a limited role of individual receptors or redundancy among them, suggesting that comprehending the interactions and signal integration of pattern recognition receptors (PRRs) in conjunction rather than isolation is essential for deciphering the communication between mycobacterial receptors and their ligands.[19] This is exemplified by TLR2, which has been demonstrated to collaborate with other TLRs, TLR-related molecules like RP105, or non-TLR receptors such as C-type lectin receptors (CLRs) to identify mycobacteria. Moreover, new findings indicate that collaboration among pattern recognition receptors (PRRs) might influence the immune response to individual mycobacterial antigens, including trehalose dimycolate (TDM, commonly referred to as cord factor). Indeed, it is also acknowledged by the CLR Mincle (macrophage-inducible C-type lectin). Furthermore, an additional layer of complexity arises from the redundancy among receptors, complicating the isolation of each receptor's contribution to anti-TB immunity.[24] In summary, Mycobacterium TB -PRR interactions may facilitate the bacillus's persistence within host phagocytes or enhance host defences by initiating immunological responses, including autophagy, phagosome maturation, apoptosis, pyroptosis, and different bactericidal processes.[25]

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TLRs are type I transmembrane proteins featuring an extracellular domain composed of leucine-rich repeats that enable the recognition of PAMPs. They possess a transmembrane domain and an intracellular toll-interleukin-1 receptor (TIR) domain essential for downstream signalling.[26] To date, ten receptors (TLR1-10) have been found in the human body, categorised into cell surface receptors (TLR1, TLR2, TLR4-6, TLR10) and intracellular receptors (TLR3, TLR7-9).[27,28] Toll-like receptors (TLRs) are extensively present on immune system cells, including monocytes, as well as on non-immune cells, such as endothelial and epithelial cells.[29] Recognition of PAMPs or DAMPs initiates the homo- or heterodimerization of TLRs, resulting in an intracellular signalling cascade that often elicits a pro-inflammatory response.[30] TLR4 is a notable instance of homodimerization, triggered by lipopolysaccharide (LPS) derived from the outer membrane of Gram-negative bacteria like Escherichia coli, whereas TLR2 is recognised for forming heterodimers with either TLR1 or TLR6, contingent upon the ligand.[31,32] Besides heterodimer formation, TLR2 may operate as a solitary receptor and in a homodimer, although the latter remains contentious.[32] Despite extensive research on TLRs, particularly TLR2, their significance in health and illness remains a focal point of investigation.

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Directly Observed Treatment Shortcourse (DOTS) is a globally endorsed approach for tuberculosis control that encompasses political commitment, case detection via microscopy, appropriate treatment of cases, continuous medication delivery, and systematic recording and reporting of cases.[33] Nigeria adopted it in 2003. Effective treatment necessitates a suitable regimen of anti-tuberculosis medications (either loose or fixed combinations, including Isoniazid, Rifampicin, and Pyrazinamide) administered consistently under direct supervision for a minimum duration of 6 months. In 2009, the global burden of illness was anticipated to exceed 9 million new cases of tuberculosis, culminating in over 14 million cases overall.[34]

1.2 Statement of the problem

Despite being preventable and treatable, TB continues to be a major contributor to morbidity and mortality in resource-constrained countries. Genotypic characterisation of tuberculosis in Nigeria has been documented concerning treatment resistance. However, there is a scarcity of reports on the genetic differences of receptor molecules between male and female individuals with pulmonary tuberculosis. The changes in receptor molecules may serve as potential facilitators or impediments to tuberculosis susceptibility, severity, and treatment outcomes. In the past decade, numerous evaluations have been published regarding the scope and significance of strain variety in Mycobacterium TB[35,36]. Large-scale DNA sequencing and other omics platforms may now examine the subject at an unparalleled scale, with the capacity to yield significant new insights. Considering the molecular intricacies of tuberculosis, it is overly simplistic to assume that different strain behaviour results from merely one or a few genetic variations.[37] While numerous studies have advocated for identifying and addressing men as a high-risk demographic for tuberculosis interventions, the influence of gender may vary significantly across diverse contexts and groups. This Nigeria-based study examines gender-related genetic variations in tuberculosis incidence across time, categorised by age and clinical presentation depending on the afflicted anatomical region, to elucidate the impact of gender on tuberculosis across diverse sociodemographic and clinical situations.

1.3 Justification of study

Mycobacterium tuberculosis continues to pose a public health challenge in Nigeria, despite several studies conducted on the subject. The bulk of these studies predominantly concentrate on the epidemiology and prevalence of *Mycobacterium TB* in Nigeria. To our knowledge, no sufficient research has been undertaken in the study context that examine gender differences concerning genetic variation among patients positive for *Mycobacterium TB*. This study aims

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to address the disparity in genetic variation across genders in individuals positive for *Mycobacterium TB*.

1.4Aim and Objectives of Study

Aim of Study

The aim of the study was to assess toll like receptor 2 (TLR2), prevalence of *mycobacterium tuberculosis (*mtb*)* and associated risk factors between male and female tuberculosis positive patients in Ogun state Nigeria.

Specific Objectives of the Study

The specific objectives of this study were to;

i. To determine the socio-demographic characteristics of the participants.

ii. To Compare the specificity and sensitivity microscopy detection of TB with GeneXpert.

iii. To determine the prevalence of *Mycobacterium tuberculosis* among male and female subjects

iv. To identify the receptor molecules of *Mycobacterium tuberculosis* among male and female *Mycobacterium tuberculosis* -positive patients.

v. To determine if any variation in the hosts' receptor molecules in both gender is associated with TB susceptibility, severity and or outcome of treatment.

1.5 Research Questions

1. How is the socio-demographic characteristics of the participants determined?

2. Do the specificity and sensitivity with microscopy detection of TB and GeneXpert comparable?

3. Is there any prevalence of *Mycobacterium tuberculosis* among male and female subjects?

4. How is the identification of the receptor molecules of *Mycobacterium tuberculosis* among male and female *Mycobacterium tuberculosis* -positive patients done?

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5. Does the hosts' receptor molecules in both gender associated with TB susceptibility, severity and or outcome of treatment have variation?

1.6 Significance of the study

Despite being preventable and treatable, tuberculosis continues to be a major cause of illness and death in resource-constrained countries like Nigeria. It has recently re-emerged in affluent countries because to its association with human immunodeficiency virus/acquired immune deficiency syndrome, demographic shifts, and human migrations. Based on tuberculin reactivity, approximately one third of the global population is estimated to be infected with latent tuberculosis. Consequently, these infected individuals are susceptible to developing tuberculosis later in life as their immunity diminishes due to ageing or co-infection with HIV, given that tuberculosis is a significant opportunistic illness in those with HIV.

While numerous studies have advocated for the acknowledgement and targeting of gender, particularly men, as a high-risk demographic for tuberculosis interventions, the influence of gender may vary significantly across diverse contexts and communities. This project will evaluate gender disparities in tuberculosis incidence across time, focussing on patterns and genetic variation to enhance understanding of gender's impact on tuberculosis within sociodemographic and clinical frameworks.

This study will elucidate the pattern and frequency of genetic diversity between genders in pulmonary tuberculosis positive cases. This study will facilitate the diagnosis, management, therapy, and prognosis of pulmonary tuberculosis by fulfilling the research aim and objectives. Understanding the genetic variances and variations in tuberculosis presentation among patients will facilitate the development of gender-specific treatments for optimal tuberculosis management.

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1.7 Scope of the study

There were many pattern recognition receptos but this study was to assess toll like receptor 2 (TLR2), prevalence of *mycobacterium tuberculosis (*mtb*)* and associated risk factors between male and female tuberculosis positive patients in Ogun state Nigeria.

1.8 Limitation of Study

This study consideres the determinations of the TLR2 in Mycobacterium Tuberculosis positive patients and negative patients as control. The high cost of the reagents and laboratory analysis is a major set back in this study.

1.9 Operational Definition of Terms

Mycobacterium Tuberculosis: The bacterium responsible for causing TB.

Pattern Recognition: The ability of TLRs to recognize specific molecular patterns associated with M. tuberculosis.

Signal Transduction: The process by which TLRs transmit signals to downstream molecules, leading to the activation of immune responses.

Immune Activation: The process by which TLRs activate immune cells, such as macrophages and dendritic cells, to respond to M. tuberculosis infection.

Cytokine Production: The release of cytokines, such as TNF-α, IL-12, and IFN-γ, by immune cells in response to TLR-mediated recognition of M. tuberculosis.

Phagocytosis: The process by which immune cells, such as macrophages, engulf and digest M. tuberculosis bacteria.

Autophagy: The process by which cells recycle damaged or infected cellular components, including M. tuberculosis bacteria.

Apoptosis: The process of programmed cell death, which can be induced by TLR-mediated recognition of M. tuberculosis.

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Inflammation: The complex biological response to M. tuberculosis infection, involving the activation of immune cells and the release of cytokines and chemokines.

Toll-like Receptor 2(TLR2): Recognizes Mycobacterium tuberculosis (M. tuberculosis) components, such as lipoteichoic acid and peptidoglycan.

MyD88: A key adaptor response gene protein involved in TLR signaling pathways.

TRIF: Another adaptor response gene protein involved in TLR signaling pathways.

Latent TB: A state in which M. tuberculosis is present in the body but not actively replicating.

Active TB: A state in which M. tuberculosis is actively replicating and causing disease.

TB Granuloma: A complex structure formed by immune cells in response to M. tuberculosis infection.

Th1 Response: A type of immune response characterized by the production of cytokines such as IFN-γ and TNF-α, which are important for controlling M. tuberculosis infection.

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Endnotes

1. A. Amir, K. Rana, A. Arya, N. Kapoor, H. Kumar, & M.A. Siddiqui, *Mycobacterium tuberculosis H37Rv: In Silico Drug Targets Identification by Metabolic Pathways Analysis*, **International Journal of Evolutionary Biology**, 28, 2014, 41-70.

2. F. Yu, J. Lee, M. Wang, H. Hsu, T. Chen, C. Cheng, Y. Yang, G. Wang, & M. Yu, *Evaluation of a modified direct agar proportion method for testing susceptibility of Mycobacterium tuberculosis from MGIT samples*, **Journals of Microbiology and Immunology Infection**, 1182(14), 2014, 15-21.

3. WHO (World Health Organization), *WHO reveals leading causes of death and disability worldwide: 2000-2019*. **Geneva, Switzerland World Health Organization** 2020.

4. WHO (World Health Organization), *Global tuberculosis report 2021. Geneva: World Health Organization*, 2021. Available Online; https://www.who.int/publications/i/item/9789240037021/2021.

5. WHO (World Health Organization), 2010/2011 *Tuberculosis Global Fact Sheet*, Available Online; www.who.int/tb/publications/2010/factsheet_tb_ 2012.pdf

6. J.R. Hargreaves, D. Boccia, C.A. Evans, M. Adato, M. Petticrew, & J.D.H Porter, *The social determinants of tuberculosis: from evidence to action*, **American Journal of Public Health**, 101, 2011, 654–662.

7. S. Katrak, & J. Flood, *Latent tuberculosis and current health disparities*, **Journal of Leukocyte and Biology**, 3, 2018, 234-239.

8. F. Mauvais-Jarvis, N. Bairey-Merz, & P.J. Barnes, *Sex and gender: modifiers of health, disease, and medicine*, **Lancet**, 396, 2021, 565–582.

9. A. Thorson, & V.K. Diwan, *Gender inequalities in tuberculosis: aspects of infection, notification rates, and compliance*, **Current Opinion on Pulmonary Medicine**, **7**, 2001, 165–169.

10. K.C. Horton, P. MacPherson, R.M.G.J. Houben, R.G. White, & E.I. Corbett, *Sex differences in tuberculosis burden and notifications in low- and middle-income countries: a systematic review and meta-analysis*, **Public Library of Science Medicine**, 13, 2016, e1002119.

11. G.R. Bauer, *Incorporating intersectionality theory into population health research methodology: challenges and the potential to advance health equity*, **Social Science Medicine**, 110, 2014, 10–17.

12. X. Zhang, A.B. Andersen, & T. Lillebaek, *Effect of sex, age, and race on the clinical presentation of tuberculosis: a 15-year population-based study*, **American Journal of Tropical Medicine and Hygiene**, 85, 2011, 285.

13. J. Bruchfeld, M. Correia-Neves, & G. Kallenius, *Tuberculosis and HIV coinfection*, **Cold Spring Harbour Perspective Medicine**, **5**, 2015, a017871.

Lead City University Ibadan DO NOT COPY

14. O. Takeuchi, & S. Akira, *Pattern Recognition Receptors and Inflammation*, **Cell**, **140**, 2020, 805–820.

15. J.S. Roh, & D.H. Sohn, *Damage-Associated Molecular Patterns in Inflammatory Diseases*, **Immune Net**work, 18, 2023, e27.

16. D. Li, & M. Wu, *Pattern Recognition Receptors in Health and Diseases. Signal Transduct. Target*, **Therapy**, 6, 2021, 291.

17. V. Caneparo, S. Landolfo, M. Gariglio, & M. De Andrea, *The Absent in Melanoma 2-Like Receptor IFN-Inducible Protein 16 as an Inflammasome Regulator in Systemic Lupus Erythematosus: The Dark Side of Sensing Microbes*, **Frontier of Immunology**, **9**, 2023, 1180.

18. T. Hu, M. Pan, Y. Yin, C. Wang, Y. Cui, & Q. Wang, *The Regulatory Network of Cyclic GMP-AMP Synthase-Stimulator of Interferon Genes Pathway in Viral Evasion*, **Frontier of Microbiology**, **12**, 2021, 790714.

19. E.K. Jo, *Intracellular signalling cascades regulating innate immune responses to Mycobacteria: branching out from Toll-like receptors*, **Cell Microbiology**, 9(5). 2017, 1087-1098.

20. J.J. Garcia-Vallejo, & Y. van-Kooyk, *Endogenous ligands for C-type lectin receptors: the true regulators of immune homeostasis*, **Immunology Review**, 230, 2019, 22–37.

21. J.J. Yim, H.W. Lee, H.S. Lee, Y.W. Kim, S.K. Han, Y.S. Shim, & S.M. Holland, *The association between microsatellite polymorphisms in intron II of the human Toll-like receptor 2 gene and tuberculosis among Koreans*; **Genes Immunology**, 7, 2016, 150–155.

22. J.L. Casanova, L. Abel, & L. Quintana-Murci, Hu*man TLRs and IL-1Rs in host defense: natural insights from evolutionary, epidemiological, and clinical genetics*, **Annual Review Immunology**, 29, 2021, 447–491.

23. A. Fortin, L. Abel, J.L. Casanova, & P. Gros, *Host genetics of mycobacterial diseases in mice and men: forward genetic studies of BCG-osis and tuberculosis*, **Annual Review of Genomics and Human Genetics**, 8, 2017, 163–192.

24. N. Reiling, S. Ehlers, & C. Holscher, *MyDths and un-TOLLed truths: sensor, instructive and effector immunity to tuberculosis*, **Immunology**, 116, 2018, 15–23.

25. A. Tanne, B. Ma, F. Boudou, L. Tailleux, H. Botella, E. Badell, F. Levillain, M.E. Taylor, K. Drickamer, J. Nigou, K.M. Dobos, G. Puzo, D. Vestweber, M.K. Wild, M. Marcinko, P. Sobieszczuk, L. Stewart, D. Lebus, B. Gicquel, & O. Neyrolles, *A murine DC-SIGN homologue contributes to early host defense against Mycobacterium tuberculosis*, **Journal of Experimental Medicine**, 206, 2019, 2205–2220.

26. T. Kawai, & S. Akira, *The Role of Pattern-Recognition Receptors in Innate Immunity: Update on Toll-like Receptors*, **Nature and Immunology**, **11**, 2020, 373–384.

Lead City University Ibadan DO NOT COPY

27. K. Takeda, A& S. kira, *Toll-like Receptors in Innate Immunity*, **International Immunology**, **17**, 2020, 1–14.

28. N.A. Lind, V.E. Rael, K. Pestal, B. Liu, & G.M. Barton, *Regulation of the Nucleic Acid-Sensing Toll-like Receptors*, **Nature Review and Immunology**, **22**, 2022, 224–235.

29. M.E. Simpson, & W.A. Petri, *TLR2 as a Therapeutic Target in Bacterial Infection*, **Trends in Molecular Medicine**, **26**, 2020, 715–717.

30. P.N. Moynagh, *TLR Signalling and Activation of IRFs: Revisiting Old Friends from the NF-KB Pathway*, **Trends in Immunology**, **26**, 2020, 469–476.

31. R. Núñez, J. Wong, J.F. Westoll, H.J. Brooks, L.A.J. O'Neill, N.J. Gay, C.E. Bryant, & T.P. Monie, *A Dimer of the Toll-like Receptor 4 Cytoplasmic Domain Provides a Specific Scaffold for the Recruitment of Signalling Adaptor Proteins*, **Public Library of Science ONE**, **2**, 2022, e788.

32. L. Oliveira-Nascimento, P. Massari, & L.M. Wetzler, *The Role of TLR2 Ininfection and Immunity*, **Frontier of Immunology**, **18**, 2022, 79.

33. J. Hayden, *Introduction to Health Behaviour Theory*, **Jones and Bartlett Learning**. 2, 2013, 13.

34. WHO (World Health Organization), 2010/2011 *Tuberculosis Global Fact Sheet*, Available Online; www.who.int/tb/publications/2010/factsheet_tb_ 2012.pdf

35. M. Coscolla, & S. Gagneux, *Does M. tuberculosis genomic diversity explain disease diversity*? **Drug Discovery Today and Disease Mechanism**, **7**, 2010, e43–e59.

36. I. Parwati, R. van-Crevel, & D. van-Soolingen, *Possible underlying mechanisms for successful emergence of the Mycobacterium tuberculosis Beijing genotype strains*, **Lancet of Infectious Disease**, 10, 2010, 103–111.

37. I. Comas; M. Coscolla, & T. Luo, *Out-of-Africa migration and Neolithic coexpansion of Mycobacterium tuberculosis with modern humans*, **Nature and Genetics**, 45(10), 2023, 1176–1182.

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Chapter Two

Literature Review

2.1 Tuberculosis (Tb)

Tuberculosis is caused by the bacteria Mycobacterium tuberculosis. M. tuberculosis is transmitted via aerosol and initially resides in the lungs, potentially leading to both pulmonary and extrapulmonary illness either immediately or following a varied latent phase. The initiation of the disease is generally subacute or chronic, resulting in the characteristic signs of cough, weight loss, and nocturnal diaphoresis. The standard treatment necessitates a regimen of four medications to avert the emergence of resistance, administered over six months to mitigate the risk of relapse from dormant persister germs that remain untouched by the initial therapy.[1] Tuberculosis accounted for approximately 25% of all fatalities in industrialised nations during the 18th and 19th centuries. Its incidence initially declined in the 20th century; however, a resurgence occurred, partly due to heightened vulnerability to infection from the HIV/AIDS epidemic and rising drug resistance, culminating in the declaration of a global emergency in 1993.[2] This ignited a revitalised emphasis and investment in the disease, resulting in a substantial decline in mortality rates over the past twenty-five years, the proliferation of quick diagnostics even in poor and middle-income nations, and the availability of numerous novel medications for drug-resistant strains. Notwithstanding this, much work remains, since the general incidence continues to decline gradually at a mere 2% year, drug-susceptible treatment persists as protracted and has been static for over forty years, and an increasing number of drug-resistant patients are being detected.[1]

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2.1.1 Microbiology

Tuberculosis is caused by obligatory pathogenic microorganisms belonging to the *Mycobacterium tuberculosis* complex. This encompasses *Mycobacterium tuberculosis* sensu stricto, the principal human pathogen, along with other related species that infect humans and animals, including *M. africanum* (predominantly infecting humans in West Africa), *M. bovis* (cattle), and *M. caprae* (goats). Mycobacteria are distinguished by their thick cell wall, composed of three macromolecules and a lipopolysaccharide, as well as their slow growth rate, exhibiting an in vitro generation period of 24 hours.[1] They are typically cultivated on solid media, such Middlebrook 7H10/7H11 or Lowenstein-Jensen medium. Liquid medium, such as Middlebrook 7H9, are predominantly utilised for sub-culturing. The BACTEC 960 system (BD, MD, USA) employs liquid culture mycobacterial growth indicator tubes (MGIT), formulated with Middlebrook 7H9, which emit fluorescence as M. tuberculosis respiration depletes oxygen in the medium, facilitating automated and expedited growth detection and reporting.[1]

2.1.2 Epidemiology

Tuberculosis (TB), a transmissible infectious illness, ranks as the second biggest cause of mortality from infectious diseases globally, following human immunodeficiency virus (HIV). In 2015, the Global Tuberculosis Report 2016 documented approximately 10.4 million new tuberculosis cases and around 1.8 million tuberculosis-related fatalities.[3] In 2015, the majority of incident cases were reported in Asian (61%) and African (26%) countries, whereas the Eastern Mediterranean, European, and American regions collectively accounted for only 13% of the incidence. India constitutes over a quarter of the global tuberculosis cases and fatalities. India is among the countries with the highest incidence of relapse and multi-drug resistant tuberculosis (MDR-TB) patients.[3]

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The Mycobacterium TB complex has likely been responsible for human disease for 5,000 to 10,000 years. The tuberculosis epidemic peaked in the 18th and early 19th centuries in Western Europe and North America, emerging as the primary cause of mortality due to the Industrial Revolution, rural to urban migration, high population density, and inadequate nutrition, with incidence rates reaching 500-800 per 100,000 in Western Europe. The incidence of tuberculosis began to decline in the latter part of the 19th century as a result of enhanced living conditions, greater nutrition, and the segregation of contagious individuals. The introduction of the BCG vaccine in the 1950s and the subsequent availability of combination chemotherapy significantly contributed to the decline in case numbers.[3]

The 1990s witnessed a significant resurgence of tuberculosis, characterised by rising incidence in numerous countries, attributed to diminished emphasis on tuberculosis control programs and the proliferation of the HIV/AIDS epidemic, especially in sub-Saharan Africa.[2] Although this prompted heightened attention towards enhancing national tuberculosis programs and funding research, the global incidence of tuberculosis had escalated to roughly 175 per 100,000 by 2000, then declining at a mere rate of 1-2% annually, resulting in an estimated incidence of 132 per 100,000 in 2018. Recent estimates indicate that there were 10.0 million new tuberculosis infections in 2018, with about 1 million involving HIV co-infection, and 1.5 million fatalities.[1]

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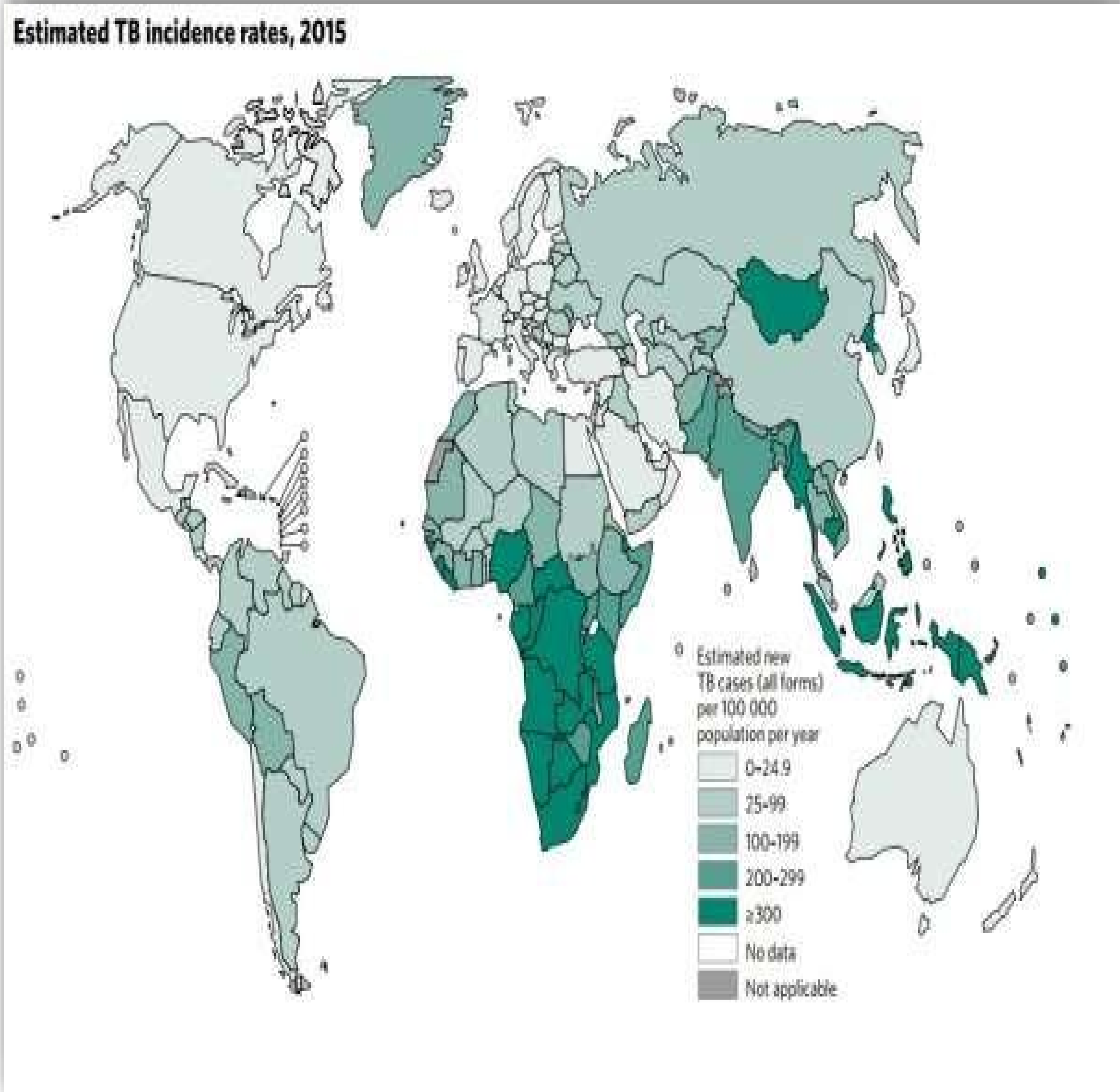

Figure 2.1: Worldwide estimated TB (all forms) incidence in 2015.[3]

2.2 Natural History of Tb

2.2.1 Initial Infection

Mycobacterium TB is transferred through the aerosol route from one infected person to another via dehydrated exhaled respiratory droplets (droplet nuclei) that can remain airborne for several hours. The infectious dose has been documented to be as minimal as one to five bacilli.[4] Upon deposition in the airways, the bacteria begin to proliferate and are phagocytosed by alveolar macrophages, eliciting an immunological response and resulting in the formation of a granuloma composed of macrophages, monocytes, and neutrophils. Granulomas may expand and merge, forming a nodular lesion or tubercle. The predominant initial infections are currently managed by cell-mediated immunity, resulting in the bacteria entering a dormant, non-replicating state. Nevertheless, if pulmonary replication is unregulated, germs may disseminate via the lymphatic system to the draining mediastinal lymph nodes, resulting in their enlargement. In roughly 5% of individuals, bacterial proliferation may transpire, resulting in a moderate, self-limiting primary illness characterised by a low-grade fever.[4] Bacterial reproductions infrequently develops uncontrolled, potentially resulting in mycobacterium tuberculosis bacteraemia and dissemination to other organs, leading to miliary TB, predominantly observed in children, or progressive primary illness impacting the lungs and pleura, more prevalent in adults.

2.2.2 Latent Infection

After primary infection, the majority of humans will successfully manage bacterial proliferation and enter a state of latent infection with dormant germs, while some individuals seem capable of resisting or swiftly eliminating the infection.[5] It is estimated that around one-quarter of the global population is latently infected with Mycobacterium tuberculosis.[6] Merely 2-4% of individuals with latent infection advance to active disease within five years, with advancement predominantly occurring in the initial year post-infection.[7] The risk of

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progression may be heightened by co-morbidities including HIV infection, chronic renal disease, diabetes, and immunosuppression.

2.2.3 Active Disease

The predominant kind of adult tuberculosis worldwide is post-primary pulmonary tuberculosis. In post-primary tuberculosis, a previously dormant bacterial population within a granuloma commences active replication. The mechanisms that drive reactivation remain poorly known; nevertheless, in certain patients, it may occur subsequent to immunosuppression, such as that caused by HIV infection, advancing age, or the administration of anti-TNFα treatment. In contrast to progressive primary disease, pathology is generally more confined, and spread is infrequent.[7] A significant portion of the pathology induced by tuberculosis is attributable to the host's immunological response during active infection. Although the majority of tuberculosis (TB) is pulmonary, dissemination during the main infection may affect several additional organs, resulting in extrapulmonary TB (EPTB). Extrapulmonary tuberculosis (EPTB) may manifest simultaneously with pulmonary disease or in isolation following the reactivation of a dormant extrapulmonary infection. The predominant locations for extrapulmonary tuberculosis (EPTB) include the lymph nodes (notably in the cervical region), pleura, bones and joints (especially the vertebral column), and central nervous system (tuberculous meningitis).[5]

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2.3 Clinical Features

Tuberculosis is an airborne communicable illness spread when aerosols (1-4 μm in size) containing the germs *Mycobacterium tuberculosis*, expelled by an infected people, are inhaled by healthy persons.[8]

2.3.1 Pulmonary Disease

The classical clinical manifestations include cough, weight loss, and night sweats, typically presenting with a subacute onset over a duration of weeks to months. Patients typically

present with a dry cough initially, which may subsequently progress to the production of purulent sputum as lung infiltrates grow and cavities form, resulting in the expectoration of bacteria and the possibility for transmission. Minor haemoptysis may arise from the deterioration of bronchial vessels.[6] Chest radiography typically reveals infiltrates and lung cavities in the upper lobes, frequently bilaterally; but, in cases of HIV co-infection, more widespread alterations and reduced cavitation are commonly observed. Clinical manifestations frequently coincide with those of other lung infections, complicating diagnosis and necessitating a heightened level of suspicion together with the results of pertinent investigations, including sputum microscopy, culture, and fast molecular diagnostics.[5]

2.3.2 Extrapulmonary Tuberculosis

Extrapulmonary tuberculosis (EPTB) can manifest in numerous locations, and its diagnosis may be more difficult than that of pulmonary illness due to the diverse signs and symptoms, which sometimes overlap with other conditions. Cervical lymph nodes represent the predominant location for extrapulmonary tuberculosis (EPTB). Lymph nodes may undergo considerable enlargement and may result in the formation of discharge sinuses on the skin. Pleural effusions are typically unilateral and consist of highly cellular fluid, however the visualisation of Mycobacteria is infrequent. TB meningitis manifests similarly to other types of meningitis, however typically with a more gradual start. Diagnosis may prove difficult without suitable investigations because of the overlap of clinical characteristics with different conditions.[7]

2.4 Diagnostic Considerations

Active tuberculosis is conclusively identified through the isolation of Mycobacterium tuberculosis. In instances of pulmonary tuberculosis, specimens are typically obtained from lung secretions, such as spontaneously generated sputum samples or bronchoalveolar lavage fluid, but may also be derived via biopsies of pleural or lung tissue. Extrapulmonary

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tuberculosis (EPTB) is firmly diagnosed through the study of pertinent specimens, such as pleural fluid (for pleural TB), cerebrospinal fluid (for meningeal TB), or lymph node biopsy. The definitive diagnosis is established through the microbiological culture of M. tuberculosis from the appropriate specimen.[1] Nonetheless, although culture is highly specific for M. tuberculosis, it is very sluggish, frequently requiring several weeks, and sensitivity may be restricted in certain situations, such as HIV-associated pulmonary illness. Automated liquid cultures are regarded as the benchmark for tuberculosis diagnosis and initial medication susceptibility assessment. Nonetheless, their expense and the necessity for highly educated personnel and adequately equipped laboratories limit their application in low-resource nations.[9] Alternative cultural techniques and pharmacological sensitivity assessments, including solid culture, microscopically observable drug susceptibility, and nitrate reductase assay, are more economically viable in resource-limited nations.[9,10] Despite requiring weeks to yield findings, solid culture remains the sole approach for assessing sensitivity to second-line medicines.[11]

A prevalent diagnostic method is the staining of clinical specimens, such as sputum smears, for acid-fast bacilli. This method is more rapid, cost-effective, and simpler than culture; however, it is less sensitive, fails to differentiate between Mycobacterial species, and cannot distinguish between live and dead bacteria.[5] Resource-constrained nations, which bear approximately 90% of the tuberculosis burden, predominantly depend on sputum smear microscopy and chest radiography as major techniques for active TB detection.[9] Approximately three-quarters of adults with active tuberculosis can be identified using smear microscopy. The grade, in accordance with WHO criteria, is as follows: No microorganisms observed in 100 immersion fields: Negative; 1 to 9 AFB observed in 100 immersion fields: Positive scanty, precise count documented; 10-99 AFB in 100 immersion fields: 1+; 1 to 10 AFB per field in 50 fields: 2+; More than 10 AFB per field in 20 fields: 3+.11 The method is

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cost-effective and necessitates minimal biosafety regulations, hence holding significant value in tuberculosis diagnosis, despite its low sensitivity. However, it does not furnish any information regarding the drug susceptibility of the microorganisms.[11] Microscopy findings are typically augmented by chest X-rays, which may reveal distinctive fluffy upper zone shadowing indicative of alveolar macrophages, lymphadenopathy, and cavitation.[12] Radiography can be beneficial in assessing dubious cases of tuberculosis when microscopy or PCR testing yield negative outcomes. Chest X-rays exhibit a sensitivity of 98% and a specificity of 75% when utilised independently as a diagnostic technique. Consequently, this approach cannot be solely depended upon for diagnosing TB.[13] scoring methods have been developed that may assist in excluding PTB, albeit they exhibit less specificity.[14]

Recent advancements in quick molecular diagnostics have been established to detect mycobacterium TB DNA with sensitivity nearing that of culture, and can identify frequently observed resistance mutations within hours. Examples include the Xpert Mycobacterium tuberculosis/RIF (Cepheid, CA, USA) assay, which detects the presence of M. tuberculosis and rifampicin resistance, and MDRTBsl (Hain Lifescience, Germany), which identifies mutations associated with fluoroquinolone and injectable drug resistance. The limitations include an inability to distinguish between live and dead bacteria, rendering them unsuitable for monitoring treatment response, and their dependence on a predefined, restricted list of resistance-associated single nucleotide polymorphisms (SNPs), which inadequately reflects the existing understanding of the relationship between genotypic and phenotypic drug resistance.[5]

Additionally, latent TB infection (LTBI) can be identified using tuberculin skin tests, although these tests cannot differentiate between mycobacterium tuberculosis infection and other mycobacterial exposures, such as BCG. The latest approach encompasses the IFNγ release assays (IGRAs).[9] The tuberculin skin test exhibits comparable sensitivity to IGRAs,

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although demonstrates lower specificity. Nevertheless, due to its low costs, it is the predominant approach employed in low-income countries for the detection and treatment of LTBI.[10] Latent infection constitutes a significant reservoir for potential disease, necessitating the screening of high-risk groups, including recent contacts of active TB patients, individuals from high-prevalence regions, and patients with HIV or diabetes, to ensure timely and appropriate treatment.[10] The diagnosis of extrapulmonary tuberculosis (EPTB) is more complex, as smear microscopy is largely ineffective in this context. The study of microbiological and histological specimens from probable infection sites is advised. Xpert® mycobacterium tuberculosis/RIF testing on cerebrospinal fluid is recommended for conditions such tuberculous meningitis, when prompt identification is critical.[15] A prompt diagnosis of tuberculosis has historically been a persistent issue. The increasing popularity of personalised therapy for infectious diseases necessitates individual risk assessment utilising genetic markers, such as SNPs, to ascertain susceptibility or resistance status.[16]

2.4.1 Drug susceptibility testing

Analogous to diagnostics, drug susceptibility testing (DST) of *M. Tuberculosis* has traditionally depended on culture-based methodologies (termed phenotypic DST) that assess bacterial proliferation in the presence and absence of a treatment. The existing WHO-sanctioned phenotypic drug susceptibility testing (pDST) methodologies are solid media testing utilising the 1% agar proportion method with Lowenstein-Jensen or 7H10/7H11 media, and liquid media testing employing the BACTEC MGIT 960 system.[15] Resistance is shown when bacteria are detected that proliferate in the presence of a medicine at a concentration exceeding a predetermined threshold that distinguishes resistant from susceptible isolates. The minimum inhibitory concentration (MIC) is the medication concentration necessary to suppress 99% of bacterial proliferation. The optimal threshold would be the MIC that establishes a clinical breakpoint based on patient outcome data to

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determine bacterial susceptibility and resistance. Comprehensive patient outcome statistics are typically lacking for the majority of tuberculosis medications, partly due to the prolonged duration of treatment, the utilisation of drug combinations, and the challenges in determining clinical results. Consequently, critical concentrations are employed to distinguish between wild-type susceptible isolates and those expected to exhibit resistance, typically based on the identification of isolates possessing probable resistance-conferring genetic changes.[15] Defining critical concentrations may be challenging when there is an overlap of minimum inhibitory concentrations (MICs) between species lacking known or potential drug resistance-associated variations (RAVs), i.e., genetically wild type, and those possessing RAVs, as exemplified by bedaquiline.Fifteen The limitations of phenotypic DST (pDST) parallel those of culture-based diagnostics. Both necessitate initial bacterial culture, succeeded by subculturing in the presence of each antibiotic, a procedure that is protracted (lasting weeks to months), technically intricate, and costly. Rapid molecular diagnostic procedures, such as Xpert Mycobacterium tuberculosis/RIF and line probe assays, can detect the presence of M. TB DNA and identify prevalent resistance-associated variations (RAV). These tests are now accessible in numerous poor and middle-income countries.[15]

Nevertheless, as previously outlined, their detection is constrained to a limited array of pre-defined SNPs. This is generally sufficient for the few well-characterized SNPs associated with rifampicin and isoniazid resistance, but it is inadequate for identifying the numerous SNPs and insertions and deletions (indels) that frequently impart resistance to medications like pyrazinamide and bedaquiline. Consequently, whole genome sequencing (WGS) is widely regarded as the optimal test for drug susceptibility testing (DST), particularly due to the growing understanding of the relationship between genotype and phenotype.[17] Whole Genome Sequencing (WGS) is also advantageous for deducing transmission networks.

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Whole Genome Sequencing (WGS) is currently the standard initial drug susceptibility testing (DST) approach in England, as well as in select centres in the USA and the Netherlands.

2.5 Treatment and Care

The conventional treatment protocol for active drug-susceptible tuberculosis includes four medications: rifampicin (H), isoniazid (R), pyrazinamide (Z), and ethambutol (E), with Streptomycin (S) occasionally included, administered during an initial two-month induction phase. Throughout this interval, the majority of rapidly proliferating microorganisms are anticipated to be eradicated. The induction phase is succeeded by a consolidation phase during which isoniazid and rifampicin are administered for an additional four months.[43] The extended treatment regimen frequently results in patient non-adherence due to several variables, including drug toxicity, social stigma, and the misconception that the illness is resolved after the cough subsides and no bacteria are detected in the sputum.[18] Directly Observed Therapy The Directly Observed Treatment, Short-course (DOTS) strategy for tuberculosis management, endorsed globally by the World Health Organisation, facilitates adherence to treatment by ensuring that the entire regimen is conducted under meticulous supervision, wherein a designated individual (health worker, volunteer, or family member) directly observes the patient ingesting the medication.[15] The patient is deemed cured if the sputum smear yields a negative result at the conclusion of treatment and at least once prior.[1] New patients are often regarded as having drug-susceptible tuberculosis, except in instances when the prevalence of isoniazid resistance in the population is elevated or when there is a probable exposure to a patient with multidrug-resistant tuberculosis. In these instances, new patients are administered ethambutol alongside two other medications throughout the continuing period.[1] Relapse instances are more prone to being affected by one or more first-line medications. In these instances and in situations of MDR-TB, it is advisable to do drug sensitivity testing (DST) for every patient. In numerous nations lacking sufficient laboratory

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equipment, the results of the DST test are not readily accessible. Empirical regimens are advised for usage in these instances until the test results are obtained.[18] For MDR-TB infections, the WHO advises a minimum of twenty months of chemotherapy, including an extended intense phase of around nine months utilising more toxic second-line medications. Extrapulmonary tuberculosis (EPTB) is managed in the same manner as pulmonary tuberculosis (PTB). Certain doctors advocate for an extended treatment duration for tuberculosis impacting the central nervous system, bones, or joints.Individuals with latent tuberculosis infection (LTBI), children under five years of age who are close contacts of active tuberculosis (TB) patients, and HIV patients without active TB infection are advised to get isoniazid for six months as prophylactic treatment.[15] The shift from planned to personalised medicinal therapeutic management for tuberculosis is impeded by the absence of suitable genetic markers, among other issues. Single nucleotide polymorphisms (SNPs) facilitate the mapping of complex genetic traits, and their correlation with susceptibility and medication response can advance the objective of delivering personalised medicines.[16.19]

2.5.1 Drug-susceptible tuberculosis

The treatment for drug-susceptible tuberculosis (TB) continues to be the six-month "short course" established by clinical trials conducted by the British Medical Research Council in the 1970s and 1980s. Shorter treatment durations result in unacceptably high relapse rates due to the prolonged time required to eradicate dormant bacteria with minimal metabolic activity.[20] The six-month standard regimen includes daily rifampicin and isoniazid throughout, supplemented by daily pyrazinamide and ethambutol for the initial two months, applicable to all pulmonary and extrapulmonary tuberculosis without central nervous system (CNS) involvement. In instances of CNS involvement, such as TB meningitis, the conventional treatment regimen is prolonged to 12 months of rifampicin and isoniazid, supplemented with pyrazinamide and ethambutol for the initial two months.[20] Rifampicin is the essential

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medication that enabled the reduction of treatment duration to six months, owing to its robust bactericidal and sterilising properties that eradicate both reproducing and dormant germs. Isoniazid is a potent bactericidal drug that accounts for the predominant bacterial eradication during the initial phase of treatment. Pyrazinamide is an effective sterilising drug believed to predominantly target intracellular bacteria in an acidic milieu during the initial phase of treatment. Ethambutol does not directly enhance the efficacy of this regimen in completely drug-susceptible disease; nonetheless, it is incorporated to fortify the regimen and mitigate the danger of developing additional resistance in the event of drug resistance.

2.5.2 Drug-resistant tuberculosis

Since ethambutol is a non-essential element of drug-susceptible tuberculosis (DSTB) therapy, infections caused by ethambutol monoresistant Mycobacterium tuberculosis can be managed as DSTB, provided that susceptibility to the other three drugs is verified. Management of isoniazid or pyrazinamide monoresistance involves discontinuing the resistant medicine and prolonging the treatment duration from six to nine months, which includes an extra three months of rifampicin and isoniazid.[20] Resistance to rifampicin and isoniazid constitutes multidrug-resistant tuberculosis (MDR-TB) and necessitates treatment with second-line medications. As rifampicin is the essential drug on which the usual treatment regimen depends, rifampicin resistant TB (RR-TB) is treated similarly to multiple drug resistant TB (MDR-TB). The management of multidrug-resistant tuberculosis (MDR-TB) has evolved markedly in the past ten years. The WHO guidelines for drug-resistant tuberculosis (DR-TB) in 2011 advised a treatment regimen utilising second-line medications, comprising a fluoroquinolone and an injectable agent (kanamycin, amikacin, or capreomycin) for a duration of 20 months.[20] Subsequent to the release of the "Bangladesh regimen," which indicated favourable clinical results with a modified nine to twelve month treatment protocol in 2010, and the evidence of non-inferiority compared to the standard (long) regimen, the

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WHO now recommends this as an alternative for patients not previously treated with second-line drugs.[1] The recommended composition of the extended regimen has been revised to prioritise bedaquiline, the first novel antituberculous agent in four decades, and linezolid, a potent drug against gram-positive infections with antimycobacterial properties, alongside the fluoroquinolones levofloxacin and moxifloxacin, replacing the injectable agents.[1] This was attributed to new information indicating decreased mortality linked to these medications, and the considerable ototoxicity resulting from injectable treatments.[21] The ideal formulation of pharmacological intervention for MDR-TB remains unresolved, with multiple clinical trials now in progress.[22] The primary enquiries to address are whether substituting injectable drugs with bedaquiline in a modified nine to twelve-month short Bangladesh regimen is non-inferior to the currently recommended regimens (under evaluation by STREAM stage 2), and whether alternative combinations of new drugs permit additional treatment shortening.[23] TB PRACTECAL is assessing if treatment duration can be shortened to six months with the administration of bedaquiline, linezolid, and the novel medication pretomanid, with or without the inclusion of moxifloxacin or clofazimine.[24] Treatment for tuberculosis resistant to one or both primary second-line medications (fluoroquinolones or injectable agents) has traditionally been classified as preextensively drug-resistant tuberculosis (preXDR-TB) or extensively drug-resistant tuberculosis (XDR-TB), respectively. However, the importance of injectable resistance has diminished, as it is less commonly employed in multidrug-resistant tuberculosis (MDR-TB) treatment protocols. Presently, preXDR/XDR-TB necessitates an individualised extended treatment regimen, customised to medication susceptibility profiles, lasting a minimum of 18 months as per WHO standards.[23] Promising results from the Nix-TB trial indicate that favourable clinical outcomes can be attained in individuals with XDR-TB through six months of treatment with bedaquiline, linezolid, and pretomanid.[24]

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2.6 Mycobacterium Tuberculosis Genome

2.6.1 Key Features

The whole genome sequence of H37Rv, the predominant laboratory strain of *Mycobacterium tuberculosis*, was published in 1998.[25] The latest annotation indicates a length of 4,411,532 base pairs, surpassing the majority of bacterial genomes but falling short of Escherichia coli, and has 3,906 coding genes, a significant portion of which are implicated in fatty acid metabolism. It is highly abundant in guanine and cytosine residues, and, in contrast to most other bacteria, there is no indication of recombination or an auxiliary genome. Approximately 10% of the genome is allocated to a specific group of glutamate-rich proteins known as the PE and PPE gene families, named after the amino acids proline and glutamic acid that comprise them. They are heterogeneous and comprise multiple tandem repeats, complicating their resolution using short-read sequencing, and are hypothesised to function as surface antigens that mediate contact with the host immune system.[25]

2.6.2 Evolutionary Origins

The *mycobacterium tuberculosis* complex (MTBC) likely originated from the evolution of an ancestor of the environmental mycobacterium *M. canetti* into a specialised human pathogen, characterised by a reduction in genome size and the loss of genetic recombination or gene transfer capabilities. The initial divergence of the MTBC likely occurred in Africa and subsequently disseminated worldwide through human migration, with animal-adapted strains of the MTBC being transmitted from humans, as demonstrated by comparative genomic studies indicating gene loss from Mycobacterium tuberculosis sensu stricto to other MTBC members.[26] Genetic research indicates that MTBC likely started approximately 5,000 to 10,000 years ago, aligning with archaeological findings of *mycobacterium tuberculosis* DNA and lipids in skeletal remains dating back 9,000 years.[27-29]

2.6.3 Global lineages

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The contemporary MTBC consists of seven human-adapted lineages and many animal-adapted strains. Lineages 1, 2, 3, 4, and 7 are conventionally classified as *Mycobacterium tuberculosis sensu stricto*, whereas lineages 5 and 6 are identified as *M. africanum.* Lineages 5 and 6 are exclusive to West Africa, whereas lineage 7 is exclusive to East Africa, indicating their particular adaptation to local host populations. Lineages 1 to 4 are scattered worldwide, with lineages 2 and 4 being the most dominant.[30] Lineage 2 is linked to elevated mutation rates, increased virulence, and a higher prevalence of treatment resistance.[31]

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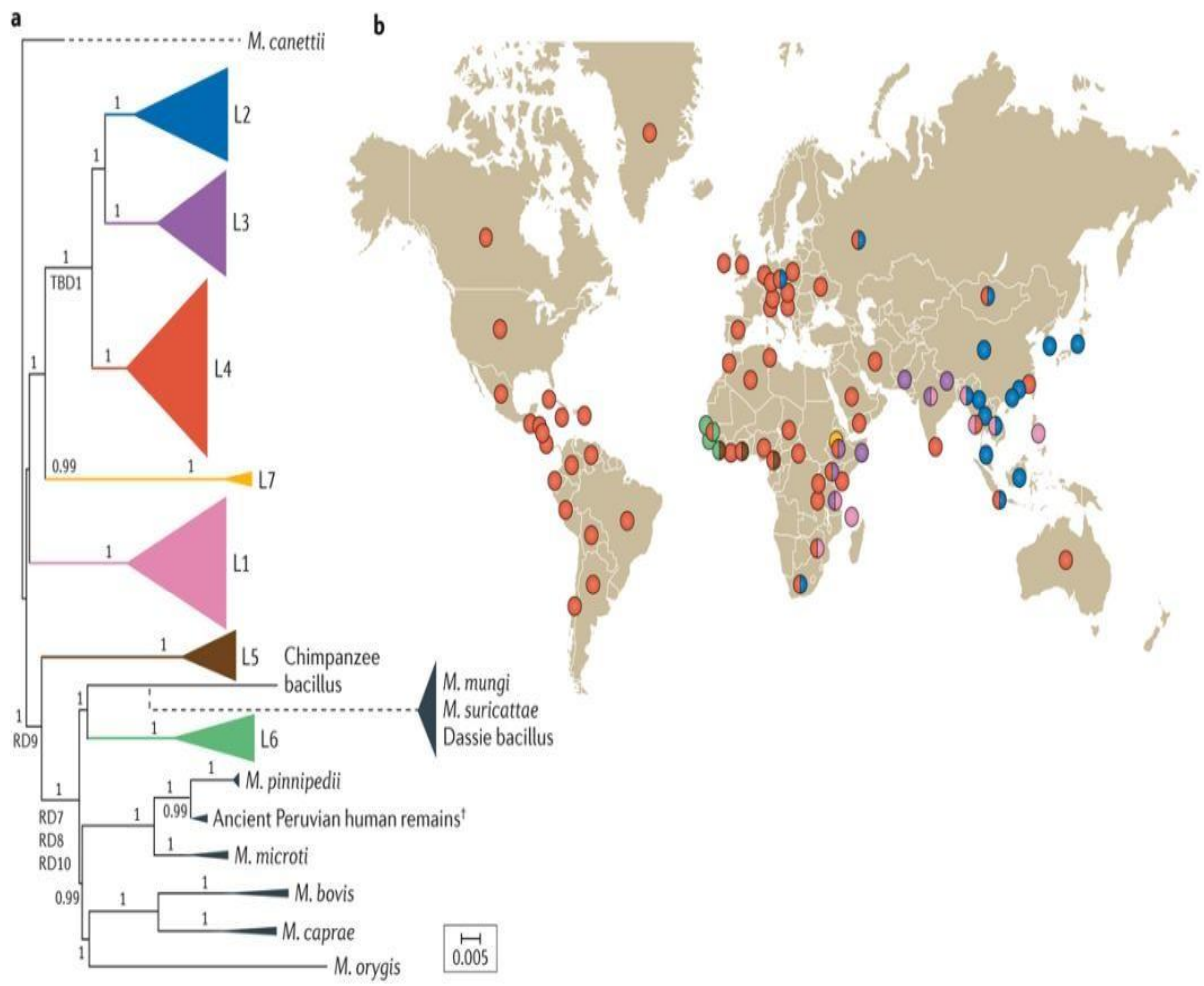

Figure 2.2. a) Phylogenetic analysis of the Mycobacterium tuberculosis complex (MTBC) based on genomic data, anchored by *Mycobacterium canettii.*

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The MTBC consists of seven human-adapted lineages (in colour) and many lineages adapted to various wild and domestic animals (in grey). Branches of the primary lineages are condensed for enhanced clarity (denoted by triangles). *Mycobacterium tuberculosis*-specific deletion 1 (TBD1) signifies that all lineage 2, lineage 3, and lineage 4 strains possess this chromosomal deletion. Correspondingly, the removal of regions of difference 7 (RD7), RD8, RD9, and RD10 is specified under the appropriate branches. Bootstrap confidence intervals are specified. The scale bar indicates the quantity of nucleotide changes per site. b) The worldwide distribution of the seven principal human-adapted MTBC lineages.[30]

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2.6.4 Genomic Changes in Drug Resistance

M. tuberculosis cannot do horizontal gene transfer; thus, drug resistance arises from chromosomal alterations, predominantly single nucleotide polymorphisms (SNPs), but may also include tiny insertions or deletions (indels) and, on rare occasions, massive deletions. In high incidence settings, most novel drug-resistant infections arise via the transmission of resistant strains rather than from mutations acquired during the infection process.[32] Research indicates that drug resistance has developed in a sequence corresponding to their introduction into clinical practice, beginning with streptomycin and isoniazid, followed by pyrazinamide, injectables, ethambutol, rifampicin, and subsequently fluoroquinolones.[33] Variants associated with drug resistance (RAVs) may impart resistance through alterations in the drug target site, drug-activating enzymes, or efflux pumps that expel antibiotics from the cell. Isoniazid serves as a prodrug, with resistance potentially arising from SNPs or, less frequently, indels in the activating enzyme encoded by katG, or from SNPs in the target inhA gene responsible for mycolic acid synthesis. Although any katG loss of function might theoretically result in isoniazid resistance, the S315T mutation is the predominant variant, as it confers resistance while preserving enzymatic activity and reducing bacterial fitness loss.[34] Rifampicin

resistance mostly arises from single nucleotide polymorphisms (SNPs) in the rifampicin resistance determination region of the rpoB gene, which encodes the β subunit of the target RNA polymerase. In contrast, resistance to pyrazinamide and bedaquiline may arise from numerous distinct SNPs and indels in the pncA and Rv0678 genes, respectively. The pncA gene encodes an activating enzyme for pyrazinamide, which, unlike katG, is not critical for bacterial fitness. Similarly, Rv0678 encodes a negative regulator of an efflux pump that also seems to have no impact on bacterial fitness.

2.6.5 Within-Host Diversity

Contemporary epidemiological studies indicate that *Mycobacterium tuberculosis* exhibits a stable mutation rate of 0.3-0.5 mutations per genome annually, significantly slower than that of most other bacteria, attributable to its prolonged generation time and the purifying selective pressure imposed by its ecological role as a specialised human pathogen.[35] Nonetheless, the comparatively slow mutation rate coupled with the absence of horizontal gene transfer poses a challenge in reconciling the extraordinary capacity of Mycobacterium tuberculosis to develop resistance to all drugs employed against it, including recent additions like bedaquiline and delamanid introduced in this decade.[36] These mutation rate estimates from between-patient longitudinal data likely obscure the growing evidence of heightened within-host diversity, which may be swiftly eliminated but could be enhanced when a selection advantage is present. The within-host genetic variety of Mycobacterium TB may result from mixed infections with genetically diverse strains or from the within-host evolution of a singular infection strain.[31] Numerous reports indicate that *M. tuberculosis* exhibits within-host genetic diversity at drug resistance-conferring loci during single strain infections, and it is hypothesised that the early detection of low-frequency mutations may forecast which patients will develop resistance and experience treatment failure.[37,38] The detection of low-frequency variations is hindered by the difficulty of distinguishing them from technological

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errors, because sequencing cultured samples may overlook the genetic diversity contained in the original clinical sample.[39] Within-host genetic diversity detected in sputum may indicate various geographically segregated bacterial subpopulations within the lung.[40, 41]

2.7 Host- Mycobacterium Tuberculosis Interaction

The formation of tuberculosis is the consequence of interactions among the host, pathogen, and environment. The pathogenesis and disease prognosis are contingent upon both bacterial and host features. Certain research indicate that a specific mix of host and *Mycobacterium TB* genotypes correlates with heightened risk and disease severity.[42] While certain individuals can combat the germs and eradicate or contain it, a portion develops the active disease. Nevertheless, the determinants influencing the onset and advancement of the sickness remain inadequately comprehended.[9]

2.7.1 Mycobacterium Tuberculosis Virulence

Mycobacterium tuberculosis is an aerobic, acid-fast, non-motile, non-encapsulated, and non-spore-forming bacillus. The cell wall possesses a significant lipid content, rendering it resistant to basic dyes.[9] The robust cell wall hinders nutrition absorption, resulting in sluggish development, while also safeguarding the bacterium from stress, several antibiotics, and destruction.[43] Thus, its sluggish replication rate and capacity to remain in a latent state pose difficulty in the prompt diagnosis and treatment of tuberculosis.[9] The mycobacterial cell wall comprises an inner and an outer layer. The inner layer comprises mycolic acids, arabinogalactan, and peptidoglycan. The external layer consists of lipoproteins and lipopolysaccharides, including mannose-capped lipoarabinomannan (ManLAM), lipomannan (LM), phthiocerol dimycocerosate, trehalose-1,6-dimycolate (TDM), sulfolipids, and phosphatidylinositol mannosides (PIMs). A few of these molecules are soluble and function as pathogen-associated molecular patterns (PAMPs) that interact directly with the immune system.[44]

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The remarkable intracellular survival capability of Mycobacterium tuberculosis is due to its capacity to reprogram macrophages post-infection, thereby evading elimination, initiating granuloma formation, and allowing the bacteria to remain isolated from host immune responses while sustaining a dormant state that is highly resistant to immune attacks and pharmacological interventions.[45] In addition to several proteins and enzymes essential for survival, mycobacterial surface lipids significantly contribute to its pathogenicity. The depletion of surface lipids correlates with a reduction in pathogenicity.[3] Mannose-capped Lipoarabinomannan (ManLAM) and Trehalose-6,6-dimycolate (TDM) are two highly effective immunomodulators warranting particular attention. ManLAM, typically found exclusively in slow-growing mycobacteria, can engage with many pattern recognition receptors (PRRs), including the mannose receptor (MR), dendritic-cell-specific intracellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN), and CD14. ManLAM has demonstrated the capacity to impede phagosome formation and IL-12 release in dendritic cells (DCs), as well as to suppress apoptosis and IFNγ signalling in macrophages.[40] It may also engage with cells in a receptor-independent manner by directly integrating into the cell membrane, thereby recently demonstrating the ability to block T-cell signalling.[45] TDM, or the cord factor, is the predominant soluble component of the cell wall in pathogenic mycobacteria. TDM is involved in the suppression of phagosome-lysosome fusion and the development of granulomas.[10] Recent findings indicate its interaction with a subset of pattern recognition receptors known as C-type lectin receptors (CTLRs), including macrophage inducible C-type lectin (Mincle) and macrophage C-type lectin (MCL), which elicit Th1/Th17 immune responses.[21]

2.7.2 Host Response against Mycobacterium Tuberculosis

Mycobacterium tuberculosis and humans are believed to have co-evolved in early human history, potentially explaining the bacterium's effective armament against the human immune

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system's countermeasures. *Mycobacterium tuberculosis* can exploit both the innate and adaptive immune responses to its advantage. The prevailing model of mycobacterium tuberculosis infection and its management posits that the bacterium is internalised by macrophages, where it proliferates. This results in either the death of the macrophage, allowing the bacteria to infect healthy cells, or the activation of antigen-specific CD4+ T cell responses that facilitate bacterial eradication or inhibit its proliferation.[11] The defining characteristic of tuberculosis infection is the formation of granulomas, which are caseous aggregates of immune cells. This dynamic structure is believed to be sustained by adaptive immunity in an effort to confine and limit the spread of the bacterium.46 Activated CD4+ T cells, along with IL-12, IFNγ, and TNFα, are essential for the regulation of tuberculosis. Nevertheless, numerous more host factors influence the determination of whether an active disease will manifest.[41] The insufficient understanding of the protective immune response in the lungs, particularly the function of CD4+ T cells in this response, constitutes a significant obstacle in the pursuit of developing a more effective vaccine for tuberculosis control.[11] Research indicates that the Bacillus Calmette-Guérin (BCG) vaccination is more efficacious in preventing disseminated infections than lung infections in both people and murine models.[45] The strain also has a deficiency in numerous virulence genes relative to *Mycobacterium TB*.

2.7.2.1 Early Response

After aerosol transmission, alveolar macrophages (AMs) in the lung alveoli are the initial host cell types targeted by mycobacteria. Macrophages function as the primary cellular habitat and reservoir for bacterial survival and transmissibility. The macrophage employs strategies to eliminate phagocytosed bacteria, including phagosome acidification following phagosome-lysosome fusion, which activates protein and lipid degrading enzymes, as well as the production of reactive nitrogen and oxygen species (RNS and ROS), microbial peptides,

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and hydrolytic enzymes. All these events collaborate to eradicate the bacteria by inhibiting its metabolism and altering its DNA and cell wall. The cell may also engage in autophagy to eliminate the germs and prevent future dissemination.[40] The survival of the bacterium is contingent not only upon the host's immunological status but also significantly influenced by the virulence of the mycobacterium tuberculosis responsible for the infection.[52] Mycobacterium TB is highly proficient in inhibiting phagosome development, hence significantly enhancing its potential for intracellular persistence.[45] Infection of alveolar macrophages induces their activation and the secretion of several cytokines, including TNF-α, IL-1β, IL-6, and IL-12. Collectively, these cytokines facilitate the recruitment and activation of macrophages and neutrophils to the infection site, promoting the T helper Type 1 (Th1) response, the proliferation of cytotoxic T cells (Tc), and NK cells, as well as granuloma formation. The Th1 response induces the synthesis of IFNγ, which is crucial for defence against intracellular infections.[40] IFNG-deficient mice exhibit a quick decline in health following tuberculosis infection compared to control mice.[45] Furthermore, IFNγ levels have been observed to be elevated in individuals with latent infections.[45] The Th17 response, mostly triggered by γδ T cells in reaction to IL-6 and marked by the release of IL-17, promotes leukocyte recruitment and has been demonstrated to contribute to the early stages of *Mycobacterium TB* infection. However, they have also been implicated in tissue damage associated with tuberculosis due to excessive neutrophil infiltration.[11]

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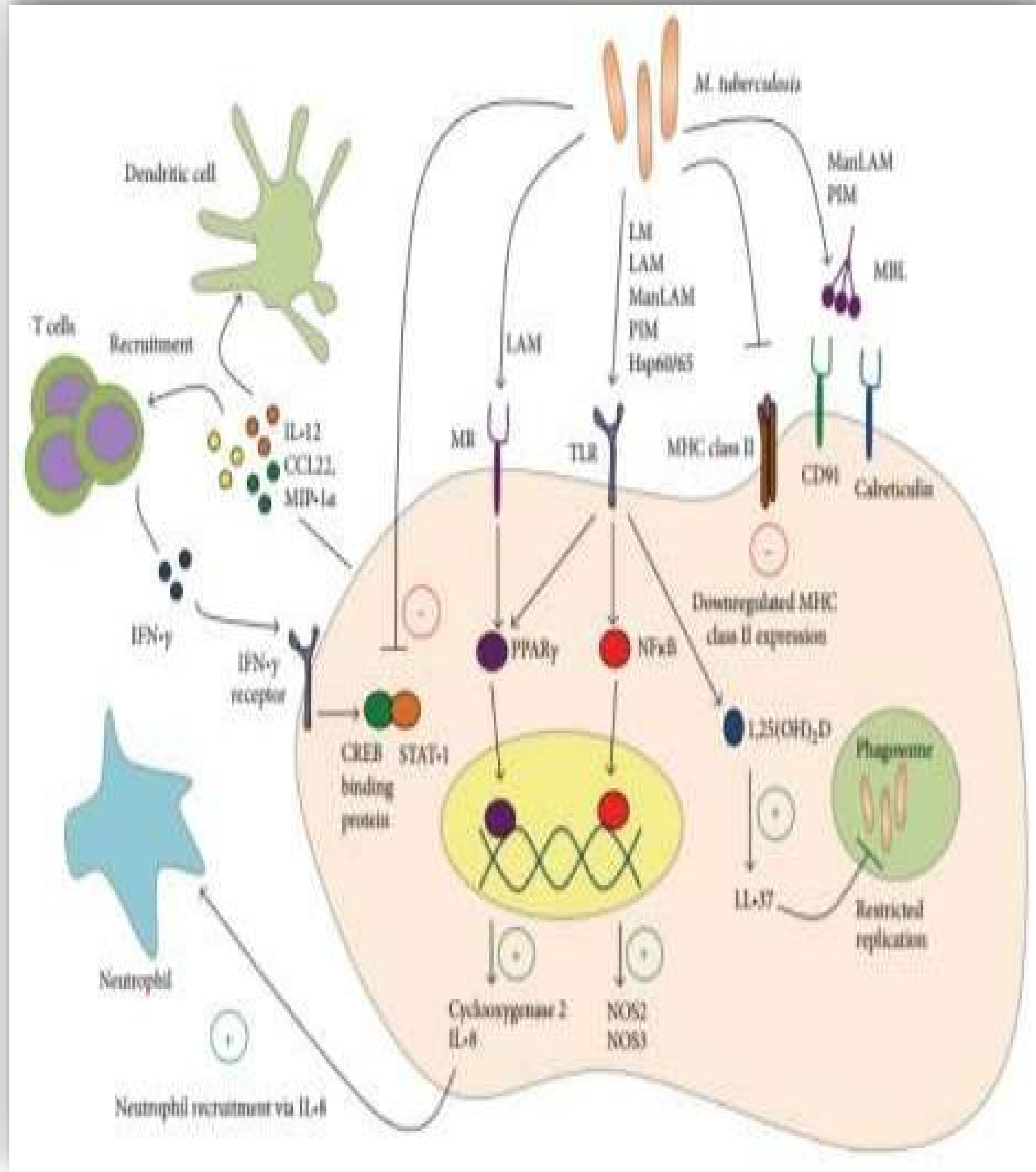

Figure 2.3: Innate immune response against *mycobacterium tuberculosis* as it is encountered by alveolar macrophages.[40]

2.7.2.2 Granuloma formation

In addition to Alveolar Macrophages (AMs), resident Dendritic Cells (DCs) also phagocytise *Mycobacterium tuberculosis* and transport them to the nearby lymph nodes to activate T-cells through MHC class II-mediated antigen presentation. Consequently, dendritic cells function as a bridge between innate and adaptive defence, while also potentially facilitating the spread of germs beyond the main infection site.[45] Upon activation of T-cells and initiation of the adaptive immune response, these cells migrate to the infection site in response to chemokines produced by infected cells, predominantly influenced by macrophages and neutrophils, a phenomenon referred to by some authors as a 'innate granuloma.[40] The recruited CD4+ T-cells encircle the uninfected macrophages and neutrophils, which in turn encircle the infected cells, effectively isolating them and inhibiting their growth, mobility, and dissemination.[44] Consequently, a structured dynamic formation, referred to as a granuloma, is established. The granuloma is considered advantageous for the host by limiting bacterial transmission, leading to a latent infection state.[7]

2.8 Pattern Recognition Receptors (PRRS) in Mycobacterium Immunity

Antigen-presenting cells, such as macrophages, contain several pattern recognition receptors (PRRs) on their surfaces that facilitate the recognition and phagocytosis of microorganisms. The activation of the receptor also initiates particular downstream pathways that result in the production of cytokines and chemokines. *Mycobacterium tuberculosis* is identified by many receptors, including those from the Toll-like receptor (TLR) and Nucleotide Oligomerisation Domain (NOD)-like receptor (NLR) families. Recently, many CTLRs have been identified that recognise and modulate signalling in response to *Mycobacterium TB*.[42] The ligation of various pattern recognition receptors (PRRs) elicits unique responses at different phases of infection, hence contributing to a complex network of coordinated interactions among diverse cells and receptors, which ultimately influences disease outcomes.[40]

2.9 Toll-Like Receptors (TLRs)

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Among the pattern recognition receptors, Toll-like receptors have been thoroughly investigated and precisely defined regarding their interaction with *Mycobacterium tuberculosis* and their involvement in modulating bacterial pathogenicity. They are transmembrane proteins expressed on the cell surface or on endocytic vesicles in immune cells. Among the ten known members in humans, primarily TLR2, TLR4, and TLR9 are engaged in the recognition of *Mycobacterium TB* ligands, including PIMs, lipoproteins, LAM, LM, and the 38-kDa and 19-kDa glycoproteins.[46] Signalling via TLRs is facilitated by TIR domain-containing adaptors, including myeloid differentiation primary response gene 88 (MyD88) and TIR-domain-containing adapter-inducing interferon-β (TRIF), which trigger a cascade of events resulting in NF-κB activation and the ensuing inflammatory response.[46] TLR2 is likely the most extensively studied among TLRs in mycobacterial infections. TLR2-/- mice exhibit heightened susceptibility to H37Rv, a virulent strain of Mycobacterium tuberculosis, characterised by an impaired granulomatous response, diminished bacterial clearance, and ultimately succumbing to the infection.[47] A comparable response was also observed in TLR4 -/- mice.TLR-2, in conjunction with TLR-1 and TLR-6, can activate signalling in response to certain ligands from *Mycobacterium tuberculosis*.[46] Double knockout mice for TLR2 and TLR9 exhibit increased vulnerability to aerosol infection by Mycobacterium TB and demonstrate modified lung histology.[48] In contrast, many knockout mouse experiments indicate a redundant function of TLRs and show that numerous responses depending on Mycobacterium tuberculosis do not necessitate specific TLR activation. TLR2 is essential for the post-translational regulation of TNF-α release, but it is not required for transcriptional activation.[48] No disparities in pulmonary diseases were noted in TLR2-, TLR4-, and TLR6-deficient animals following aerosol infection with *Mycobacterium TB*. There is evidence that mycobacterium tuberculosis identification may mediate cellular responses in a MyD88-independent manner.[46] Mice defective in Myeloid Differentiation

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main response gene 88 (MYD88) exhibited comparable NF-κB activity to wild-type mice during aerial *Mycobacterium TB* infection.[47] The discrepancies in the outcomes of knockout mice experiments may be ascribed to various reasons, including the strains of mice and bacterial pathogens employed, as well as genetic variations; nonetheless, they also imply that the function of TLRs in mycobacterium tuberculosis infection is superfluous. Nucleotide-binding Oligomerisation Domain-like (NOD-like) receptors are multi-domain pattern recognition receptors (PRRs) situated intracellularly, hence involved in the detection of intracellular infections. In humans, there are 23 identified members of the NLR family, comprising NLRs and LRPs.[48] NOD-2, or CARD15, identifies the muramyl dipeptide inside the peptidoglycan layer of bacterial cell walls, and its function has been examined in relation to immunity against *Mycobacterium TB* infection.[49] Upon ligand binding, NOD-2 binds receptor-interacting protein 2 (Rip2/RICK) through CARD-CARD contacts, resulting in the nuclear translocation of NF-κB through a number of steps.[49] NOD-2 defective mice demonstrated a diminished inflammatory response to aerosol infection with Mycobacterium TB and displayed an increased bacterial load compared to their wild-type counterparts six months post-infection.[50] The NLRP3 protein can identify the ESAT-6 antigen of Mycobacterium tuberculosis and initiate the release of protective IL-1β and IL-18 through the activation of inflammasome assembly.[49] TDB, an analogue of the mycobacterial cord factor, has recently demonstrated the ability to activate the NLRP3 inflammasome.[50] Numerous investigations have revealed such discrepancies in the context of TLRs in mouse research.[43] In addition to increasing the complexity of mycobacterial pathogenicity, the discrepancies suggest the possibility of receptors beyond TLRs and NOD-Like Receptors (NLRs) that may also significantly contribute to the recognition of Mycobacterium tuberculosis and the immune response.

2.9.1 Discovery of TLRs

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Toll-like receptors (TLRs) derive its nomenclature from their resemblance to the protein encoded by the toll gene, which was discovered during the embryonic development and dorsoventral polarisation of Drosophila in 1985 by Christiane Nüsslein-Volhard. She observed an unusual phenotype in mutant fly larvae and exclaimed, “Das war ja toll!” which translates to “That was strange or weird!” in German; hence, the protein was named “Toll.”[51,52] The protein produced by the "Toll" gene functions as a receptor on cells and is crucial for the host's innate immunity against fungal infections in adult flies.[52]

2.9.2 Structure and characterization of TLRs

TLRs are type I transmembrane proteins characterised by 20–27 external leucine-rich repeats (LRR) that facilitate the recognition of PAMP/DAMP, along with transmembrane domains and intracellular toll–interleukin 1 (IL-1) receptor (TIR) domains essential for the activation of downstream signalling pathways.[51] The extracellular domains of TLR possess glycosyl moieties that function as binding sites for ligands.[52] The precise processes governing glycan-mediated ligand recognition remain unclear.[80] The primary attributes that differentiate various TLRs are ligand selectivity, signalling transduction routes, and subcellular localisation.[51]

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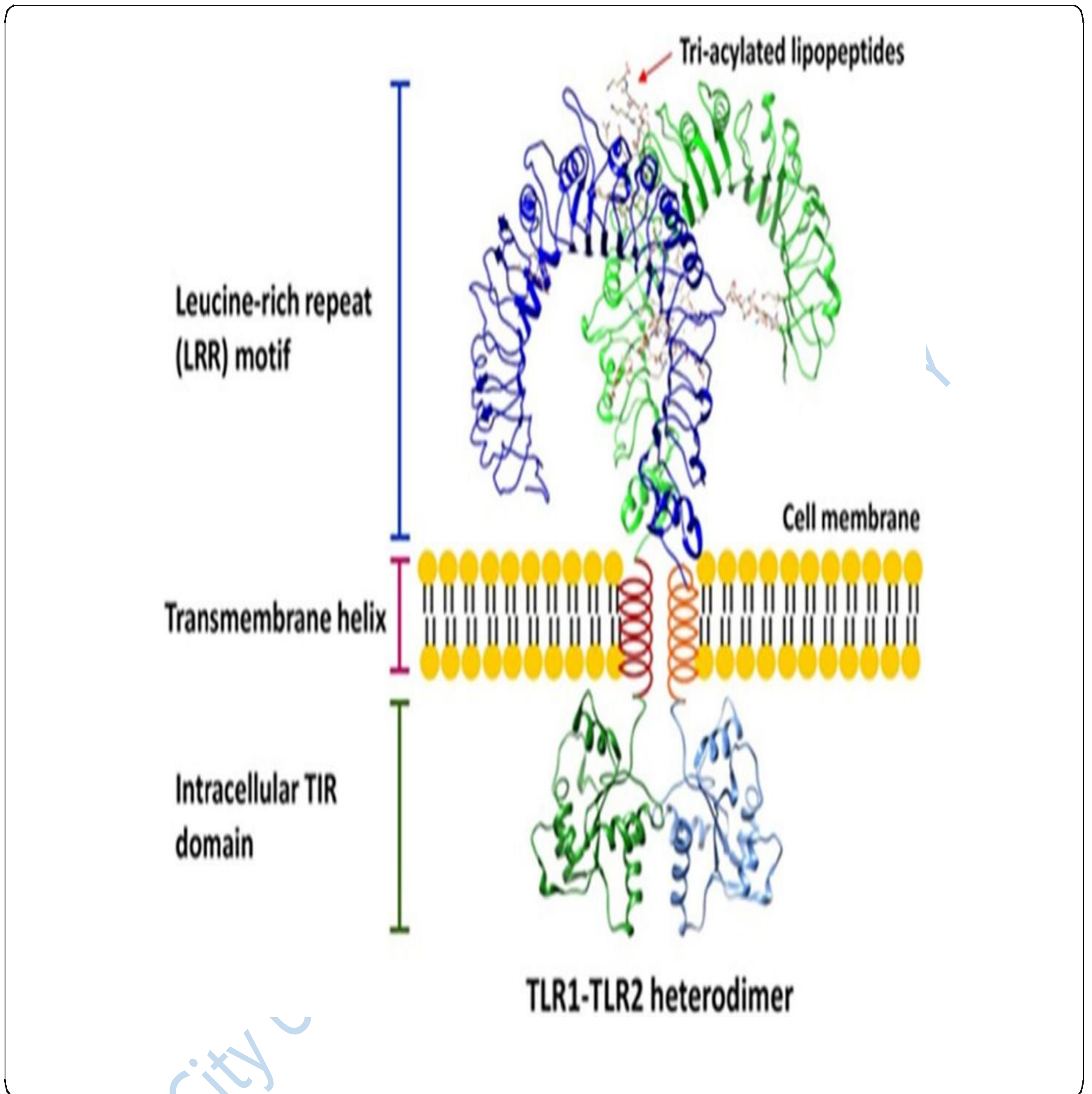

Figure 2.4: Structure of TLRs.[52]

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2.9.3 Members of TLR family

Ten members of the human TLR family and thirteen members of the mouse TLR family have been found, while TLR10 is non-functional in mice.[53] TLR4 was the inaugural toll-like receptor homolog identified in humans, demonstrating the capacity to promote the expression of genes associated with inflammatory reactions.[50] Its function was corroborated with the identification of a single mutation in the TLR4 gene within a mouse strain that exhibited unresponsiveness to lipopolysaccharides (LPS).[50] TLRs are categorically categorised into: I. Cell membrane TLRs, which include heterodimers of TLR2 with TLR1 and TLR6, as well as TLR4, TLR5, and TLR10, are expressed on the cell surface.[50] II. Intracellular TLRs or nucleic acid sensors (TLR3, TLR7, TLR8, and TLR9) that are situated in the endoplasmic reticulum (ER), endosomes, and lysosomes.[53]

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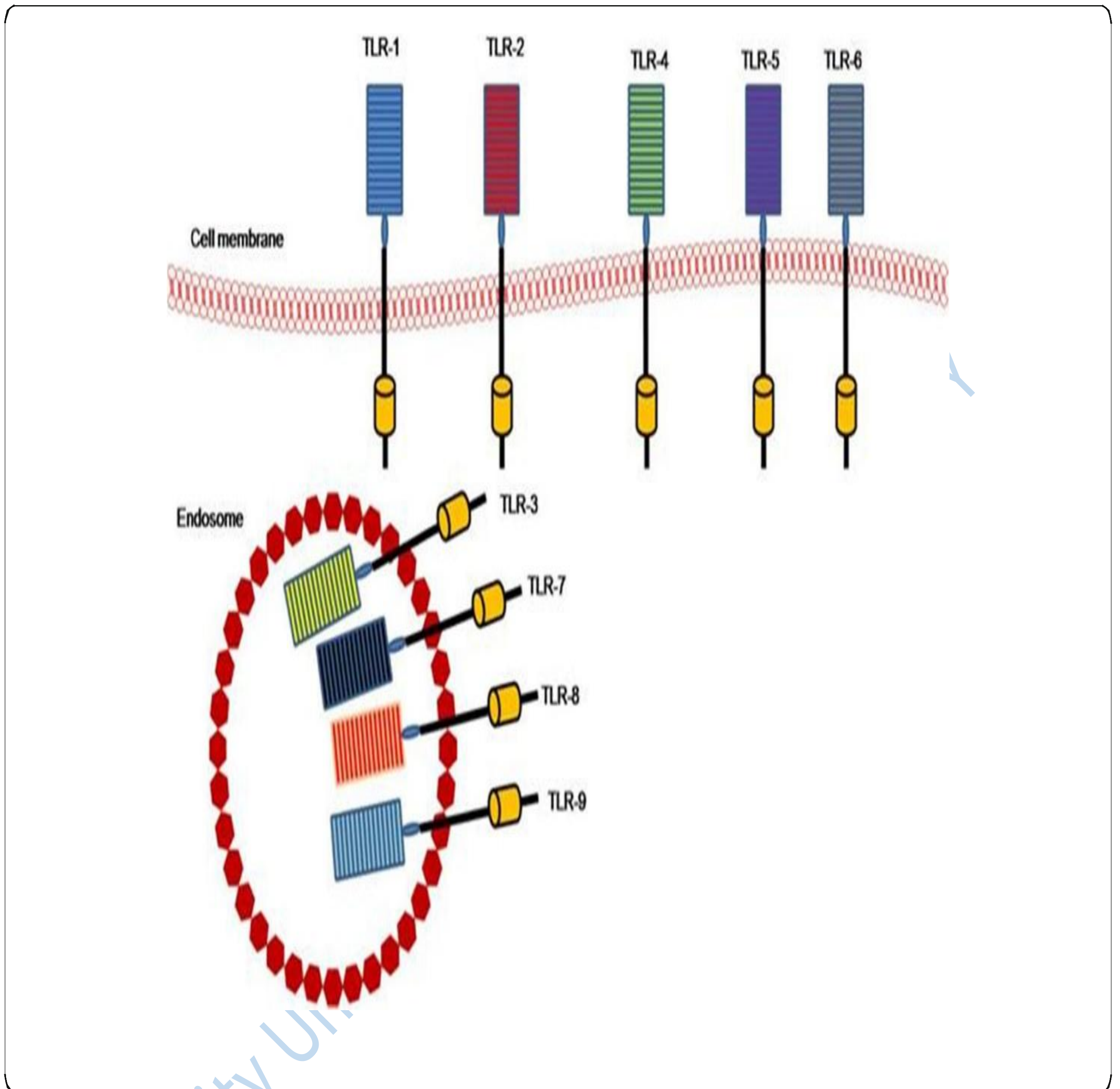

Figure 2.5: Members of TLRs family and their location.[50]

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2.9.4 Expression of TLRs

The expression of TLR mRNA is remarkable as it is not confined to immune tissues such as the spleen, thymus, tonsils, lymphatic vessels, and lymph nodes, but is instead disseminated throughout various tissues, including peripheral blood leukocytes, heart, liver, pancreas, colon, small intestine, lung, kidney, ovary, placenta, testis, prostate, skeletal muscle, and brain.[49] They are present on all innate immune cells, including macrophages, NK cells, dendritic cells, and circulating leukocytes such as monocytes and neutrophils, as well as on adaptive immunity cells like T and B lymphocytes, and on non-immune cells such as epithelial, endothelial cells, and fibroblasts.[49]

2.9.5 TLR signaling pathways

Innate immunity was formerly seen as a nonspecific immune response. The discovery of TLRs revealed the significant specificity of innate immunity and its ability to differentiate between self and nonself.[49,50] Cell surface TLRs (TLR1, TLR2, TLR4, TLR5, TLR6, and TLR11) primarily identify microbial membrane constituents to elicit an inflammatory response.[51] In contrast, intracellular TLRs (TLR3, TLR7, TLR8, and TLR9) primarily detect microbial nucleic acids from bacteria or viruses, eliciting Type I IFN responses and inflammatory reactions. The erroneous identification of self-nucleic acids may lead to autoimmune disorders.[51]

Ligand-mediated dimerisation of TLR ectodomains, upon binding to certain ligands, leads to the simultaneous dimerisation of the cytosolic TIR domains of each TLR.[52-53] Dimerised receptor TIR domains are recognised by two receptor-proximal membrane adaptor proteins: the TIR domain-containing adapter protein (TIRAP; sometimes referred to as MAL) and the TIRAP-inducing IFN-b (TRIF)-related adaptor molecule (TRAM).[52] Peripheral membrane proteins examine the inner leaflets of plasma and endosomal membranes via the N-terminal phosphoinositide binding domain of TIRAP or the bipartite localisation domain of TRAM, which comprises an N-terminal myristoylation motif and a phosphoinositide-binding motif.[54]

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TIRAP and TRAM can additionally attract myeloid differentiation main response protein 88 (MyD88) and TRIF, respectively, and promote the formation of a substantial oligomeric scaffold known as Myddosome or Triffosome.One hundred four These supramolecular complexes comprise downstream signalling components and kinase enzymes.[54] Elevated local concentrations of signalling molecules enhance the inherently weak allosteric connections and trigger cytosolic signal transmission.[54] TLR signalling pathways can be primarily categorised into MyD88-dependent pathways, which facilitate the production of inflammatory cytokines, and TRIF-dependent pathways, which are responsible for the induction of Type I IFN and inflammatory cytokines, based on the specific supramolecular complexes formed.[54]

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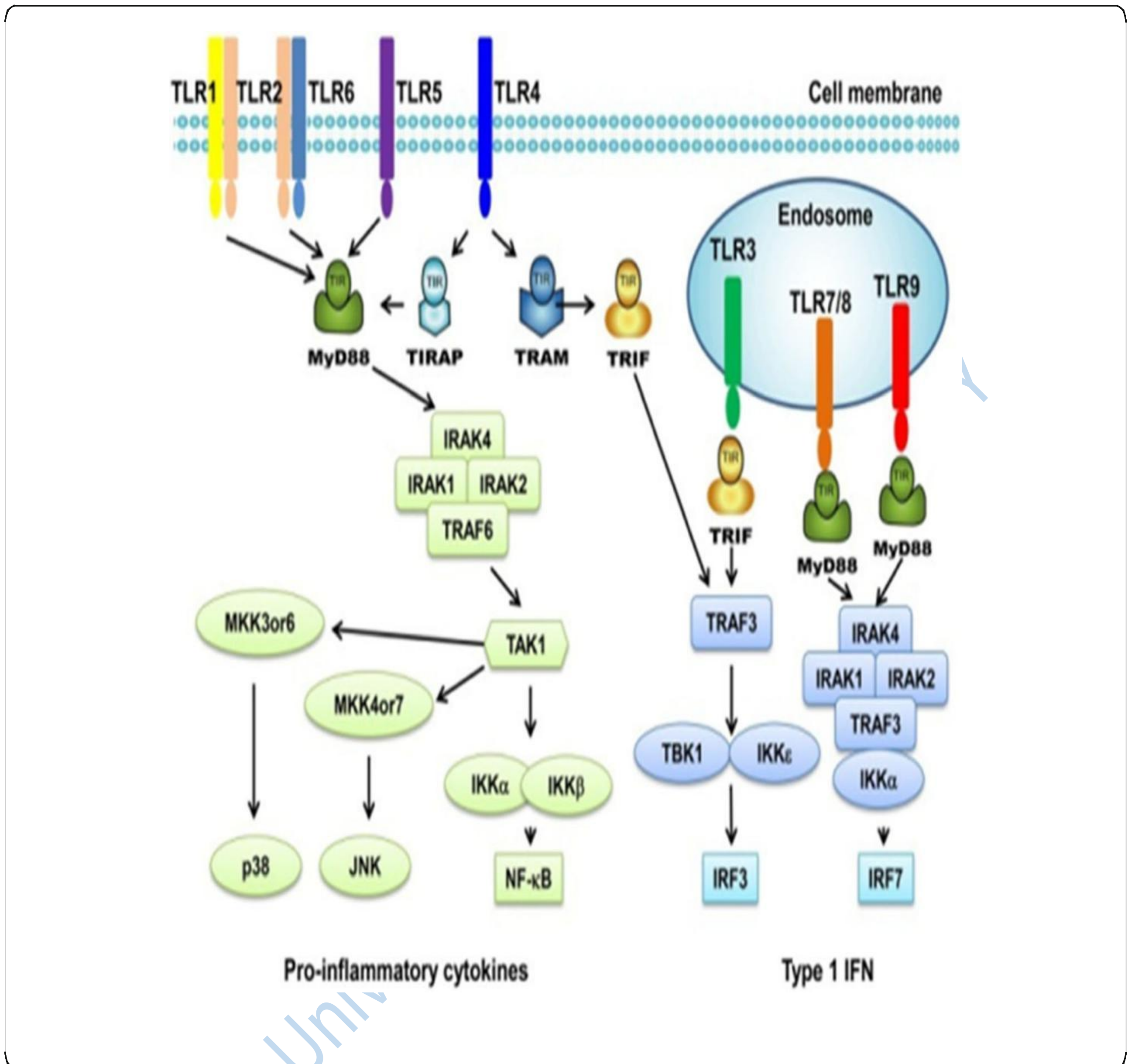

Fig. MYD88-dependent pathway

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2.9.6 MYD88-dependent pathway

MyD88 is the inaugural recognised member of the TLR family; it is utilised by all TLRs except TLR3 and stimulates the NF-kB signalling pathway.[93] Upon activation by particular ligands, MyD88 attracts IL-1 receptor-associated kinases (IRAK)—IRAK4, IRAK1, IRAK2, and IRAK-M—which assemble into a complex with IRAK kinase family members, known as the Myddosome.[54] During Myddosome formation, IRAK4 is initially activated by MyD88 via its N-terminal death domain, which is also present in IRAK4. Like MyD88, IRAK4 is crucial for the activation of NF-kB and mitogen-activated protein kinases (MAPKs) within the MyD88-dependent pathway.[55] Activated IRAK4 progressively activates IRAK1 and IRAK2, which then undergo autophosphorylation at many locations.[54] While the activation of both kinases is essential for the vigorous activation of TLR-induced NF-kB and MAPK signalling, the relative significance of IRAK1 and IRAK2 may vary between humans and mice.[56] Activated IRAK1 can engage with tumour necrosis factor (TNF) receptor-associated factor 6 (TRAF6), an E3 ligase that facilitates the formation of Lys63 (K63)-linked polyubiquitin, leading to the activation of TRAF6. TRAF6, in conjunction with E2 ubiquitin-conjugating enzymes Ubc13 and Uev1A, facilitates the formation of K63-linked polyubiquitin chains and enhances K63-linked polyubiquitination of TRAF6 and IRAK1. Preliminary research indicated that K63-linked polyubiquitination of TRAF6 and IRAK1 could function as a scaffold for the activation of downstream TGFb-activated protein kinase 1 (TAK1) or IkB kinase (IKK).[57,58] Nonetheless, clear biochemical evidence is lacking, and conflicting studies indicate that the ubiquitination of TRAF6 may be unnecessary for the activation of downstream protein kinases.[54] Consequently, it remains to be determined whether the K63-linked polyubiquitination of TRAF6 and IRAK1 directly activates downstream protein kinases or only functions as an indicator of signalling pathway activation.[59] Recent biochemical investigations demonstrated that the unconjugated K63 polyubiquitin chains

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produced by TRAF6 and Ubc13/Uev1A can directly activate TAK1 in vitro by interacting with the newly identified zinc finger-type ubiquitin-binding domain of TAB2 and TAB3, resulting in proximity-dependent transphosphorylation of TAK1 at Thr-187. Nonetheless, the manner in which these free polyubiquitin chains activate downstream protein kinases in vivo is still to be elucidated. Phosphorylated TAK1 subsequently activates the IKK complex-NF-kB pathway and the MAPK pathway.[60]

The IKK complex consists of the catalytic subunits IKKa and IKKb, together with the regulatory subunit nuclear factor-kB essential modulator (NEMO), also known as IKKg.[54] K63 polyubiquitin chains may connect TAK1 to the IKK complex, facilitating the phosphorylation of IKKb by TAK1 due to their spatial closeness, resulting in the activation of the IKK complex.[60,61] Recent findings indicate that Met1-linked ubiquitin dimers, or linear ubiquitin dimers,[61] exhibit a binding affinity to NEMO that is 100-fold greater than that of K63-linked ubiquitin, suggesting that linear ubiquitination, facilitated by the linear ubiquitin chain assembly complex (LUBAC), plays a role in the activation of IKK.[62] The activated IKK complex can phosphorylate the NF-kB inhibitory protein IkBa, leading to its proteasomal breakdown, which permits NF-kB to translocate into the nucleus and initiate proinflammatory gene expression.[60]

In the MAPK pathway, active TAK1 concurrently activates the MAPK family members Jun N-terminal kinases (JNKs) and p38 via promoting the activation of MAPK kinases 4/7 (MKK4/7) and MKK3/6. IKKb catalyses the phosphorylation of p105, leading to its degradation by the Skp1-Cul1-F-box ubiquitin ligase (SCFbTrCP) complex, resulting in the production of p50 and the release of tumour progression locus 2 (TPL2), which activates MKK1/2, subsequently phosphorylating and activating extracellular signal-regulated protein kinase 1 (ERK1) and ERK2. The MAPKs subsequently phosphorylate cyclic AMP-responsive element-binding protein (CREB) and the activator protein 1 (AP-1) transcription

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factors, which are composed of a heterodimer of c-Fos and c-Jun subunits, to modulate inflammatory responses.[54] TAK1 is a pivotal element in MyD88-dependent NF-kB and MAPK signalling pathways. A prior study indicated that TAK1 is essential for the activation of the NF-kB and MAPK signalling pathways in mouse embryonic fibroblast cells, B cells, and T cells.[135,139] Nevertheless, we discovered that TAK1 functions as a negative regulator in murine neutrophils.[63] Conversely, TAK1 may function as a positive regulator in human neutrophils, indicating a cell type-specific involvement for TAK1 in TLR-mediated signalling.[64] Recent findings indicate that Tak1 loss in mice modifies the intestinal microbiome, which may enhance protective immunity against colitis and colorectal cancer.[65]

In the signalling pathways of TLR7, TLR8, and TLR9 in plasmacytoid dendritic cells (pDCs), MyD88 stimulates NF-kB signalling and engages with interferon regulatory factors (IRF)-5 and IRF-7 to induce proinflammatory cytokines or Type I interferons (IFN-a and IFN-b) responses.[65,66] IRF7 is mostly expressed by plasmacytoid dendritic cells (pDCs), which can associate with the Myddosome comprising IRAK4, TRAF6, TRAF3, IRAK1, and IKKa.[148] IRAK1 and IKKa subsequently phosphorylate the IRF7 protein, resulting in its separation from the Myddosome and subsequent dimerisation. The IRF7 homodimer translocates to the nucleus and induces IFNa expression.[47] According to the contract, IRF5 undergoes phosphorylation by IKKb at Ser462, facilitating proinflammatory cytokine transcription, but does not influence IFNa production.[67]

2.9.7 TRIF-dependent pathway

In macrophages and conventional dendritic cells (cDCs), the production of IFN triggered by TLR3 or TLR4 is not reliant on MyD88 but is instead mediated by TRIF, together with the proteins TRAM and TRAF3.[68] Upon the identification of dimerised TLR4 in endosomes, TRAM is believed to engage with TRIF to facilitate the assembly of the hypothesised Triffosome,[54] wherein TRIF interacts with TRAF6 and TRAF3. Activated TRAF6 recruits

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the kinase receptor-interacting protein 1 (RIP1), which then recruits and activates the TAK1 complex and IKK complex, resulting in the activation of NF-kB and MAPKs and the production of inflammatory cytokines.[69] A prior study indicated that TRAF6 may facilitate the ubiquitination of RIP1.[69] Nonetheless, TRAF6 has been indicated as non-essential for TRIF-dependent TLR signalling, suggesting that other E3 ligases may be accountable for RIP1 ubiquitination. Recently, the E3 ubiquitin ligase Peli1 was discovered to enhance TRIF-dependent TLR signalling and proinflammatory cytokine production by promoting the ubiquitination of RIP1, suggesting that Peli1 may have a redundant function with TRAF6.[59] TRIF further facilitates the TRAF3-dependent activation of the IKK-related kinase TANK-binding kinase 1 (TBK1). TRAF3 activates TBK1 and IKKi, in conjunction with NEMO, facilitating the phosphorylation and dimerisation of the interferon-inducing transcription factor IRF3. Thereafter, the IRF3 homodimer migrates from the cytoplasm to the nucleus, where it induces the production of Type I IFN genes and IFN-stimulated genes (ISGs).[67] A 39-amino-acid pLxIS motif has recently been found in TRIF (but not in MyD88), which is subject to phosphorylation by TBK1. The phosphorylated motif can attract IRF3, resulting in the phosphorylation and activation of IRF3 by TBK1.[68] Consequently, TLR4 utilises TRIF rather than MyD88 to facilitate IRF3-mediated IFN production within the endosome. In contrast to TLR4, TRAM is unable of interacting with TLR3 or modulating TLR3 signalling, suggesting that TLR3 may directly engage with TRIF or utilise an alternative sorting adapter to connect TRIF to TLR3.

2.9.8 Function of TLRs

The primary purpose of TLRs is to identify various infections.[78] Each immune system cell possesses a distinct set of TLR that perform specialised activities in identifying PAMP/DAMP and facilitating immune responses.[70] The function of TLR in innate immunity involves the stimulation of antibacterial activity and the synthesis of inflammatory

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cytokines.[70] Upon activation by PAMPs or DAMPs, TLRs recruit adapter proteins that serve as a platform for the recruitment of IL-1R-associated protein kinases (IRAK) 1, 2, 4, and M, as well as TAB2 and TNF receptor-associated factor 6 (TRAF6), ultimately resulting in the nuclear translocation of the pro-inflammatory transcription factors nuclear factor kappa-B (NF-kB), activator protein 1 (AP-1), and interferon regulatory factor 3 (IRF3).[71] Each transcription factor governs the transcription of specific genes that encode distinct sets of proteins, including pro-inflammatory cytokines (tumour necrosis factor alpha [TNF-α], interleukin [IL]-1β, and IL-6), type 1 interferons (IFN-α and IFN-β), chemokines (CXCL8 and CXCL10), and antimicrobial peptides.[72]

2.9.9 Consequences of the released cytokines

A major feature of TLRs activation is the secretion of proinflammatory cytokines and type1 interferon. Cytokines are short-lived, long-range mediators that act on different tissues to mount the systemic response. Although TLRs are playing important role in host defense, proinflammatory cytokines such as TNF-α and IL-1β have varied effects on the host body where they are the master contributors for the behavioral changes during infection such as weakness, lethargy, fatigue, and anorexia.[73] They affect the physiological processes as they direct the metabolic processes towards catabolism, preventing muscle protein synthesis, boosting muscle wasting, and inducing liver anabolism. They induce also peripheral lipolysis, upregulate de novo fatty acids synthesis and hepatic triglyceride production, and promote secretion of VLDL-cholesterol, all of which increase serum triglycerides levels.[74]

2.9.10 Importance of TLRs

1. TLRs serve as bridging molecules, as they are expressed on dendritic cells that connect innate and adaptive immunity.[47] TLRs identify pathogens and provide signals to antigen-presenting cells, which eliminate germs through phagocytosis.[75] The presence of a complete set of TLRs on immature DCs facilitates their maturation process.[75]

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2. Their significance arises from their capacity to identify invading pathogens, PAMPs, and endogenous damage-associated molecules, DAMPs, to initiate self-healing and tissue repair processes.[75] The significance of TLR1, TLR2, and TLR6 is in their capacity to identify bacterial lipoproteins and glycolipids.[75] TLR7, 8, and 9 detect nucleic acids, including bacterial and viral single-stranded RNA (ssRNA) and unmethylated CpG DNA motifs; TLR3 differentiates viral double-stranded RNA (dsRNA); TLR4 identifies fibronectin and lipopolysaccharides (LPS); TLR5 recognises bacterial flagellin; TLR11 and 12 detect profilin; and the function of TLR10 remains unidentified.[76]

3. TLRs induce apoptosis in infected cells. It halts protein synthesis, restricts the infection, and downregulates the immune response in dendritic cells during the inflammatory state of sepsis.[77]

4. Toll-like receptors (TLRs) are significant risk factors in the pathogenesis of various diseases, and the manipulation of their signalling has been proposed for disease management and treatment.[180] For instance, TLR3, TLR7, and TLR8 play crucial roles in allergen identification and the subsequent development of allergic disorders such as allergic rhinitis.[72]

2.9.11 Negative feedback regulators of TLRs

In TLR signalling, various negative regulators have been found that impede ligand-receptor binding, degrade target proteins, and hinder the recruitment or transcription of intermediates.[73] Soluble versions of TLRs (sTLRs) are pivotal in modulating inflammation across many situations. They comprise soluble variants of TLR2 and TLR4 that serve as a feedback mechanism to limit excessive TLR activation. The sTLR4 markedly suppresses LPS-induced TNF-α generation and NF-κB activation.[74] The sTLR2 suppresses the production of IL-8 and TNF produced by bacterial lipopeptides.[75] Both membrane-bound receptors, suppression of tumorigenicity 2L (ST2L) and single immunoglobulin interleukin-1 receptor-related protein (SIGIRR), function as membrane-associated TLR regulators. ST2L

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interacts with MyD88 and MAL, while SIGIRR associates with TLR4, IRAK4, and TRAF6. Both block the MyD88-dependent pathway.[76-80] Additional intracellular TLR regulators, including as sMyD88, A20, and small heterodimer partner (SHP), have demonstrated a negative regulatory effect on TLR signalling. MyD88 replaces MyD88 to antagonise the MyD88-dependent pathway by inhibiting IRAK4 recruitment; IRAKM reduces IRAK1 phosphorylation by targeting the IRAK1-IRAK4 complex. A20 is an inducible deubiquitinating enzyme that deubiquitinates TRAF6 to end TLR signalling.[81-84] SHP acts as a regulator of NF-κB and an inhibitor of TRAF6 ubiquitination.[85]

2.10 Toll Like Receptor 2 (Tlr2)

Toll-like receptors (TLRs), the mammalian counterparts of the Drosophila melanogaster Toll receptor identified in 1985, are highly conserved and predominantly involved in pattern recognition.[86] TLR2, in conjunction with TLR4, is among the most extensively researched pattern recognition receptors; unlike some other receptors in this family, TLR2 is recognised for its ability to create diverse heterodimers with TLR1, TLR4, TLR6, and TLR10.[87] Recent studies indicate that TLR2 may be the most adaptable Toll-like receptor, as it recognises a wide array of ligands from diverse pathogen sources and interacts with numerous other receptors.[88]

TLR2/1 heterodimers detect triacylated lipopeptides (LPs) from Gram-negative bacteria or mycoplasma, such as lipoarabinomannans and lipomannans, while TLR2/TLR6 heterodimers identify diacylated LPs, including lipoteichoic acid (LTA) from Gram-positive bacteria and mycoplasma.[86,88] Depending on the ligand, low-endotoxic atypical LPS can facilitate TLR2/TLR4 heterodimerization, while the TLR2/TLR10 heterodimer has been identified as involved in the identification of Helicobacter pylori LPS.Additional coreceptors, such as clusters of differentiation 14 (CD14), have been identified that facilitate the physical closeness of CD14 with TLR2 and TLR1 by binding lipoproteins to initiate signalling.[89] The

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mRNA expression of TLR2, unlike TLR1, TLR4, and TLR6, was significantly elevated in alveolar macrophages following extended LPS exposure (24 hours).[90] In addition to heterodimerization, there is some evidence suggesting the occurrence of TLR2 homodimerization; nonetheless, this remains a subject of controversy.[89] Frequently utilised TLR2 ligands for in vitro and in vivo investigations include synthetic di- and triacylated lipoproteins such as Pam2CSK4, Pam3CSK4, derived from Escherichia coli, and FSL-1, which exemplifies a pathogen-associated molecular pattern of lipoprotein extracted from Mycoplasma fermentans or Mycoplasma salivarium.[90] Additionally, endogenous ligands recognised as danger signals for TLR2 have been found, including heat shock proteins, human β-defensin-3, high mobility group box 1 protein (HMGB1), and hyaluronan fragments.[91] Amyloid-β (Aβ) and α-synuclein (αSyn) are additional ligands of TLR2 that significantly contribute to neurodegenerative diseases.[86] Consequently, alongside PAMPs, the endogenous activation of TLR2 by host-derived danger signals is a significant contributor to the pathophysiology of inflammation and associated illnesses.

Ligand-induced dimerisation initiates TLR2 signal transduction. Dimerisation positions the TIR domains of the cytoplasmic tails in close proximity, facilitating signalling by adaptor molecules containing TIR domains.[86] TLR2 predominantly depends on the myeloid differentiation factor 88 (MyD88) and the Toll-interleukin-1 receptor domain-containing adaptor protein (TIRAP) for signalling.[91] The MyD88-dependent pathway entails death-domain interactions that facilitate intracellular signalling sequentially. The recruitment of MyD88 is indirect and facilitated by TIRAP.[92] MyD88 activates and then induces the phosphorylation of interleukin-1 receptor-associated kinase 4 (IRAK4), IRAK1, and IRAK2 in succession.[92] The IRAK complex interacts with tumour necrosis factor (TNF) receptor-associated factor 6 (TRAF6), which experiences K63-linked autoubiquitination and subsequently ubiquitinates nuclear factor kappa B (NFκB) essential modulator (NEMO). The

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assembly of transforming growth factor-β-activated kinase-1 (TAK1), TAK1-binding protein 2 (TAB2), and TAB3 is subsequently activated. Subsequently, TAK1 phosphorylates IkappaB kinase alpha (IKKα) and IKKβ, which in turn phosphorylate IκB, marking it for degradation. This process ultimately facilitates the production of pro-inflammatory cytokines through NF-κB and activating protein-1 (AP-1), as well as the activation of mitogen-activated protein kinase (MAPK), thereby modulating cell proliferation and survival.[92,93] The connection between TIRAP and IRAK1 and IRAK4 can induce the degradation of TIRAP after its phosphorylation and ubiquitination, hence inhibiting TLR2 signalling.[93]

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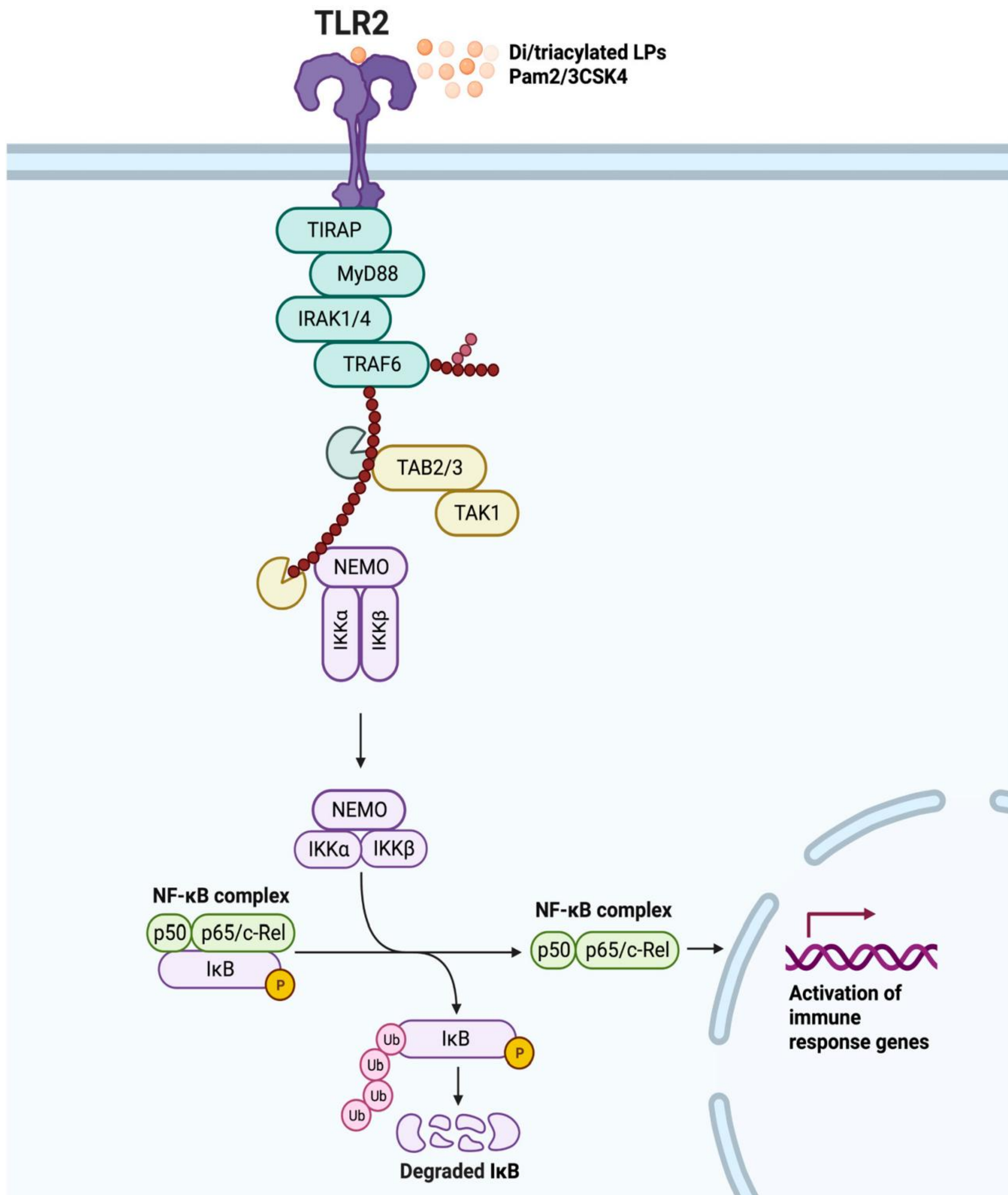

Figure 2.7. TLR2 MyD88-dependent signalling cascade.

Dimerisation is initiated by ligand binding (diacylated lipopeptides (LPs) or triacylated LPs, and synthetic Pam2CSK4 or Pam3CSK4), resulting in a signalling cascade that commences with Toll-interleukin-1 receptor domain-containing adaptor protein (TIRAP) binding to TLR2, subsequently leading to the recruitment of MyD88. After the activation of interleukin-1 receptor-associated kinase (IRAK) 4, IRAK1, and IRAK2, TNF receptor-associated factor 6 (TRAF6) experiences K63-linked autoubiquitination and subsequently ubiquitinates nuclear factor kappa B (NF-κB) essential modulator (NEMO). The complex comprising transforming growth factor-β-activated kinase 1 (TAK1), TAK1-binding protein 2 (TAB2), and TAB3 is subsequently activated to phosphorylate IkappaB kinase alpha (IKKα) and IKKβ, which in turn phosphorylate IκB, tagging it for degradation and liberating the NF-κB complex (composed of p50 and p65/c-Rel). This ultimately results in the activation of immune response genes, including the synthesis of pro-inflammatory cytokines through the transcription factor NF-κB. Derived from "NF-KB signalling pathway".[93]

2.10.1 Dimerization and Additional Co-Receptors of TLR2

2.10.1.1 Homodimerization

Numerous papers in recent years have referenced the existence of TLR2 homodimers; nonetheless, the role of this homodimer remains contentious.[86,94] In vitro investigations, like those by de Groot et al., indicate that TLR2 operates as a homodimer, although it may necessitate co-receptors to attain an active state. Specifically, they demonstrated that TLR2 signalling was diminished in the TLR1- and TLR6-deficient reporter systems, however remained detectable in response to Mycoplasma pneumoniae and Streptococcus pneumoniae.[94] In a separate study, another research team determined that LTA-activated TLR2 through the TLR2 homodimer is probably less effective than LPs activating the TLR2 heterodimer, which is restricted to the induction of IRAK-M.[90] It has been discovered that diprovocim-1 as an efficacious inducer for the production of TLR2/TLR1 heterodimers and

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TLR2 homodimers in vitro.[89] Contradictory findings indicate that the proline-proline-glutamic acid 18 (PPE18) protein of Mycobacterium TB promotes TLR2 homodimerization, hence initiating anti-inflammatory responses that enhance the activation of mammalian p38 MAPK.[95] Additionally, a CRISPR/Cas9-mediated knockout of endogenous TLR6 in JE6-1 TLR2/6 reporter cells has recently demonstrated the expression of TLR2 homodimers on the cell surface. Nonetheless, these reporter cells exhibited no response to any of the bacterial TLR2 agonists evaluated, nor were they detectable following diprovocim-1 induction. Reporter cell activation transpired exclusively in cells harbouring TLR2/1 heterodimers.[86] The latest findings indicate that the functionality of a TLR2 homodimer is significantly reliant on undiscovered ligands and co-receptors. Innovative methodologies and strategies may clarify the putative functions of the TLR2 homodimer to resolve these unresolved enquiries.

2.10.1.2 TLR2 Heterodimers

The capacity of TLR2 to dimerise with itself and various TLRs significantly broadens the range of identifiable diseases. Ozinsky et al. were among the pioneers in identifying that TLR2 necessitates heterodimerization with either TLR1 or TLR6 to trigger appropriate cellular activation and a pro-inflammatory response.[95] Structural investigations of the heterodimers have corroborated the significance of ligand binding in stabilising TLR2/1 and TLR2/6 dimerisation for subsequent signalling.[95] Nevertheless, given that the heterodimerization of TLR2 with TLR1 or TLR6 has been well examined, we will concentrate on discoveries regarding alternative TLR2 heterodimerizations. Evidence is mounting that TLR10 can form functional heterodimers with TLR2.[90,95] This is credible as TLR10, in mammals, belongs to the TLR1 subfamily and possesses several paralogous genes in common with TLR1, TLR2, and TLR6.[92-95] A study evaluated the capacity of the TLR2/10 heterodimer to trigger downstream signalling by analysing mRNA expression levels of pro-

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inflammatory cytokines following exposure to Helicobacter pylori or LPS, revealing a notable upregulation of interleukin-1β (IL-1β) in TLR2/10-transfected HEK293 cells.[96] In a separate investigation, TLR10 was suppressed in the human colon adenocarcinoma cell line HT-29 and in monocytic THP-1 cells, resulting in enhanced vitality of Listeria monocytogenes. The researchers identified that the TLR2/TLR10 heterodimer was responsible for the activation of NF-κB.[96] NC1-N87 gastric cells cultured with Helicobacter pylori, concluding that the TLR2/TLR10 heterodimer enhances NF-κB activation more significantly than other TLR2 heterodimers.[87] A recent research report demonstrated that TLR10 modulates TLR2-induced cytokine production in monocytes derived from Parkinson's disease patients.[90] Nonetheless, the precise ligand(s) and function of the TLR2/10 heterodimer remain inadequately defined. A 2014 paper indicated that the hemoglobin-induced TLR2/4 heterodimer facilitates inflammatory damage in intracerebral haemorrhage in both in vivo and in vitro models.[89] An in vitro study show that in HEK293 cells, matrix metalloproteinase 2 (MMP2) interacts with both TLR2 and TLR4 for signalling, indicating the potential formation of a heterodimer between TLR2 and TLR4, which may initiate a MyD88-dependent signalling cascade.[97] A study further validated the presence of functional TLR2/4 heterodimers by demonstrating that low-endotoxic atypical LPS derived from Ochrobactrum intermedium serves as a potent agonist for TLR2/TLR4, a finding corroborated through molecular docking analysis and fluorescence resonance energy transfer.[97] Nonetheless, the intricate mechanism of these diverse TLR2 heterodimers remains uncertain, necessitating further research and particular ligands to validate these findings.

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2.10.2 Other Co-Receptors Supporting TLR2

Accessory receptors, which serve as additional co-receptors, are frequently necessary to enhance TLR2 ligand delivery, pattern recognition, and several other activities.[187,193,232] Notable adaptors comprise CD14 and CD36, glycoproteins mostly expressed on monocytes and macrophages that facilitate TLR2-dependent inflammation.[98] Immunoprecipitation and sugar inhibition tests demonstrated that the physical contact between ArtinM, a TLR2 agonist, and TLR2 as well as CD14 is essential for the activation of M1 macrophages by ArtinM.[98] A further study indicated that the administration of anti-CD36 to LPS-incubated human microglia modulated inflammatory cytokine levels in the brains of neonatal mice, leading to a notable reduction in TLR2 expression, although TLR4 expression remained unchanged.[97] Alongside CD14 and CD36, the hyaluronan receptor CD44 was considerably increased in THP-1 wild-type cells compared to TLR2 knockout cells, as determined by quantitative mass spectrometry.[98] Several publications in recent years have revealed an interaction between TLR2 and CD44.[97,98] A notable category of TLR2 co-receptors comprises heterodimeric integrin receptors, which are essential for promoting cell–cell and cell–extracellular matrix adhesion during inflammatory processes.[99] They consist of an α and a β subunit, enabling the recognition of many ligands pertinent to cell adhesion and migration.[99] Multiple study papers indicate that β1, β2, and β3 directly or indirectly engage with TLR2 to activate signalling pathways that result in cytokine production.[98] A 2019 in vitro study revealed that α-1-acid glycoprotein enhanced TLR2-dependent β2-integrin-mediated neutrophil adhesion.[99] Conversely, it has been demonstrated that Pam3CSK4 elevated α3β1 integrin levels, an effect that was suppressed by TLR2 silencing. Paracoccidioides brasiliensis infection resulted in elevated TLR2 levels, while concurrently, α3β1 integrin levels were downregulated in human lung epithelial cells.[99] These findings reiterate that TLR2 regulation is contingent upon the ligand and cell type.

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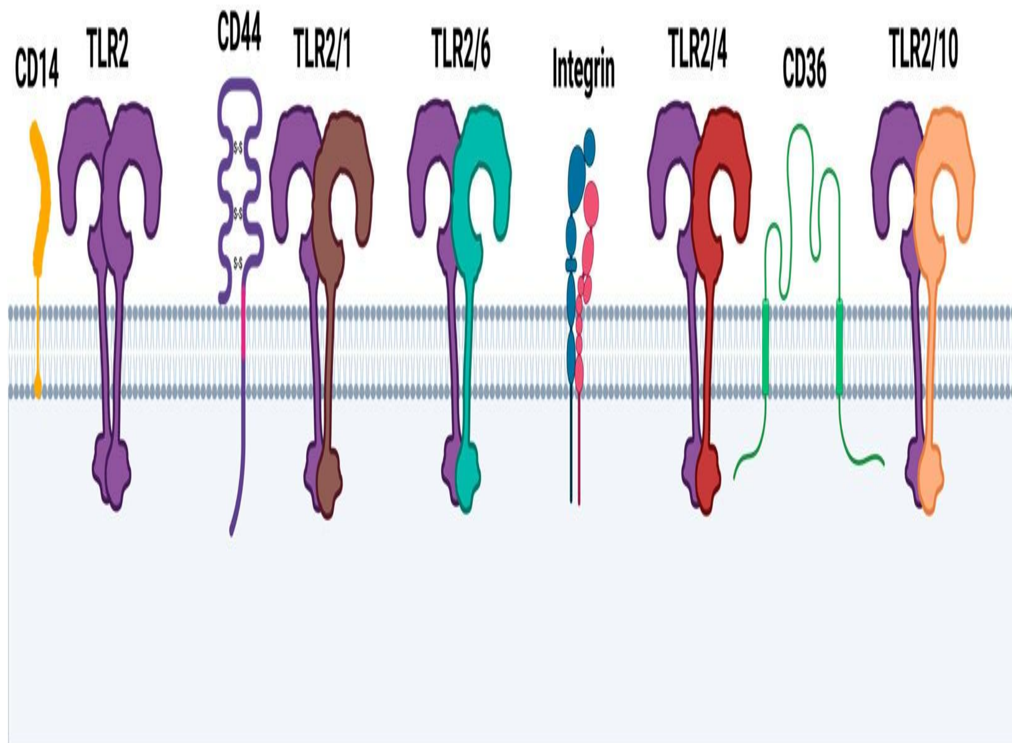

Figure 2.8: TLR2 homo- and heterodimers and additional co-receptors. Upon ligand binding, dimerization is initiated, often requiring additional co-receptors such as cluster of differentiation 14 (CD14), CD44 receptor, integrin receptors, or CD36.[99]

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2.10.3 Optogenetics, an Innovative Approach to Study TLR Dimerization and Signaling

Traditional methods for identifying and characterising dimerisation and signalling pathways, such as pharmacological manipulation, ligand exposure, knockout models, or biochemical assays, may not necessarily be the most suitable approach. Models utilising TLR2 knock-outs underscore the significance of TLR2, although they fail to elucidate the intricate signalling mechanisms or connections with other coreceptors. Nonetheless, novel experimental methods, including optogenetics, are being developed that do not depend on ligand binding to activate cellular receptors. Optogenetics was initially presented by Deisseroth in 2006 within neuroscience, employing light-sensitive ion channels to remotely regulate action potentials and, consequently, neural networks.[99] This biotechnological approach use genetic engineering to merge an effector protein with a light-sensitive protein domain derived from microbial, fungal, or plant photoreceptors, enabling precise regulation of specific cell activities through light.[100] In recent years, various optically controlled receptors, including as G protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs), have been designed to facilitate the activation and inactivation of signalling pathways.[100] A study employed the light–oxygen–voltage (LOV) sensor domain to facilitate receptor tyrosine kinase (RTK) dimerisation through light activation.[100] LOV domains in several eukaryotic and prokaryotic proteins bind a flavin chromophore to serve as blue light sensors. To employ these LOV domains for optogenetic applications, they were extracted from various organisms, including Arabidopsis thaliana and Chlamydomonas reinhardtii.[99] Our research group has developed light-inducible TLR4 cellular models utilising human pancreatic cancer and endothelial cell lines.[101] The LOV domain, extracted from the yellow-green alga Vaucheria frigida aureochrome 1, was fused at the C-terminus to the whole TLR4 protein. This advanced technology facilitates accurate on/off switching with temporal and spatial regulation, potentially providing valuable insights into TLR2 homodimerization and signalling.

2.10.4 TLR2 Mediates Both Pro- and Anti-Inflammatory Responses

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Activation of TLR2 can elicit both pro-inflammatory and anti-inflammatory responses, which are significantly influenced by the specific cell type, cellular compartment, ligand, and co-receptors involved. This section will highlight new results that demonstrate the various and complex impacts of TLR2 activation. The established heterodimerization of TLR2 with TLR1 or TLR6 induces a conventional pro-inflammatory response across various cell types, although the anti-inflammatory response, functioning as an immune regulator, appears to be more diverse. A investigation shown that the elevated quantity and blockwise distribution of certain methyl esters of pectins were accountable for a TLR2/1-dependent anti-inflammatory impact.[101] The flavonoid baicalin, derived from Scutellaria baicalensis Georgi, suppressed inflammation in rats with chronic obstructive pulmonary disease through the TLR2/MyD88/NFκB pathway.[102] The anti-inflammatory characteristics of TLR2 were demonstrated in response to polysaccharide A (PSA) from Bacteroides fragilis in B cells and T cells, leading to the generation of IL-10 and interferon-γ (IFN-γ).M2 macrophages from healthy individuals or rheumatoid arthritis patients exhibited diminished anti-inflammatory activity in the presence of elevated TLR2 levels. Soluble TLR2 (sTLR2), produced through proteolytic cleavage of the TLR2 transmembrane protein, referred to as ectodomain shedding, has been proposed as a potential biomarker for infections and systemic inflammation.[102,103] In this regard, it has been demonstrated that macrophages release soluble and full-length TLR2 under anti-inflammatory settings, suggesting that vesicle-bound full-length TLR2 possesses decoy features that may contribute to immune suppression.[90]

While pattern recognition is the predominant function of TLR2, it is not its exclusive biological role. Our previous research shown that TLR2 significantly contributes to the adhesion and migration of monocytes.[100] We conducted multiple functional cell-based experiments utilising wild-type, TLR2 knockout, and TLR2 knock-in THP-1 cells. Our findings indicate that TLR2 facilitates a more rapid and robust adherence of monocytes to the

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endothelium, as well as a more significant breach of the endothelial barrier following endothelial activation. Previous studies have demonstrated the significance of ligand-activated TLR2 in cell adhesion and migration; however, we highlighted that the activation of endothelial cells is adequate to elicit a unique function of TLR2 expressed on monocytes.[103] Numerous studies have established the role of TLR2 in coagulation, leading to enhanced monocyte adhesion, elevation of tissue factor, increased extrinsic pathway coagulation, active platelet recruitment, and the development of platelet–monocyte aggregates.[104,105] Notably, TLR2 was identified in early endosomes, late endosomes/lysosomes, and Rab-11-positive compartments, but absent in the Golgi apparatus and endoplasmic reticulum of monocytes.Recent studies indicate that IL-10 release in response to Listeria monocytogenes infection, both in vitro and in vivo (mouse model), is predominantly TLR2 reliant, while immunological suppression by phagosome-confined bacteria in vivo primarily relies on endosomal TLRs.[106]

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Table 1. TLR2-dependent pro- and anti-inflammatory responses.

TLR Dimer	Ligand	Cytokines/Chemokines
TLR2/1	Triacyl LPs Pam3CSK4	IL-6, IL-8, TNF-α, IL-1α, IL-1β, IL-12, IL-13, IFN-α, IFN- β
TLR2/6	Diacyl LPs Pam2CSK4 FSL-1	IL-6, IL-8, IL-12, TNF-α, IFN-α, IFN- β, CXCL-10
TLR2/?	*Listeria monocytogenes*	IL-10
TLR2/?	*Bacteroides fragilis* PSA	IL-10, IFN-γ
TLR2/?	LPS	TNF, CXCL10, IL10
TLR2/4	Hemoglobin, atypical LPS	IL-12, IL-6, TNF-α
TLR2/10	*Helicobacter pylori*	TNF- α, IL-1β

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2.10.5 The Role of TLR2 in Infectious and Neurodegenerative Diseases

TLR2 and its signalling pathways have been associated with numerous inflammatory illnesses, including rheumatoid arthritis, inflammatory bowel disease, asthma, and multiple sclerosis.[107] This discussion will focus on sepsis and COVID-19, both of which can result in chronic inflammation and neuroinflammation, potentially contributing to neurodegenerative illnesses such as Alzheimer's disease (AD) and Parkinson's disease (PD).

2.10.5.1 Sepsis

Sepsis is characterised as a systemic dysregulated immune response to an infectious reaction induced by pathogens, such as bacteria, viruses, fungi, and parasites.[107,108] This perilous illness injures the body's tissues and organs, ultimately resulting in organ failure and death, with fatality rates ranging from 20% to 70% if untreated.[108] Progress in medical healthcare has enabled the majority of patients to endure the early hyperinflammatory period of sepsis. However, the shift to the subsequent immunosuppressive phase remains crucial, as 30% of patients succumb to secondary infections.[107] Recently, TLR2 and TLR4, present on immune cells including monocytes, endothelial cells, and platelets, have garnered interest regarding human sepsis.[109] Due to TLR2's fundamental involvement in acute and chronic inflammation, it must be meticulously regulated to prevent an aberrant host response. Failure of this regulation can precipitate sepsis through infections that activate platelets, which subsequently engage with endothelial and immune cells, resulting in the rupture of the endothelium barrier, fluid extravasation, and eventually, tissue destruction and organ failure.[110] Another scenario illustrates the activation of the endothelium by pathogens, leading to the secretion of chemokines and cytokines (cytokine storm) that initiate the recruitment of platelets and immune cells, ultimately resulting in a hyperinflammatory response that induces apoptosis, disrupts the endothelial barrier, causes fluid leakage, and exacerbates tissue damage.[110] Research indicates that Streptococcus pneumoniae triggers platelet activation through TLR2, and its suppression entirely prevents platelet aggregation, suggesting TLR2's role in the

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thrombotic problems associated with sepsis.[111] A separate research group examined 59 patients with Staphylococcus aureus bacteremia and discovered that TLR2 downregulation, together with elevated IL-6 and IL-10 levels, suggestive of immunological dysregulation in the early stages of bacteremia, may correlate with mortality.[109] Moreover, A study demonstrated that TLR2 is significantly increased during the immunosuppressive phase of systemic inflammatory response syndrome (SIRS) and in patients with sepsis.[90] In accordance with prior studies, the experimental model of polymicrobial sepsis caused by cecal ligation and puncture demonstrated elevated TLR2 levels in the kidney and intestine in one group.[111] In the identical sepsis animal paradigm, another group has recently shown that TLR2-deficient mice have diminished levels of IL-10 and decreased caspase-3 activation in the spleen.[112] Collectively, our data highlight the complex involvement of TLR2 in both hyperinflammation and sepsis-induced immunosuppression.

2.10.5.2 COVID-19

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has posed significant challenges to human health and public safety in recent years, belongs to the Coronaviridae family of β-coronaviruses, characterised as positive-stranded, enveloped RNA viruses.[113] Clinical characteristics encompass an elevation in inflammatory monocytes and neutrophils, alongside a vigorous inflammatory environment characterised by cytokines such as IL-1β, IL-6, and TNF-α.[113] Cytokines are integral to innate immunity and crucial for pathogen clearance; nevertheless, their unregulated release can induce hyperinflammation, resulting in cellular apoptosis and tissue injury.[114] thousand two hundred ninety-six Zheng et al. established that TLR2 can identify the SARS-CoV-2 envelope (E) protein and is essential for the production of inflammatory cytokines during β-coronavirus infection.[113] Furthermore, it was discovered that the SARS-CoV-2 E protein can elicit TLR2-dependent inflammation in the pulmonary tissues of mice.[115] In accordance with this, an additional study shown that the

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interaction between E protein and TLR2 results in the subsequent activation of the transcription factor NF-κB, which promotes the synthesis of IL-8.[115] It has been suggested that prophylactic intranasal delivery of a TLR2/6 agonist diminishes SARS-CoV-2 transmission and offers protection against Covid-19. Sepsis and COVID-19 patients exhibit numerous pathophysiological and clinical characteristics, including thrombocytopenia, coagulopathy, vascular microthrombosis, elevated cytokine levels, septic shock, multiorgan failure syndrome, fever, leukopenia, hypotension, and leukocytosis.[113] The observations underscore the significance of timing reliance regarding TLR2, which may result in either protective or detrimental effects.

2.10.5.3 Neuroinflammation

TLR2 is expressed on many immune cells as well as on neuronal and glial cells in the central, peripheral, and enteric nervous systems, allowing them to function as immune cells.[116] Numerous recent papers indicate that TLR2 plays a crucial role in neurological and neurodegenerative illnesses by affecting brain cells and plasticity. Neuroinflammation is defined by the activation and proliferation of microglia and astrocytes, together with elevated concentrations of pro-inflammatory cytokines, chemokines, and cytotoxic substances.[117,118] This ultimately results in the permeabilization of the blood-brain barrier, infiltration of peripheral immune cells, and neuronal cell death.[118] While neuroinflammation serves as a crucial mechanism for safeguarding the central nervous system, a sustained inflammatory response, referred to as chronic inflammation, may result in neuronal damage and death, increasing susceptibility to neurodegenerative disorders such as Alzheimer's disease or Parkinson's disease.[119]

2.10.5.4 Alzheimer's Disease

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Alzheimer's disease (AD), the most common neurodegenerative disorder among the elderly, is pathologically defined by the aggregation of Aβ plaques, hyperphosphorylated tau in neurofibrillary tangles, and resultant increased neuroinflammation.[120] Given that inflammation substantially influences the aetiology of Alzheimer's disease (AD), various inflammatory mediators have been suggested as potential indicators for AD, including IL-1β, TNF-α, NF-κB, and TLR2.Three hundred twelve A substantial body of evidence indicates that inflammation can induce Aβ or tau accumulation, thereby eliciting inflammatory responses that perpetuate a cycle of neuroinflammation and disease.[120] Microglia, the primary cellular element of the brain's innate immune system, exhibit elevated levels of TLR2, rendering them a significant target in the onset, advancement, and maintenance of Alzheimer's disease.[121] TLR2, in conjunction with CD14 and TLR4, is crucial for the activation of microglia triggered by fibrillar Aβ.[120] A study demonstrated that selectively inhibiting the TLR2–MyD88 connection ameliorated Alzheimer's disease pathogenesis, encompassing hippocampus glial activation and diminished Aβ accumulation.[120] In the context of TLR2, a delicate distinction exists between acute, protective inflammation and chronic, neurotoxic inflammation, necessitating the identification of an appropriate framework for therapeutic strategies.

2.10.5.5 Parkinson's Disease (PD)

Parkinson's disease is the most prevalent type of α-synucleinopathy and the second most frequent neurodegenerative disorder.[122] The clinical characteristic of Parkinson's disease is Lewy bodies, which are proteinaceous inclusions primarily composed of misfolded and aggregated α-synuclein.[121] Analogous to Alzheimer's Disease, the dissemination and mechanism of αSyn misfolding remain ambiguous; nonetheless, in vivo methodologies along with diverse in vitro investigations have yielded compelling evidence that environmental and genetic variables, along with inflammation, are significant contributors.[123] TLR2 recognises

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alarmins, including αSyn, produced from injured neuronal cells, prompting cytokine production and the recruitment of other immune cells, such as T cells and B cells, to initiate an adaptive immune response.[124] TLR2 is proposed to be elevated in the aetiology of synucleinopathies in the brains of Parkinson's disease patients, indicating that TLR2 mediates both pro-inflammatory and neurotoxic effects of extracellular α-synuclein aggregates. Kim et al. demonstrated that inhibiting TLR2 decreased αSyn accumulation in neuronal and astroglial cells, as well as neuroinflammation, neurodegeneration, and functional impairments in a Parkinson's disease mice model.[124] In accordance with prior research, current findings indicate that neuronal TLR2 activation abruptly disrupts the lysosomal autophagy system and exacerbates αSyn pathology induced by α-Syn produced fibrils in human cell cultures. Moreover, the ablation of TLR2 in a human neuroblastoma cell line, along with the application of the small molecule TLR2 inhibitor (NPT1220-321) in induced pluripotent stem cell-derived neurones from a Parkinson's disease patient, has shown that αSyn pathology can be mitigated.[124] A recent study indicates that targeted suppression of the TLR2 interaction region of MyD88 and the NEMO binding domain can impede αSyn propagation both in vitro and in vivo.[120] Collectively, multiple studies suggest TLR2's involvement in the pathogenesis of PD; nevertheless, the precise mechanism by which TLR2 influences αSyn pathophysiology remains ambiguous.[125]

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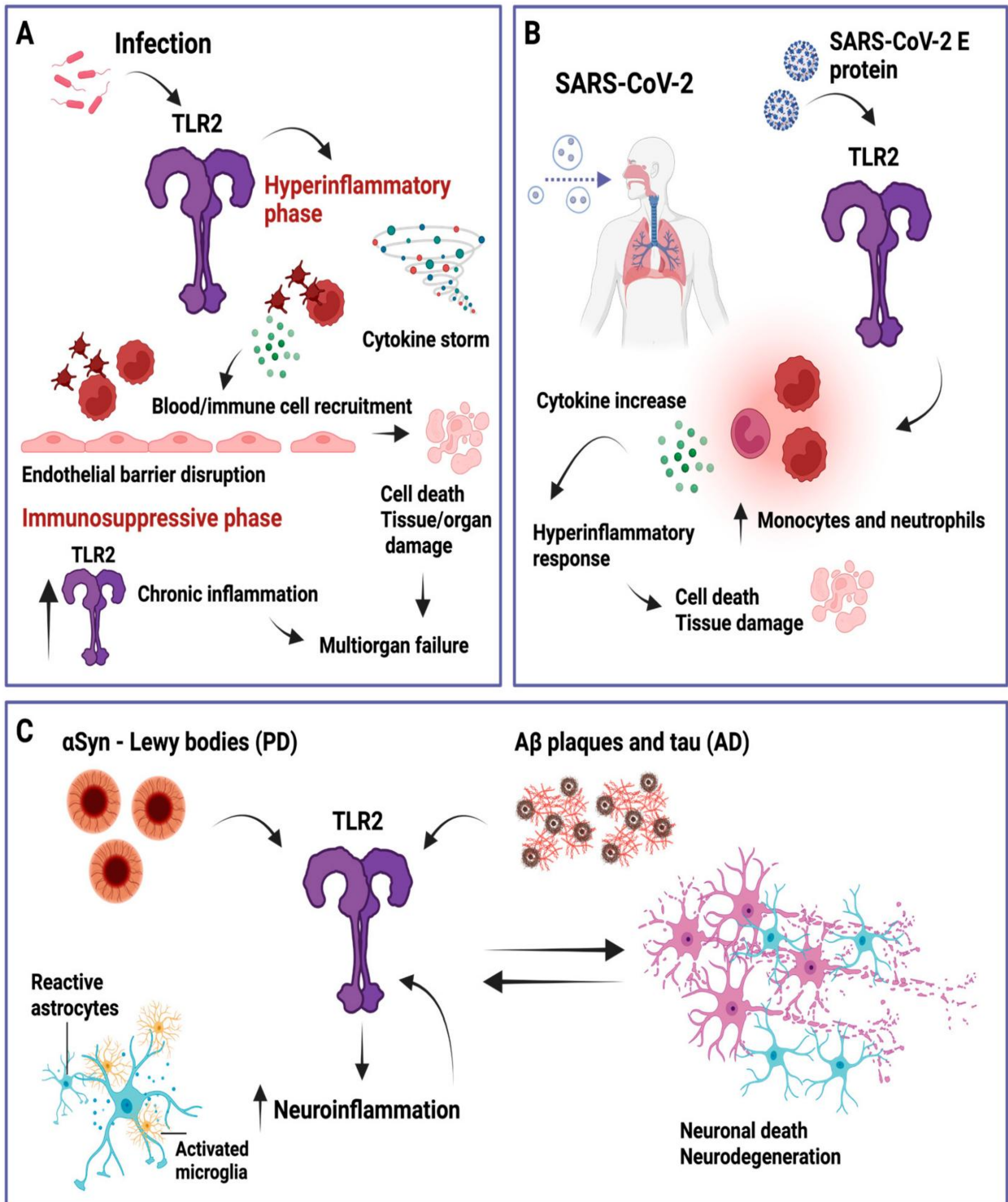

Figure 2.9: Concise summary of TLR2's role in many pertinent inflammatory disorders. Sepsis. Immune regulation failure can induce a hyperinflammatory state characterised by increased cytokine production (cytokine storm), which may trigger sepsis through TLR2 activation. This process further stimulates platelets and other immune cells, resulting in interactions with endothelial cells that cause endothelial barrier disruption, cellular apoptosis,

and subsequent tissue and organ damage. Furthermore, TLR2 has been demonstrated to be upregulated during the immunosuppressive phase of sepsis, potentially resulting in multiorgan failure. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). TLR2 can identify the SARS-CoV-2 envelope (E) protein, resulting in an elevation of inflammatory monocytes and neutrophils, which triggers a hyperinflammatory response that may result in cellular death and tissue injury. Parkinson's disease (PD) and Alzheimer's disease (AD). TLR2 can identify α-synuclein (αSyn), amyloid-β (Aβ) plaques, and tau. This induces neuroinflammation marked by reactive astrocytes and activated microglia, which then elevate TLR2 levels, resulting in a feedback loop of neuronal cell death, more TLR2 overexpression, and neuroinflammation.[124]

2.10.6 Future Perspectives

In the past two decades, a significant revelation in medicine has been the association of the immune system and consequent inflammatory processes with not just a limited number of specific diseases but also a wide array of mental and physical health disorders.[125] Chronic inflammatory disorders are increasingly recognised as the primary cause of mortality globally, accounting for almost 50% of all deaths attributable to inflammation-related diseases, including stroke, cancer, ischaemic heart disease, autoimmune diseases, and neurodegenerative diseases.[126] TLR2 is a pivotal component in inflammation-mediated processes and is among the most thoroughly examined pattern recognition receptors.[126] A significant number of in vitro and in vivo investigations have been performed to thoroughly clarify the function of this protein. Among all identified TLRs, TLR2 is distinctive due to its ability to heterodimerise with various TLRs, hence expanding the ligand spectrum and demonstrating both pro- and anti-inflammatory properties.[86,90] Evidence has accumulated indicating that TLR2 signalling is activated by SARS-CoV-2, resulting in significant pro-inflammatory cytokine expression that may exacerbate severe COVID-19. Additionally,

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TLR2 has been recognised as a crucial mediator of neuroinflammation, which is significantly pertinent to the pathogenesis of neurodegenerative disorders.[127] Notwithstanding these advancements, significant gaps remain in our understanding of TLR2 processes in both health and illness. We have merely begun to explore the mechanistic connection between TLRs and neurodegenerative disorders, for instance. Additional investigations, particularly utilising patient-derived samples, are essential to better evaluate the complexity. Moreover, focus must be made on in silico approaches, which offer valuable tools for both drug design and discovery methodologies, as well as for cellular models of diverse illnesses.[127] It is imperative to address these deficiencies to formulate therapeutic solutions for inflammation-related diseases, particularly neurodegenerative disorders, which will increasingly burden society as the population ages. Recent findings emphasise that neurological illnesses present a significant challenge to the sustainability of global healthcare.[127]

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Endnotes

1. WHO (World Health Organization), *Treatment of Tuberculosis- guidelines*. 2019

2. WHO (World Health Organization), *TB: a global emergency, WHO report on the TB epidemic*. Geneva: Available Online: https://apps.who.int/iris/bitstream/handle/10665/58749/WHO_TB_94.177/1991

3. WHO (World Health Organization), *Global Tuberculosis Report* 2016.

4. E. Balasubramanian, E.H. Wiegeshaus, B.T. Taylor, & D.W. Smith, *Pathogenesis of tuberculosis: pathway to apical localization, Tuberculosis Lung Disease*, 75(3), 2014, 168–178.

5. L.L. Lu, M.T, Smith, & K.K.Q. Yu, *IFN-γ-independent immune markers of Mycobacterium tuberculosis exposure*, **Nature Medicine**, 25(6), 2019, 977–987.

6. R.M.G.J. Houben, & P.J. Dodd, *The Global Burden of Latent Tuberculosis Infection: A Re-estimation Using Mathematical Modelling*, **Public Library of Science Medicine**, 13(10), 2016.

7. I. Abubakar, F. Drobniewski, & J. Southern, *Prognostic value of interferon-γ release assays and tuberculin skin test in predicting the development of active tuberculosis (UK PREDICT TB): a prospective cohort study*, **Lancet of Infectious Diseases**, 18(10), 2018, 1077–1087.

8. N.M. Schluger, & W.N. Rom, *The host immune response to tuberculosis*, **American Journal of Respiratory and Critical Care Medicine**, 157(1), 2018, 679-691.

9. S.D. Lawn, & A.I. Zumla, *Tuberculosis*, **Lancet**, 378(9785), 2021, 57-72.

10. A. Zumla, *Tuberculosis*, **New England Journal of Medicine**, 368(8), 2023, 745-755.

11. WHO (World Health Organisation), *Global Tuberculosis Report*, 2015. Available Online: http://www.who.int/tb/publications/global_report/en/2015.

12. I.A. Campbell, & O. Bah-Sow, *Pulmonary tuberculosis: diagnosis and treatment*, **British Medical Journal**, 332(7551), 2016, 1194-1197.

13. WHO (World Health Organization), *Systematic Screening of Active tuberculosis-Principles and recommendations*, 2023

14. L.M. Pinto, *Scoring systems using chest radiographic features for the diagnosis of pulmonary tuberculosis in adults: a systematic review*, **European Respiratory Journal**, 42(2), 2023, 480-494.

15. WHO (World Health Organization), *International Standards for Tuberculosis Care*, 3, 2018.

16. M. Mirsaeidi, *Personalized medicine approach in mycobacterial disease*, **International Journal of Mycobacteriology**, 1(2), 2022, 59-64.

Lead City University Ibadan DO NOT COPY

17. L.J. Pankhurst, O. Jo, C. Elias, & A.A. Votintseva, *Rapid, comprehensive, and affordable mycobacterial diagnosis with wholegenome sequencing: A prospective study*, **Lancet of Respiratory Medicine**, 4(1), 2016, 49–58.

18. C.R. Horsburgh, C.E. Barry, & C. Lange, *Treatment of Tuberculosis*, **New England Journal of Medicine**, 373(22), 2015, 2149-2160.

19. H.J. Salzer, *Personalized Medicine for Chronic Respiratory Infectious Diseases: Tuberculosis, Nontuberculous Mycobacterial Pulmonary Diseases, and Chronic Pulmonary Aspergillosis*, **Respiration**, 92(4), 2016, 199-214.

20. WHO (World Health Organization), *Guidelines for the Programmatic Management of Drug-Resistant Tuberculosis*, **Geneva**, 2011.

21. A. Van-Deun, A.K.J. Maug, & M.A.H. Salim, *Short, highly effective, and inexpensive standardized treatment of multidrug-resistant tuberculosis*, **American Journal of Respiratory Critical Care Medicine**, 182(5), 2020, 684–692.

22. A.J. Nunn, P.P.J. Phillip, & S.K. Meredith, *A trial of a shorter regimen for rifampin-resistant tuberculosis*, **New England Journal of Medicine**, 380(13), 2019, 1201–1213.

23. N. Ahmad, S.D. Ahuja, & O.W. Akkerman, *Treatment correlates of successful outcomes in pulmonary multidrug-resistant tuberculosis: an individual patient data meta-analysis*, **Lancet**, 392(10150), 2018, 821–834.

24. F. Conradie, A.H. Diacon, & N. Ngubane, *Treatment of Highly DrugResistant Pulmonary Tuberculosis*, **New England Journal of Medicine**, 382(10), 2020, 893–902.

25. S.T. Cole, R. Brosch, & J. Parkhill, *Deciphering the biology of mycobacterium tuberculosis from the complete genome sequence*, **Nature**, 393(6685), 1998, 537–544.

26. I. Comas, M. Coscolla, & T. Luo, *Out-of-Africa migration and Neolithic coexpansion of Mycobacterium tuberculosis with modern humans*, **Nature and Genetics**, 45(10), 2023, 1176–1182.

27. K.I. Bos, K.M. Harkins, & A. Herbig, *Pre-Columbian mycobacterial genomes reveal seals as a source of New World human tuberculosis*. **Nature**, 514(7253), 2014, 494–497.

28. G.L. Kay, M.J. Sergeant, & Z. Zhou, *Eighteenth-century genomes show that mixed infections were common at time of peak tuberculosis in Europe*, **Nature and Communication**; 6, 2015.

29. I. Hershkovitz, H.D. Donoghue, & D.E. Minnikin, *Detection and molecular characterization of 9000-year-old Mycobacterium tuberculosis from a neolithic settlement in the Eastern Mediterranean*, **Public Library of Science One**, 3(10), 2018.

30. S. Gagneux, *Ecology and evolution of Mycobacterium tuberculosis*, **Nature Review Microbiology**, 16(4), 2018, 202–213.

Lead City University Ibadan DO NOT COPY

31. C. Ford, K. Yusim, & T. Ioerger, *Mycobacterium tuberculosis - Heterogeneity revealed through whole genome sequencing*, **Tuberculosis**, 92(3), 2022, 194–201.

32. Q. Jiang, Q. Liu, & L. Ji, *Citywide Transmission of Multidrug-resistant Tuberculosis Under China's Rapid Urbanization: A Retrospective Population-based Genomic Spatial Epidemiological Study*, **Clinical Infectious Diseases**, 3, 2019, 23-27.

33. Y. Ektefaie, A. Dixit, L Freschi, & M. Farhat, *Tuberculosis resistance acquisition in space and time: an analysis of globally diverse M. tuberculosis whole genome sequences*, Available Online: http://biorxiv.org/content/early/2019/11/11/837096.abstract

34. A.S. Pym, B. Saint-Joanis, & S.T. Cole, *Effect of katG mutations on the virulence of Mycobacterium tuberculosis and the implication for transmission in humans*, **Infection and Immunology**, 70(9), 2012, 4955–4960.

35. V. Eldholm, & F Balloux, *Antimicrobial Resistance in Mycobacterium tuberculosis: The Odd One Out*, **Trends in Microbiology**, 24(8), 2016, 637–648.

36. A.Y. Morales-Arce, R.B. Harris, A.C. Stone, & J.D. Jensen, *Within-host Mycobacterium tuberculosis evolution: a population genetics perspective*. Available from: http://biorxiv.org/content/early/2019/12/04/863894.abstract/2019

37. S. Polsfuss, S. Hofmann-Thiel, & M. Merker, *Emergence of Low-level Delamanid and Bedaquiline Resistance during Extremely Drug-resistant Tuberculosis Treatment*, **Clinical Infectious Diseases**, 69(7), 2019, 1229–1231.

38. M. de-Vos, S.D. Ley, & K.B Wiggins, *Bedaquiline Microheteroresistance after Cessation of Tuberculosis Treatment*, **New England Journal of Medicine**, 380(22), 2019, 2178–2180.

39. J.Z. Metcalfe, E. Streicher, & G. Theron, *Mycobacterium tuberculosis subculture results in loss of potentially clinically relevant heteroresistance*, **Antimicrobial Agents and Chemotherapy**, 61(11), 2017.

40. K. Dheda, L. Lenders, & G. Magombedze, *Drug-penetration gradients associated with acquired drug resistance in patients with tuberculosis*, **American Journal of Respiratory Critical Care Medicine**, 198(9), 2018, 1208–1219.

41. Q. Liu, L.E. Via, & T. Luo, *Within patient microevolution of Mycobacterium tuberculosis correlates with heterogeneous responses to treatment,* **Science of Reproduction**, 5, 2015.

42. A. O'Garra, *The immune response in tuberculosis*, **Annual Review of Immunology**, 31, 2023, 475-527.

43. J. Kleinnijenhuis, *Innate immune recognition of Mycobacterium tuberculosis*, **Clinical Development and Immunology**, 1, 2021, 405310.

Lead City University Ibadan DO NOT COPY

44. H.E. Hett, & E.J. Rubin, *Bacterial growth and cell division: a mycobacterial perspective*, **Microbiology and Molecular Biology Review**, 72(1), 2018, 126-156.

45. M. Gengenbacher, & S.H.E Kaufmann, *Mycobacterium tuberculosis: success through dormancy*, **Fems Microbiology Reviews**, 36(3), 2022, 514-532.

46. B.W. Jones, *Different Toll-like receptor agonists induce distinct macrophage responses*, **Journal of Leukocyte and Biology**, 69(6), 2021, 1036-1044.

47. T.K. Means, *Differential effects of a Toll-like receptor antagonist on Mycobacterium tuberculosis-induced macrophage responses*, **Journal of Immunology**, 166(6), 2021, 4074-4082.

48. E.K. Jo, *Intracellular signalling cascades regulating innate immune responses to Mycobacteria: branching out from Toll-like receptors*, **Cell Microbiology**, 9(5). 2017, 1087-1098.

49. M.B. Drennan, *Toll-like receptor 2-deficient mice succumb to Mycobacterium tuberculosis infection*, **American Journal of Pathology**, 164(1), 2018, 49-57.

50. B. Abel, *Toll-like receptor 4 expression is required to control chronic Mycobacterium tuberculosis infection in mice*, **Journal of Immunology**, 169(6), 2022, 3155-3162.

51. Y. Bulut, *Cooperation of Toll-like receptor 2 and 6 for cellular activation by soluble tuberculosis factor and Borrelia burgdorferi outer surface protein A lipoprotein: role of Tollinteracting protein and IL-1 receptor signaling molecules in Toll-like receptor 2 signaling*, **Journal of Immunology**, 167(2), 2021, 987-994.

52. A. Bafica, *TLR9 regulates Th1 responses and cooperates with TLR2 in mediating optimal resistance to Mycobacterium tuberculosis*, **Journal of Experimental Medicine**, 202(12), 2015, 1715-1724.

53. S. Shi, *Expression of many immunologically important genes in Mycobacterium tuberculosis-infected macrophages is independent of both TLR2 and TLR4 but dependent on IFN-alphabeta receptor and STAT1*, **Journal of Immunology**, 175(5), 2015, 3318-3328.

54. I. Sugawara, *Mycobacterial infection in MyD88-deficient mice*, **Microbiology and Immunology**, 47(11), 2023, 841-847.

55. L. Franchi, *Function of Nod-like receptors in microbial recognition and host defense*, **Immunology Review**, 227(1), 2019, 106-128.

56. S.E. Girardin, *Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection*, **Journal of Biological Chemistry**, 278(11), 2023, 8869-8872.

57. M. Divangahi, *NOD2-Deficient Mice Have Impaired Resistance to Mycobacterium tuberculosis Infection through Defective Innate and Adaptive Immunity*, **Journal of Immunology**, 181(10), 2018, 7157-7165.

Lead City University Ibadan DO NOT COPY

58. B.B. Mishra, *Mycobacterium tuberculosis protein ESAT-6 is a potent activator of the NLRP3/ASC inflammasome*, **Cellular Microbiology**, 12(8), 2020, 1046-1063.

59. K. Schweneker, *The mycobacterial cord factor adjuvant analogue trehalose-6,6 '-dibehenate (TDB) activates the Nlrp3 inflammasome*, **Immunobiology**, 218(4), 2023, 664- 673.

60. K.V. Anderson, L. Bokla, & C. Nusslein-Volhard, *Establishment of dorsal ventral polarity in the Drosophila embryo: the induction of polarity by the Toll gene product*, **Cell,** 42, 2020, 791–798.

61. D.S. Schneider, K.L. Hudson, T.Y. Lin, & K.V. Anderson, *Dominant and recessive mutations define functional domains of Toll, a Trans membrane protein required for dorsal-ventral polarity in the Drosophila embryo*, **Genes Dev,** 5, 2021, 797–807.

62. B. Lemaitre, E. Nicolas, L. Michaut, M. Reichhart, & L.A. Hoffmann, *The dorsoventral regulatory gene cassette spätzle/Toll/cactus controls the potent antifungal response in Drosophila adults*, **Cell,** 86, 2020, 973–983.

63. W. Gao, Y. Xiong, Q. Li, & H. Yang, *Inhibition of Toll-like receptor signaling as a promising therapy for inflammatory diseases: a journey from molecular to nano therapeutics*, **Front Physiol**, 8, 2022, 508–517.

64. S. Akira, S. Uematsu, & O. Takeuchi, *Pathogen recognition and innate immunity*, **Cell,** 124, 2021, 783–801.

65. J.M. Isaza-Correa, Z. Zheng-Liang, A. van den Berg, A. Arjan-Diepstra, & V.L. Lydia, *Toll-like receptors in the pathogenesis of human B cell malignancies*, **J Hematol Oncol,** 7, 2019, 57.

66. K. Singh, S. Kant, S.K. Gupta, & V.K. Singh, *Toll-like receptor 4 polymorphisms and their haplotypes modulate the risk of developing diabetic retinopathy in type 2 diabetes patients*, **Mol Vision**. 20, 2019, 704–713.

67. R.S. Mahla, *Sweeten PAMPs: role of sugar complexed PAMPs in innate immunity and vaccine biology*, **Front Immunol**, 4, 2023, 248.

68. M. Yamamoto, & K. Takeda, *Current views of toll-like receptor signaling pathways*, **Gastroenterol Res Pract**, 240365, 2020.

69. R. Medzhitov, P. Preston-Hurlburt, & C.A. Janeway, *A human homologue of the Drosophila Toll protein signals activation of adaptive immunity*, **Nature,** 388, 2022, 394–397.

70. A. Poltorak, X. He, I. Smirnova, M.Y. Liu, C. Van-Huffel, & X. Du, *Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in TLR4 gene*, **Science,** 282, 2023, 2085–2088.

Lead City University Ibadan DO NOT COPY

71. N.J. Gay, M.F. Symmons, M. Gangloff, & C.E. Bryant, *Assembly and localization of Toll-like receptor signalling complexes*, **Nat Rev Immunol**, 14, 2019, 546–558.

72. G. Sellge, & T.A. Kufer, *PRR-signaling pathways: learning from microbial tactics*, **Semin Immunol**, 27, 2020, 75–84.

73. K.A. Zarember, & P.J. Godowski, *Tissue expression of human Toll-like receptors and differential regulation of Toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines*. **J Immunol**, 168(2), 2022, 554–561.

74. Y. Delneste, C. Beauvillain, & P. Jeannin, *Innate immunity: structure and function of TLRs*, **Med Sci (Paris),** 23(1). 2022, 67–73.

75. R. Medzhitov, & C.A. Janeway, *Innate Immunity: The Virtues of a Nonclonal System of Recognition*, **Cell,** 91(3), 2022, 295–298.

76. J.A. Hoffmann, F.C. Kafatos, C.A. Janeway, & R.A.B. Ezekowitz, *Phylogenetic Perspectives in Innate Immunity*, **Science,** 284(5418), 2019, 1313–1318.

77. A. Aderem, & R.J. Ulevitch, *Toll-Like Receptors in the Induction of the Innate Immune Response*, **Nature,** 406(6797), 2020, 782–787.

78. T. Kawai, & S. Akira, *The Role of Pattern-Recognition Receptors in Innate Immunity: Update on Toll-Like Receptors*, **Nat Immunol**, 11(5), 2020, 373–384.

79. A.L. Blasius, & B. Beutler, *Intracellular Toll-Like Receptors*, **Immunity,** 32(3), 2020, 305–315.

80. E. Latz, A. Verma, A. Visintin, M. Gong, C.M. Sirois, & D.C.G. Klein, *LigandInduced Conformational Changes Allosterically Activate Toll-Like Receptor 9*, **Nat Immunol**, 8(7), 2022, 772–779.

81. K.A. Fitzgerald, E.M. Palsson-McDermott, A.G. Bowie, C.A. Jefferies, A.S. Mansell, & G. Brady, *Mal (MyD88-Adapter-Like) Is Required for Toll-Like Receptor-4 Signal Transduction*, **Nature,** 413(6851), 2021, 78–83.

82. T. Horng, G.M. Barton, & R. Medzhitov, *TIRAP: An Adapter Molecule in the Toll Signaling Pathway*, **Nat Immunol,** 2(9), 2021, 835–841.

83. M. Yamamoto, S. Sato, H. Hemmi, S. Uematsu, K. Hoshino, & T. Kaisho, *TRAM is Specifically Involved in the Toll-Like Receptor 4-Mediated MyD88-Independent Signaling Pathway*, **Nat Immunol** 4(11), 2023, 1144–1150.

84. K.A. Fitzgerald, D.C. Rowe, B.J. Barnes, D.R. Caffrey, A. Visintin, & E. Latz, *LPS-TLR4 Signaling to IRF-3/7 and NF-Kappa B Involves the Toll Adapters TRAM and TRIF*, **J Exp Med** 198(7), 2023, 1043–1055.

Lead City University Ibadan DO NOT COPY

85. J.C. Kagan, T. Su, T. Horng, A. Chow, S. Akira, & R. Medzhitov, *TRAM Couples Endocytosis of Toll-Like Receptor 4 to the Induction of Interferon-Beta*, **Nat Immunol**, 9(4), 2023, 361–368.

86. K.I. Iwami, T. Matsuguchi, A. Masuda, T. Kikuchi, T. Musikacharoen, & Y. Yoshikai, *Cutting edge: naturally occurring soluble form of mouse Toll-like receptor 4 inhibits lipopolysaccharide signaling*. **J Immunol,** 165, 2021, 6682–6686.

87. E. LeBouder, J.E. Rey-Nores, N.K. Rushmere, M. Grigorov, S.D. Lawn, & M. Affolter, *Soluble forms of Toll-like receptor (TLR)2 capable of modulating TLR2 signaling are present in human plasma and breast milk*. **J Immunol,** 171, 2023, 6680–6689.

88. E.K. Brint, D. Xu, H. Liu, A. Dunne, A.N. McKenzie, & L.A. O'Neill, *ST2 is an inhibitor of interleukin 1 receptor and Toll-like receptor 4 signaling and maintains endotoxin tolerance*, **Nat Immunol**, 5, 2019, 373–379.

89. K. Kobayashi, L.D. Hernandez, J.E. Galán, C.A. Janeway, R. Jr, Medzhitov, & R.A. Flavell, *IRAK-M is a negative regulator of Toll-like receptor signaling*, **Cell,** 110, 2022, 191–202.

90. D.L. Boone, E.E. Turer, E.G. Lee, R.C. Ahmad, M.T. Wheeler, & C. Tsui, *The ubiquitinmodifying enzyme A20 is required for termination of Toll-like receptor responses*, **Nat Immunol,** 5, 2019. 1052–1060

91. O. Leavy, *Innate immunity: SHP regulates TLR signaling*, **Nat Rev Immunol,** 11, 2021, 502.

92. L. Oliveira-Nascimento, P. Massari, & L.M. Wetzler, *The Role of TLR2 Ininfection and Immunity*. **Frontier of Immunology**, **18**, 2022, 79.

93. C. Hashimoto, K.L. Hudson, & K.V. Anderson, *The Toll Gene of Drosophila, Required for Dorsal-Ventral Embryonic Polarity, Appears to Encode a Transmembrane Protein*. **Cell**, **52**, 2023, 269–279.

94. K.V. Anderson, G. Jürgens, & C. Nüsslein-Volhard, *Establishment of Dorsal-Ventral Polarity in the Drosophila Embryo: Genetic Studies on the Role of the Toll Gene Product*. **Cell**, **42**, 2020, 779–789.

95. A. Ozinsky, D.M. Underhill, J.D. Fontenot, .M. Hajjar, K.D. Smith, C.B. Wilson, L. Schroeder, & A. Aderem, *The Repertoire for Pattern Recognition of Pathogens by the Innate Immune System Is Defined by Cooperation between Toll-like Receptors*. **Proc. Natl. Acad. Sci**, **97**, 2020, 13766–13771.

96. Y.C. Wang, Y. Zhou, H. Fang, S. Lin, P. Wang, X. Xiong, J. Chen, X. Xiong, F. Lv, & Q. Liang, *Toll-like Receptor 2/4 Heterodimer Mediates Inflammatory Injury in Intracerebral Hemorrhage*. **Annals of Neurology**, **75**, 2024, 876–889.

Lead City University Ibadan DO NOT COPY

97. S. Su, L. Tao, Z. Deng, W. Chen, S. Qin, & H. Jiang, *TLR10: Insights, Controversies and Potential Utility as a Therapeutic Target*. **Scand. Journal of Immunology 93**, 2021, e12988.

98. J. van Bergenhenegouwen, T.S. Plantinga, L.A.B. Joosten, M.G. Netea, G. Folkerts, A.D. Kraneveld, J. Garssen, & A.P. Vos, *TLR2 & Co: A Critical Analysis of the Complex Interactions between TLR2 and Coreceptors*. **J. Leukoc. Biol.**, **94**, 2023, 885–902.

99. M.B.T. Keshavarz-Fathi, & N. Rezaei, *Chapter 3—Vaccines, Adjuvants, and Delivery Systems*; **Academic Press: Cambridge, MA**, 2019; pp. 45–59, ISBN 978-0-12-814039-0.

100. V.J. Quesniaux, D.M. Nicolle, D. Torres, L. Kremer, Y. Guérardel, J. Nigou, G. Puzo, F. Erard, & B. Ryffel, *Toll-like Receptor 2 (TLR2)-Dependent-Positive and TLR2-Independent-Negative Regulation of Proinflammatory Cytokines by Mycobacterial Lipomannans*. **J. Immunol.**, **172**, 2024, 4425–4434.

101. E.M. Long, B. Millen, P. Kubes, & S.M. Robbins, *Lipoteichoic Acid Induces Unique Inflammatory Responses When Compared to Other Toll-like Receptor 2 Ligands*. **PLoS ONE**, **4**, 2019, e5601.

102. S. Francisco, J.M. Billod, J. Merino, C. Punzón, A. Gallego, A. Arranz, S. Martin-Santamaria, & M. Fresno, *Induction of TLR4/TLR2 Interaction and Heterodimer Formation by Low Endotoxic Atypical LPS*. **Front. Immunol.**, **12**, 2021, 748303.

103. H. Nagashima, S. Iwatani, M. Cruz, J.A. Jiménez Abreu, T. Uchida, V. Mahachai, R.K. Vilaichone, D.Y. Graham, & Y. Yamaoka, *Toll-like Receptor 10 in Helicobacter Pylori Infection*. **J. Infect. Dis.**, **212**, 2020, 1666–1676.

104. M. Manukyan, K. Triantafilou, M. Triantafilou, A. Mackie, N. Nilsen, T. Espevik, K.H. Wiesmüller, A.J. Ulmer, & H. Heine, *Binding of Lipopeptide to CD14 Induces Physical Proximity of CD14, TLR2 and TLR1*. **Eur. J. Immunol.**, **35**, 2020, 911–921.

105. J. Hoppstädter, A. Dembek, R. Linnenberger, C. Dahlem, A. Barghash, C. Fecher-Trost, G. Fuhrmann, M. Koch, A. Kraegeloh, & H. Huwer, *Toll-Like Receptor 2 Release by Macrophages: An Anti-Inflammatory Program Induced by Glucocorticoids and Lipopolysaccharide*. **Front. Immunol.**, **10**, 2019, 1634.

106. H. Zheng, Z. Tan, T. Zhou, F. Zhu, Y. Ding, T. Liu, Y. Wu, & W. Xu, *The TLR2 Is Activated by Sporozoites and Suppresses Intrahepatic Rodent Malaria Parasite Development*. **Sci. Rep.**, **5**, 2020, 18239.

107. T. Oosenbrug, M.J. van de Graaff, M.E. Ressing, & S.I. van Kasteren, *Chemical Tools for Studying TLR Signaling Dynamics*. **Cell Chem. Biol.**, **24**, 2022, 801–812.

108. C.J. Kurkjian, H. Guo, N.D. Montgomery, N. Cheng, H. Yuan, J.R. Merrill, G.D. Sempowski, W.J. Brickey, & J.P. Ting, *The Toll–Like Receptor 2/6 Agonist, FSL–1 Lipopeptide, Therapeutically Mitigates Acute Radiation Syndrome*. **Sci. Rep.**, **7**, 2022, 17355. [CrossRef]

Lead City University Ibadan DO NOT COPY

109. K.A. Scheibner, M.A. Lutz, S. Boodoo, M.J. Fenton, J.D. Powell, & M.R. Horton, *Hyaluronan Fragments Act as an Endogenous Danger Signal by Engaging TLR21*. **J. Immunol**., **177**, 2021, 1272–1281.

110. N. Funderburg, M.M. Lederman, Z. Feng, M.G. Drage, J. Jadlowsky, C.V. Harding, A. Weinberg, & S.F. Sieg, *Human β-Defensin3 Activates Professional Antigen-Presenting Cells via Toll-like Receptors 1 and 2*. **Proc. Natl. Acad. Sci**., **104**, 2022, 18631–18635.

111. C. Erridge, *Endogenous Ligands of TLR2 and TLR4: Agonists or Assistants*? **J. Leukoc. Biol**., **87**, 2020, 989–999.

112. K.E. Marks, K. Cho, C. Stickling, & J.M. Reynolds, *Toll-like Receptor 2 in Autoimmune Inflammation*. **Immune Netw.**, **21**, 2021, e18.

113. S. Liu, Y. Liu, W. Hao, L. Wolf, A.J. Kiliaan, B. Penke, C.E. Rübe, J. Walter, M.T. Heneka, & T. Hartmann, *TLR2 Is a Primary Receptor for Alzheimer's Amyloid β Peptide to Trigger Neuroinflammatory Activation*. **J. Immunol**., **188**, 2022, 1098–1107.

114. C. Kim, D.H. Ho, J.E. Suk, S. You, S. Michael, J. Kang, S. Joong-Lee, E. Masliah, D. Hwang, & H.J. Lee, *Neuron-Released Oligomeric α-Synuclein Is an Endogenous Agonist of TLR2 for Paracrine Activation of Microglia*. **Nat. Commun**., **4**, 2024. 1562.

115. M. Yamamoto, S. Sato, H. Hemmi, H. Sanjo, S. Uematsu, T. Kaisho, K. Hoshino, O. Takeuchi, M. Kobayashi, & T. Fujita, *Essential Role for TIRAP in Activation of the Signalling Cascade Shared by TLR2 and TLR4*. **Nature**, **420**, 2022, 324–329.

116. S. Rajpoot, K.K. Wary, R. Ibbott, D. Liu, U. Saqib, T.L.M. Thurston, & M.S. Baig, *TIRAP in the Mechanism of Inflammation*. **Front. Immunol**., **12**, 2021, 697588.

117. S. Mantovani, B. Oliviero, S. Varchetta, A. Renieri, & M.U. Mondelli, *TLRs: Innate Immune Sentries against SARS-CoV-2 Infection*. **International Journal of Molecular Science**, **24**, 2023, 8065.

118. T. Kawasaki, & T. Kawai, *Toll-like Receptor Signaling Pathways*. **Front. Immunol**., **5**, 2024, 461.

119. A. Dunne, S. Carpenter, C. Brikos, P. Gray, A. Strelow, H. Wesche, N. Morrice, & L.A.J. O'Neill, *IRAK1 and IRAK4 Promote Phosphorylation, Ubiquitination, and Degradation of MyD88 Adaptor-like (Mal)*. **J. Biol. Chem**., **291**, 2021, 24802.

120. W. Piao, L.W. Ru, & V.Y. Toshchakov, *Differential Adapter Recruitment by TLR2 Co-Receptors*. **Pathog. Dis**., **74**, 2021, ftw043.

121. B.R. Sahoo, M. Basu, B. Swain, M.R. Dikhit, P. Jayasankar, & M. Samanta, *Elucidation of Novel Structural Scaffold in Rohu TLR2 and Its Binding Site Analysis with Peptidoglycan, Lipoteichoic Acid and Zymosan Ligands, and Downstream MyD88 Adaptor Protein*. **Biomed Res. Int**., 2013, 185282.

Lead City University Ibadan DO NOT COPY

122. S. Shukla, E.T. Richardson, M.G. Drage, W.H. Boom, & C.V. Harding, *Mycobacterium Tuberculosis Lipoprotein and Lipoglycan Binding to Toll-Like Receptor 2 Correlates with Agonist Activity and Functional Outcomes*. **Infect. Immun**., **86**, 2023, e00450-18.

123. R.C.A. de Groot, H. Zhu, T. Hoogenboezem, A.C.J.M. de Bruijn, E. Eenjes, A.E.J. Jong, A.L. Belo, S.C. Estevão, J.J. Bajramovic, & R.J. Rottier, *Mycoplasma Pneumoniae Compared to Streptococcus Pneumoniae Avoids Induction of Proinflammatory Epithelial Cell Responses despite Robustly Inducing TLR2 Signaling*. **Infect. Immun**., **90**, 2022, e00129-22.

124. J. Im, J.E. Baik, D. Lee, O.J. Park, D.H. Park, C.H. Yun, & S.H. Han, *Bacterial Lipoproteins Induce BAFF Production via TLR2/MyD88/JNK Signaling Pathways in Dendritic Cells*. **Front. Immunol**., **11**, 2020, 564699.

125. L. Su, Y. Wang, J. Wang, Y. Mifune, M.D. Morin, B.T. Jones, E.M.Y. Moresco, D.L. Boger, B. Beutler, & H. Zhang, *Structural Basis of TLR2/TLR1 Activation by the Synthetic Agonist Diprovocim*. **J. Med. Chem**., **62**, 2019, 2938–2949.

126. A. Udgata, R. Qureshi, & S. Mukhopadhyay, *Transduction of Functionally Contrasting Signals by Two Mycobacterial PPE Proteins Downstream of TLR2 Receptors*. **J. Immunol**., **197**, 2021, 1776–1787.

127. K. Radakovics, C. Battin, J. Leitner, S Geiselhart, W. Paster, J. Stöckl, K. Hoffmann-Sommergruber, & P. Steinberger, *A Highly Sensitive Cell-Based TLR Reporter Platform for the Specific Detection of Bacterial TLR Ligands*. **Front. Immunol**., **12**, 2022, 817604.

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Chapter Three

Material and Methods

3.1 Study site

This study was conducted in three (3) TB treatment centres (DOTs) in Abeokuta, Ogun State (FMC Abeokuta, State Hospital Ijaiye, Abeokuta and Sacred Heart Catholic Hospital, Lantoro, Abeokuta

Abeokuta is the largest city and capital of Ogun state in South-West Nigeria. It is situated on the East bank of River Ogun, near a group of rocky outcrops in a wooded savannah; 48 miles

[77km] North of Lagos by railway, or 81 miles [130km] by water. Abeokuta produces timber, rubber, yams, rice, cassava, maize, cotton, and Shea butter which are chief articles of trade. In addition, it is a principal player in the exportation of cocoa, palm, and associated products, kola nuts, and fruits. Prominent disease outbreaks in this area include malaria, schistosomiasis and other soil-transmitted helminths, HIV/AIDS, leprosy, diabetes, tuberculosis, and cholera.

3.2 Study participants

This comprised of males and females consenting participants diagnosed of pulmonary tuberculosis and accessing treatments at the DOTS clinics in FMC Abeokuta, Ogun state. Participants that are negative for pulmonary tuberculosis were enrolled as negative controls.

3.3 Sample size

The sample size (N) was determined using sample size determination formula for health studies. The prevalence of TB in Ogun state is 8.0%.[1]

$$N = \frac{Z^2P\ (1 - P)}{D^2} + 10\%\ \text{attrition rate}$$

N = sample size required

Z = confidence level at 95% (standard value of 1.96)

P = estimated prevalence of TB in Ogun state is 8.0% (0.08)

D = margin of error at 5% (standard value = 0.05)

$$N = \frac{1.96^2 \times 0.08\ (1-0.08)}{0.05^2}$$

$$\frac{3.8416 \times 0.08(0.92)}{0.0025}$$

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N =113

Note: Population of TB positive patients in Ogun State is 300.

Using the Finite population correction formula

$$n_{adj} = \frac{n}{1 + \frac{(n-1)}{N}}$$

$$n_{adj} = \frac{113}{1 + \frac{(113-1)}{300}}$$

$$n_{adjusted} = \frac{113}{1 + \frac{112}{300}}$$

$$n_{adjusted} = \frac{113}{1 + 0.3733}$$

$$n_{adjusted} = \frac{113}{1.3733}$$

$$n_{adjusted} = 82.27$$

Rounding up the adjusted sample size would be approximately 83. Therefore the adjusted sample size for the population of 300 was 83.

A total of 130 participants were recruited for the present study comprising of 83 TB positive participants and 47 TB negative healthy individuals forming the control group.

3.4 Study Design

This was a cross-sectional prospective quantitative study.

Sampling technique

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The sampling technique that was adopted for the purpose of this research was the purposive sampling technique which was used to select 130 participants.

3.5 Sample Collection and Transportation

One spot sputum sample was collected in a labelled standard sterile screw-capped leak proof sputum container with specific clinic identification and study numbers according to the National TB program guidelines. Sputum smear microscopy was performed at the study sites, and AFB smear-positive and negative participants was identified. Five milliliters (5mL) of venous blood was obtained from the anterior cubital vein of qualified patients using sterile venipuncture procedure using 5ml disposable, sterile syringe. The blood was collected in ethylene diamine tetra acetic acid anticoagulated sample bottle for genomic analysis and was stored between -20^{0}C to -80^{0}C until required for analyses. The samples were labelled P with corresponding number (P1, P2, P3 …… P83) for samples from TB positive participants . Also the samples were labelled N with corresponding number (N1, N2, N3 …… N47) for samples from TB negative participants.

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3.6 Laboratory analysis

3.6.1: Determination of TB status by microscopy

Determination of TB status by microscopy was done employing Ziehl Neelsen’s Staining.

3.6.1.1 Principle: Ordinary aniline dye solutions do not readily penetrate the substance of the tubercle bacilli and therefore it is not suitable for staining. By the use of powerful staining solution that contains phenol and with the application of heat, which act as a mordant, it can be made to penetrate the bacillus. Once stained the tubercle bacillus will withstand the action of powerful decolorizing agents, thus retains the stain.[2]

3.6.1.2 Requirements: Strong carbol fuchsin, 20% H_2SO_4, 95% alcohol or acid alcohol, methylene blue solution, distilled water, spirit lamp, compound microscope and cedarwood oil/liquid paraffin.

3.6.1.3 Composition of strong carbol fuchsin: Basic fuchsin - 10 g Absolute alcohol - 100 mL 5% phenol in water - 1000 mL

3.6.1.4 Procedure:

A drop of the mucopurulent sputum was smeared on a clean grease free slide using a applicator stick and it was air dried for 15-30minutes. When dry, the smear facing upward it was fixed by applying heat from below.

Primary staining and mordanting: The fixed smear was flooded with strong Carbol fuchsin for 5 minute and heated intermittently. Until the steam rises, taking care to see that the stain does not boil and that the smear does not get charred. The smear was washed well with distilled water. The basic fuchsin in strong Carbol fuchsin, basic stain while carbolic acid acts as a mordant. On heating, the mycolic acid in the cell wall of the acid fast organisms is liquefied and the basic stain imbibed by the organism is fixed by the mordant. On washing, the smear was cleared of excess of the stain or any deposits.

Decolorisation: The slide was covered with 20% H_2SO_4 for 2–3 minutes and washed with water. This step was repeated till the smear becomes colorless. With this, only acid fast organisms retain the basic stain, while the cells and other organisms are rendered colorless. After that it was decolorized with 95% alcohol. By decolorising with alcohol; saprophytes can be differentiated from Mycobacterium tuberculosis, as saprophytes are only acid fast whereas mycobacterium tubercle bacilli is acid as well as alcohol fast.

Counter Staining: The slide was covered with methylene blue or 1–2 minutes, washed with water and blotted to dry. Acid fast organisms (M.tb) remain pink while the other organisms and cells, take up the counter stain and turn blue.

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A drop of cedarwood oil or liquid paraffin was placed on the stained smear. The microscope was adjusted for increased light by raising the condenser and the smear was examined under the oil immersion objective using the plane mirror.

3.6.2: Determination of M.tb by GeneXpert

The sputum sample was mixed with sample reagent (SR) and shaked manually for 10 to 15times and it was incubated for 15minutes at room temperature.

2ml of the processed sample was transferred to the test cartridge, the barcode was scanned and it was loaded into the Gene Xpert instrument following the manufacturer's instructions.

After 2hours of sample loading, the results was interpreted by the Gene Xpert diagnosis system from the measured fluorescent signals which was displayed automatically as detected, not detected or invalid/error for M.tb.[3]

3.6.3: Determination of M.tb drug susceptibility by GeneXpert

The sputum sample was mixed with sample reagent (SR) and shaked manually for 10 to 15times and it was incubated for 15minutes at room temperature.

2ml of the processed sample was transferred to the test cartridge, the barcode will be scanned and it was loaded into the Gene Xpert instrument following the manufacturer's instructions.

After 2hours of sample loading, the results was interpreted by the Gene Xpert diagnosis system from the measured fluorescent signals which was displayed automatically as susceptible or resistant to rifampicin.[3]

3.6.4:Participants'Genomic analysis

Genomic DNA was isolated from EDTA anticoagulated whole blood using the standard protocol (NIMR Kit).[4]

Reagent preparation

1. 8mls of absolute ethanol was added to each bottle of wash buffer 1 concentrate.

2. 32mls of absolute ethanol was added to each bottle of wash buffer 2 concentrate.

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3.6.4.1 Procedure

1. 100µl of specimen was added into a microcentrifuge tube.

2. 500µl of the lysis buffer was added.

3. The mixture was votexed and incubated at 56^{0}C for 10minutes.

4. It was centrifuged at 10,000 RPM for 1minute.

5. 200µl of absolute ethanol was added to the tube

6. The mixture was transferred into the spin column and centrifuged at 10,000 RPM for 30seconds.

7. The flow-through was discarded and the collection tube was blotted on a tissue paper.

8. 500µl of wash buffer 1 was added to the spin column.

9. It was centrifuged at 10,000RPM for 30seconds.

10. The flow-through was discarded and the collection tube was blotted on a tissue paper.

11. 500µl of wash buffer 2 was added to the spin column.

12. It was centrifuged at 10,000 RPM for 1minute.

13. The flow-through was discarded and the collection tube was blotted on a tissue paper.

14. The spin column was centrifuged at 12,000-14,000RPM for 3minutes to remove all traces of ethanol.

15. The spin column was placed into another microcentrifuge tube.

16. 50µl of elution buffer or nuclease free water was added to the center of the column.

17. It was incubated at room temperature for 1-2minutes.

18. It was centrifuged at 10,000RPM for 1minute to elute the DNA.

19. Step 16 to 18 was repeated.

20. The DNA was stored at -20 to -80^{0}C.

3.6.5 Genotyping of Toll Like Receptors (TLRs)

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A PCR-based restriction fragment length polymorphism (PCR–RFLP) analysis was performed for TLR1 (Rs Number: rs7656411), using genomic DNA isolated from peripheral blood. The forward (F: ACGTTGGATGCCTTTAAATTACTGTGTATC) and reverse (R:ACGTTGGATGGTACATGTGAGCTAAATAG) primer sequences, chromosome position (chr4:154627655), gene location (ear gene-3) and restriction enzymes (BspL I) was used to detect the restriction digestion patterns for the different alleles.[5-8] Each of the PCRs consisted of a pre-denaturation step of 4minutes at 94°C and 40cycles each of 30seconds denaturation at 94°C, 30seconds annealing at 55°C and 30seconds elongation at 72°C. This was followed by a post-elongation step of 7minutes at 72°C.

3.7 Ethical considerations

3.7.1 Approval from health research ethics committee

Ethical approval to conduct the research was obtained from "Federal Medical Center and State Hospital Abeokuta Research and Ethical Review Committee". Written informed consent was obtained from the participants before the administration of questionnaires (for collection of respondent's bio-data) and collection of sample. The participant's decision to participate in the study was solely voluntary.

3.7.2 Confidentiality of the Participant's Data

Confidentiality was ensured as information obtained from participants was solely handled by the researchers and used for this study. The lack of identifiers guaranteed respondents' privacy (anonymity).

3.8 Data Management and Analysis

The result obtained were organized and subjected to appropriate statistical analysis. Statistical Package for Social Science (SPSS) version 26.0 was used in all the statistical analyses. Results were described in percentages and proportions and displayed in appropriate tables

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and figures. For the eliciting association between variables Chi-square test, ANOVA, or student t-test were used and the $p<0.05$ was considered statistically significant.

3.9 Budget

This study is a self-sponsored program. The price of transportation, reagent, and tools purchased was covered by the researcher. The researcher covered the majority of the research study's estimated cost 920,000

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3.10 Work-plan and budget presentation

Table 4: Work-plan and budget presentation

Task	Materials required	Cost	Duration
Ethical approval,	Protocol for submission to research ethics committee	20,000	7th December to 6th January, 2024
Participant recruitment, sample collection , transportation, and laboratory	Sputum and blood samples containers for sputum and blood	100,000	January -April, 2024

analysis	samples collection from participants after obtaining informed consents		
	Primers and other molecular biology reagent & bioinformatics	500,000	January-February, 2024
Analysis of data	Generated research data	50,000	7th January to 14th April, 2024
Report and thesis writing	Draft of thesis for final correction	100,000	15 January to 31st May 2024
Submission of completed thesis	Corrected thesis	50,000	1st February to 8th June, 2024
	Publication of two manuscript(s)	100,000	July-August, 2024
	Total cost	**920, 000**	

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Endnotes

1. T.I. Babajide, V.U. Nwadike, D.A Ojo, O.A Onasanya, K.C Ojide, & I.E Kalu, *Prevalence of tuberculosis amongst patients attending two secondary hospitals in Abeokuta Ogun state*, **African Journal of Clinical and Experimental Microbiology**, 15(3), 2014, 144-150.

2. N.H. Smith, & P. Upton, *Naming spoligotype patterns for the RD9-deleted lineage of the Mycobacterium tuberculosis complex*; www.Mbovis.org. **Infectious Genetic Evolution**, 12(4), 2012, 873–876.

3. M. Folwaczny, J. Glas, H.P. Torok, O. Limbersky, & C. Folwaczny, *Toll-like receptor (TLR) 2 and 4 mutations in periodontal disease*, **Clinical and Experimental Immunology**, 135(2), 2014, 330–335.

4. D. Nilsson, A.K. Andiappan, C. Hallden, W. De-Yun, T. Sall, & C.F. Tim, *Toll-like receptor gene polymorphisms are associated with allergic rhinitis: a case control study*, **BioMedical Central Medicine and Genetics**, 13, 2020, 66.

5. P. Selvaraj, M. Harishankar, B. Singh, M.S. Jawahar, & V.V. Banurekha, *Toll-like receptor and TIRAP gene polymorphisms in pulmonary tuberculosis patients of South India*, **Tuberculosis**, 90(5), 2020, 306–310.

6. B. Langmead, & S.L. Salzberg, *Fast gapped-read alignment with Bowtie 2*. **Nature Methods**, 9(4), 2022, 357-359.

7. A. McKenna, *The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data*, **Genome Research**, 20(9), 2020, 1297-1303.

8. S. Li, X. Chen, G. Hao, X. Geng, W. Zhan, & J. Sun, *Identification and characterization of a novel NOD-like receptor family CARD domain containing 3 gene in response to extracellular ATP stimulation and its role in regulating LPS-induced innate immune response in Japanese flounder (Paralichthys olivaceus) head kidney macrophages*, **Fish & Shellfish Immunology**, 50, 2016, 79-90.

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Chapter Four

Results and Discussion

4.1 Results

Table 4.1, shows the socio-demographic characteristics of the participants. In relation to age, 16.9% of the participants were within the age group 20-30years, 31-40years (40.0%), 41-50years (20.8%) while the remaining 22.3% were above 50years. Majority (60.0%) of the participants were males while the remaining 40% were females. Majority (68.5%) of the participants were Christians, Muslims (29.2%) while the remaining 2.3% were traditionalists. Majority (71.0%) of the participants were of the Yoruba ethnic group, Igbo (19.2%), Hausa

(8.5%) while the remaining 2.3% were of other ethnic group in the study setting. Majority (60.8%) of the respondents were married, single (31.5%), divorced (6.2%) while the remaining 1.5% were widowed. 20.0% of the participants were artisans, traders (40.0%), civil servants (30.8%) while the remaining 9.2% were unemployed. Majority (66.9%) of the respondents had attained tertiary educational level, none (4.6%), primary (8.5%), while the remaining 20.0% had only attained secondary educational level. 36.9% of the participants had a family size less than 4, 4-6 (54.6%) while the remaining 8.5% had a family size above 6. 34.6% of the participants stays in a self-contain, flat (51.5%) while the remaining 13.9% stays in a single room apartment. 43.9% of the participants had a history of alcohol consumption. 35.4% of the participants had a history of smoking. Majority (63.8%) of the participants stated they were TB positive while the remaining 36.2% stated they were TB negative.

Table 4.2, shows the Comparison of the sensitivity and specificity of microscopy result and GeneXpert screening of TB. The result of the analysis shows a 100% specificity of the result of microscopy detection of TB compared to the result of GeneXpert detection of TB and also a 100% sensitivity of the result of microscopy detection of TB compared to the result of GeneXpert detection of TB.

Figure 4.1, shows the prevalence of MTB among TB patients in relation to their gender. The prevalence of MTB was higher among male subjects (58%) compared to female subjects (42%),

Findings from the analysis of *Mycobacterium tuberculosis*-positive patients (n=83; 48 males and 35 females) in Ogun State, Nigeria. The study investigates TLR2 polymorphisms to:

1. Identify receptor molecules in male and female patients.
2. Examine gender-based variations in receptor molecules and their association with TB susceptibility, severity, and treatment outcomes.

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4.2 Receptor Molecule Polymorphisms and Their Frequencies

The receptor molecules analyzed were TLR2 gene fragments, identified through PCR and restriction digestion. Three key polymorphisms were observed:

- **CC Allele**: Single band at 50 bp.
- **TT Allele**: Single band at 113 bp.
- **TC Allele**: Double bands at 50 bp and 113 bp.

4.3 Distribution of TLR2 Polymorphisms Between Genders

The frequencies of these polymorphisms in male and female participants are summarized in Table 3.

4.4.1 Gender-Based Differences

The result of the analysis shows a 100% specificity of the result of microscopy detection of TB compared to the result of GeneXpert detection of TB and also a 100% sensitivity of the result of microscopy detection of TB compared to the result of GeneXpert detection of TB. The prevalence of MTB was higher among male subjects (58%) compared to female subjects (42%),

CC Allele (Single band at 50 bp):

- Detected in 22.9% of the population (12 males, 7 females).
- Slightly more prevalent in males than females.
- Associated with higher TB susceptibility and severity.

TT Allele (Single band at 113 bp):

- Found in 8.4% of participants (5 males, 2 females).
- Lower prevalence and equally distributed across genders.
- Linked to a protective effect against TB severity.

TC Allele (Double bands at 50 bp and 113 bp):

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- The most common polymorphism, detected in 55.4% of cases (24 males, 22 females).
- Slightly higher frequency in males but with minimal gender variation.
- Associated with moderate susceptibility and better treatment outcomes.

No Band:

- Present in 13.3% of participants (7 males, 4 females).
- Indicates absence of the receptor molecule gene.

Association With TB Susceptibility and Severity

- The **CC allele** was strongly associated with increased TB susceptibility and severity, reflecting compromised immune response due to reduced TLR2 functionality.
- The **TT allele** was associated with reduced TB severity, highlighting its protective role in immune regulation.
- The **TC allele** demonstrated moderate susceptibility and better treatment outcomes, likely due to balanced immune modulation.

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Association with Multi-Drug Resistant TB (MDR-TB)

- **CC Allele**: Higher association with MDR-TB cases, indicating reduced immune efficacy.
- **TC Allele**: Associated with lower MDR-TB rates, reflecting effective immune response modulation.
- **TT Allele**: Negligible association with MDR-TB, suggesting robust immune control.

Figure 4.2, Labeled electrophoresis gel showing allelic interpretation of samples after restriction enzyme digestion. A 100 bp ladder was used as a molecular weight marker to confirm fragment sizes. Lane annotations indicate the following allele interpretations: a 50 bp

band alone represents the **CC allele**, a 113 bp band alone represents the **TT allele**, and the presence of both 50 bp and 113 bp bands corresponds to the **TC allele**

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Table 4.1: Socio-demographic characteristics of the participants.

Variables	**Options**	**Frequency (n)**	**Percentage (%)**
Age (years)	20-30	22	16.9
	31-40	52	40.0
	41-50	27	20.8
	Above 50	29	22.3
Gender	Male	78	60.0
	Female	52	40.0

Religion	Christianity	89	68.5
	Islam	38	29.2
	Traditionalist	3	2.3
Ethnicity	Yoruba	91	71.0
	Igbo	25	19.2
	Hausa	11	8.5
	Others	3	2.3
Marital Status	Single	41	31.5
	Married	79	60.8
	Divorced	8	6.2
	Widowed	2	1.5
Occupation	Artisan	26	20.0
	Trader	52	40.0
	Civil servant	40	30.8
	Unemployed	12	9.2
Education status	None	6	4.6
	Primary	11	8.5
	Secondary	26	20.0
	Tertiary	87	66.9
Number in family	Less than 4	48	36.9
	4-6	71	54.6
	Above 6	11	8.5
Nature of accommodation	Self-contain	45	34.6
	Flat	67	51.5
	Single room	18	13.9

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History of alcohol consumption	Yes	57	43.9
	No	73	56.1
History of smoking	Yes	46	35.4
	No	84	64.6
TB status	Positive	83	63.8
	Negative	47	36.2

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TABLE 4.2: Comparison of the sensitivity and specificity of microscopy result with GeneXpert result.

			GeneXpert		
			Negative	**Positive**	**Total**
Microscopy	**Negative**	Count	47	0	47
		% within GeneXpert	100.0%	0.0%	36.2%
	Positive	Count	0	83	83
		% within GeneXpert	0.0%	100.0%	63.8%
Total		**Count**	**47**	**83**	**130**
		% within GeneXpert	**100.0%**	**100.0%**	**100.0%**

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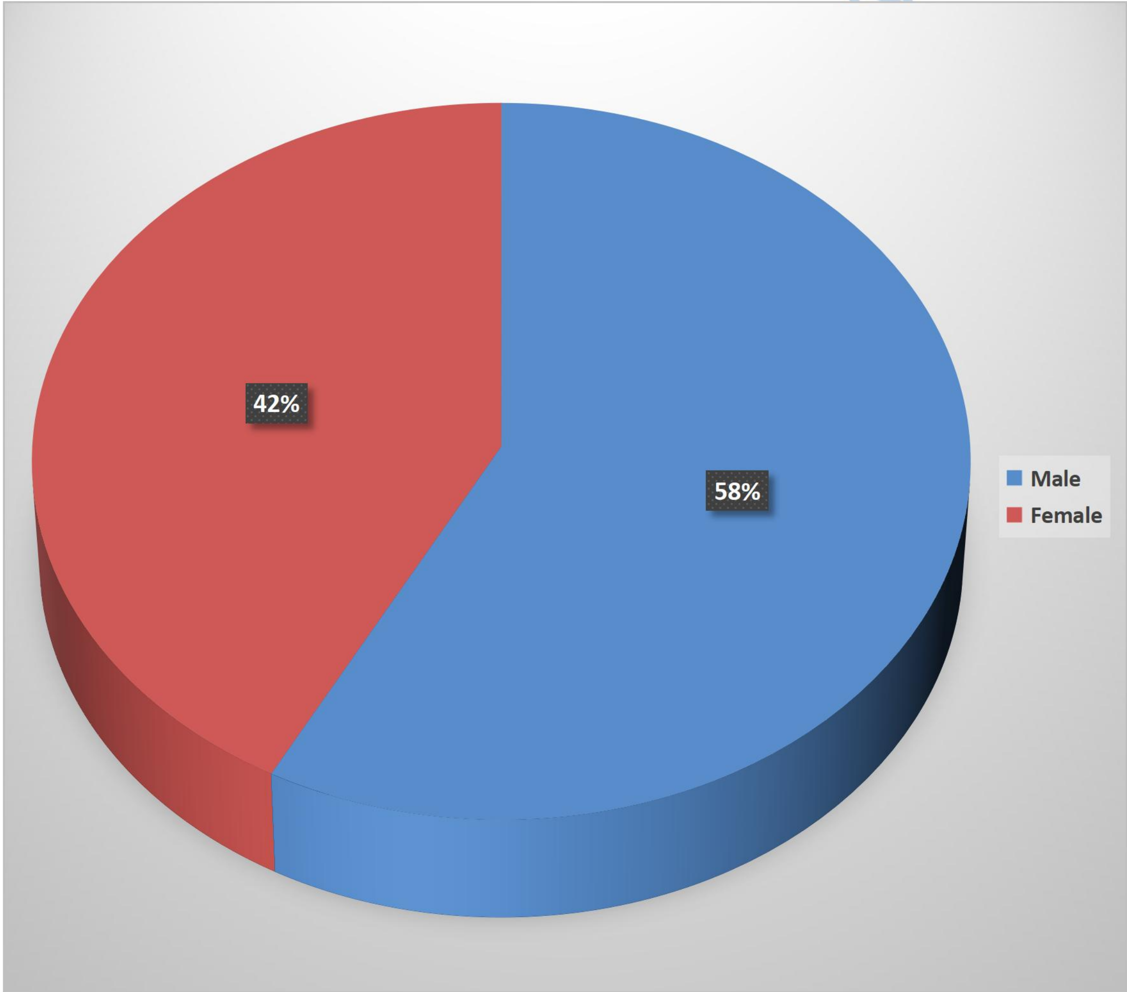

Figure 4.1: Prevalence of MTB among TB patients in relation to their gender.

Table 4.3: Distribution of Band Patterns of TLR2 Polymorphisms by Gender with Frequency.

Band Pattern	Male (n=48)	Female (n=35)	Total (n=83)	Frequency (%)
(50 bp - CC)	12	7	19	22.9
113 bp - TT)	5	2	7	8.4
(50 & 113 bp - TC)	24	22	46	55.4
No Band	7	4	11	13.3

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Table 4.3: Comparison of the distribution of band pattern of TLR-2 polymorphism between subjects and control group.

Band Pattern	Participants		Total	p-value
	Subjects (n=83)	Controls (n=47)		
(50 bp - CC)	19	2	21	
113 bp - TT)	7	4	11	
(50 & 113 bp - TC)	46	33	79	<0.0001
No Band	11	8	19	
Total	**83**	**47**	**130** **7**	

Level of significance p≤0.05

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Table 4.4: Comparison of the distribution of Band Patterns of TLR2 Polymorphisms by Gender

Band Pattern	Male (n=48)	Female (n=35)	Total (n=83)	p-value
(50 bp - CC)	12	7	19	
113 bp - TT)	5	2	7	
(50 & 113 bp - TC)	24	22	46	0.225
No Band	7	4	11	
Total	**48**	**35**	**83**	

Level of significance p≤0.05

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Table 4.5: Comparison of the distribution of band pattern of TLR-2 polymorphism among subjects in relation to alcohol consumption.

Band Pattern	Alcohol consumption		Total	p-value
	Yes (n=35)	No (n=48)		
(50 bp - CC)	14	5	19	
113 bp - TT)	5	2	7	
(50 & 113 bp - TC)	23	33	46	<0.0001
No Band	3	8	11	
Total	**35**	**48**	**83**	

Level of significance p≤0.05

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Table 4.6: Comparison of the distribution of band pattern of TLR-2 polymorphism among subjects in relation to smoke intake.

Band Pattern	Smoking		Total	p-value
	Yes (n=35)	**No (n=48)**		
(50 bp - CC)	11	8	19	
113 bp - TT)	4	3	7	
(50 & 113 bp - TC)	10	36	46	0.040
No Band	1	10	11	
Total	**26**	**57**	**83**	

Level of significance p≤0.05

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Table 4.7: Comparison of the distribution of band pattern of TLR-2 polymorphism among subjects in relation to age group.

Band Pattern	Age group (years)				Total	p-value
	20-30	31-40	41-50	Above 50		
(50 bp - CC)	3	7	4	5	19	
113 bp - TT)	2	3	1	1	7	
(50 & 113 bp - TC)	6	20	9	11	46	0.908
No Band	4	2	3	2	11	
Total	**15**	**32**	**17**	**19**	**83**	

Level of significance $p \leq 0.05$

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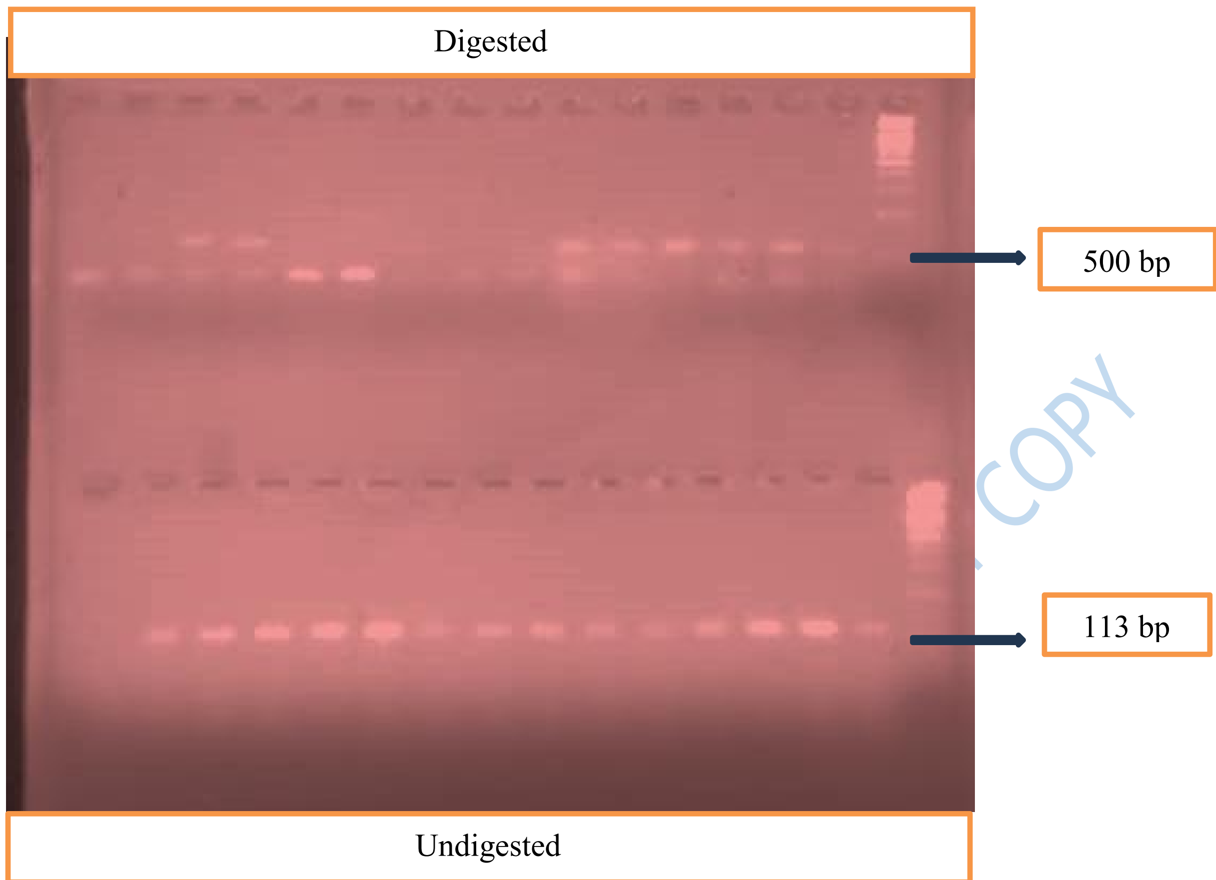

Figure 4.2: Labeled electrophoresis gel showing allelic interpretation of samples after restriction enzyme digestion.

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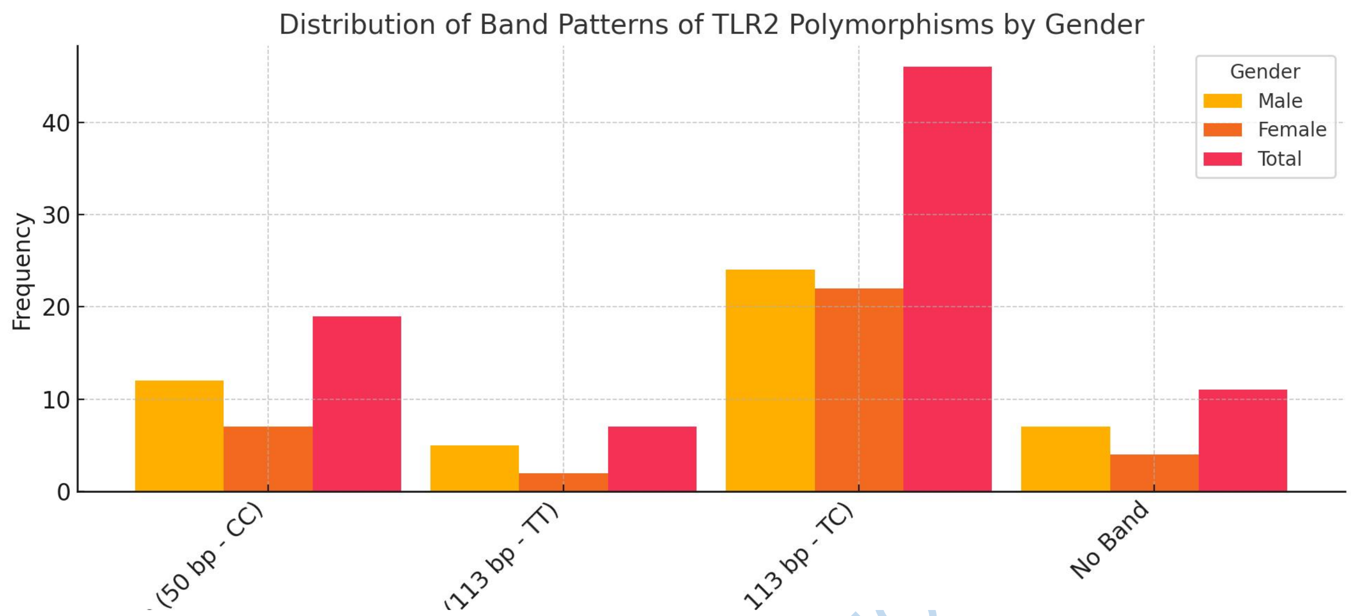

Figure 4.3: Distribution of band patterns of TLR2 polymorphisms by gender.

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4.3 Discussion

4.3.1 Socio-Demographic Characteristics of Participants

The socio-demographic attributes of participants provided significant insights into the population most impacted by tuberculosis in Ogun State, Nigeria. The bulk of participants were aged 31–40 years (40%), followed by those aged 41–50 years (20.8%), and persons over 50 years (22.3%). The age distributions underscore the unequal effect of tuberculosis on individuals during their productive years, aligning with the global epidemiological picture of tuberculosis as reported by the World Health Organisation.[1] The working-age population frequently faces elevated risks from employment settings, socio-economic difficulties, and behavioural influences that heighten vulnerability to infectious diseases such as tuberculosis.[2]

The gender distribution indicated that males (60%) were more impacted than females (40%), with a prevalence of MTB at 58% among males and 42% among females. The gender discrepancy in tuberculosis prevalence has been extensively documented in the literature.[3,4] The elevated frequency among males may be ascribed to various interconnected causes, including:

- **Behavioral Patterns:** Men are more prone to partake in hazardous activities, including smoking and excessive alcohol intake, all of which have been demonstrated to detrimentally affect pulmonary health and weaken immunological function.[4] This study found that 35.4% of participants had a history of smoking, while 43.9% reported alcohol intake, with these behaviours being disproportionately prevalent among males.
- **Occupational Exposure:** Men in the study group may have a greater propensity to engage in labour-intensive occupations with increased exposure to respiratory dangers, a conclusion corroborated by research undertaken in other locations with a high prevalence of tuberculosis.[5]

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- **Biological Differences:** Hormonal factors contribute significantly, as testosterone may suppress immunological responses in males, but oestrogen may augment immune activity in females.[6]

The ethnic and religious demography provide further background for the prevalence of tuberculosis in the research area. The predominant participants were Yoruba (71%) and Christians (68.5%), indicative of the cultural and religious demographics of Ogun State. This distribution indicates that TB risk factors transcend ethnic and religious boundaries, highlighting the global necessity for effective TB prevention programs. Education levels indicated that 66.9% of participants possessed postsecondary education, which is significant as higher education is frequently linked to enhanced health literacy and access to healthcare. Notwithstanding this, the prevalence of tuberculosis remained elevated, indicating that socio-economic and environmental factors, including housing conditions and occupational hazards, surpass the protective influence of education in this demographic.[5]

4.3.2 Comparison of Microscopy and GeneXpert in TB Detection

The comparison of microscopy and GeneXpert in detecting tuberculosis among participants demonstrated a flawless sensitivity and specificity of 100% for both diagnostic techniques. These data indicate that, under regulated settings, both approaches are dependable instruments for identifying MTB. Nevertheless, situating these findings within practical environments underscores the subtleties of each diagnostic method.

Microscopy, a longstanding and prevalent diagnostic technique for tuberculosis, is both economical and accessible, particularly in resource-constrained environments. Nonetheless, its dependence on operator proficiency and incapacity to identify smear-negative instances considerably restrict its sensitivity in standard clinical practice.[7] In contrast, GeneXpert, a molecular diagnostic instrument, has revolutionised tuberculosis diagnosis by facilitating swift and precise identification of Mycobacterium tuberculosis and drug resistance, especially

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to rifampin. The WHO has designated GeneXpert as the optimum diagnostic instrument for tuberculosis owing to its enhanced accuracy and capacity to deliver results within hours.[8]

Despite the evident advantages of GeneXpert, its substantial cost and infrastructural demands provide considerable obstacles to its extensive deployment in resource-limited environments. Previous studies underscored the necessity of investing in diagnostic infrastructure to enhance the availability of GeneXpert and guarantee fair access to sophisticated tuberculosis diagnoses.[9] This study highlights the ideal alignment between microscopy and GeneXpert, emphasising the necessity of refining microscopy methods and educating healthcare professionals to enhance its diagnostic efficacy while expanding GeneXpert implementation.

The result of the present study revealed that expression of the TT, CC and TC genotypes of TLR-2 was significantly higher among TB patients compared to controls. The result of the present study was in line with that of previous studies.[8,9] A study reported that TLR-2 polymorphism including TT and TC genotypes at specific loci, were significantly more frequent in TB patients indicating their role in susceptibility to infection.[8] Another study confirmed that polymorphisms in TLR-2 including CC, TT and TC genotypes wee significantly correlated with TB susceptibility across diverse populations.[9] The increased susceptibility associated with these polymorphism in TLR-2 can be attributed to the evidence that alteration in TLR-2 signaling can disrupt phagosome maturation, enabling MTB to evade immune responses.[9]

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4.3.3 Prevalence of MTB Among Male and Female Participants

The research revealed that 63.8% of subjects tested positive for MTB, with a greater incidence in males (58%) than in females (42%). This finding corresponds with global trends documented in the WHO Global Tuberculosis Report, which consistently indicates a greater TB burden among males.[1] A multitude of variables contributes to this gender disparity:

1. **Biological Vulnerability:** Testosterone in males has been demonstrated to inhibit specific immunological responses, thus heightening vulnerability to diseases such as tuberculosis.[4] In contrast, oestrogen in females possesses immune-enhancing characteristics, offering certain protection against the advancement of latent tuberculosis to active disease.[6]
2. **Behavioral Risk Factors:** The increased prevalence of smoking and alcohol consumption among guys raises their susceptibility to tuberculosis. Such behaviours compromise lung defences and elevate the risk of getting active tuberculosis.[3]
3. **Health-Seeking Behaviors:** Men are more prone to postponing medical attention, perhaps leading to extended infectious durations and worse illness advancement.[2]

The increased incidence of MTB in males highlights the necessity for gender-sensitive interventions. Public health initiatives must focus on behavioural risk factors and encourage early diagnosis and treatment compliance within male demographics.

4.3.4 TLR2 Polymorphisms and Their Association With TB Susceptibility and Severity

This study discovered three significant TLR2 polymorphisms (CC, TT, and TC alleles) in MTB-positive patients, demonstrating notable correlations with tuberculosis susceptibility, severity, and treatment results.

- **CC Allele (22.9%):** The CC genotype correlated with heightened susceptibility to tuberculosis and its severity, indicating impaired immunological responses attributed to diminished TLR2 activity. This discovery corroborates previous study, which indicated that the CC allele hinders the identification of MTB by immune cells, resulting in protracted pathogen clearance and exacerbated illness severity.[10]
- **TT Allele (8.4%):** The TT allele exhibited protective effects by mitigating TB severity via improved immunological modulation. A study emphasised the

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significance of particular TLR2 polymorphisms, such as TT, in facilitating vigorous immune responses, thereby constraining disease progression.[11]

- **TC Allele (55.4%):** The TC allele, the predominant variant, was linked to intermediate vulnerability and improved treatment outcomes. Their balanced immune regulation likely aids in successful pathogen clearance while reducing tissue damage.[12]

4.3.5 Gender-Based Variations in TLR2 Polymorphisms

The study identified slight yet significant gender-related variations in the distribution of TLR2 polymorphisms:

- **CC Allele:** Slightly more prevalent in males (25%) than females (20%), reflecting higher TB susceptibility among males.
- **TT Allele:** Equally distributed across genders, with protective effects against TB severity.
- **TC Allele:** The most common allele in both genders, with minimal variation.

The findings indicate that whereas hereditary variables significantly contribute to tuberculosis susceptibility, they interact with gender-specific behavioural and biological elements to affect illness outcomes. Similarly indicated that genetic predisposition interacts with socio-cultural factors to influence tuberculosis occurrence and severity among communities.[13]

4.3.6 Association of TLR2 Polymorphisms With MDR-TB

The study identified distinct associations between TLR2 polymorphisms and MDR-TB:

- **CC Allele:** Firmly correlated with MDR-TB, signifying impaired immunological responses and heightened disease severity.
- **TC Allele:** Correlated with reduced MDR-TB rates, indicating successful immunological modulation and improved treatment results.

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- **TT Allele:** Minimal correlation with MDR-TB, underscoring its preventive function against severe illness.

These results corroborate that of previous study which established that genetic variables substantially affect treatment resistance in tuberculosis. The robust correlation between the CC allele and MDR-TB highlights the necessity for focused strategies to identify and address high-risk people.[14]

Lead City University Ibadan DO NOT COPY

Endnotes

1. WHO (World Health Organization), *Global Tuberculosis Report* 2016.

2. S.D. Lawn, & A.I. Zumla, *Tuberculosis*, **Lancet**, 378(9785), 2021, 57-72.

3. WHO (World Health Organization), *Treatment of Tuberculosis- guidelines*. 2019

4. N.M. Schluger, & W.N. Rom, *The host immune response to tuberculosis*, **American Journal of Respiratory and Critical Care Medicine**, 157(1), 2018, 679-691.

5. I. Abubakar, F. Drobniewski, & J. Southern, *Prognostic value of interferon-γ release assays and tuberculin skin test in predicting the development of active tuberculosis (UK PREDICT TB): a prospective cohort study*, **Lancet of Infectious Diseases**, 18(10), 2018, 1077–1087.

6. A.M. Cooper, *Expression of memory immunity in the lung following re-exposure to Mycobacterium tuberculosis*, **Tuber Lung Disease**, 78(1), 2017, 67-73.

7. I.A. Campbell, & O. Bah-Sow, *Pulmonary tuberculosis: diagnosis and treatment*, **British Medical Journal**, 332(7551), 2016, 1194-1197.

8. WHO (World Health Organization), *International Standards for Tuberculosis Care*, 3, 2018.

9. L.M. Pinto, *Scoring systems using chest radiographic features for the diagnosis of pulmonary tuberculosis in adults: a systematic review*, **European Respiratory Journal**, 42(2), 2023, 480-494.

10. M.B. Drennan, *Toll-like receptor 2-deficient mice succumb to Mycobacterium tuberculosis infection*, **American Journal of Pathology**, 164(1), 2018, 49-57.

11. S. Shi, *Expression of many immunologically important genes in Mycobacterium tuberculosis-infected macrophages is independent of both TLR2 and TLR4 but dependent on IFN-alphabeta receptor and STAT1*, **Journal of Immunology**, 175(5), 2015, 3318-3328.

12. I. Hershkovitz, H.D. Donoghue, & D.E. Minnikin, *Detection and molecular characterization of 9000-year-old Mycobacterium tuberculosis from a neolithic settlement in the Eastern Mediterranean*, **Public Library of Science One**, 3(10), 2018.

13. B. Abel, *Toll-like receptor 4 expression is required to control chronic Mycobacterium tuberculosis infection in mice*, **Journal of Immunology**, 169(6), 2022, 3155-3162.

14. N. Ahmad, S.D. Ahuja, & O.W. Akkerman, *Treatment correlates of successful outcomes in pulmonary multidrug-resistant tuberculosis: an individual patient data meta-analysis*, **Lancet**, 392(10150), 2018, 821–834.

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Summary, Conclusion, and Recommendations

This chapter offers a summary of the findings, conclusion and and making relevant recommendations within the framework of global tuberculosis research and control initiatives.

5.1 Summary Of Findings

The present study assessed toll like receptor 2 (TLR2), prevalence of *mycobacterium tuberculosis (*mtb*)* and associated risk factors between male and female tuberculosis positive patients in Ogun state Nigeria. The result revealed a 100% specificity of the result of microscopy detection of TB compared to the result of GeneXpert detection of TB and also a 100% sensitivity of the result of microscopy detection of TB compared to the result of GeneXpert detection of TB. The prevalence of MTB was higher among male subjects (58%) compared to female subjects (42%). The result of the present study also shows the distribution of band pattern of TLR2 polymorphism among the subjects in relation to their gender. Three key polymorphisms were observed:

CC Allele: Detected in 22.9% of the population (12 males, 7 females).

TT Allele: Found in 8.4% of participants (5 males, 2 females).

TC Allele: The most common polymorphism, detected in 55.4% of cases (24 males, 22 females).

5.2 Conclusion

This study accomplished its goals, offering significant insights into the socio-demographic, genetic, and diagnostic factors of tuberculosis. The results highlight the essential interaction of behavioural, genetic, and environmental factors in determining tuberculosis susceptibility and outcomes. Principal findings encompass:

1. **Socio-Demographic Factors:** Tuberculosis disproportionately impacts males and those in their prime working years, underscoring the necessity for tailored interventions.

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2. **Diagnostic Tools:** GeneXpert exhibits enhanced precision in identifying TB and MDR-TB, whereas microscopy continues to be vital in resource-constrained environments.
3. **TLR2 Polymorphisms:** Genetic variations substantially affect tuberculosis susceptibility, severity, and treatment outcomes, with the CC allele presenting increased risks.
4. **Behavioral Risk Factors:** Smoking and alcohol consumption raise susceptibility to tuberculosis, especially in males.

5.3 Recommendations

1. **Integrate Genetic Screening:** Include TLR2 polymorphism analysis in TB management protocols to identify high-risk individuals and guide personalized treatment strategies.
2. **Expand Diagnostic Access:** Strengthen the availability of GeneXpert in rural and underserved areas to enhance early diagnosis and reduce MDR-TB rates.
3. **Promote Behavioral Interventions:** Develop public health campaigns targeting smoking and alcohol use, particularly among male populations.
4. **Enhance Gender-Sensitive Programs:** Address gender disparities in TB prevalence and outcomes by implementing tailored interventions.
5. **Invest in Research:** Explore additional genetic markers and their interactions with environmental factors to support precision medicine approaches for TB control.

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5.4 Contribution to Knowledge

1. The research indicated that in the geographical areas analysed, around 50% of the patients with MTB possess the TC allele polymorphism in their TLR2 expression.

2. Furthermore the differences in TC allele variations of the TLR2 receptor between male and female patients with MTB are not statistically significant.

5.5 Further Research

Further research that may be pursued include the followings:

1. Investigation of the impact of hormonal differences on TLR2 expression and MTB susceptibility between male and female TB patients.

2. Analysing the effect of co-infections (eg HIV, malaria) on TLR2 expression and MTB susceptibility in TB patients in Ogun state.

3. Developmen and validation of a predictive model for TB susceptibility based on TLR2 gene polymorphysms and associated risk factors in Nigerian patients.

4. Examining the effect of environmental factors (eg, smoking, alcohol consumption) on TLR2 expression and function in male and female TB patients.

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Bibliography

Books

Kagan, J.C., & Medzhitov, R. *Phosphoinositide-Mediated Adaptor Recruitment Controls Toll-Like Receptor Signaling.* **Cell,** 125(5) (2021): 943–955.

Keshavarz-Fathi, M. B. T., & Rezaei, N. (2019). *Vaccines, adjuvants, and delivery systems*. **Academic Press: Cambridge**, MA. ISBN: 978-0-12-814039-0.

O'Neill, L.A., & Bowie, A.G. *The Family of Five: TIR-Domain-Containing Adaptors in Toll-Like Receptor Signalling.* **Nat Rev Immunol**, 7(5) (2022): 353–364.

Takeda, K., & Akira, S. (2020). *Toll-like receptors.*), **Current protocols in immunology** (Vol. 109, pp. 1421–1420).

Kagan, J.C., & Medzhitov, R. *Phosphoinositide-Mediated Adaptor Recruitment Controls Toll-Like Receptor Signaling.* **Cell,** 125(5) (2021): 943–955.

Keshavarz-Fathi, M. B. T., & Rezaei, N. (2019). *Chapter 3—Vaccines, adjuvants, and delivery systems*. **Academic Press: Cambridge**, MA. ISBN: 978-0-12-814039-0.

Journals

Ahmad, N., Ahuja, S. D., & Akkerman, O. W. (2018). *Treatment correlates of successful outcomes in pulmonary multidrug-resistant tuberculosis: An individual patient data meta-analysis*. **Lancet,** 392(10150), 821–834.

Ajibade, A. A., Wang, Q. F., Cui, J., Zou, J., Xia, X. J., & Wang, M. J. (2022). *TAK1 negatively regulates NF-Kappa B and P38 MAP kinase activation in Gr1(+)CD11b(+) neutrophils*. **Immunity**, 36(1), 43–54.

Ajibade, A.A., Wang, H.Y., & Wang, R.F. *"Cell Type-Specific Function of TAK1 in Innate Immune Signaling." Trends Immunol,* 34(7) (2023): 307–316.Akira, S., Uematsu, S., & Takeuchi, O**. "Pathogen Recognition and Innate Immunity." Cell**, 124(4) (2021): 783–801.

Ajibade, A.A., Wang, Q.F., Cui, J., Zou, J., Xia, X.J., & Wang, M.J. I*"TAK1 Negatively Regulates NF-Kappa B and P38 MAP Kinase Activation in Gr1(+)CD11b(+) Neutrophils*, **." Immunity** 36(1) (2022): 43–54.

Behzadi, P., García-Perdomo, H. A., & Karpiński, T. M. (2021). *Toll-like receptors: General molecular and structural biology.* **Journal of Immunology Research**, 2021, 9914854.

Bhoj, V. G., & Chen, Z. J. (2019). *Ubiquitylation in innate and adaptive immunity*. **Nature,** 458(7237), 430–437.

Bhoj, V.G., & Chen, Z.J. "*Ubiquitylation in Innate and Adaptive Immunity*." **Nature**, 458(7237) (2019): 430–437.

Blasius, A. L., & Beutler, B. (2020). *Intracellular toll-like receptors*. **Immunity,** 32(3), 305–315.

Lead City University Ibadan DO NOT COPY

Bonham, K.S., Orzalli, M.H., Hayashi, K., Wolf, A.I., Glanemann, C., & Weninger, W. *"A Promiscuous Lipid-Binding Protein Diversifies the Subcellular Sites of Toll-Like Receptor Signal Transduction."* **Cell,** 156(4) (2019): 705–716.

Boone, D. L., Turer, E. E., Lee, E. G., Ahmad, R. C., Wheeler, M. T., & Tsui, C. (2019). *The ubiquitin-modifying enzyme A20 is required for termination of Toll-like receptor responses.* **Nature Immunology,** 5, 1052–1060.

Bresnahan, K. A., & Tanumihardjo, S. A. (2019). *Undernutrition, the acute phase response to infection, and its effects on micronutrient status indicators*. **Advances in Nutrition,** 5(6), 702–711.

Brint, E. K., Xu, D., Liu, H., Dunne, A., McKenzie, A. N., & O'Neill, L. A. (2019). *ST2 is an inhibitor of interleukin 1 receptor and Toll-like receptor 4 signaling and maintains endotoxin tolerance.* **Nature Immunology**, 5, 373–379.

Campbell, I.A., & Bah-Sow, O. *"Pulmonary Tuberculosis: Diagnosis and Treatment*." **British Medical Journal**, 332(7551) (2016): 1194–1197.

Cao, Z.D., Henzel, W.J., & Gao, X.O. "IRAK*: A Kinase Associated With the Interleukin-1 Receptor."* **Science**, 271(5252) (2021): 1128–1131.

Clark, K., Nanda, S., & Cohen, P. Molecular control of the NEMO Family of Ubiqultin-Binding Protains Nat Rev Mol Cell Biol, 14(10) (2023): 673–685.

Cole, J. E., Georgiou, E., & Monaco, C. (2020). *The expression and functions of toll-like receptors in atherosclerosis.* **Mediators Inflamm**, 393946.

Cole, S. T., Brosch, R., & Parkhill, J. (1998). *Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence*. **Nature**, 393(6685), 537–544.

Colleselli, K., Ebeyer-Masotta, M., Neuditschko, B., Stierschneider, A., Pollhammer, C., Potocnjak, M., ... & Wiesner, C. (2023). *Beyond pattern recognition: TLR2 promotes chemotaxis, cell adhesion, and migration in THP-1 cells*. **Cells**, 12, 1425.

Conradie, F., Diacon, A. H., & Ngubane, N. (2020). *Treatment of highly drug-resistant pulmonary tuberculosis.* **New England Journal of Medicine**, 382(10), 893–902.

Conze, D.B., Wu, C.J., Thomas, J.A., Landstrom, A., & Ashwell, J.D. "*Lys63-Linked Polyubiquitination of IRAK-1 is Required for Interleukin-1 Receptor- and Toll-Like Receptor-Mediated NF-kappaB Activation."* **Mol Cell Biol**, 28(10) (2023): 3538–3547.

Cusson-Hermance, N., Khurana, S., Lee, T. H., Fitzgerald, K. A., & Kelliher, M. A. (2020). *Rip1 mediates the Trif-dependent toll-like receptor 3- and 4-induced NF-kB activation but does not contribute to interferon regulatory factor 3 activation.* **Journal of Biological Chemistry**, 280(44), 36560–36566.

Lead City University Ibadan DO NOT COPY

da Silva, T. A., Zorzetto-Fernandes, A. L. V., Cecílio, N. T., Sardinha-Silva, A., Fernandes, F. F., & Roque-Barreira, M. C. (2017). *CD14 is critical for TLR2-mediated M1 macrophage activation triggered by N-glycan recognition*. **Scientific Reports**, 7, 7083.

de Barros, B. C. S. C., Almeida, B. R., & Suzuki, E. (2020). *Paracoccidioides brasiliensis downmodulates A3 integrin levels in human lung epithelial cells in a TLR2-dependent manner.* **Scientific Reports**, 10, 19483.

de Groot, R. C. A., Zhu, H., Hoogenboezem, T., de Bruijn, A. C. J. M., Eenjes, E., Jong, A. L., ... & Rottier, R. J. (2022). *Mycoplasma pneumoniae compared to Streptococcus pneumoniae avoids induction of proinflammatory epithelial cell responses despite robustly inducing TLR2 signaling.* **Infection and Immunity**, 90, e00129-22.

Dunne, A., Carpenter, S., Brikos, C., Gray, P., Strelow, A., Wesche, H., ... & O'Neill, L. A. J. (2021). *IRAK1 and IRAK4 promote phosphorylation, ubiquitination, and degradation of MyD88 adaptor-like (Mal)*. **Journal of Biological Chemistry**, 291, 24802.

Ea, C. K., Deng, L., Xia, Z. P., Pineda, G., & Chen, Z. J. (2021). *Activation of IKK by TNFα requires site-specific ubiquitination of RIP1 and polyubiquitin binding by NEMO*. **Molecular Cell,** 22(2), 245–257.

Ea, C.K., Deng, L., Xia, Z.P., Pineda, G., & Chen, Z.J. "*Activation of IKK by TNFa Requires Site-Specific Ubiquitination of RIP1 and Polyubiquitin Binding by NEMO*." **Mol Cell,** 22(2) (2021): 245–257.

Ear, T., Fortin, C.F., Simard, F.A., & McDonald, P.P. "Constitutive Association of TGF-β–Activated Kinase 1 With the IkB Kinase Complex in the Nucleus and Cytoplasm of Human Neutrophils and Its Impact on Downstream Processes." **J Immunol,** 184(7) (2020): 3897–3906.

Erridge, C. (2020). *Endogenous ligands of TLR2 and TLR4: Agonists or assistants?* **Journal of Leukocyte Biology**, 87, 989–999.

Ezz, M. A., Mansouri, A., Akthar, I., Yousef, M. S., Kowsar, R., & Miyamoto, A. (2023). *Hyaluronan regulates sperm-induced inflammatory response by enhancing sperm attachment to bovine endometrial epithelial cells via CD44: In-silico and in-vitro approaches.* **Frontiers in Endocrinology**, 14, 1134868.

Fitzgerald, K. A., McWhirter, S. M., Faia, K. L., Rowe, D. C., Latz, E., & Golenbock, D. T. (2023). *IKK epsilon and TBK1 are essential components of the IRF3 signaling pathway.* **Nature Immunology**, 4(5), 491–496.

Fitzgerald, K.A., Rowe, D.C., Barnes, B.J., Caffrey, D.R., Visintin, A., & Latz, E. "*LPS-TLR4 Signaling to IRF-3/7 and NF-Kappa B Involves the Toll Adapters TRAM and TRI*F." **J Exp Med,** 198(7) (2023): 1043–1055.

Lead City University Ibadan DO NOT COPY

Fore, F., Indriputri, C., Mamutse, J., & Nugraha, J. (2020). *TLR10 and its unique anti-inflammatory properties and potential use as a target in therapeutics*. **Immune Network,** 20, e21.

Francisco, S., Billod, J. M., Merino, J., Punzón, C., Gallego, A., Arranz, A., Martín-Santamaría, S., & Fresno, M. (2021). *Induction of TLR4/TLR2 interaction and heterodimer formation by low endotoxic atypical LPS*. **Frontiers in Immunology**, 12, 748303.

Fujita, H., Rahighi, S., Akita, M., Kato, R., Sasaki, Y., & Wakatsuki, S. "*Mechanism Underlying IkappaB Kinase Activation Mediated by the Linear Ubiquitin Chain Assembly Complex.*" **Mol Cell Biol**, 34(7) (2019): 1322–1335.

Funderburg, N., Lederman, M. M., Feng, Z., Drage, M. G., Jadlowsky, J., Harding, C. V., Weinberg, A., & Sieg, S. F. (2022). *Human β-defensin3 activates professional antigen-presenting cells via toll-like receptors 1 and 2.* **Proceedings of the National Academy of Sciences,** 104, 18631–18635.

Gadagkar, S. G., Lalancette-Hébert, M., Thammisetty, S. S., Vexler, Z. S., & Kriz, J. (2023). *CD36 neutralisation blunts TLR2-IRF7 but not IRF3 pathway in neonatal mouse brain and immature human microglia following innate immune challenge.* **Scientific Reports,** 13, 2304.

Gottipati, S., Rao, N.L., & Fung-Leung, W.P. "*IRAK1: A Critical Signaling Mediator of Innate Immunity."* **Cell Signal**, 20(2) (2023): 269–276.

Goulopoulou, S., McCarthy, C. G., & Webb, R. C. (2021). *Toll-like receptors in the vascular system: sensing the dangers within*. **Pharmacological Reviews**, 68(1), 142–167.

Granucci, F., & Zanoni, I. (2013). *Role of CD14 in host protection against infections and in metabolism regulation.* **Frontiers in Cellular and Infection Microbiology**, 3, 32.

Hashimoto, C., Hudson, K. L., & Anderson, K. V. (2023). The *Toll gene of Drosophila, required for dorsal-ventral embryonic polarity, appears to encode a transmembrane protein.* **Cell**, 52, 269–279.

Honda, K., Ohba, Y., Yanai, H., Negishi, H., Mizutani, T., & Takaoka, A. "*Spatiotemporal Regulation of MyD88–IRF-7 Signalling for Robust Type-I Interferon Induction."* **Nature**, 434(7036) (2020): 1035–1040.

Honda, K., Yanai, H., Negishi, H., Asagiri, M., Sato, M., & Mizutani, T. (2020). *IRF-7 is the master regulator of type-I interferon-dependent immune responses.* **Nature**, 434(7034), 772–777.

Hoppstädter, J., Dembek, A., Linnenberger, R., Dahlem, C., Barghash, A., Fecher-Trost, C., Fuhrmann, G., Kraegeloh, A., & Huwer, H. (2019). *Toll-like receptor 2 release by macrophages: An anti-inflammatory program induced by glucocorticoids and lipopolysaccharide.* **Frontiers in Immunology**, 10, 1634.

Lead City University Ibadan DO NOT COPY

Horsburgh, C. R., Barry, C. E., & Lange, C. (2015). *Treatment of tuberculosis*. **New England Journal of Medicine,** 373(22), 2149–2160.

Huang, Y., Temperley, N. D., Ren, L., Smith, J., Li, N., & Burt, D. W. (2021). *Molecular evolution of the vertebrate TLR1 gene family: A complex history of gene duplication, gene conversion, positive selection, and co-evolution.* **BMC Evolutionary Biology,** 11, 149.

Im, J., Baik, J. E., Lee, D., Park, O. J., Park, D. H., Yun, C. H., & Han, S. H. (2020). *Bacterial lipoproteins induce BAFF production via TLR2/MyD88/JNK signaling pathways in dendritic cells*. **Frontiers in Immunology**, 11, 564699.

Janot, L., Secher, T., Torres, D., Maillet, L., Pfeilschifter, J., Quesniaux, V. F. J., ... & Erard, F. (2023). *CD14 works with Toll-like receptor 2 to contribute to recognition and control of Listeria monocytogenes infection.* **Journal of Infectious Diseases**, 198, 115–124.

Jin, M. S., Kim, S. E., Heo, Y. J., Lee, M. E., Kim, H. M., Paik, S. G., ... & Lee, J. O. (2022). *Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide.* **Cell**, 130, 1071–1082.

Kagan, J.C., Su, T., Horng, T., Chow, A., Akira, S., & Medzhitov, R. "*TRAM Couples Endocytosis of Toll-Like Receptor 4 to the Induction of Interferon-Beta."* **Nat Immunol**, 9(4) (2023): 361–368.

Kaiser, W. J., & Offermann, M. K. (2020). *Apoptosis induced by the toll-like receptor adaptor TRIF is dependent on its receptor interacting protein homotypic interaction motif.* **Journal of Immunology,** 174(8), 4942–4952.

Kang, J. Y., Nan, X., Jin, M. S., Youn, S. J., Ryu, Y. H., Mah, S., ... & Lee, J. O. (2019). *Recognition of lipopeptide patterns by Toll-like receptor 2-Toll-like receptor 6 heterodimer.* **Immunity**, 31, 873–884. Chávez-Sánchez, L., Garza-Reyes, M. G., Espinosa-Luna, J. E., Chávez-Rueda, K., Legorreta-Haquet, M. V., & Blanco-Favela, F. (2024). *The role of TLR2, TLR4, and CD36 in macrophage activation and foam cell formation in response to OxLDL in humans*. **Human Immunology**, 75, 322–329.

Kawai, T., & Akira, S. (2023). *Toll-like receptor and RIG-1-like receptor signaling.* **Annals of the New York Academy of Sciences,** 1143(1), 1–20.

Kawasaki, T., & Kawai, T. "*Toll-Like Receptor Signaling Pathways.*" **Front Immunol,** 5 (2019): 461.

Kawasaki, T., & Kawai, T. (2024). *Toll-like receptor signaling pathways.* **Frontiers in Immunology,** 5, 461.

Kim, C., Ho, D. H., Suk, J. E., You, S., Michael, S., Kang, J., Joong-Lee, S., Masliah, E., Hwang, D., & Lee, H. J. (2024). *Neuron-released oligomeric α-synuclein is an endogenous agonist of TLR2 for paracrine activation of microglia.* **Nature Communications,** 4, 1562.

Lead City University Ibadan DO NOT COPY

Kurkjian, C. J., Guo, H., Montgomery, N. D., Cheng, N., Yuan, H., Merrill, J. R., Sempowski, G. D., Brickey, W. J., & Ting, J. P. (2022). *The toll-like receptor 2/6 agonist, FSL-1 lipopeptide, therapeutically mitigates acute radiation syndrome.* **Scientific Reports,** 7, 17355.

Lamothe, B., Besse, A., Campos, A.D., Webster, W.K., Wu, H., & Darnay, B.G. "*Site-Specific Lys-63-Linked Tumor Necrosis Factor Receptor-Associated Factor 6 Auto-Ubiquitination is a Critical Determinant of I Kappa B Kinase Activation.*" **J Biol Chem,** 282(6) (2022): 4102–4112.

Lamothe, B., Lai, Y.J., Hur, L., Orozco, N.M., Wang, J., & Campos, A.D. "*Deletion of TAK1 in the Myeloid Lineage Results in the Spontaneous Development of Myelomonocytic Leukemia in Mice.*" **PloS One,** 7(12) (2022): e51228.

Liang, J., Jiang, D., Griffith, J., Yu, S., Fan, J., Zhao, X., ... & Noble, P. W. (2022). *CD44 is a negative regulator of acute pulmonary inflammation and lipopolysaccharide-TLR signaling in mouse macrophages*. **Journal of Immunology**, 178, 2469–2475.

Liu, H.H., Xie, M., Schneider, M.D., & Chen, Z.J. "*Essential Role of TAK1 in Thymocyte Development and Activation.*" **Proc Natl Acad Sci,** 103(31) (2021): 11677.

Liu, S. Q., Cai, X., Wu, J. X., Cong, Q., Chen, X., & Li, T. (2020). *Phosphorylation of innate immune adaptor proteins MAVS, STING, and TRIF induces IRF3 activation.* **Science**, 347(6227), 1217–U17.

Liu, S., Liu, Y., Hao, W., Wolf, L., Kiliaan, A. J., Penke, B., Rübe, C. E., Walter, J., Heneka, M. T., & Hartmann, T. (2022). *TLR2 is a primary receptor for Alzheimer's amyloid β peptide to trigger neuroinflammatory activation*. **Journal of Immunology**, 188, 1098–1107.

Lo, Y.C., Lin, S.C., Rospigliosi, C.C., Conze, D.B., Wu, C.J., & Ashwell, J.D. "*Structural Basis for Recognition of Diubiquitins by NEMO.*" **Mol Cell**, 33(5) (2019): 602–615.

Long, E. M., Millen, B., Kubes, P., & Robbins, S. M. (2019). *Lipoteichoic acid induces unique inflammatory responses when compared to other toll-like receptor 2 ligands*. **PLoS ONE**, 4, e5601.

Mantovani, S., Oliviero, B., Varchetta, S., Renieri, A., & Mondelli, M. U. (2023). *TLRs: Innate immune sentries against SARS-CoV-2 infection.* **International Journal of Molecular Sciences,** 24, 8065.

Marks, K. E., Cho, K., Stickling, C., & Reynolds, J. M. (2021). *Toll-like receptor 2 in autoimmune inflammation.* **Immune Network,** 21, e18.

Moresco, E. M., LaVin, D., & Beutler, B. (2021). *Toll-like receptors*. **Current Biology**, 21(13), R488–R493.

Lead City University Ibadan DO NOT COPY

Noad, J., von der Malsburg, A., Pathe, C., Michel, M.A., Komander, D., & Randow, F. "*LUBAC-Synthesized Linear Ubiquitin Chains Restrict Cytosol-Invading Bacteria by Activating Autophagy and NF-KappaB.*" **Nat Microbiol**, 2 (2023): 17063.

Oliveira-Nascimento, L., Massari, P., & Wetzler, L. M. (2022). *The role of TLR2 in infection and immunity.* **Frontiers of Immunology,** 18, 79.

Pinto, L.M. "*Scoring Systems Using Chest Radiographic Features for the Diagnosis of Pulmonary Tuberculosis in Adults: A Systematic Review."* **European Respiratory Journal,** 42(2) (2023): 480–494.

Rahighi, S., Ikeda, F., Kawasaki, M., Akutsu, M., Suzuki, N., & Kato, R. "*Specific Recognition of Linear Ubiquitin Chains by NEMO is Important for NFkappaB Activation."* **Cell,** 136(6) (2019): 1098–1109.

Re, F., & Strominger, J. L. (2021). *Toll-like receptor 2 (TLR2) and TLR4 differentially activate human dendritic cells*. **Journal of Biological Chemistry**, 276, 37692–37699.

Rowe, D.C., McGettrick, A.F., Latz, E., Monks, B.G., Gay, N.J., & Yamamoto, M. "*The Myristoylation of TRIF-Related Adaptor Molecule Is Essential for Toll-Like Receptor 4 Signal Transduction.*" **Proc Natl Acad Sci,** 103(16) (2021): 6299–6304.

Sasaki, K., & Iwai, K. "*Roles of Linear Ubiquitinylation, a Crucial Regulator of NF-kB and Cell Death, in the Immune System.*" **Immunol Rev,** 266(1) (2020): 175–189.

Sato, S., Sanjo, H., Takeda, K., Ninomiya-Tsuji, J., Yamamoto, M., & Kawai, T. "*Essential Function for the Kinase TAK1 in Innate and Adaptive Immune Responses*." **Nat Immunol**, 6(11) (2020): 1087–1095.

Schuman, J., Chen, Y., Podd, A., Yu, M., Liu, H.H., & Wen, R. "*A Critical Role of TAK1 in B-Cell Receptor–Mediated Nuclear Factor kb Activation."* **Blood**, 113(19) (2019): 4566–4574.

Sharma, N., Akhade, A. S., & Qadri, A. (2023). *Sphingosine-1-phosphate suppresses TLR-induced CXCL8 secretion from human T cells.* **Journal of Leukocyte Biology**, 93(4), 521–528.

Shimizu, Y., Taraborrelli, L., & Walczak, H. "*Linear Ubiquitination in Immunity*." **Immunol Rev,** 266(1) (2020): 190–207.

Su, S., Tao, L., Deng, Z., Chen, W., Qin, S., & Jiang, H. (2021). TLR10: *Insights, controversies, and potential utility as a therapeutic target.* **Scandinavian Journal of Immunology,** 93, e12988.

Sumanth, M. S., Abhilasha, K. V., Jacob, S. P., Chaithra, V. H., Basrur, V., Willard, B., ... & Marathe, G. K. (2019). *Acute phase protein, α-1-acid glycoprotein (AGP-1), has differential effects on TLR-2 and TLR-4 mediated responses.* **Immunobiology**, 224, 672–680.

Lead City University Ibadan DO NOT COPY

Sun, J., Li, N., Oh, K.S., Dutta, B., Vayttaden, B.J., & Lin, B. "*Comprehensive RNAi-Based Screening of Human and Mouse TLR Pathways Identifies Species-Specific Preferences in Signaling Protein Use."* **Sci Signal**, 9(409) (2021): ra3.

Tokunaga, F. "Linear Ubiquitination-Mediated NF-kb *Regulation and Its Related Disorders."* **J Biochem**, 154(4) (2023): 313–323.

Vidya, M. K., Kumar, V. G., Sejian, V., Bagath, M., Krishnan, G., & Bhatta, R. (2022). *Toll-like receptors: Significance, ligands, signaling pathways, and functions in mammals*. **International Review of Immunology**, 37(3), 1–17.

Wan, Y. Y., Chi, H., Xie, M., Schneider, M. D., & Flavell, R. A. (2021). *The kinase TAK1 integrates antigen and cytokine receptor signaling for T cell development, survival, and function.* **Nature Immunology,** 7(8), 851–858.

Wu, C.J., Conze, D.B., Li, T., Srinivasula, S.M., & Ashwell, J.D. "*Sensing of Lys 63-Linked Polyubiquitination by NEMO is a Key Event in NF-kB Activation*." **Nat Cell Biol,** 8(4) (2021): 398–406.

Xia, Z. P., Sun, L., Chen, X., Pineda, G., Jiang, X., & Adhikari, A. (2019). *Direct activation of protein kinases by unanchored polyubiquitin chains*. **Nature**, 461(7260), 114–119.

Xia, Z.P., Sun, L., Chen, X., Pineda, G., Jiang, X., & Adhikari, A. "*Direct Activation of Protein Kinases by Unanchored Polyubiquitin Chains*." **Nature**, 461(7260) (2019): 114–119.

Yamamoto, M., Sato, S., Hemmi, H., Hoshino, K., Kaisho, T., & Sanjo, H. (2023). *Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway.* **Science**, 301(5633), 640–643.

Yuk, J. M., & Jo, E. K. (2021). *Toll-like receptors and innate immunity*. **Journal of Bacteriology and Virology,** 41(4), 225–235.

Zumla, A. "*Tuberculosis*." **New England Journal of Medicine**, 368(8) (2023): 745–755.

Proceedings and Conference Articles

Keshavarz-Fathi, M. B. T., & Rezaei, N. (2019). Chapter 3—Vaccines, adjuvants, and delivery systems. Academic Press: Cambridge, MA, pp. 45–59. ISBN 978-0-12-814039-0.

Oosenbrug, T., van de Graaff, M. J., Ressing, M. E., & van Kasteren, S. I. (2022). Chemical tools for studying TLR signaling dynamics. Cell Chemical Biology, 24, 801–812.

Ozinsky, A., Underhill, D. M., Fontenot, J. D., Hajjar, M., Smith, K. D., & Aderem, A. (2020). The repertoire for pattern recognition of pathogens by the innate immune

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system is defined by cooperation between toll-like receptors. *Proceedings of the National Academy of Sciences, 97*, 13766–13771.

Quesniaux, V. J., Nicolle, D. M., Torres, D., Kremer, L., Guérardel, Y., Nigou, J., Puzo, G., Erard, F., & Ryffel, B. (2024). Toll-like receptor 2 (TLR2)-dependent-positive and TLR2-independent-negative regulation of proinflammatory cytokines by mycobacterial lipomannans. Journal of Immunology, 172, 4425–4434.

Websites

WHO (World Health Organisation). *Global Tuberculosis Report 2015.* Available Online: http://www.who.int/tb/publications/global_report/en/2015.

WHO (World Health Organization). Systematic Screening of Active Tuberculosis - Principles and Recommendations. (2023).

World Health Organization (WHO). (2023). Systematic screening of active tuberculosis - Principles and recommendations. Retrieved from WHO website.

World Health Organization (WHO). (2018). International standards for tuberculosis care. Retrieved from WHO website.

World Health Organization (WHO). (1991). TB: A global emergency. Retrieved from WHO Website.

World Health Organization (WHO). (2011). Guidelines for the programmatic management of drug-resistant tuberculosis. Retrieved from WHO Website.

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Appendix I:

Informed Consent Form

Initials……………………………………………………………

To participate in this research study, it is necessary that you give your informed consent

By signing this form, you are indicating that you understand the nature of the research study and your role in the research, and that you agree to participate in the research (having read the subject information form).

Please consider the following points before signing

I understand that I am participating in a research

I understand that my participation will be anonymous (that is, my name will not be linked with my personal information) and that all information I provide will remain confidential I understand that I will be provided with an explanation of the research in which I am participating:

I understand that my participation in this research is voluntary, and that I may refuse to participate further at any time without having to offer an explanation

By signing this form, I am stating that I understand the above information and consent to participate in this study

________________________________ __________________________

Signature/Thumbprint of Participant/Date Signature of Investigator/Date

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APPENDIX 2

QUESTIONNAIRE

Good day Sir/Ma I am a post graduate student in the department of Biological sciences, Lead City University Ibadan, conducting a research titled "**Frequency and pattern of genetic variation between genders in Pulmonary Mycobacterium tuberculosis positive cases at DOTs clinics in Abeokuta, Ogun state**". Kindly avail me few minutes of your time to peruse and provide the information required in this questionnaire. I assured you that all information supplied shall be used only for the research purpose which is necessary for the control and prevention of complications associated diabetes mellitus. Please respond honestly to the question below. The confidentiality of your response to the questionnaire is guaranteed. Thank you.

SECTION A: DEMOGRAPHIC AND CLINICAL DATA:

Instruction: Please tick ( ) and fill in the gap as appropriate

1. Age (years): 20-30 [ ], 31-40 [ ] 41-50 [ ] Above 50 [ ]

2. Gender: Male [ ] Female [ ]

3. Religion: Christianity [ ] Islam [ ] Traditionalist [ ]

4. Ethnic group: Yoruba [ ] Igbo [ ] Hausa [ ] Others (specify) ……………….

5. Marital status: Single [ ] Married [ ] Divorced [ ] Widowed [ ]

6. Occupation: Artisan [ ] Trader [ ] Civil servant [ ] Unemployed [ ]

7. Education status: None [ ] Primary [ ] Secondary [ ] Tertiary [ ]

8. Number in family: less than 4 [ ] 4-6 [ ] above 6 [ ]

9. Nature of accommodation: self-contain [ ] Flat [ ] single room [ ]

10. History of alcohol consumption: Yes [ ] No [ ]

11. History of smoking: Yes [ ] No [ ]

12. TB Status: Positive [ ] Negative [ ]

13. If Positive, what is the duration of treatment? Baseline [ ]; two months [ ]; five months; seven months [ ] 18 months [ ] others (pls specify) [ ]

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Bio-Data

ABUBAKRE, Rodiah Opeyemi

kemta oke-odo idi-aba Abeokuta Ogun state Nigeria.

Telephone: +234(0)8112465625, +234(0)9012589821

E-mail: hazeldoyen@gmail.com

PERSONAL DETAILS

FULL NAME:	ABUBAKRE, Rodiah Opeyemi.
STATE OF ORIGIN:	Ogun State.

NATIONALITY: Nigerian

MARITAL STATUS: Single

PERMANENT HOME ADDRESS: 11, Kemta, Oke-Odo, Idi-Aba, Abeokuta.

PHONE NUMBERS: 08112465625, 09012589821

PERSONAL OBJECTIVES

What I love about science is that it has yet to offer the 'best' way for anything. Man will always find ways to do things better than before, quote: "Wherever the knowledge takes us, it will empower us to do more." I want to be part of the cutting-edge science that optimizes this for the good of medicine and man.

SKILLS AND TECHNOLOGICAL KNOW-HOW

5. Medical Laboratory Management
6. Quality laboratory practice and standard operating procedures
7. Office Package and Technology
8. Excellent Communication and Organization
9. Ability to Work with Little or No Supervision
10. Proactive, Diligent, Loyal and Dedicated to Work
11. Dedicated Team Player

EDUCATION AND QUALIFICATIONS

- ***M.Sc. Molecular Biology and Genomics*** ***In-view***

Attained from: Lead City University, Ibadan

- ***Certificate of National Service (NYSC)*** ***2022***
- 2022 Batch A

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- ***B.MLS Medical Laboratory Science*** **2020**

Attained from: Achievers University, Owo, Ondo State.

- **Second Class Degree** **2022**

- ***West Africa Examination Council Secondary School Certificate Examination*** **2014**

Attained from: Aminat International College

6. Nine credits (including Mathematics and English)

7.

- ***Primary School Leaving Certificate*** **2007**

Attained from: Ima International School

TRAININGS ATTENDED

8. 21st Century Biomedical Sciences Conference **2022**
9. Annual AMLSN Scientific Conference and Workshop **2021**
10. YMLS Annual Conference, Akure, Ondo State. **2019**

CLINICAL POSTING

- State Hospital, Ijaye, Abeokuta **2016-2017**
- Federal Medical Center, Owo **2017-2020**
- Federal Medical Center, Abeokuta **2019-2020**

WORK EXPERIENCE

- Federal Medical Center, Abeokuta **2021-2022**

3. ***Intern***

- Federal Medical Center, Abeokuta **2022-2023**

4. ***Medical Laboratory Science Corp Member***

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LEADERSHIP POSITIONS HELD

- **Member** **2016-2018**

Student Representative Council, NIMELSSA, Achievers University Chapter.

- **Speaker of House** **2019-2020**

Achievers University Student Representative Council.

- **Ameerah (Chairperson)** **2018-2020**

Muslim Student Society of Nigeria, Achievers University Chapter.

- **Project Director** **2022-2023**

Drug free community development service NDLEA Abeokuta.

PUBLICATIONS

Emokpae, M.A., Ogboru, E. and Abubakre, R.O. (2020). Relationship between Luteinizing Hormone and Follicle Stimulating Hormone Ratio and Body Mass Index in Women with Polycystic Ovarian Syndrome. *Journal of Medical Laboratory Science;* **30** (4): 1-10. http://doi.org/10.5281/zenodo.4364696

HOBBIES

Reading, research and serving humanity.

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The University Compliance Certification

This is to certify that, this thesis was written by **Rodiah Opeyemi ABUBAKRE** with Matriculation Number **LCU/PG/003249** Department of Biological Sciences, Faculty of Natural and Applied Sciences, Lead city university, Ibadan, Oyo State, in full compliance with the approved University format and style.

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Signature **Date**