Chikungunya infection and immunity: an overview

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Abstract: Background: Chikungunya infections are a major concern because of their persistent recurrence in the last few decades. Chikungunya virus (CHIKV) is a mosquito borne alpha virus that causes an acute febrile illness accompanied by body rashes, myalgia and polyarthralgia; which may persists for years. Chikungunya fever, affect millions of people in Africa, Asia, including the Indian subcontinent and lately in several regions of the Americas. No specific antivirals and vaccines are currently available to treat or prevent the disease.

Methods: Understanding the mechanisms of host immune responses to CHIKV and the immunopathology of the disease is essential for the development of vaccines and diagnostics. Many studies have demonstrated the role of the immune response in the immunopathology of the disease and in the host’s incapability to clear the virus efficiently. In this review the excellence of the referenced papers were evaluated using standard tools.

Results: Total seventy-four papers were included in the review. Majority of them were highlighted the importance of understanding the immunopathology of virus, contributing factor and control measures of the epidemics. Nineteen papers were provided the current occurrence and re-emergence of the disease. The diagnostic importance (sensitivity and specificity) of developed diagnostics for the early detection were also provided in eight papers.

Conclusion: In the current review we highlighted the importance of conceptual mechanism of the host immune responses to CHIKV and the immunopathology of this alpha virus. The presented review provides an update on the infection, its vector, and the disease transmission, research development, and administration for avoidance of Chikungunya disease.

Keywords: Chikungunya virus, transmission, infection, Aedes mosquito, immunity, diagnosis

1. INTRODUCTION

Chikungunya virus (CHIKV) is an alpha virus; that causes an acute infection characterized by high fever, body rashes and polyarthralgia [1]. The disease is usually self-limiting, but in some patients, debilitating joint pain can persist for years. The word Chikungunya signifies "twists up" in the Makonde dialect of northern Mozambique and southeastern Tanzania. In 1953, it was first isolated from the blood sample of infected patients in Tanzania [2]. The infection has been identified as the etiologic operator of sporadic epidemics in Africa and Asia. In 2004, it was reappeared in Indian ocean islands, Italy, and France [3, 4]. In the year 2006, CHIKV infection was re-emerged in Southeast Asia, infected 1.3 million humans [5]. The flare-up of the disease is further confirmed due to single virus mutation, the vector finds better approaches to disseminate and the host needed immunity to conflict the disease. CHIKV continues to cause large epidemics outbreaks worldwide. The increased frequency of chikungunya outbreaks in the past few years described its more severe forms than the previously reported ones [6]. The current outbreaks of CHIKV, Zika virus and Japanese encephalitis [7] highlighted the importance of understanding the immunopathology of virus, contributing factor and control measures of the epidemics. Currently, no specific treatment or vaccine is available to prevent the disease, however, several candidates are under development. In this review, we are providing an update on the infection, its vector, and the disease transmission, clinical characterization, research development, and administration for the avoidance of Chikungunya disease.

2. EPIDEMICS: PAST AND PRESENT

The first outbreak of Chikungunya was reported, in 1953 in Makonde, Tanzania [1]. In Asia, the first outbreak was reported in Thailand in 1958 [2]. The following years, CHIKV re-emerged causing massive outbreaks in different regions of Eastern, Southern and West Africa [8]. Between 1999 and 2000, 50,000 cases were reported in Congo [9] and 1,722 cases were reported on the French island of Reunion in the Indian Ocean between 2005 to 2006 [10]. In India, the first outbreak of Chikungunya occurred in 1964, affecting...
man in Pondicherry, Chennai and Vellore, which was followed by another outbreak in Nagpur and Barshi in 1973 [5]. According to the National Vector Control Board, New Delhi, the disease has re-emerged after 32 years in 2006 and affected many states of India that includes Andhra Pradesh, Andaman and Nicobar Islands [11]. In 2013, the spread of Chikungunya was reported for the first time in the United States of America with 66 confirmed cases and around 181 suspected cases by the end of 2014 [12]. In 2014, the Centers for Disease Control and Prevention reported the first Chikungunya case in Florida among the travelers returning from the affected areas [6, 13]. By the year 2014, the Pan American Health Organization (PAHO) reported thousands of suspected Chikungunya cases in the Caribbean [14] and in the year 2015-2016, a total of 3,49,936 suspected and 1,46,914 confirmed cases were documented by PAHO [15] (Fig. 1).

3. THE VIRUS, THE VECTOR AND DISEASE TRANSMISSION

3.1. The Virus and Genome

CHIKV is an enveloped RNA alphavirus belongs to the family Togaviridae. The single-stranded positive-sense genomic RNA is approximately 12kb long acts as mRNA for the synthesis of nonstructural polyproteins (nsP1–nsP4). A subgenomic RNA referred to as 26S RNA translates into the capsid protein (p130 polyprotein) and four distinct membrane-embedded proteins (E3, E2, 6K and E1) [16]. Like other alphaviruses, the genomic organization of CHIKV is also considered to be: 5’ capnsP1- nsP2-nsP3-nsP4- (junction region)-C-E3-E2-6KE1-poly (A) 3’ (Fig. 2). A specific mutation was detected in E1 (Ala226Val) envelope protein in some tropical CHIKV isolates from the Indian Ocean outbreak. This specific mutation had immense significance in the epidemiology of viral transmission by the mosquitoes. Initially, the mutations were absent, but later >90% of mutations were observed in the viral strains [17] that might be one of the reasons for the progression of chikungunya epidemics. It is also demonstrated by Schuffenecker et al. that the genetic change at position E1: A226 V reduce their cholesterol dependence to infect mosquito hosts and might helped in the replication and transmission of CHIKV [3, 18].

The E1 and E2 envelope protein are modules of spikes and extend the viral surface in the pattern of membrane-anchored types. These spike proteins facilitate attachment to host cell surfaces and viral entry into the mosquito midgut. The E1 is a type II glycoprotein that mediates low pH-triggered membrane fusion during virus infection [19]. The E2 envelope protein, on the other hand, is a type I transmembrane glycoprotein, that is responsible for receptor binding during the course of the alphavirus attachment to the host cell [20]. The proficiency of alphavirus entry depends on the level of cholesterol composition in the host cell membrane. The E1 (Ala to Val) mutation at position 226 increases the viral infection for A. albopictus and leads to the proficient propagation into the midgut epithelium, that is one of the core sites of infection to CHIKV isolates [21, 22]. Interestingly, this mutation did not affect the viral replication in A. aegypti [21]. Recently, other mutations have been identified in E2 proteins that regulate CHIKV adaptation inside the mosquito. E1 and E2 proteins are liable for the
3.2. The Vector

*Anopheles* are the main mosquito species likely for the transmission of disease in Indian epidemic, Indian Ocean islands and in Asia [3, 24]. However, *Aedes furcifer*, *A. luteocephalus*, and *A. taylori* are accountable for disease transmission in Africa [8]. *Anopheles* is a prime circulating vector species in the rural regions of Orissa and Madhya Pradesh and *Aedes albopictus* in Tamil Nadu and Southeast Asia [10, 11]. Due to the wide abundance of this vector, the transmission rate of infection is also very high.

An enzootic sylvatic cycle and an endemic/epidemic urban cycle are two distinct transmission cycles reported for CHIKV. The sylvatic cycle involves a number of arboreal *Aedes* mosquitos’ species as vectors (*A. furcifer*, *A. vittatus*, *A. fulgens*, *A. luteocephalus*, *A. dalzieli*, *A. vigilax*, *A. camptorhynchites*) and non-human primates as reservoir hosts [25]. *A. furcifer* vector transmits the virus from monkeys to humans [8]. Numerous characteristics make *A. aegypti* an efficient vector for CHIKV due to its high susceptibility to the virus, preferences to survive near to human habitat and blood meal requirements during the day time [25].

3.3. Phylogensis

Genetic studies of CHIKV suggest that its origin was in tropical Africa [26], and later on, it was described to have three genotypes such as West African, East/ Central/ South African and Asian genotypes [3]. Out of these genotypes, Asian genotypes were found to have a high degree of nucleic acid homology among themselves, whereas African strains showed wider sequence diversity due to genetic microevolutions during the course of epidemics [27]. One strain named East Africa genotype was isolated from the epidemic took place in the year 2005 in Indian Ocean islands which are currently circulating in India [28]. On the other hand, past outbreaks in India occurred due to Asian genotypes. Three distinct CHIKV phylogroups known to exist which were the partial E1 sequences from African and Asian isolates. The first phylogroup contained isolates from West Africa, second phylogroup were the isolates from Asia, and the third one corresponds to East, Central, and South African isolates [3]. All alphaviruses are antigenically related and exhibit a worldwide dispersion.

3.4. Interactions and Transmission between Virus and Vector

There are numerous theories proposed which explain the 2006 epidemic in the Indian Ocean islands and India. Approximately 90% of viral sequences from there had E1 gene mutation at 226 positions [21] that permit the virus for invasion and then thriving inside the cells lacking cholesterol (e.g. Mosquito cells) [19]. Thus, an increased chance of infecting vector (*A. albopictus*) leads to higher probability of virus transmission in humans. The travelling of Chikungunya confirmed cases from India to the neighbouring countries having a prevalence of filarial parasite infection could be the major factor in re-emergence of Chikungunya [29]. Importantly, the affected population lacked herd immunity.

*Anopheles* vector being a short distant flyer is unable to spread infection across any large geographic area; hence people mobilization from one part to other part of the world due to trade and industrialization is probably mostly responsible for the broad diffusion of the virus. The epidemic in the Indian Ocean islands of Comoros, Mauritius, Seychelles and Reunion lends credence to this suggestion. These islands due to their popularity attract about 1.5 million tourist every year [30], and in the year 2005 after a major epidemic several tourists while returning from these islands to Italy [31], southern France and Spain were found viraemic. Concerns have been voiced about the establishment of *A. albopictus* in southern Europe, which would help the virus to find its way into the territories which were previously uninfected.

3.5. Virus Propagation and Target Organs

CHIKV enters the subcutaneous capillaries post-intradermal inoculation by infected mosquitoes. Its replication starts by infecting susceptible cells such as macrophages or fibroblasts and endothelial cells. During an incubation period followed by 3 to 7 days, blood circulating viral load increases rapidly and reached more than $10^{10}$ copies/ml, which is not common in other viral infection. Through the circulatory system, viral particle is transported to secondary lymphoid organ and then dispersed to different organs, including liver, muscular tissue, joints and brain. Due to the abundance of macrophages in these tissues, it acts...
as Trojan horses for the virus to spread to other parts of the body (Fig. 3). Tissue-specific pathological events include hepatocyte apoptosis and adenopathy, whereas mononuclear cell infiltration and viral replication in the muscles and joints are associated with severe pain, with some patients presenting arthritis [32].

### 3.6. Clinical Features and Complications Associated with Chikungunya Infection: A Two-Stage Disease

Chikungunya infection is described on the basis of information obtained during various epidemics throughout the world at regular intervals (International Classification of Diseases-10, code A.92.0). Febrile illness, a major clinical symptom of CHIKV infection is clinically related to the symptoms of Dengue virus infection [32]. CHIKV infection starts with an initial silent incubation period of approximately 2 – 4 days (range 1 – 12 days) (Fig. 4). It is a self-resolving disease and fever can be controlled using mild antipyretics.

### 3.7. The Acute Stage

After CHIKV infection, the symptoms appear within 2 to 6 days and the symptoms start abruptly which lasts for about a week till immunity plays its role to overcome infection. The acute phase is set for the first 10 days after onset of disease [33]. The most frequent symptoms include high fever, arthralgias, back pain and headache (World Health Organization 2015). A high fever, responsive to antipyretics, sickness accompanied with intense fatigue, anorexia, myalgias, nausea, and vomiting in adults, and even transient confusion in elderly patients are the symptoms. In some cases, asymptomatic infection of Chikungunya was also reported [34]. Peripheral joints especially interphalangeal joints, wrists and ankles usually very painful and swollen. Some of the other symptoms include transient maculopapular rashes, sometimes edematous can be observed on the trunk of half of the infected patients [35]. In addition, miscellaneous cutaneous and mucosal changes have been described during the acute phase of the disease viz. photosensitivity, stomatitis, oral cavity ulcers, exfoliate dermatitis, vesicles, bullae, purpura [33] and gastrointestinal symptoms are common. Initial biological changes are transient such as leukopenia and lymphopenia, mild thrombocytopenia, low wage increase in C-reactive protein, and hepatic cytolysis [36]. The most frequent complications are convulsions, meningoencephalitis, and Guillain-Barré syndrome, in which the direct role of CHIKV is evident during early manifestations and sometimes minor bleedings were also observed [37]. Adults and children share a similar clinical resemblance to CHIKV infection but sometimes in children, it gets complicated by neuropsychological changes, including lethal meningoencephalitis with myocarditis or extensive epidermolysis [38].

### 3.8. The Chronic Stage

The symptoms of the chronic stage are related to that of high CHIK viral load in the acute stage. After infection, chronic stage in an adult can persist for months or even years, while they are not common in children [39]. During the initial
3 months, patients show discomfort, inflammation in the joints and tendons and an increased impairment in daily life [40]. The impairment in the extremities occurs due to severe polyarthritis involving most distal joints, and multiple hypertrophic tenosynovitides that are sometimes responsible for carpal or tarsal tunnel syndromes [41]. Reversions are common during the chronic stage, often triggered due to the low-temperature exposure that includes mild fever, increased inflammation of joints, and sometimes also affects new joints [42]. Chikungunya infection induces an inflammatory response similar to rheumatoid arthritis [43, 44]. Continuous inflammation of joints in response to viral antigen causes arthralgia, evidenced by the fact that the viral RNA has been detected in perivascular macrophages up to 18 months [42].

The persistence of Chikungunya infection during the chronic phase, stimulate a large number of cytokines, chemokines, growth factors and a variety of interleukines II-6 and IL-15 in the plasma of infected individuals [45]. IL-1 alpha and TNF-alpha shows a synergistic effect on the production of IL-6 and IL-8 [46]. To promote the immune cell activation, Th1 cytokine response is crucial; however, they might cause tissue injuries. Increased IFN-γ and IL-12 also shows synergistic effects to promote innate immune cell activation in the majority of CHIKV-infected patients. Although, regulatory inflammatory response prevents the establishment of a chronic disease, any dysregulation of inflammatory response may lead to the development of chronic arthralgia or arthritis.

3.9. Chikungunya Virus Life Cycle in Host Cells

Alphaviruses enters into the target cells by endocytosis with the help of some receptors such as dendritic cell-specific ICAM3-grabbing non-integrin 1 (CD209), liver and lymph node-SIGN (L-SIGN), heparin sulfate, laminin and integrins; which have been implicated in this process, but their exact roles have not been defined well [47]. Inside the endosomes, conformational changes in viral envelope take place due to an acidic environment that exposes the E1 protein of virus to mediate virus-host cell membrane fusion. This results in cytoplasmic delivery of the core, thereby releasing viral genome. The precursors of non-structural proteins (nsPs) are translated from the viral mRNA, and its cleavage produces nsP1-nsP4. nsP1 causes deduction of negative strand of viral RNA and has RNA capping properties [48], nsP2 is having RNA helicase, RNA triphosphatase and protease properties; thus involved in the shutting off the host cell transcription [48], nsP3 is part of the replica unit and nsP4 is the viral RNA polymerase. The assembly of these proteins constitutes viral replication complex which synthesizes a full-length negative-strand RNA intermediate.

This functions as the template for the deduction of both subgenomic (26S) and genomic (49S) RNAs. The subgenomic RNA drives the look of the C–pE2–6K–E1 polyprotein precursor, which is processed by an autoproteolytic serine protease. The processing of mRNA leads to the generation of pE2 and E1 glycoproteins which are exported to plasma membrane via Golgi complex where pE2 gets cleaved into E2 (involved in receptor binding) and E3 (pE2 folding and its subsequent association with E1) with the release of capsid (C). Binding of viral nucleocapsid to the viral RNA and recruitment of the membrane-associated envelope glycoproteins leads to the formation of viral assembly where these assembled alpha virus particles having icosahedral core starts budding on cell membrane [49].

3.10. Host Immune Response to Chikungunya Virus

Innate immunity is the first host defense mechanism against virus multiplication in target organs. Infected monocytes play critical role during the early phase of CHIKV infection and migrate while differentiating in inflammatory macrophages to the synovial tissues of chronically CHIKV-infected individuals to limit virus replication [42]. On the other hand, migrating infected monocytes can also propagate infectious virions in to the nervous system and other organs, leading to the development
of clinical manifestations. Wauquier and colleagues (2011) demonstrated that CHIKV infection in humans prompts strong innate immune responses involving over production of IFN-γ together with inflammatory chemokines and cytokines [50]. During the acute phase of chikungunya infection, IFN-α detected on the first day of viral infection and its concentration is correlated with plasma viral load, which is significantly higher in elderly patients [51]. While plasma concentrations of Th1 and Th2 cytokines remain low, the inflammatory response to CHIKV infection most often leads to virus elimination and clinical recovery [45, 52].

Chikungunya Fever (CHIKF) is a self-limiting disease, with a duration of clinical symptoms of 7-10 days. Viral clearance is associated with a strong adaptive immune response that confers protection against re-infection. Nevertheless, in some instances, chronic disease including arthralgia may occur. In some cases, chronic symptoms remain even after virus clearance. During the CHIK outbreaks in the La Réunion, various markers of inflammation (IFN-α, IL-6, monocyte chemotactic protein-1/CCL-2, IL-8, and matrix metalloproteinase-2) were found in the synovial fluid of a patient suffering from chronic pain [45]. Hoarau et al. (2010) also reported higher plasma concentrations of IL-12 and IFN-α mRNA in blood mononuclear cells in patients during and even after the convalescent phase for up to 6 months to a year after infection [42]. Wauquier N (2011) and Teo TH (2013) have also documented increased levels of Th1-type cytokines during chronic disease [52, 53]. During this period, active regulatory mechanisms responsible for the resolution of inflammation take place [54].

3.11. Differential Diagnosis of CHIKF

Chikungunya is accompanied by anaemia, leukopenia and high serum aminotransferase, but unfortunately, none of these laboratory findings are specific for the diagnosis of chikungunya infection [54]. The specific diagnosis of Chikungunya can be obtained by serological tests, molecular methods or viral cultures. Serological tests detect anti-CHIK antibodies using IgM enzyme-linked immuno-sorbent assay (ELISA) [55]. However, anti-CHIK IgM antibodies appear only 4-5 days after the onset of fever, and thereafter fever gets settled in most of the cases. In addition, paired sera (acute- and convalescent-phase serum spaced at least 2 weeks apart) are needed for accurate serological diagnosis.

The usefulness of peptides for the diagnosis of viral, bacterial, parasitic and autoimmune diseases has been shown by many researchers [56]. Synthetic peptides offer the advantage of enhanced specificity and eliminate non-specific reactions by keeping off the cross-reactive sequences from other arboviruses. In the previous work, we have developed a peptide-based detection of anti-CHIK antibodies with improved sensitivity and specificity than the existing serological assays using the specified sequences [57, 58].

Molecular diagnosis of Chikungunya by RT-PCR is also another strategy adopted by many researchers these days. Patients with Chikungunya tend to have viraemia that can stay in the bloodstream up to 6 days. The virus can be detected by PCR [59]. In one of the surveys, a single step RT-PCR was developed using 2, 7-diamino-1, 8-naphthyridine derivative (DANP) - labeled cytosine bulge hairpin primers to amplify the nsP2 (non-structural protein) region of CHIKV genome that is a potential clinical molecular diagnostic assay for CHIKV in acute phase patient’s serum [60]. Parida et al. reported the diagnostic accuracy of reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay targeting the E1 gene [61]. Viral culture, the reference standard for the diagnosis of Chikungunya infection [62] is expensive, time-consuming and technology-intensive, and thus cannot be applied in field settings.

3.12. Treatment and Preventions

Various advances have been contracted to produce an efficient vaccine against CHIKV infections. Unfortunately, no vaccine or specific antiviral drugs are currently available for CHIKF. Taking rest, plenty of fluids and the medications such as ibuprofen, naproxen, acetaminophen, or paracetamol is the only treatment to relieve the discomfort of fever and pain. Chloroquine phosphate was reported to be efficacious in the treatment of chronic CHIKV arthritis [63]. Ribavirin, an antiviral agent was used to treat CHIKV induced arthritis in a small cohort of patients and was found to be beneficial in resolving joint and soft tissue swelling [64]. Briolant et al. [65] reported a synergistic effect of ribavirin and interferon-α in the inhibition of CHIKV and SFV replication in cell culture. Recently, Rulli et al. [66] also reported the beneficial effects of bindarit, a small molecule anti-inflammatory drug in rodent model to effectively treat CHIKV infection induced arthritis. Another drug Arbidol, which is used against influenza and other respiratory infections was also found efficacious in inhibiting CHIKV replication in Vero and MRC-5 cell lines (IC50<10μg/ml)b [67].

In a recent development, Immunization with virus-like particles (VLPs) and DNA vaccines caused immunogenicity and thus found to protect the mice and non-human primates against CHIKV infection [68]. Many compounds have been reported that interferes with virus replication by the inhibition of heat shock protein 90 (HSA90) and other cell signaling pathways [69, 70]. In our previous work, we developed a peptide-based immunogen using selected immunodominant B and T cell epitope from E1, E2 and Capsid proteins of CHIKV using two permissible adjuvants encapsulated in nanoparticles and showed high humoral and cellular responses. The antibodies generated were found to be neutralizing during in vitro assay. This was further modified as multiple peptide-based immunogens using many B and T cell epitopes to enhance the immunogenicity [71].

Recently, Chattopadhyay et al. developed a chimeric vaccine using a vascular stomatitis virus (VSV) backbone and CHIKV structural proteins (VSV [increment]G-CHIKV) [72]. This VSV [increment] G-CHIKV chimeric virus induced a good neutralizing antibody response in mice against CHIKV infection. Recently, another vaccine is undertaking for preclinical trials in non-human primates that were designed by Plante et al. after presenting the EMCV IRES sequence into the CHIKV subgenomic promoter region [73].
CONCLUSION AND FUTURE PROSPECTS

The enzootic East, Central and South African (ECSA) lineage in Africa was affected by explosive Chikungunya outbreaks in the 1950s and later in 2004. A. aegypti and A. Albopictus, the two vectors that probably facilitate endemicity in most regions of the tropics and subtropics had affected the large population in many adolescent regions in the predictable future; although the essence of the disease is typically hard to estimate because CHIKV and DENV infections show overlapping clinical symptoms [74].

The persistence of CHIKV in joints may, therefore, contribute to chronic local inflammation, causing severe pain. High plasma levels of IL-12, IFN-α [42] and high levels of Th1-type cytokines [52] in the sera after the recovery phase has been identified in many patients even a year after the chronic infection. Regulatory mechanisms seem to be taken to anticipate the formation of chronic disease, weeks or even months after viral clearance from the lineage. Thus, it would be interesting to determine the cytokine profiles associated with chronic disease in a large group of infected patients. The modifications in these immunological profiles if any, would help us to develop a powerful analytical tool, facilitating preventive and targeted immunomodulatory treatment to the infected patients.

Future emergence from enzootic African cycles will also remain a threat that is increasing more with air travel and international commerce. Similarly, the deployment of promising human vaccines/ newer drugs probably offers the best promise for creating a major impact in restricting CHIKV circulation and preventing human disease. Further, the diagnostic accuracy for Chikungunya infection can help us in early detection of disease without any cross-reactivity with other viral diseases. Thus, it is an urgent need to implement strong evidence-based interferences to help in preventing future epidemics of Chikungunya infection.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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