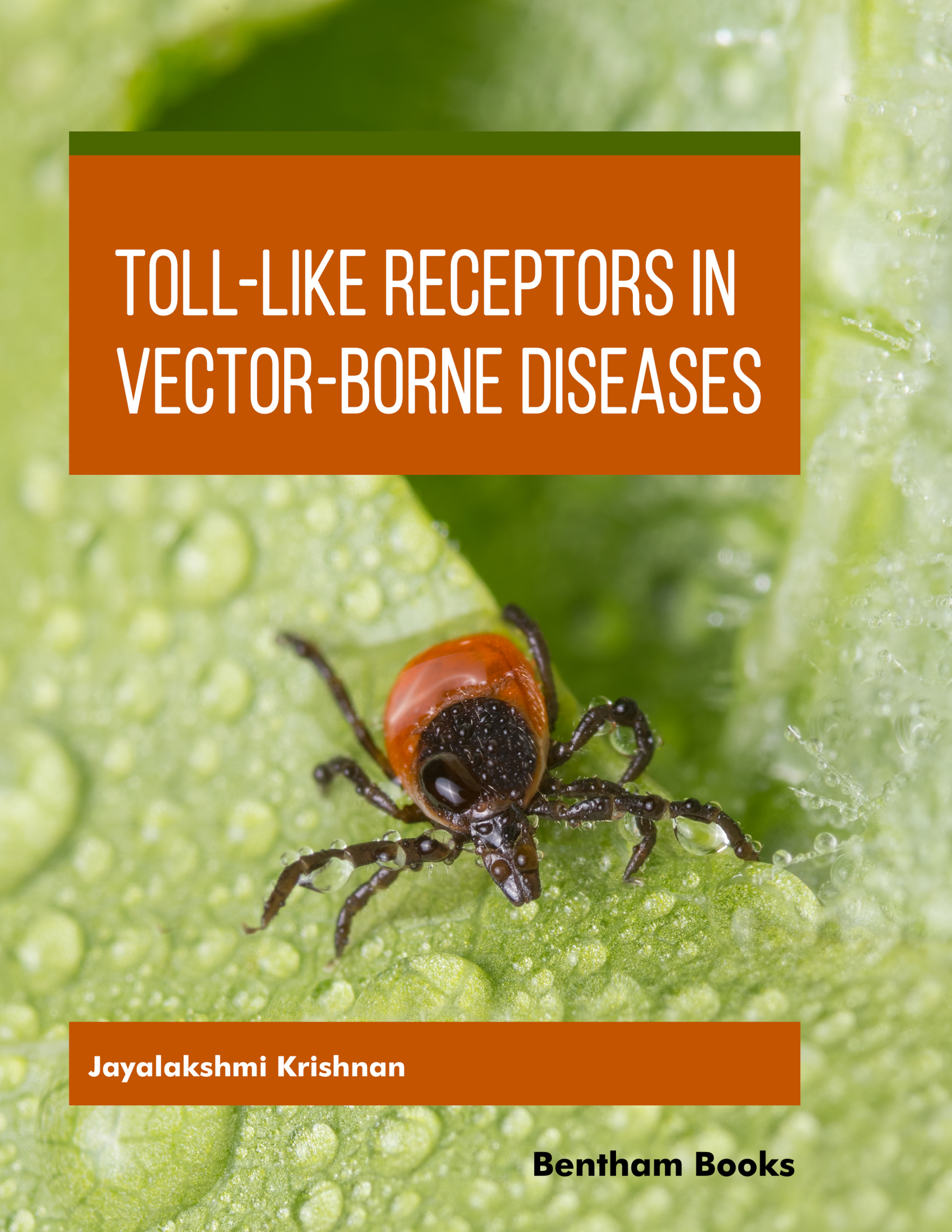
TOLL-LIKE RECEPTORS IN VECTOR-BORNE DISEASES



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Toll-Like Receptors in Vector-Borne Diseases

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ISBN (Online): 978-981-5124-54-5

ISBN (Print): 978-981-5124-55-2

ISBN (Paperback): 978-981-5124-56-9

First published in 2023.

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FOREWORD

This topic is appropriate when we talk about the control of vector-borne diseases as a top priority in the world. Vector-borne diseases are a huge public health problem all over the world. Vectors are organisms that transmit pathogens from infected human to animal or from animal to human, accounting for 17% of Vector-borne diseases (VBDs). Vector-borne diseases such as Malaria, Dengue, Chikungunya, Human African Trypanosomiasis, Leishmaniasis, Japanese encephalitis, Chaga's diseases, Yellow fever, Leishmaniasis, and Onchocerciasis have become major public health concern affecting more than one billion cases and one million deaths globally. There is an urgent need to control these challenges and state of art techniques and science and technology will take it forward. Toll-like receptors are the primary pattern recognition receptors in the human systems and in eliciting innate immune signalling. Cytokines produced through toll-like receptors activation act as a bridge to elicit an adaptive immune response as well. I appreciate this book's title on toll-like receptors in vector-borne diseases as this book can be read by many researchers, industry persons, policymakers, and academicians and can cater to the needs of the research on these vector-borne diseases.

I convey my best wishes to the editor and hope this book will be a great success.

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PREFACE

Organisms/animals that transmit diseases are called vectors. They cause serious health problems to the human population such as illness and death. Famous vectors that cause diseases include fleas, ticks, mites, and mosquitos. Mostly, the vectors are invertebrate arthropods and non-living fomites. A disease that can be transmitted from an arthropod or a fomite to a human or animal or plant is called a vector-borne disease (VBDs). Vectors are able to carry and transmit various infectious organisms such as parasites, bacteria and viruses. Vector-borne diseases in a given country affect the socio-economic status and have a huge impact on the Global disease burden.

Ironically, despite decades of research on VBDs, still much remains to be discovered on the complicated relationships between vector, host, and pathogen in their internal environment. The emergence of new diseases such as Zika possess more questions on the complicity of host-pathogen-vector interaction. Any effective vaccine/intervention/ depends on the complete information on the molecules that perform interaction between host-pathoge--vector. Hence, a complete understanding is very much essential. Vector-borne diseases are a threat to the community worldwide. Each year 2.5 billion people in over 100 countries (WHO reports) die of such diseases. Brain inflammation, coma, cerebral leakage, meningitis, neuronal and glial cell degeneration, endothelial dysfunction, blood-brain barrier leakage, and disturbance in Cerebro Spinal Fluid (CSF) circulation have all been noted in various vector-borne diseases such as dengue, Chikungunya, Malaria, West Nile fever, Filariasis and Japanese encephalitis (JE) . I wish our readers can be satisfied with many questions which they feel excited to find the answer for research questions on the etiology of neurological sequale of vector-borne diseases in this book.

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CHAPTER 1

Introduction to Vector Borne Diseases

Abstract: Vector-borne diseases(VBDs) are reported to represent amount 17% of all infectious diseases. The geographical distribution of vectors depends upon various climatic factors, and social factors. In the recent past, the spread of VBDs across the world is so rapid that it is associated with a change in climatic factors, global warming, travel and trade, unplanned urbanization, deforestation *etc*. Amongst the vector-borne diseases notable is West Nile fever (WNF) caused by West Nile Virus (WNV). WNF belongs to the family of Flaviviridae, which is part of the Japanese encephalitis antigenic group. WNV is transmitted from infected birds to human beings by mosquito bites. WNV is readily reported in Africa, Europe, the Middle East, North America and West Asia. Looking at the history, WNV was first isolated in a woman in the West Nile district of Uganda in 1937. It was identified in birds (crows and columbiformes) in the Nile delta region in 1953. Over the past 50 years, human cases of WNV have been reported in various countries.

Keywords: Chikungunya fever, Dengue, Leishmaniasis, Malaria, Vector-borne diseases (VBDs), West Nile fever (WNF).

INTRODUCTION

Vector-borne diseases(VBD)s are reported to represent amount 17% of all infectious diseases. The geographical distribution of vectors depends upon various climatic factors, and social factors. In the recent past, the spread of VBDs across the world is so rapid that it is associated with change in climatic factors, global warming, travel and trade, unplanned urbanization, deforestation *etc*.

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CHIKUNGUNYA

For joint pain caused by chikyngunya medicines such as nacproxen, ibuprofen, and acetaminophen can be tried. Joint pain caused by chikungunya persists for several weeks even after the fever has been cured. As of now there is no vaccine for the treatment of chikyngunya and no antiviral treatment is available. Using insect repellent, sleeping under the mosquito net, breeding places control, wearing full clothes can be used as preventive options for chikungunya.The word Chikungunya is coined from the makondae language which means bends up or contorted "walk with bent over". This disease was reported in 1952 after an outbreak in Makonde Plateau in eastern Tanzania. Initially, this disease was reported seen in Africa and Asia but after 2007, it is also reported to occur from various European countries as well. Currently, more than 60 countries are reporting this fever. The mosquitos which spread Chikungunya fever are day bitters, they spread the disease from one infected person to another normal person when they bite. Symptoms last for 2 to 12 days after the infection begin.The fever is divided into acute and chronic phases,in which the acute phase is known as the febrile phase. Fever is the main symptom in the acute phase and in the chronic phase and is characterised by inflammatory joint pain in some patients up to years it can extend.

Chikungunya fever is a viral disease like WNV that belongs to the genus Alphavirus from the family of *Togaviridae*. Chikungunya fever is transmitted to human beings by the infected mosquito of *Ad aegypti,* and *Ae albopictus*. Chikungunya fever was first described during an outbreak in southern Tanzania in 1952. At present, CHIKV is reported in 50 countries.

LEISHMANIASIS

Leishmaniasis is a protozoan parasitic infection that is transmitted to human beings through the bite of an infected female sandfly. There are three types of Lieshmaniasis, cutaneous, visceral and mucocutaneous. Amongst these, Visceral Lieshmaniasis is progressing rapidly in east Africa with the highest mortality and morbidity. Visceral leishmaniasis if not treated can lead to high rates of mortality and epidemics. Cutaneous leishmaniasis is observed in Afghanistan and Syrian Republic. According to WHO, in 2014, more than 90% of new cases were reported to WHO from six countries: Brazil, Ethiopia, India, Somalia, South Sudan and Sudan. Strikingly, a vast major case of cutaneous Leishmaniasis is reported from Pakistan, Peru, Saudi Arabia and the Syrian Arab Republic, Afghanistan, Algeria, Brazil, Colombia, and the Islamic Republic of Iran. Mucocutaneous leishmaniasis is reported in Bolivia, Brazil and Peru, somewhere around 90%. Surprisingly, the control programs for Kala-azar are successful in

South-East Asia Region (SEARO) countries.

MALARIA

Malaria is caused by one of four species of the *Plasmodium* parasite transmitted by female *Anopheles* spp mosquitoes. Malaria vector control methods have been proven successful in the past which is one of the deadliest vector-borne diseases. Malaria is one of the life-threatening diseases. According to WHO, in 2015 an estimated 212 million cases of malaria occurred worldwide and 429,000 people died, mostly children in the African Region. According to CD, about 1,500 cases of malaria are diagnosed in the United States each year. In India, malaria is a well-known reported public health problem. According to National Vector Bone Diseases Control Programme (NVBDCP), in 2017, among the total of 1,98, 303 cases of malaria, it was reported that *Plasmodium falsiparum* is seen in 1,42,152 cases.

DENGUE

Dengue is the most important arboviral human disease,*Ae. aegypti,* and *Ae. albopictus*, the dengue vectors can be easily influenced by changing humidity, temperature, rainfall, degree of urbanization, and control measures taken by various countries. According to WHO before 1970, dengue was reported in nine countries only, however, now the spread is so rapid that dengue is reported in more than 100 countries, and such a situation is very alarming. In 2016, 1,29,166 cases have been reported in India by the National Vector Borne Disease Control Programme (NVBDCP).

JE was first reported in Uttar Pradesh, the main JE epidemic area in the northern state of India in 1978. The *Cx.vishnui* subgroup, and the *Cx. tritaeniorhynchus, Cx. seudovishnui* and *Anopheles subpictus*, were the main mosquito vectors and secondary vectors in India. In 2016, a total of 1676 cases of JE is reported from India according to NVBDCP. The virus is a single-stranded RNA virus.Pigs and birds are the intermediate hosts of the virus. Human beings are considered dead-end hosts. Upon bite of the infection the virus replicates in the lymph nodes and then viremia develops. After this, the virus enters the central nervous system to effect. The virus has the capacity to alter neurodevelopment also. There is no effective treatment for this viral infection but there can be supportive care and fluids given. Southeast Asian nations and west pacific nations are at higher risk of reporting the cases. Avoiding mosquito bites is the best prevention for this disease.

CHAPTER 2

Pattern Recognition Receptors in Brain: Emphasis on Toll Like Receptors and their Types

Abstract: The immune system is highly complex; it senses foreign invaders, thus protecting the body. The adaptive arm of the immune system confers long-term protection, whereas the innate immune system confers immediate protection. In the case of the immune system, the pattern recognition receptors offer various modes of sensing the pathogen-associated molecular patterns present in pathogens. The receptors that sense invading pathogens are called Pattern recognition receptors [1]. The adaptive immune system is very sophisticated, as it is trained to identify only the "specific antigen", but PPRs are customised to sense a wide array of "common patterns" present in the pathogens. Cerebral pericytes are the cells that are seen as embedded in the basement membrane of capillaries. Matzinger [2] gave a new insight into the recognition of pathogens by PRRs as those that recognise PAMPs and DAMPs (Damage Associated Molecular Patterns). While PAMPs can be presented as exogenous ligands to the receptor, DAMPs are presented as endogenous ligands. Once these PRRs are activated either by PAMPs or DAMPs, they lead to the production of inflammation to clear the infection. However, over-activation during chronic conditions leads to pathological changes.

Keywords: CD 14 cofactor, DAMPs, NF-κB, TLRs.

INTRODUCTION

TLRs can be present in the membrane or in the intracellular system, wherever they are located they can recognise the PAMPs. They are a part of the surveillance system in the cells along with other mechanisms. Viral, bacterial, parasitic and any other pathogenic components can be recognised by TLRs.

There are 13 TLRs that are currently identified and all of them use MyD88 as their adaptor molecule except TLR3 [1, 2]. This activation can produce inflammatory responses through the recruitment of various kinases and adaptor molecules. These include the activation of NF-κB and MAPKs, such as stress-activated protein kinase/JNK and p38. In humans, TLR1-10 are present, whereas in Mice along with the homologues of TLR1-9 there are additional TLRs, *i.e.*, TLR 11 to 13 are present [3 - 5]. Further, ten chicken (avain) functional TLRs are

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identified [6]. Among these TLRs, TLR3, TLR7-9 and TLR11-13 can be seen in endosomes which are intracellular organelles.

TLR1, 2, 4, 5, 6 and 10 are present in the cell surface. These TLRs differ in their ability to bind and recognise pathogens. Most bacterial ligands can be identified by TLR1, 2, 4, 5, 6, and 10 in which TLR 2 and 4 (CD 14 as a cofactor) can also recognise viral surface proteins [7, 8]. Sometimes, upon pathogenic binding TLRs form either homo or heterodimers [9]. TLR-induced pathways can be bifurcated as two, based on their ability to use myeloid differentiation factor 88 (MyD88). Except for TLR3 all TLRs use the MyD88-dependent pathway, TLRs use the TRIF pathway to entice the inflammatory events.

Brain and Pathogen Associated Molecular Patterns

Brain pericytes have been shown to express a variety of PRRs including TLRs, NOD, and NLRs [10]. Pericytes have been known to control various neurovascular functions such as cerebral blood flow, angiogenesis, and permeability of the BBB blood-brain barrier) [11]. In the brain, several DAMPs are reported to be released after an injury that leads to the activation of TLRs. Such DAMPs include Heat Shock Proteins, HMGB1, fibronectin, hyaluronic acid, nucleic acids such as mRNA and miRNAs, mitochondrial DNA, and N-formyl peptides [12 - 16]. Microglia express a full repertoire of TLRs in the brain, and microglial cells express mRNA for TLRs 1-9. Astrocytes also express TLRs 3,4, and 2 upon activation. Under resting and activating conditions astrocytes express TLR3. But TLR2 and 4 can be expressed upon activation only. Oligodendrocytes express both TLR2 and TLR3. Several TLRs can be expressed by neurons as well.TLR singaling through neurons is found to be involved in nociception. TLR4 can be seen as expressed in macrophages of CNS.

Cerebral Malaria and TLRs

It was long thought the brain is immunologically inert. The blood circulation in the brain also is supplied with T cells. These cells have been shown to experimentally sequester and activated in cerebral malaria during *Plasmodium berghei* infection. During experimental induction of Cerebral Malaria (CM), the sequestered αβ $CD8^{+}$ T cells have a pathological effect [17, 18].

Further experimental studies on Cerebral Malaria, indicated specific proteins on Malaria induction in the brain such as PKC-θ signalling. This is very much essential for the recruitment of CD8(+) T cells to the brain thus resulting in pathological alterations of brain microvasculature. PKC-θ thus can be considered as one of the target molecules for CM [19] . Huggins *et al*., 2017 [20] , have done a murine experimental CM, which showed CD8T cells, are responsible for

BBB breakdown. This BBB breakdown is associated with the reduction of claudin-5 and occuldins the tight junctional proteins.

Using TRIF-deficient mice it was shown that, in cerebral malaria, TLR 2 and TLR 9 mediated pathways in a MyD88-dependent manner have played a critical role in combating Pba infection [21]. Using Plamsmodium berghi model it is proven that the mice that are infected but deficient with TLR9 are not protected [22]. There are studies that point out that TLR7 deletion can protect against cerebral malaria in mice by altering cytokine production [23]. In Ugandan children, it was found that *TLR2* δ22 polymorphism is causing protection against cerebral malaria [24]. Experimental cerebral malaria is prevented by blocking the nucleic acid-sensing TLRs such as TLR9 [25]. Using meta-analysis it was shown that TLRs such as TLR1,4 and 9 are associated with high paracytemia and TLRs such as 2 and 6 are associated with the severity of the disease [26].

TLRs are synthesized in the endoplasmic reticulum, after which it goes to the Golgi apparatus. After some modification from Golgi apparatus either it goes to the plasma membrane or to the endosomes.The nucleic acid sensing TLRs are transported to the endosomes. Adaptors play an important role in the signaling of TLRs. There are various adaptors which are having TIR domains, these adaptors are recruited by various TLRs, the names of the adaptors are MyD88, TRIF, TIRAP/MAL, or TRAM. Myd88 is a common adaptor which is used by all the TLRs. Based on the usage of MyD88 TLR signaling pathways are divided as MyD88 dependent pathways and MyD88 independent pathways. In 1985, Toll gene was identified in drosophila by https://en.wikipedia.org/wiki/Christiane_N%C3%BCsslein-Volhard Christiane Nüsslein-Volhard. The study in drosophila has revealed that the gene that is involved in dorsoventral polarization is very similar to a gene that participates in innate immune signaling in the mammals. Due to this similarity the Toll is named after. In fruitfly embryos the toll pathways is very similar to mammalian IL-1R pathway indicating in fruitfly also Toll pathway can control the activities such as developmental patterning. The experiments with Toll mutant drosophila have revealed that the fly is not able to elicit immune responses against the fungal infections.

TLRs are transmemebrane proteins that contain 20–27, Extracellular Leucine-Rich Repeats (LRR). Each TLR can be recognized by their location, using of signal transduction molecules and signal transduction pathways. The MyD88 independent pathway is also called as TRIF pathways. Ubiquitination, phosphorylation and protein modifications play an important role in TLR mediated signaling. However, mutations in TLR signaling can lead to the development of various autoimmune diseases, and other inflammatory diseases.CD14 a co-receptor plays very important role in TLR signaling and

CHAPTER 3

Malaria

Abstract: The World Health Organization (WHO) defines cerebral malaria (CM) as an otherwise unexplained coma in a patient with asexual forms of malaria parasites on the peripheral blood smear. Malaria is a severe, devastating illness characterised by respiratory distress, severe anemia, and cerebral malaria (CM). Altered consciousness, convulsions, ataxia, hemiparesis, and other neurologic and psychiatric impairments are noted in cerebral malaria. Thus, cerebral malaria is defined as a condition in which a human has *Plasmodium falciparum*, a parasite in peripheral blood, followed by neurological complications of any degree. CM accounts for 300,000 deaths per year, and almost any survivors there display severe neurological manifestations. Coma is the outcome of CM, which is again due to brain hypoxia due to inflammation, edema, Brain swelling, and vascular blockage, are all due to the sequestration of pRBCs in brain microvasculature [1, 2]. In Ugandan children with CM infected with *P.falciparum*, severe cognitive impairment, behaviour problems such as hyperactivity, inattentiveness, aggressive behaviour, loss of speech, hearing loss, blindness, and epilepsy were noted (Irdo *et al.* , 2010). Heme offered protective responses to ECM, by dampening the activation of microglia, astrocytes, and expression of IP10, TNFa, and IFNg [3].

Keywords: Blood brain barrier (BBB), Glycosylphosphatidylinositol (GPI) anchors, *Plasmodium falciparum*, *Plasmodium berghei*, Postmalaria neurological syndrome (PMNS).

INTRODUCTION

MALARIA: PATHOLOGY IN THE BRAIN BY NEUROIMAGING

Malaria is the leading cause of death among infectious diseases globally. *Plasmodium falciparum* infection in children and adults causes cerebral malaria which is a primary cause of death in both groups [1]. Research on Cerebral Malaria (CM) is still elusive.

Both animal and human studies reveal various complicated features for the development of CM such as, increased pro-inflammatory cytokines, adhesion molecules, cytoadherence of parasite-infected erythrocytes, platelets, WBCs in the microvasculature of CNS [2 - 8].

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Plasmodium falciparum infection in humans causes hearing loss in adults [8] and mental health disorders in children [2, 9 - 12]. Past evidence in people affected with cerebral malaria suggest that even after recovery they display brain injury in terms of cognitive deficits and neurological deficits in almost 25% of patients [9, 10, 12].

RBCs are parasitized (pRBCs), get adhere to the endothelium, and cause vasospasm. This is due to a reduced supply of Nitric Oxide. Once the blood vessel is blocked, it leads to the rupture of schizonts, leading to the accumulation of products of haemolysis. This in turn leads to more depletion of NO. All these mechanisms contribute to severe cerebral vasospasm. This further leads to brain swelling along with vaso-occulsion causing neuronal cell damage and death in CM [13].

Pathogenesis of cerebral malaria (CM), is conferred as microvascular obstruction, endothelial dysfunction, brain swelling, and impairment of blood-brain barrier, vasogenic edema, and venous congestion in both adults and children in Indian patients [13]. Basal nuclei consistency was also observed in MRI of patients suffering from CM. Further, studies also found in Patients that CM causes acute haemorrhagic infarctions were also seen in the brain stem, cerebellum, cerebral white matter, and insular cortex. This is followed by bithalamic infarctions with or without haemorrhages [14].

MRI in adults with severe *falciparum* malaria, revealed diffuse cerebral swelling, in adult Bangladeshi patients. These patients also showed focal extracellular oedema, cytotoxic oedema, and mildly raised brain lactate in MRI. In addition, these patients showed retinal whitening in patients with coma. However, subtle differences were noticed in children with cerebral edema in Malawian children, where severe effects of *falciparum* malaria were observed. Adult CM and coma are characterised by disturbances in microcirculation, and ischemia. This is attributed to sequestered parasites. These mechanisms can be taken into account for explaining the pathology of cerebral malaria in adults [15].

Plasmodium falciparum, the deadliest malarial parasite, kills 10 to 30% of people every year, it affects various organs of the body. Cytoadherence of parasite-infected erythrocytes in brain endothelium causes complications in CNS. Though adults and children both get the disease, the clinical manifestation and pathological occurrence entirely differ between the two groups, while coma remains a common symptom of both groups [16, 17]. On the other hand, in the case of the absence of severe or cerebral malaris in adults, MRI studies reveal acute cerebral injury with a lesion in the corpus callosum [18]. Cerebral venous sinus thrombosis is a complicated form of brain pathology in various infectious

diseases, including malaria. Severe *falciparum* malaria resulted in increased intracranial pressure, and sinus thrombosis with venous infarction in a 43 old Thai patient [18].

Postmalaria neurological syndrome (PMNS), is reported to occur after two months of *falciparum* infection clearance. A 54-year-old Japanese man is reported to have PMNS with incoherent speech and disturbed and uncooperative behaviour with a high fever. Interestingly this patient displayed negative for malarial parasites in the Peripheral blood smear. Further, no organisms were found in CSF and MRI studies revealed no acute disseminated encephalomyelitis. Thus, PMNS remains an answered area of research [19]. Post cerebral malaria motor deficit is noted [20], and increased intracranial pressure followed by brain swelling is noted in children who died of malaria albeit not noted in survivors [21]. Comparative studies reveal in children with retinopathy-negative children display severe neurological deficits as observed by MRI in comparison with retinopathy-positive CM [22]. Atypical neurological manifestations were observed in patients infected with *P.falciparum* with cerebral venous thrombosis [21].

NOVEL MALARIA BIOMARKERS

Increased lactate and alanine concentrations are noted along with decreased aspartate and adenosine triphosphate levels which is due to hypoxia and ischemia whichin turn is due to clogged RBSc in brain microcirculation [2, 4]. interferon-γ, chemokine CXCL10, and lymphotoxin-α, in astrocytes, contribute to the development of cerebral malaria in murine as well as human primary astrocyte culture in experimental cerebral malaria [23]. Cerebral Malaria in children is also characterised by the high level of Osteoprotegrin (OPG), which is a protein stored in Weibel-Palade (WP) bodies. These are intracellular storage organelles in endothelial cells, during EC activation they get to fuse with the extracellular membrane and release their content. One such content is Osteoprotegrin (OPG), which is highly elevated in children with CM. Experimental cerebral malaria in mice has conformed to the elevated level of Osteoprotegrin (OPG), in mice [23]. Thus making it a new tool for malaria pathology. Thus identification of biomarkers for cerebral malaria becomes essential for therapeutic approaches. Apolipprotein E, present in the brain has been reported to be involved in various neurological diseases. The absence of Apoliproteptein E has protected the mouse brain from cerebral malaria, in which reduced T cell sequestration and parasites were seen [24]. Proteomic profiling of ECM, brains of C57/Bl6N mice infected with *P. berghei* ANKA has revealed potential biomarkers which could be the underlying cause of neurological manifestations [15].

CHAPTER 4

TLRs in Lymphatic Filariasis

Abstract: Lymphatic filariasis is one of the neglected tropical diseases and also a disfiguring vector-borne disease. Parasitic nematodes such as *Wuchereriabancrofti*, *Brugiamalayi*, and *Brugiatimori* are the three types of parasites that cause lymphatic pathology in terms of *hydrocele, lymphedema, and elephantiasis* [1]. Among these three parasites, *Wuchereriabancrofti* is the principal parasite, which causes around 90% of infections. These nematodes impair the lymphatic system, thus leading to considerable morbidity in the affected people. The life cycle of this adult-stage lymph-dwelling parasites is complex in nature. Once they start infecting the lymphatics, they cause swelling, dilatation, and thickening of lymph vessels.

Keywords: Bancroftian filariasis, *B. malayi*, Proinflammatory cytokines, *Wolbachia* lipoproteins, *Wuchereria bancrofti*.

INTRODUCTION

Immune cell interaction with a parasite is of paramount importance as they could just lead to immunopathogenesis. Dendritic cells are known as antigen-presenting cells as they can mount the T cell-derived immune responses.

The sheath antigen protein which is of 70kDa from *Wuchereriabancrofti* binds with the human dendritic cells and causes the maturation of the cells along with proinflammatory cytokine secretion *via* toll-like receptor 4 signaling [1, 2]. TLR polymorphisms are involved in making the disease susceptible to the pathogen. TLR 2 -196 to -173 polymorphism in the 5' untranslated region is reported from the blood samples collected from the Tanga region with bancroftianfilariasis [3]. Unlike viral and bacterial infections filarial infections cause immunomodulation, for example, it has been shown that pre-infection with filarial parasites, protects the mice from both types of diabetes [4]. In line with these studies, filariasis, and *Mycobacterium tuberculosis* confection studies also have shown that filarial antigens cause immunomodulation by inhibiting the TLR2 and TLR9 expression [4]. *Wolbachia* is a symbiotic bacteria living inside the filarial nematode and WSP (Wolbachia Surface protein) from these species induces a strong inflammatory process through TLR2 and TLR4 in dendritic cells and macrophages [5]. There are studies that point out that *Wolbachia* lipoproteins augment the immune respo-

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nse to *B. malayi* by stimulating both innate and adaptive immune response through TLR2 and 6 [6]. Diminished TLR (TLR1, TLR2, TLR4, and TLR9) expression is seen in macrophages during filarial infection but not in monocytes [7]. Lacto-N-fucopentose III (LNFPIII)and phosphorylcholine-decorated glycans from filarial parasite act as pattern recognition receptors to TLR4 [8]. MF-exposed monocyte-derived human DCs (mhDCs) showed decreased expression of TLR4 and TLR3 mRNA in comparison with unexposed cells [7]. T cell expression of TLR9 was not diminished during the filarial infection but TLR1, TLR2, and TLR4 expression was lowered [8]. Filarial antigen from Brugiapahangi (BpA) triggers apoptosis of normal human monocytes through TLR 4 [8].

Proinflammatory cytokines are the key inducers of lymphatic filariasis-induced pathology. Attempts have been made to understand the impact of lymphatic filariasis in triggering the TLRs. Cytokine responses in patients of chronic, subclinical pathology and uninfected; individuals have shown that TLR2,7 and TLR 9n mediated inflammatory responses were higher in the chronically infected group [8]. This response is mediated by extracellular signal-regulated kinase 1/2 (ERK1/2) and p38 mitogen-activated protein (MAP) kinases (MAPK) hyperphosphorylation. NF-κB also hyperphosphorylated in chronically infected individuals. In Thailand, an investigation of SNPs was found to be associated with TLR2 −196 to −173 del, +597 T>C, and +1350 T>C polymorphisms in asymptomatic bancroftian filariasis [9].

When the discovery of endosymbiotic *Wolbachia* bacteria was made the approach towards LF has been changed. *Wolbachia* in *B. malayi* activates TLR2-TLR6 interactions which in turn triggers the adaptor molecules MyD88 and TIRAP/Mal causing innate inflammatory events (Hise *et al.,* 2004). Purified major Wolbachia surface protein (rWSP) acts as a ligand to TLR2 and TLR4 (Table **3**) [10].

Table 3. Ligands that bind with TLRs from filarial parasites.

Ligand	Pathogen	TLR	Reference
sheath antigen protein	*Wuchereriabancrofti*	**TLR 4**	Mukherjee, *et al.,* 2019 [10]
Lacto-N-fucopentose III	filarial parasite	**TLR 4**	Goodridge, *et al.,* 2005) [10]
phosphorylcholine-decoratedglycans	filarial parasite	**TLR 4**	Goodridge, *et al.,* 2005) [10]
Brugiapahangi antigen (BpA)	*Brugiapahangi*	**TLR4**	(Alok Das Mohapatra, *et al.,* 2014). [10]

(Table 3) cont.....

Ligand	Pathogen	TLR	Reference
purified major Wolbachia surface protein (rWSP)	endosymbionts of the genus Wolbachia (Rickettsiales) of filarial nematodes	TLR2 and TLR4	Norbert W. Brattig *et al.,* 2004[10]

Lymphatic Filariasis and Inflammation

In PBMCs of patients with lymphatic pathology, it was shown that vascular endothelial growth factor and angiopoietin levels were increased. Lymphangiogenesis is a process by which new blood vessels form and aggravate the disease. TLRs in lymphatic pathology tend to increase this process and contribute to the pathology [11]. TLR2 and TLR4 are involved in the pathogenesis created by a major surface protein of wolbachia species [12]. Human embryonic skin cells expressing the TLRs are stimulated by *Brugia malayi*. In C57BL/6 and TLR4(-/-) mice filarial extract leads to the production of cytokine production but not from TLR2(-/-) or TLR6(-/-) mice [13]. Murine peritoneal-elicited macrophages were exposed to *Brugia malayi* female worms (bmfe), associated with down-regulation of TLR4 but upregulation of cd14, cd40, and TLR2. But in a TLR2 and MyD88-dependent manner macrophage tolerance is established creating an immunological phenotype that is responsible for human filariasis [14]. In individuals with chronic lymphatic pathology, TLRs have increased production of TH1 in patients with chronic lymphatic pathology. TLR2 and TLR9 are shown to mediate this kind of proinflammatory cytokine production in lymphatic filariasis [15]. In B cells and monocytes of filarial-infected individuals, TLRs expression was lower in the B cells but not in monocytes [16]. Filarial coinfection with latent TB has been shown to diminish the expression of antigen-specific TLR2 and 9 levels in human patients [17]. Mf-Exposed Monocyte-Derived Human Dcs (Mhdcs) Upon Infection With filarial in humans, has shown diminished expression of TLR3 and TLR4 mRNA levels along with the downregulation of various interferons and interleukins [18]. TLR2 And TLR6 are involved in the *Brugia malayi* female extract-induced inflammation [19]. TLR 2 -196 to -173 del polymorphisms is also found to be involved in the bancroftian filariasis in western tanzania.In filarial-infected individuals, TLR expression is monitored in B cells and monocytes in comparison with uninfected individuals and it was found that B cells are having less TLR expression [20]. Dendritic cells also tend to express lesser TLR 3 and 4 upon infection with live filarial parasites . This may be due to the novel protection mechanisms in the development of filarial pathology .

CHAPTER 5

TLRs and Visceral *Leishmania*sis

Abstract: Sandly bites transmit the *Leishmania* parasites under the skin, and the disease remains a major public health problem in infected countries. There are two types of *Leishmania*sis, 1) Visceral *Leishmania*sis 2) cutaneous *Leishmania*sis. Among these two types, Visceral *Leishmania*sis is fatal, and, if not treated, leads to mortality. It is observed that approximately 90% of cases come from India, Bangladesh, Sudan, South Sudan, Ethiopia, and Brazil. These diseases are caused by *L. major*, *L. mexicana, L. guyanensis*, *L. amazonensis*, *L. braziliensis*, and visceral *Leishmania*sis by *L. donovani*, and *L. chagasi*. Experimental studies in KO of TLR2 and TLR4 have shown larger lesions and higher parasite loads upon infection with *L. mexicana* than the control mice [1]. *Leishmania* DNA is recognised as a PAMP by TLR9 [2]. These parasites are rapidly phagocytosized by neutrophils, macrophages, and dendritic cells. Different parasites of *Leishmania* elicit different kinds of responses in the host, which in turn depends on the genetics and immune responses of the host.

Keywords: *L. braziliensis*, *L.chagasi*, *L. donovani*, *L. major*, *L. major*, Pegylated bisacycloxypropylcysteine.

INTRODUCTION

TLR 2 is activated upon cutaneous *Leishmania*sis and cytokine overexpression is also noted during visceral *Leishmania*sis [1, 2]. Purified *L. major* lipophosphoglycan (LPG), is a PAMP to TLR2 that activates NK cells [3]. Immunohistochemical expression of TLRs 2,9, and 4 from skin lesions of 40 patients infected with *Leishmania* (V.) braziliensis and *Leishmania*(L.) amazonensis were investigated.This study points out that both pathogens differed in their ability to induce TLRs expression. For example, *L.* (*V.*) *braziliensis* induced higher expression of TLR2 and TLR4 whereas *L.* (*L.*) *amazonensis* showed a strong correlation with the TLR9 [4], Further studies corroborate that active *Leishmania* cases display increased expression of TLR2 and TLR4 along with other cytokines such as TNF-α, IL-10, and TGF-β [5]. However, after drug treatment, there was a sliding of cells from pro and anti-inflammation even under the expression of TLRs.

Monocyte cell lines infected with *Leishmania* parasites showed downregulation of TLR2 and TLR4, causing an increase in IL-10 and IL-12p40 production [6]. The

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parasite uses this kind of mechanism for its survival, also the study showed that parasite structural integrity is important for its modulation of pathways *via* TLR2 [7]. However, at the initial stages of infection with *L.chagasi*there is an increase in the TLR2-4, IL-17, TNF-α and TGF-β mRNA expression however at later stages of infection there is a decreased expression leading to immunomodulatory events.

Some natural ligands for TLRs act as a protective mechanism for *Leishmania* infection.

For example, Pegylated Bisacycloxypropylcysteine, a Diacylated Lipopeptide (BPPcysMPEG,) provides anti-*Leishmania*l protection by inducing the IL-12, T regulatory cells and Nitric oxide in BALB/c-derived peritoneal macrophages upon infection with *L. major* [8, 9]. For any therapeutic approaches, this kind of mechanism can further be explored. Progmastigotes and amastigotes differ in their capacity to display Glycocalyx. Amastigotes do not possess the glycocalyx layer, suggesting that promastigotes would preserve this structure, whereas amastigotes lose it during intracellular transformation.

Through adaptor molecule MyD88 of TLRs produce IL-1*α* in macrophages. These findings came from the studies which were done on MyD88-/- cells (Hawn *et al.,* 2002). T cell, maturation, and DC priming are dependent on MyD88 and TLRs during *Leishmania* infection. *L. donovani*, *L. braziliensis*, *L. major,* and *L. mexicana* were found to induce DC maturation during the presence of MyD88, whereas the absence of MyD88 attenuated these process (De Trez *et al.,* 2004). Two antigens from *L. donovani* (65 and 98 kDa, in combination) in the macrophage cell line have upregulated TLR2 expression (Srivastava *et al.,* 2011). In human primary macrophages, *L. panamensis, infection caused an upregualtion various TLRs such as* TLR1, TLR2, TLR3, and TLR4 along with the secretion of TNF. Both amastigotes and promastigotes of *L. panamensis,* have failed to induce the TNF production in MyD88/TRIF$^{-/-}$ murine bone marrow-derived macrophages and mouse macrophage cell lines [10]. Interestingly, at the early stages of parasite infection, the absence of TNFa in TLR4-/- macrophage cells caused increased parasite survival. TLR2 absence does not alter the production of TNFa to the infection by *L. panamensis,* whereas TLR4, as well as endosomal TLRs, are indispensible for macrophage activation (Table **4**).

Table 4. Visceral *Leishmania*sis: Ligand and TLRs.

Ligand	TLR	Ref
L. donovani promastigotes	TLR2 and 3	Flandin *et al*., 2006
LPGs of *L. major*, *L. mexicana*, *L. aethiopica*, and *L. tropica*	TLR2 ligands	de Veer *et al.,* 2003, Srivastava*et al.,* 2013
L. donovani antigens (65 and 98 kDa, in combination)	TLR2	Srivastava *et al.,* 2011 [11]

Mice lacking MyD88, an adaptor protein of TLR pathways, seems to be more susceptible to *L. major* infection than the wild-type C57BL/6 MICE [12]. By TLR2 pathways, the lipophosphoglycan (lpg) i from *L. major* is involved in eliciting the immune response. TLKRs not only recognize protozoan PAMPs but also they can recognize bacterial and fungal ligands as well. TLR2 can be activated upon binding with T.cruzi *via* glycosylphosphatidylinositol (gpi) anchors [13]. Mice lacking TLR2,4, 1 infected with *L. major* have displayed a significant increase in parasitic burden along with large lesions by promoting TH2 immunity [14]. *L. Major* IR75 infection with MyD88 -/- is causing the susceptibility of the mice to the infection due to impaired Th1 responses [15]. TLR9 knockout mice displayed the highest susceptibility to T.cruzi infection due to the IL12/IFN responses in antigen-presenting cells [16]. Anti*Leishmania*l responses are mediated by decreased expression of TLR9 in BLAB/c mice [17]. In susceptible balb/c mice, it was observed that TLR has interdependency on each other for eliciting immune responses upon infection with *Leishmania Major* [18] .

The same observations were made in susceptible balb/c mice have elicited strong anti-Leishmanial function through CD40 along with TLR4 to *Leishmania Major* [19]. Infection of macrophages with *L. major* has resulted in reducing TLR9-induced anti Leishmanial responses by downregulating TLR9 *via* interaction with lipophosphoglycan and TLR2 [20]. C57bl/6 mice defective in TLR3,7 and 9 are tested for their capacity for autophagy in macrophages after Leishmania infection and it was found that the cells undergo autophagy and lead the path to the resistance of the parasite . In C57bl/6 Mice, TLR3,4 And 9 By inducing Interferon Interleukin responses cause resistance to the *L. Major* infection [21]. *L. major* promastigotes infection in TLR9 deficient macrophages display decreased expression of TLR1,2 and 3 also these macrophages display less CD40 and interleukin 12 expressions [22]. In macrophages treated with *L. major* infection has reduced CD 40 expression which in turn leads to reduced infection through the N-RAS activation [23]. *Leishmania major*-infected macrophages cause interleukin-dependent T regulatory cells induced anti-Leishmanial protection for pegylated bisacycloxypropylcysteine a TLR6 ligand . In mouse peritoneal

CHAPTER 6

Dengue Virus and Toll-Like Receptors

Abstract: Dengue is one of the most important arboviral diseases recorded in the world. Dengue, a public health problem in tropical and subtropical countries, is spread by female *Aedes* mosquito bites. Among *Aedes* mosquitoes, *Aedesaegypti* is the primary vector and *Aedesalbopictus* is the less infective secondary vector [1]. Dengue hemorrhagic fever (DHF) is a severe form of the disease, that causes differential expression of the TLRs in dendritic cells (DCs). TLR3 and TLR9 in DCs of patients with early onset of dengue fever were unregulated, whereas in severe cases, poor expression of TLR3 and TLR9 is observed [2]. This kind of alteration in the TLR expression during dengue may alter the clinical manifestation of the disease. However, this can be considered for further research on therapeutics.

Keywords: Human peripheral blood mononuclear cells (PBMC), NF-kB mediated pathways.

INTRODUCTION

In blood monocytes, the TLR2 is able to sense the dengue virus infection and modulate the immune responses. TLR2 inhibition to block before dengue infection resulted in the inhibition of NF-kB protein in paediatric patients. Similar studies on severe cases of dengue and other febrile illness have shown differential expression of TLRs.

In cultured hepatoma cells, induction of IFN beta abrogates the type II dengue virus infection which is mediated or activated by TLR3 [3]. This kind of protection mediated by TLR3, can be further explored for therapeutic and clinical prevention of the disease[3]. In the Human peripheral blood mononuclear cells (PBMC), dengue NS1 protein-induced IL6 and TNF production *via* the activation of TLR2 and TLR6. Further, animal studies on $TLR6^{-/-}$ mice treated with DV NS1 protein, have shown higher survivability [4].

The interaction between the virus and the receptor is very complex. Dengue virus infection leads to plasma leakage and increased vascular permeability. There are several groups trying to understand these mechanisms. One such mechanism of immunomodulatory action of the TLR 2 receptor seems to be through the abrogation of NF-kB mediated pathways during TLR2 blockage in blood mono-

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cytes [5]. Blocking TLR2 prior to DNEV infection prevents the vascular damage caused by the virus by reducing or completely preventing the activation of vascular endothelium.

During NS1 protein stimulation Endocan mRNA levels were higher in the endothelial cell lines [6]. However, the blockade of TLR4 prevented Endocan production in endothelial cells during NS1 activation. Endothelial cell monolayer integrity is disrupted by NS1 (dengue virus non-structural protein) which acts as PMAP by a TLR4-dependent mechanism [7]. This NS1 also acts like LPS-induced inflammatory patterns which are a TLR4 agonist. NS1 protein may induce chemokines and cytokines leading to a vascular leak in dengue-affected patients, thus TLR4 antagonists can offer a therapeutic option.

Almost all human cells express TLRs, especially TLR3, 7, and 9 are seen in various cells of the body such as neurons, epithelial cells, dendritic cells (DCS), lining the airway, genital tract, biliary tract, and intestine. TLRs are always vulnerable to SNPs and these SPNs contribute to the manifestation of various viral and non-viral diseases [8 - 11].

TLR9 (rs187084, rs5743836) and TLR7 (rs179008, rs179009) SNPs are significantly related to the dengue disease in comparison to non-febrile patients. Clinical cases of dengue in Veracruz, Mexico display genetic polymorphism in TLR3, TLR4, TLR7, and TLR8 [12]. The production of IL-6 and TNF-α induced by Dengue virus NS1 protein was found to be reduced when TLR2 and TLR6 receptors were blocked in human PBMC [4]. The same studies extend to animal models such as mice, has also shown the same results. In comparison with Wild type mice infected with Dengue virus NS1 protein, DV NS1 protein-treated TLR6-/- mice have displayed a higher survival rate. NS1 was also found to disrupt the endothelial cell monolayer integrity in *in vitro* model of the vascular leak by activating TLR4 in PBMcs.

Mouse models also suggest that TLR4 antagonists can reduce capillary leak [13, 14]. In comparison with DHF lower expression of LTR2 was found in DF patients. Reduced stimulation of TLR2, TLR9, and no alterations was noticed in TLR4 levels in the Monocytes of patients with severe dengue fever [15]. DENV-antibody complex infection FcR region has downregulated TLRs gene expression and upregulated various other molecules leading to increased viral load [13, 14].

As per a study conducted in human monocytic cells U937 cells, it is shown that IL-8 is secreted after dengue viral detection by TLR3 [16]. Upon the entry of the dengue virus inside the cell, not only TLRs like TLR3 and TLR7, but there are other receptors that sense the dengue virus . These two TLRs are present in endosomes. The other receptors which sense the dengue virus are cytoplasmic

retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5). RIG-I, MDA5, and TLR3 combination is crucial in limiting the dengue viral replication and host defense [16].

The knockout studies conducted in HUH-7 cells (silencing RIG-I and MDA5) have led to the high susceptibility of the cell to the virus. Similar observations were made in the same cells when TLR3 is knocked out, leading to high susceptibility. On the other hand, when these cells were silenced only for RIG-I and MDA5, high production of IFN-β was observed. Few other studies confirm the cooperation of RIG-I, MDA5, and TLR3 combination in eliciting strong antiviral responses [17]. In mouse ovarian granulosa cells stimulated with Polyinosinic-polycytidylic acid [poly(I:C)], a TLR3 agonist, lead to the upregulation of TNF-α and IL-6, and type I interferons (IFN-α/β) which are proinflammatory cytokines.TLR3 knockout studies in these cells compromised the production of antiviral responses, also complete abrogation of antiviral response was seen in MDA5/RIG-I signal blocking.

Viral recognition by TLRs triggers a cascade of signalling events such as myeloid differentiation primary response gene 88 (MyD88) and TIR-domain-containing adapter-inducing IFNβ (TRIF) pathways. The result of this pathway activation is to produce pro-inflammatory cytokines such as IFNs. This production of IFNs occurs through the activation of IRF3/IRF7 and nuclear factor-κB (NF-κB). TLR3 and TLR4 are different in sensing ssRNA and dsRNA components. For example, TLR3 binds with dsRNA components and TLR7 binds with ssRNA components. TLR7 activates the myeloid differentiation primary response gene 88-dependent signal pathway whereas TLR3 acts through the TIR-domain-containing adapter-inducing IFNβ. However, the non-structural protein recognition by TLR4 can induce severe vascular damage. Interestingly, dengue virus-infected cells can recognize the viral components through microRNAs [18]. These microRNAs can also be recognized by TLRs, in turn activating innate immune responses. Anti-dengue status can be established in cells through the production of type I IFNs, which are mediated by TLR signaling *via* the activation of transcription factors such as IRF-3, IRF -7 and NF-κB [4, 19].

CONCLUDING REMARKS

Cytokine production is the hallmark of any viral disease and dengue fever is not an exception. Endocan, is a biomarker for endothelial cell activation which result in lymphopenia, and thrombocytopenia results in disease progression. TLR SNPs are reported in various types of diseases such as malaria, TB, lymphatic filariasis, HCV, and cancer.

CHAPTER 7

Chikungunya Virus and Toll like Receptors

Abstract: Infected mosquitoes of Aedes species spread Chikungunya fever upon the biting of the mosquitoes. Chikungunya fever first came to the limelight upon an outbreak in southern Tanzania in 1952. These days almost all countries in the world are reporting Chikungunya fever. There is no vaccine for the Chikungunya virus. The infection causes severe joint pain, nausea, vomiting, conductivities, headache, and muscle pain, followed by fever. Clinical manifestations occur after 2-7 days of the mosquito bite. This chapter addresses key issues on Chikungunya viral infection in brain cells with reference to the triggering of events associated with toll-like receptors.

Keywords: Chikungunya fever (CHIKF), Chikungunya virus(CHIKV), IFN-α levels, TLR polymorphism in Dengue and CHIKV.

INTRODUCTION

Chikungunya virus (CHIKV) belongs to the family of *Togaviridae* and the genus of alphaviruses [1]. This virus is transmitted by *Aedes* mosquitoes and the disease caused by the virus became a global public health threat. In 1952 in Tanzania of East Africa first human cases of CHIKV infection have been documented since then there are a lot of countries have reported this virus with endemicity [2]. This virus targets almost all types of cells in the body, such as neuronal cells, dermal cells, fibroblasts, macrophages and monocytes [3 - 5]. Chikungunya fever (CHIKF), is a disease caused by the Chikungunya virus belonging to the family of *Togaviridae*, genus Alphavirus. This is a single-stranded positive virus, upon infection in humans the disease is manifested as myalgia and severe acute or chronic arthralgia [1, 2]. This disease also clinically manifested with immunopathology and the production of alarming levels of pro-inflammatory factors. Among the TLRs, TLR3 seems to contribute or involve in innate immune responses to various viruses such as murine cytomegalovirus, respiratory syncytial virus, influenza virus, Chikungunya virus(CHIKV) and herpes simplex virus 2 [3 - 5].

Type I interferon levels and cytokines are elevated after Chikungunya virus infection as a part of innate immune responses *via* the activation of TLRs. There are studies that explored if genetic variations can be a cause of the development

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of Chikungunya. One such genetic attribution is due to the presence of the SNPs in genes of TLR8 and TLR7. The presence of SNPs in these receptor genes leads to the patient being more susceptible to Chikungunya [6]. Not only that, possessing the SNPs also renders the cells to make more interferon-alpha. Patients with the rs179010-CC genotype have shown significant production of IFN-α levels [6].

The immunopathological events associated with the CHICK infections seem to be caused by the overproduction of TNFa, and proinflammatory interleukins IL) 1β (IL1β), IL6, IL12p70, and chemokines (CCL2 to CCL 10). The role of TLRs in these immunopathological events is decoded especially through TLRs. It was found that interleukin 27 (IL27) levels were higher in the serum of the patients with chronic chikungunya fever (CHIKF) rather than the acute ones. A study by Valtes-Lopez *et al.,* 2022 has evinced that binding of CHICKV PAMP to TLR1/2-MyD88 activates NF-κB-complex thus leading to EBI3 mRNA which is a heterodimer of IL27. The other pathways during the replicating period of the virus lead to the production of the dsRNA, this binds with TLR3-TRIF leading to the activation of IRF1. This complex in turn leads to the production of IL27 mRNA.

Cytokines and chemokines which are regulated through TLRs have shown differential expression in CSF and serum in patients with and without neurological complications. In patients with neurological complications, in CSF there was an increase in IL-6, IL-8, TNF-α, IFN-α,MCP-1, RANTES, MIG and TARC levels. In patients, without neurological complications, there was a high production of IL-17A, IL-8, IL-1β, MCP-1, RANTES, IP-10 and TARC [7]. This clearly indicates that the TLR system gets activated and sends the inflammatory responses associated with CHIKV infection. In an interesting study by Thangamani *et al.,* 2010 [7], needle-injected CHIKV has upregulated TLR3 transcription and interferon-γ in CD-1 mice, whereas uninfected and mosquito bite has downregulated TLR3 levels. This difference might be due to the composition of the saliva of the mosquito.

There were studies on TLR polymorphism in Dengue and CHIKV co-and mono infections. The study has been done by using eight SNPs of TLR8 (rs5744080-chrX:12919685, rs3764879-chrX:12906578, rs3764880-chrX:12906707) TLR7 (rs179008-chrX:12885540, rs5741880-chrX:12869297, rs179010-chrX:128 84 766, rs3853839-chrX:12889539) and TLR3 (rs3775290-chr4:186083063). In comparison to healthy controls, the co-infected patients display high CC genotypes of TLR8 and 8 SNPs thus rendering the patients to be more susceptible to coinfection [8]. Vitamins are known to establish the antiviral state in a cell, especially a vitamin D3 has been known as an immunomodulator. When the

human monocytes (VD3-Mon) and monocyte-derived macrophage cells were infected with CHICKV and Vitamin D3 it has been known to alter the TLR mRNA expression and other proinflammatory cytokines as well. This study highlights the importance of vitamins in the regulation and modulation of innate immunity through TLR signalling [5, 9, 10]. In febrile patients coinfected with Dengue and CHICKV, TLR SNPs are reported to be the CC genotype that causes the susceptible coinfections where as specific genotypes offered protection from both the viral coinfections [11]. In CHICKV-infected macrophages TLR3 induces TRIF a downstream signaling molecule that can cause IRF 1 and TIL27 mRNA expression [12]. Dengue and CHIKCV confection along with various serotypes and especially the dengue 2 serotypes was found to the circulating in the Indian population from west Bengal [13]. Asian lineage CHIKV is reported to suppress DENV replication in infants from mexico [14]. Upon investigation of TLR-induced cytokines in CHICKV patients in serum samples of human patients, it was found that various chemokines and cytokines were without any neuro symptoms. This study proves that TLRs are involved in CHICKV infections [15]. CHICKV susceptibility also increases due to the polymorphisms in TLR-7 and TLR-8 SNPs [16]. Not only that but polymorphisms in TLR3, DC-SIGN, and TNF-α genes were also reported to be involved in the CHICKV infection as reported from the patients from Nicaragua [17]. The same type of study extended to Dengue virus infection is associated with SNPS from rs3775290 for TLR3, followed by SNPS from s8192284 for IL6R, and also rs7248637 for DC-SIGN was reported from the Colombian population [18]. In human and mouse fibroblast cells it was reported that SNPS from TLR on rs6552950 region leads to the severity of the infection [19]. POLY IC an agonist of TLR3 treatment in Human bronchial epithelial cells, inhibited the replication of CHICKV [20]. *TLR7* rs3853839 in male patients causes lymphopenia and increases the risk of infection [21]. TLR polymorphism is reported to be the one form of the manifestations of the disease.

EPIDEMIOLOGY OF CHIKV

CHIKV is reported to be present in the plasma samples of children , and also it was detected in the cerebrospinal fluid of the central nervous system confirming the infection to the nervous system. This was reported from India in 2006 [22]. In La Réunion islands also CHIKV was reported in CSF and plasma the antibodies were found but the antibodies were not seen in the synovial samples and maternal milk [23]. In Salvador, Brazil, out of the many patients admitted due to the fever the screening has resulted in the identification of Zika and CHIKV in at least 20 samples [24]. In the border areas between Colombian and Venezuelan places it was identified that out of the 157 serum samples, the infection of CHIKV was found in 29% of blood samples that is 47 people. This disease was also found to

CHAPTER 8

West Nile Virus and Toll-like Receptors

Abstract: West Nile Fever is transmitted by West Nile Virus (WNV), which is a single-stranded RNS *flavivirus*. This disease is transmitted by the bite of mosquitoes. This disease is endemic in various countries in Africa, Asia, Europe and North America [1, 2]. There is no vaccine yet for this disease which is displayed by various symptoms in humans varying from neurological squealae (encephalitis) and meningitis. Apart from this, patients report fever, headache, and myalgia as well.

Keywords: MicroRNAs, MyD88, Myeloid differentiation factor 88-deficient (*Myd88*$^{-/-}$), NF-κB, STAT1, TRAF3.

INTRODUCTION

TLRs 7 and 3 can act to prevent or aggravate the pathogenicity of WNV infection. TLR3 depended on promoted entry of WNV in brain pathogenesis followed by neuronal injury in mice [3]. On the other hand, it was demonstrated that there was no difference in the susceptibility of Mice to WNV in TLR-/- and wild-type mice [4].

To effective WNV clearance upon infection, TLR7 and IL23 intact condition is required. However, TLR7 -/- and myeloid differentiation factor 88-deficient (*Myd88*$^{-/-}$) mice failed to detect the WNV which lead to a further increase of viremia causing the susceptibility [5]. The resident tissue macrophages such as kupffer cells in the liver and microglia in the brain, sense the WNV by TLR and through the IL-23 signalling pathway they recruit more immune cells to the infected organs. This mechanism is compromised in TLR7-/- mice leading to high viremia. This TLR7 and IL23 interaction are necessary for immune cell homing to the affected /infected cells, thus pharmacotherapy can consider these molecules for a therapeutic approach to WNV infection.

West Nile virus was infected in equine PBMCs and was analyzed for the presence of TLR signature. The findings revealed the upregulation of TLR1, 3, 5, 7-9 transcripts followed by TRAF3, MyD88, STAT1, NF-κB, and 2, ISG15, IRF3, and 7, SOCS1, and 3 in the infected cells in comparison with the control cells [6]. Stingingly, post-infection of 24 hrs raised the viral titer in the cells but further

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replication of the virus was stopped in the subsequent hours. The rise of the TLR genes and their subsequent signal transduction proteins could be the reason why the replication of the virus was not noted after 24 hrs in equine PBMCs [6].

TLR8 is not explored properly for its role in WNV infection. A study found that the TLR8-/- murine cells were resistant to the WNV infection [7, 8]. TLR7 mediates antiviral responses against WNV. Overexpression of *TLR7* and IFN-stimulated gene-56 expression was observed in TLR8-/- mice. This overexpression of *TLR7* has caused the effective clearance of the WNV virus. SOCS 1 is associated with TLR8 and not with TLR7, the selective knockdown of SOCS 1 resulted in higher IFN responses followed by *TLR7*expression [7, 8]. These findings clearly indicate that TLR8 is blocking the expression of TLR7-mediated WNV clearance and anti-viral responses by coupling with SOCS1.

Human studies on WNV infection and the responses of the primary human macrophages have shown surprising findings. In humans, the elderly are very prone to WNV infection causing death in them due to poor immune responses. When the primary human macrophages of the elderly and young donors after MWN infection were investigated it was found that there were higher TLR3 levels and cytokine levels in the elderly than in the young donor cells [9]. This is due to the presence of a signal transducer and activator of the transcription 1 (STAT1)-mediated pathway. Glycosylated WNV envelope protein from WNV upon binding with C-type lectin dendritic cell-specific intercellular adhesion molecule 3 (ICAM3) and DC-SIGN cause the downregulation of TLR3 this is mediated *via* STAT1. In elderly patients, there is a cytokine storm due to the high levels of TLR3 and it leads to blood-brain barrier disruption which causes these brain infections of WNV.

MicroRNAs and West Nile Virus

MicroRNAs have been investigated and found to be very reliable biomarkers for TLR activation. A study has been conducted in HEK293-NULL and HEK293-TLR3 cells infected with WNV to understand the pattern of innate immune responses. NF-kB gene signature revealed that TLR3 was solemnly responsible for WNV-induced gene signature. The study further explored the miRNA expression events in the TLR3 knockout condition. There were 70 microRNAs produced upon WNV infection *via* TLR3 signaling. These microRNAs were found to be involved in regulating cell death, viral pathogenesis, and immune cell trafficking [10].

TLR3 participates actively in triggering the pathogenesis of the West Nile Virus. In the brain, the neuronal injury to the WNV is caused by the leaking of blood-brain barrier by the activation of TNFa produced *via* TLR3 [3, 11]. TLR3 seems

to do cross-priming of immune cells, which plays an important role in CD8+ T cell responses to proteins. TLR3 activation is a must for the antigen-presenting cells such as dendritic cells to act, as this activation increases the phagocytosis of infected material [12]. In the CNS, the knockout of TLR3 and the intracranial injection of WNV caused increased viral replication in primary cortical neurons [13].

MyD88-/- and TLR-/- and WNV infection

When compared to wild type mice MyD88/Trif−/− displayed high viral burden leading to increased susceptibility to the virus [14]. Inflammatory events, microglail activation and astrogliosis were also witnessed due to high viral burden [14]. TLR3 and microRNAs role have been investigated in the WNV infection in HEK293-NULL and HEK293-TLR3 cells.In a TLR3 independent manner microRNAs (70 of them) have been induced which are responsible for cell death, immune cell trafficking and viral pathogenesis [15]. Upon investigation with TLR7-/- and MyD88 deficient mice after WNV infection they had reduced interleukin responses [16]. TLR3-/- mice is investigated for its role in west nile virus infection.In primary cortical neuronal cultures this deficiency of TLR3 is causing enhanced viral replication. In CNS tissues deficiency of IRF 7 leads to increased viral load and also in various cells such as cortical neurons, DCs, fibroblasts, and macrophages [17]. Various TLRs mRNA were increased along with signalling pathway genes in equine cells upon exposure to west nile virus infection [18]. There are studies which prove that genetically knockout conditions with TLRs to WNV, has increased viral load and increased susceptibility to the infection [19 - 22]. Not only TLRs but RLRs pathways are also involved in controlling the infections caused by WNV [23]. Following intradermal injection of WNV in TLR7-/- mice, Langerhans cells were reduced not in TLR7 minus mice but in wild type mice. Even the infection with WNV was higher in keratinocytes of TLR7 minus than the wild type keratinocytes. TLR7 modulates the expression of various cells in the body by that it can contribute to the systemic infection [24]. In old (21- to 22-month) mice the susceptibility to WNV virus was assessed in comparition with young (6- to 10-week-old) mice. Impaired TLR signalling in old mice contributes to the loss of innate and adaptive immune system that lead to susceptibility to the virus [25]. NS4B-P38G mutant west nile virus was investigated in the MyD88 -/- and TLR7 -/-mice and it was found that they were more susceptible than the wild type mice [26]. Upon WNV infection, TLR3 knockout mice were very much resistant to the virus. Not only resistant but it also has increased viral load in various tissues including brain causing neuropathological changes [27]. When exposed to west nile virus, TLR3-/- neuronal cells were showing increased expression in comparison with wild type cells upon west nile virus infection. Further, there was increased viral burden was

CHAPTER 9

Japanese Encephalitis and Toll-like Receptors

Abstract: Viral encephalitis is a major pathological situation. It can be caused either by DNA or RNA viruses. Japanese encephalitis belongs to the member of *flavivirus* and it is a mosquito-borne disease, causing viral disease. Japanese encephalitis can be prevented by a vaccine. TLR3 and TLR4 signal pathways are activated due to JE Japanese encephalitis infection. TLR3 and Retinoic acid-inducible I also participate in mediating inflammation owing to Japanese encephalitis infection. In this kind of virus infection first, the cells are infected, causing primary viremia, subsequently infecting the CNS tissues as well. More than 60% of the world's population is living in JE endemic places.

Keywords: CNS inflammation, Flavirius infections, Murine microglia cells, TLR7 mRNA levels.

INTRODUCTION

Due to climate change and global warming, this disease is now spreading to various parts of the world where previously it was not reported [1]. Approximately 60% of the population is living in endemic countries. There are four genera of *flaviviruses* that are very lethal to human beings, namely, *Pegvirus*, *Pestivirus*, *Flavivirus*, and *Hepacivirus*. Viruses such as dengue virus (DENV), West Nile virus (WNV), Zika virus (ZIKV), and Japanese encephalitis virus (JEV) are pathogenic to humans and fall in *flaviviruses*. As per WHO, approximately 67,000 cases occur in JE endemic countries every year despite vaccination progress [2]. JE can cause mortality in 30% of cases, and survivors of this disease face cognitive impairment, and motor paralysis as a result of neurological squeal [3].

TLRs in Japanese encephalitis (JE) have not been well explored. There was a study in which TLR knockout mice displayed differential immune responses [4]. It was found that TLR3 -/- was highly susceptible to JE infection whereas TLR4-/- mice displayed resistance.In most cases of flavirius infections, TLR 7 plays the first line of defence by recognising the pathogen. In systemic infection, TLR7 activation produces Type I interferon production, which in turn protects the mice from the JE virus by creating an antiviral state [4]. Post-JEV infection, TLR7

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mRNA levels were elevated,this further was confirmed by TLR-/- mice, wherein TLR7-/- mice displayed a very high viral load [5]. TLR8 was found to be highly regulated in TLR7-/- knockout mice, which may act as a parallel immune protecting effect to JE infection in the absence of TLR7. TLR polymorphism plays an important role in the susceptibility of JE patients to the virus. For example, Leu412Phe polymorphism, with high frequency was noted in JE patients in contrast to the control group [6].

Type I interferon production creates an antiviral state in the JEV-infected cells through TLR7 thus offering a protective effect [7]. Several studies on TLR-/- were done in mice to understand the effects of JEV. Mice with TLR 3 KO condition display high susceptibility whereas mice with TLR 4 KO condition have shown high resistance to JE. These differences primarily lie in eliciting pathological reactions. Severe CNS inflammation and infiltration of monocytes ($CD11b^{+}Ly\text{-}6C^{high}$) were attributed to the increased viral burden in the TLR3 KO condition. In addition, there was increased Blood Brain Barrier permeability leading to cytokine and chemokine expression in various cells [4]. On the other hand, TLR4 KO mice display less CNS inflammation by reduced pro-inflammatory cytokine expression characterised by less viral burden and little leukocyte infiltration. Although JE can be curable by Vaccination, still much research needs to be done on its interaction with the immune system. Toll-like receptors 7 and 8 play an important role in sensing the viral components of JEV thus priming the immune cells [8]. In brain cells such as microglial cells TLRs 3, 2 and 7 interact with the JE virus [9].

In murine microglia cells, KO of TLR3 and infection with JEV lead to high viral load [10]. TLR3 and retinoic acid-inducible gene I (RIG-I) in microglia cells play an important role in JEV pathogenesis. These two molecules can recognise ds RNA which in turn leads to the upregulation of extracellular signal-regulated kinase (ERK) and p38 mitogen-activated protein kinase (p38MAPK) along with TLR3 expression. These molecules were abrogated along with activator protein 1 (AP-1), and nuclear factor κB (NF-κB) when TLR3 and RIG-I were attenuated. The effects of TLR 3 and RIG 1 KO is: Increased viral proliferation and reduction of TNF-α, IL-6, and CCL-2 secretion usually induced by JEV [10].

Endosomal compartments have TLR7 and TLR8, these receptors sense the ssRNA viruses. Along with TLRs, RNA helicases also take part in sensing the viral accumulation in the cytosol. For example, RIG-I (retonic acid inducible gene -1) recognises all RNA viruses such as paramyxoviruses, influenza virus, and Japanese encephalitis virus and other viruses such as paramyxoviruses and influenza virus [11, 12]. JEV also stops the cross-presentation of both soluble and cell-associated antigens by activating the TLR2-MyD88 and p38 MAPK-

signalling [13, 14]. This suppression of cross-presentation can lead to diminished $CD8^+$ T-cell responses to antigens. In order to plan an effective vaccine strategy, understanding these TLR-mediated responses would be better.

JEV and Inflammation Through TLRs

During systemic infection, using knock-down mice (TLR7(KD), it was demonstrated that JEV infections lead to the production of type I IFN production leading to protection by establishing an antiviral state.The role of microRNAs has been also identified in causing microglial activation in the Japanese Encephalitis virus (JEV) infection. In JEV-infected microglial cells, let-7a and let-7b (let-7a/b) have been overexpressed leading to the caspase activation and cell death which was mediated by the NOTCH-TLR7 pathway [15]. In human microglial cells also the same kind of observation was made that the JE virus uses microRNAs to achieve cell death [16]. In a study, JEV-infected neuro2a cell line and adult mice were used to understand the patterns of innate immune mechanisms. In TLR3 gene silencing conditions in both cell lines and mice, there was a higher replication of JEV irus with MyD88 and IRF 7 overexpression [17]. CNS of JEV-infected mice upon microarray studies showed higher expression of TLR2, TLR4, and TLR7. In humans, upon mild and severe infection with JEV, it was found that significant upregulation of chemokine and TLR7 was observed that may be considered as significant tools for viral inflammation [18]. Compensatory roles for TLRs knockout were also identified in mice. It was found that in TLR7-/- mice, there was a significant upregulation of TLR8, indicating that there is a compensatory role created by the TLR7 knockout condition. Among mild and severely infected JE patients it was shown that differential expression of Chemokine ligands and TLRs are noted as higher. Using the infected dendrite cells and macrophages the JE virus enters the brain through newly produced virion particles. Culex mosquitoes spread the JE infection from bird to bird and also to other animals and humans in tropical countries [19]. In the temperate regions vertical transmission of JEV is also higher [20, 21]. In $CD8^+$ T cells, TLR2-MyD88 and p38 MAPK signal pathway mediate the cross-presentation of soluble antigens from the JE virus [22]. Dendritic cells were impaired due to JE infection with very poor CD 4 and CD8 cell responses. These effects are mediated by both MyD88-dependent and independent pathways leading to viral survival. In macrophages JE induced chemokine and proinflammatory cytokines mRNA.

MicroRNAs and JEV

There was an involvement of microRNAs in JEV infection to regulate the inflammation creased by JEV virus. The *in vitro* and *in vivo* studies on miRNA show that miR-19b-3p has been elevated in the cultured cells and in mice brain

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