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Review Article

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CATASTROPHIC EFFECT OF *Aedes aegypti*: AN INSECT BORNE VIRUS FROM TOGAVIRIDAE FAMILY

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ABSTRACT

Chikungunya fever is a viral disease transmitted to humans by the bite of infected mosquitoes. The virus is a member of the genus Alphavirus, in the family Togaviridae. Chikungunya fever is a viral disease transmitted to humans by the bite of infected mosquitoes. Chikungunya virus is a member of the genus Alphavirus, in the family Togaviridae. The fever is diagnosed based on symptoms, physical findings (e.g., joint swelling), laboratory testing, and the possibility of exposure to infected mosquitoes. There is no specific treatment for chikungunya fever; care is based on symptoms. Chikungunya infection is not usually fatal. Steps to prevent infection with chikungunya virus include use of insect repellent, protective clothing, and staying in areas with screens. Chikungunya virus was first isolated from the blood of a febrile patient in Tanzania in 1953, and has since been cited as the cause of numerous human epidemics in many areas of Africa and Asia and most recently in limited areas of Europe.

Keywords: Alphavirus, virion, genome, nucleocapsid, aedes aegypti, biomarker, insect repellants, diagnosis, prognosis and macrophage.

INTRODUCTION

In biology and immunology, an alphavirus belongs to the group IV Togaviridae family of viruses, according to the system of classification based on viral genome composition introduced by David Baltimore in 1971. Alphaviruses, like all other group IV viruses have a positive sense single stranded RNA genome. There are 27 alphaviruses, able to infect various vertebrates such as humans, rodents, fish, birds, and larger mammals such as horses as well as invertebrates. Transmission between species and individuals occurs mainly via mosquitoes making the alphaviruses a contributor to the collection of Arboviruses – or Arthropod Borne Viruses. Alphaviruses particles are enveloped have a 70 nm diameter, tend to be spherical (although slightly pleomorphic), and have a 40 nm isometric nucleocapsid.

Genome

The genome of alphaviruses consists of a single stranded positive sense RNA. The total genome length ranges between 11,000 and 12,000 nucleotides, and has a 5' cap, and 3' poly-A tail. There are two open reading frames (ORF's) in the genome, non-structural and structural. The first is non structural and encodes proteins for transcription and replication of viral RNA, and the second encodes four structural proteins: Capsid protein C, Envelope glycoprotein E1, Envelope glycoprotein E2, and Envelope glycoprotein E3. The expression of these proteins and replication of the viral genome all takes place in the cytoplasm of the host cells.

There are many alphaviruses distributed around the world with the ability to cause human disease. Infectious arthritis, encephalitis, rashes and fever being the most commonly observed. Larger mammals such as humans and horses are usually dead-end hosts or play a minor role in viral transmission, however in the case of Venezuelan equine encephalitis the virus is mainly amplified in horses. In most other cases the virus is maintained in nature in mosquitoes, rodents and birds.

Table 1. Pathogenesis and immune response

Virus	Human Disease	Vertebrate Reservoir	Distribution
Sindbis virus	Rash, arthritis	Birds	Europe, Africa, Australia
O'nyong'nyong virus	Rash, arthritis	Primates, Humans	Africa
Chikungunya virus	**Rash, arthritis**	**Primates, humans**	**Africa, India, SE Asia**
Mayaro virus	Rash, arthritis	Primates, humans	South America
Ross River virus	Rash, arthritis	Mammals, humans	Australia, South Pacific
Barmah Forest virus	Fever, malaise, rash, joint pain, muscle tenderness	Humans	Australia
Eastern equine encephalitis virus	Encephalitis	Birds	Americas
Western equine encephalitis virus	Encephalitis	Birds, mammals	North America
Venezuelan equine encephalitis virus	Encephalitis	Rodents, horses	Americas

Alphavirus infections are spread by insect vectors such as mosquitoes. Once a human is bitten by the infected mosquito, the virus can gain entry into the bloodstream,

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causing viremia. The alphavirus can also get into the CNS where it is able to grow and multiply within the neurones. This can lead to encephalitis, which can be fatal. When an individual is infected with this particular virus, its immune system can play a role in clearing away the virus particles. Alphaviruses are able to cause the production of interferons. Antibodies and T cells are also involved. The neutralizing antibodies also play an important role to prevent further infection and spread.[1]

Diagnosis, prevention and control

Diagnoses are based on clinical samples from which the virus can be easily isolated and identified. There are no alphavirus vaccines currently available. Vector control with repellents, protective clothing, breeding site destruction, and spraying are the preventive measures of choice.

Research

Alphaviruses are of interest to gene therapy researchers, in particular the Ross River virus, Sindbis virus, Semliki Forest virus, and Venezuelan equine encephalitis virus have all been used to develop viral vectors for gene delivery. Of particular interest are the chimeric viruses that may be formed with alphaviral envelopes and retroviral capsids. Such chimeras are termed pseudotyped viruses. Alphaviral envelope pseudotypes of retroviruses or lentiviruses are able to integrate the genes that they carry into the expansive range of potential host cells that are recognized and infected by the alphaviral envelope proteins E2 and E1. The stable integration of viral genes is mediated by the retroviral interiors of these vectors. There are limitations to the use of alphaviruses in the field of gene therapy due to their lack of targeting, however, through the introduction of variable antibody domains in a non-conserved loop in the structure of E2, specific populations of cells have been targeted.

Furthermore, the use of whole alphaviruses for gene therapy is of limited efficacy both because several internal alphaviral proteins are involved in the induction of apoptosis upon infection and also because the alphaviral capsid mediates only the transient introduction of mRNA into host cells. Neither of these limitations extends to alphaviral envelope pseudotypes of retroviruses or lentiviruses. However, the expression of Sindbis virus envelopes may lead to apoptosis, and their introduction into host cells upon infection by Sindbis virus envelope pseudotyped retroviruses may also lead to cell death. The toxicity of Sindbis viral envelopes may be the cause of the very low production titers realized from packaging cells constructed to produce Sindbis pseudotypes. Another branch of research involving alphaviruses is in vaccination. Alphaviruses are apt to be engineered to create replicon vectors which efficiently induce humoral and T-cell immune responses. They could therefore be used to vaccinate against viral, bacterial, protozoan and tumor antigens. Makonde, also known as ChiMakonde in the language, is the language spoken by the Makonde, an ethnic group in southeast Tanzania and northern Mozambique. Makonde is a central Bantu language closely related to Yao. Chikungunya, the name of a mosquito-borne viral fever, is derived from the Makonde word meaning "that which bends up," after the disease was first identified on the Makonde plateau. The derivation of the term is generally falsely attributed to Swahili. Chikungunya (in the Makonde language "that which bends up") virus (CHIKV) is an insect-borne virus, of the genus *Alphavirus*, that is transmitted to humans by virus-carrying *Aedes* mosquitoes. There have been recent breakouts of CHIKV associated with severe illness. CHIKV causes an illness with symptoms similar to dengue fever. CHIKV manifests itself with an acute febrile phase of the illness lasting only two to five days, followed by a prolonged arthralgic disease that affects the joints of the extremities. The pain associated with CHIKV infection of the joints persists for weeks or months, or in some cases years.

Figure 1. Chikungunia virion, *Aedes aegypti*

Signs and symptoms

The incubation period of Chikungunya disease is from two to five days. Symptoms of the disease include a fever up to 40°C (104°F), a petechial or maculopapular rash of the trunk and occasionally the limbs, and arthralgia or arthritis affecting multiple joints. Other nonspecific symptoms can include headache, conjunctival Infection, and slight photophobia. Typically, the fever lasts for two days and then ends abruptly. However, other symptoms—namely joint pain, intense headache, insomnia and an extreme degree of prostration—last for a variable period; usually for about 5 to 7 days. Patients have complained of joint pains for much longer time periods; some as long as 2 years, depending on their age.[2]

Diagnosis

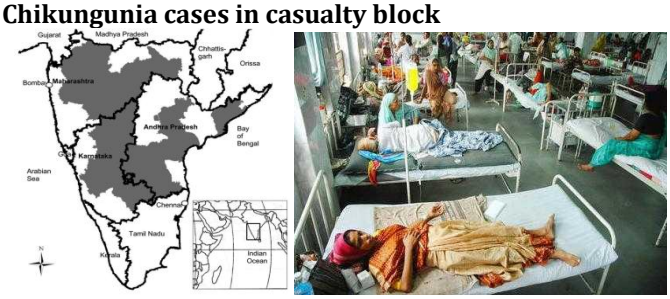
Common laboratory tests for chikungunya include RT-PCR, virus isolation, and serological tests.

- Virus isolation provides the most definitive diagnosis but takes 1–2 weeks for completion and must be carried out in Biosafety level 3 laboratories. The technique involves exposing specific cell lines to samples from whole blood and identifying chikungunya virus-specific responses.
- RT-PCR using nested primer pairs to amplify several Chikungunya-specific genes from whole blood. Results can be determined in 1–2 days.
- Serological diagnosis requires a larger amount of blood than the other methods and uses an ELISA assay to measure Chikungunya-specific IgM levels. Results require 2–3 days and false positives can occur with infection via other related viruses such as O'nyong'nyong virus and Semliki Forest Virus.[3]

Causes

Chikungunya virus is indigenous to tropical Africa and Asia, where it is transmitted to humans by the bite of infected mosquitoes, usually of the genus *Aedes*. Chikungunya virus belongs to alphavirus genus of the Togaviridae family. It is an "Arbovirus" (Ar-arthropod, bo-borne). CHIK fever epidemics are sustained by human-mosquito-human transmission. The word "chikungunya" is thought to derive from description in local dialect of the contorted posture of patients afflicted with the severe joint pain associated with this disease.[4] The main virus reservoirs are monkeys, but other species can also be affected, including humans.

Figure 2. Chikungunia prone region in India, Chikungunia cases in casualty block



PATHOPHYSIOLOGY

This has been poorly researched to date. Human epithelial and endothelial cells, primary fibroblasts and monocyte-derived macrophages are susceptible to infection. Lymphoid and monocytoid cells, primary lymphocytes and monocytes and monocyte-derived dendritic cells are not susceptible to infection. Viral entry occurs through pH-dependent endocytosis. Infection is cytopathic and associated with the induction of apoptosis in the infected cell. Infection is highly sensitive to the antiviral activity of type I and II interferon.

GENOME STRUCTURE

The virion consists of an envelope and a nucleocapsid. The genome consists of a single-stranded, positive-sense RNA molecule of approximately 12000 nucleotides long. The 5' end is capped with a 7-methylguanosine while the 3' end is polyadenylated. A subgenomic positive-strand RNA referred to as 26SRNA is transcribed from a negative-stranded RNA intermediate. This RNA serves as the mRNA for the synthesis of the viral structural proteins. Alphaviruses have conserved domains that play an important role in the regulation of viral RNA synthesis. These domains are found at the 5' and 3' ends as well as at the intergenic region. Virions are spherical and measure about 70nm in diameter. Surface projections are glycoprotein spikes covering the surface evenly.

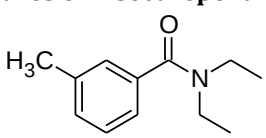
Genome Organization and Replicaton

Virions located on the surface of the cell membrane enter the host cells by fusion and endocytosis of the viral envelope. The uncoating of the virions occurs in the cytoplasm. The site of mRNA transcription is in the cell cytoplasm. Replication is not restricted to a particular tissue or organ of the host so the virus replication occurs in various organs. The insect host initiates the virus replication. The genome replication is done in the cytoplasm.

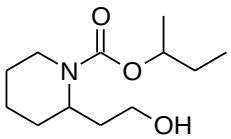
PREVENTION

The most effective means of prevention are protection against contact with the disease-carrying mosquitoes and mosquito control. These include using insect repellents with substances like DEET (N,N-Diethyl-meta-toluamide; also known as N,N'-Diethyl-3-methylbenzamide or NNDB), icaridin (also known as picaridin and KBR3023, hydroxyethyl isobutyl piperidine carboxylate), PMD (p-menthane-3,8-diol, a substance derived from the lemon eucalyptus tree), or IR3535 (ethyl 3-[acetyl(butyl)amino]propanoate).

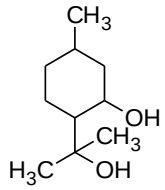
Structures of insect repellants



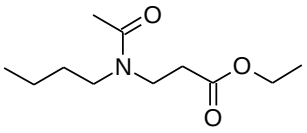
N,N-Diethyl-meta-toluamide



Icaridin



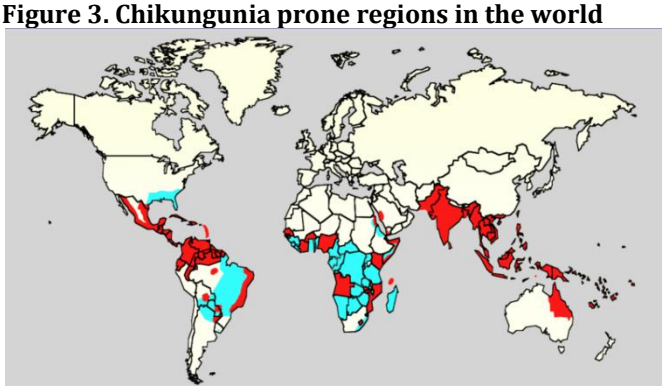
p-Menthane-3,8-diol



IR-3535

Wearing bite-proof long sleeves and trousers (pants) also offers protection. In addition, garments can be treated with pyrethroids, a class of insecticides that often has repellent properties. Vaporized pyrethroids (for example in mosquito coils) are also insect repellents. Securing screens on windows and doors will help to keep mosquitoes out of the house. In the case of the day-active *Aedes aegypti* and *Aedes albopictus*, however, this will have only a limited effect, since many contacts between the vector and the host occurs outside.[5]

Figure 3. Chikungunia prone regions in the world



TREATMENT

There are no specific treatments for Chikungunya. There is no vaccine currently available. A Phase II vaccine trial, sponsored by the US Government and published in the *American Journal of Tropical Medicine and Hygiene* in 2000, used a live, attenuated virus, developing viral resistance in 98% of those tested after 28 days and 85% still showed resistance after one year.[6] A serological test for Chikungunya is available from the University of Malaya in Kuala Lumpur, icul. Chloroquine is gaining ground as a possible treatment for the symptoms associated with Chikungunya, and as an anti-inflammatory agent to combat the arthritis associated with Chikungunya virus. A University of Malaya study found that for arthritis-like symptoms that are not relieved by aspirin and non-steroidal anti-inflammatory drugs (NSAID), chloroquine phosphate (250 mg/day) has given promising results. Research by an Italian scientist, Andrea Savarino, and his colleagues together with a French government press release in March 2006 have added more credence to the claim that chloroquine might be effective in treating chikungunya. Unpublished studies in cell culture and monkeys show no effect of chloroquine treatment on reduction of chikungunya disease. The fact sheet on Chikungunya advises against using aspirin, ibuprofen, naproxen and other NSAIDs that are recommended for arthritic pain and fever.[7]

Vaccine development

A virus-like particle based vaccine has protected monkeys from Chikungunya virus infection, and passive immunization from these monkeys protected immunodeficient mice against exposure to a dose of virus that would otherwise be lethal, demonstrating that the humoral response was highly protective.[8] A DNA vaccine candidate is also being tested for Chikungunya virus. The vaccine cassette was designed based on CHIKV Capsid and

Envelope specific consensus sequences with several modifications, including codon optimization, RNA optimization, the addition of a Kozak sequence, and a substituted immunoglobulin E leader sequence. These constructs induced humoral and cellular immune responses in mice.[9]

Passive immunity

Antibodies isolated from patients recovering from Chikungunya fever have been shown to protect mice from infection.[15]

Prognosis

Recovery from the disease varies by age. Younger patients recover within 5 to 15 days; middle-aged patients recover in 1 to 2.5 months. Recovery is longer for the elderly. The severity of the disease as well as its duration is less in younger patients and pregnant women. In pregnant women, no untoward effects are noticed after the infection. Ocular inflammation from Chikungunya may present as iridocyclitis, and have retinal lesions as well.[10] Pedal oedema (swelling of legs) is observed in many patients, the cause of which remains obscure as it is not related to any cardiovascular, renal or hepatic abnormalities.

Epidemiology

Chikungunya virus is an alphavirus closely related to the O'nyong'nyong virus the Ross River virus in Australia, and the viruses that cause eastern equine encephalitis and western equine encephalitis.[11] Chikungunya is generally spread through bites from *Aedes aegypti* mosquitoes, but recent research by the Pasteur Institute in Paris has suggested that Chikungunya virus strains in the 2005-2006 Reunion Island outbreak incurred a mutation that facilitated transmission by *Aedes albopictus* (Tiger mosquito).[12]

Concurrent studies by arbovirologists at the University of Texas Medical Branch in Galveston, Texas, confirmed definitively that enhanced chikungunya virus infection of *Aedes albopictus* was caused by a point mutation in one of the viral envelope genes (E1). Enhanced transmission of chikungunya virus by *Aedes albopictus* could mean an increased risk for chikungunya outbreaks in other areas where the Asian tiger mosquito is present. A recent epidemic in Italy was likely perpetuated by *Aedes albopictus*. In Africa, chikungunya is spread via a sylvatic cycle in which the virus largely resides in other primates in between human outbreaks.[13] On 28 May 2009 in Changwat Trang of Thailand where the virus is endemic, the provincial hospital decided to deliver by Caesarean section a male baby from his Chikungunya-infected mother-Khwanruethai Sutmueang, 28, a Trang native—in order to prevent mother-foetus virus transmission. However, after delivering the baby, the physicians discovered that the baby was infected with Chikungunya virus, and put him into intensive care because the infection had left the baby unable to breathe by himself or to drink milk. The physicians presumed that Chikungunya virus might be able to be transmitted from a mother to her foetus; however, there is no laboratory confirmation for this presumption.

History

The name is derived from the Makonde word meaning "that which bends up" in reference to the stooped posture developed as a result of the arthritic symptoms of the disease. The disease was first described by Marion Robinson and W.H.R. Lumsden in 1955, following an outbreak in 1952 on the Makonde Plateau, along the border between Mozambique and Tanganyika (the mainland part of modern day Tanzania).[14] According to the initial 1955 report about the epidemiology of the disease, the term 'chikungunya' is derived from the Makonde root verb *kungunyala*, meaning to dry up or become contorted. In concurrent research, Robinson glossed the Makonde term more specifically as "that which bends up." Subsequent authors apparently overlooked the references to the Makonde language and assumed that the term derived from Swahili, the lingua franca of the region. The erroneous attribution of the term as a Swahili word has been repeated in numerous print sources. Many other erroneous spellings and forms of the term are in common use including "Chicken guinea", "Chicken gunaya “and” Chickengunya". Since its discovery in Tanganyika, Africa, in 1952, chikungunya virus outbreaks have occurred occasionally in Africa, South Asia, and Southeast Asia, but recent outbreaks have spread the disease over a wider range.

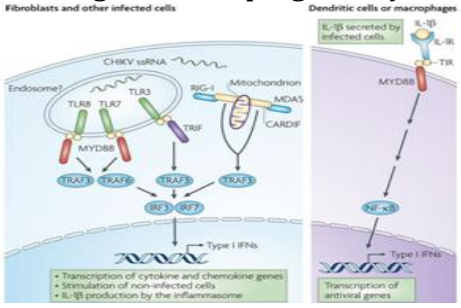
Use as a biological weapon

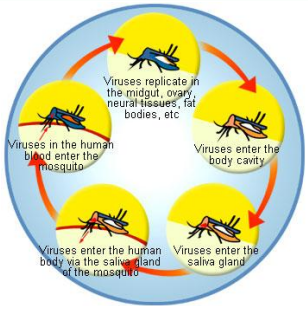
Chikungunya was one of more than a dozen agents that the United States researched as potential biological weapons before the nation suspended its biological weapons program.[15]

Chikungunya Fever Symptoms and Treatment

Chikangunya is a viral disease that is transmitted to human beings by mosquito bites. It is not possible to contract the chikungunya fever from another human being. The condition is not a fatal one and for now there are no specific vaccines or medicines available but mosquito control is the best method of prevention of chikungunya. Symptoms of Chikungunya fever include chills, nausea, vomiting, fever, joint pains and headaches. Chikungunya may happen suddenly and often is accompanied with some rashs on the skin. However the most painful symptom is severe joint pain. Mouth ulcers, conjunctivitis and los of taste may also hassle patients of chikungunya. It is not a fatal condition, but the effects can last for years. The fever will subside early but joint pains may remain for many, many years. There are no drugs available to treat this fever however, the symptoms can be treated. When one suffers from this condition, it is ideal that one consults their doctor and prescribes to the treatment that they suggest. Plenty of rest and lots of fluids are advisable. In terms of prevention, vaccines for the chikungunya fever are still under experimentation. The best method is too preventing mosquito bites. Making sure that breeding grounds for mosquitoes are destroyed is the best idea. Keep your home clean and stay hygienic and you can ensure that the fever does not harm anyone in your family. Eat healthy, stay healthy and make sure your immune system can fight this viral disease. The condition may not be fatal, but it can leave scars for life.[16]

Figure 4. Chikungunia macrophages lifecycle





Chikungunya fever infects eyes too

The chikungunya virus now reportedly affects eyes too. According to the scientists who have analyzed multiple patients, have confirmed that chikungunya fever not only affects your joints, but also causes irritation of the eye, and blurring of vision. Doctors advise visiting an ophthalmologist immediately if you experience any irritation, swelling or blurring of vision. Chikungunya is a fever which is caused by the bite of a mosquito of the type *aedes*. A bite of the mosquito infects you with the virus causing fever, headache and irritation of the eye.[1]

Chikungunia is much deadlier than imagination

Finally reports are coming from various research labs that the chikungunya virus is fast becoming deadly and cases where patients are dying with other complications such as multiple organ failure and heart attacks. The following places recently reported positive for Chikungunya: Sri Lanka, Australia, Singapore, Taiwan, China, and Hong Kong. While in the good news section to a cure/treatment for chikungunia, Scientists have now found a way to learn how the chikungunya disease spreads in the human body by using a genetic variation of mice. A special variety of mice was created to reduce their inherent capability to fight the virus. According to the scientists, "It is hoped that further studies with the mice will explain how the virus spreads from the muscle and skin tissue to the central nervous system in the more severe cases of the disease, and how the virus penetrates the placenta barrier when it is spread from mother to child. The team hopes that the work may also help to explain how viruses are transmitted across tissue barriers for other diseases too."

Chikungunya Virus Still a Threat in Europe

Today the health experts who first studied the outbreak of Chikungunya have confirmed that the world has not seen the last of this disease. If nothing is done to check the population of mosquitoes and other factors causing this disease, it might as well spread all across the U.S.A and the European Union. The outbreak of Chikungunya in Emilia-Romagna region of north eastern Italy was confirmed in the late August this year. Soon later, a panel of researchers and analysts has been finding out the potential damage that can be caused by this disease. They have recently in October disclosed the report, informing WHO and the world that the potential mass outbreak is possible by the next spring in 2008 if nothing is done to control it immediately, since the mosquitoes eggs hatch by then. Though as we here at chikungunia.com already know that this disease is not life threatening, but fatigue or pain in the joints can be disturbing your well being for anywhere from several weeks to months. There is no vaccine or specific antiviral treatment for the illness, which is caused by an *Alphavirus.* Also some cases have been found in the United States also, mostly from visitors to Italy and other Asian countries. But the local health authorities there believe that the outbreak there is not a threat due to the lifestyle there. Mostly air-conditioned buildings, cars and other limited exposure mean that the contact with the mosquitoes is limited.[18]

Treatment: Ayurvedic Cure for Chikungunya?

With the virus now in Europe, mainly in Italy, Chikungunia is gaining fast attention around the world. On the treatments front, Ayurveda has been doing wonders in the treatment of Chikunguya (commonly mispelt as Chikungunia). If you already know about the treatments of this disease, you will know that there is not such thing as a proper cure or treatment for this disease. Recently it was discovered that Triphala, an ayurvedic composition of 3 fruits namely Harada (haritaki), Amla (amalaki) and Behada (bibhitaki) is a good medicine for Chikungunya. Also powdered Sunflower seed taken along with honey is a good supplement precribed by the Ayurvedic docters at my place in Cochin, India. This mixture must be taken about 3 times a day along with your regular medicines to gain relief from joint pains which is a major after effect of this illness. Drinking loads of water will also help in reducing the pain and suffering from this disease.

Severity and Occurrence of the Disease

Infection can affect the nervous system or the musculo-skeletal system. General symptoms include fever, headache, maculopapular rash, arthralgia, myalgia, photophobia and lymphadenopathy. Infection is usually acute or chronic; more than 12% of patients with Chikungunya develop chronic joint pain. Prevalence of viral infection is seasonally dependent and incidences of the virus are usually noticeable in summer and wet seasons. The incubation period lasts usually two to four days followed by a recovery in five to seven days. The virus can be detected during the first 48 hours of disease and maybe detected as late as four days in some patients. The virus is known to occur in tropical regions. Viral host lives under aerobic conditions and lives in the atmosphere where it is wet. During 2005-2006 twelve cases of Chikungunya fever were diagnosed in the United States (ProMED 2006a). In India there have been at least hundred thousand cases reported with two confirmed fatalities. Infections may occur in areas that are not considered endemic but, travel and globalization increases the possibility of epidemic outbreaks in other regions around the globe.[19]

Below is a list of some Chikungunya outbreaks:

- Tanzania in 1953 (first recoded outbreak)
- Kolkata, India in 1963
- Port Klang, Malaysia in 1999
- 237 deaths and 33% of people infected in Reunion islands in 2006-2007
- Italy in 2007
- Kerala, India in 2007

Diagnosis

The best way to diagnose the disease is by distinguishing the Chikungunya strain by kinetic hemagglutination inhibition tests. Even monoclonal antibodies can be used in test such as the ELISA. The ELISA, Enzyme-linked immunosorbent assay, is a technique used in immunology to detect presence of an antibody or an antigen in a blood sample. Recently, a reverse transcriptase PCR technique has also been used for the diagnosis of Chikungunya virus. PCR results can be available within one to two days.

Prevention

Currently there is no prevention for this virus causing disease. There are no anti-viral medications available

although one's own immune system is the best fighter for this virus. Mosquito repellents can be used to avoid from getting bitten and wearing clothing that is covering your extremities is a big help. Eliminating mosquito-breeding sites is another key prevention measure. The mosquito bites in the day time and reduces its humming of the wings while it is approaching the target and it will bite from beneath the arms.

Phylogenetic Relationships

There are very few reports on the molecular relationship between Chikungunya and other members of the Alphavirus genus. It is very closely related to the O'nyong nyong virus. Chikungunya and O'nyong nyong virus have 85% similarities in their genome.

CURRENT RESEARCH

How chikungunya has spread to new vectors and new locations?

Researchers from the University of Texas Medical Branch have discovered how a key protein switch allows Chikungunya virus to spread to new vectors. Since Chikungunya virus cases have increased globally in the recent years this study also focused on another types of vector that carries this virus. It was spotted in the Indian Ocean islands where Aedes aegypti the primary carrier is not found. In this area another vector named Aedes albopictus, a relative of the Asian tiger mosquito was found carrying the virus. In earlier studies done from the epidemic on islands of the Indian Ocean the Chikungunya strain had enveloped protein gene, E1-A226V. The researchers cloned the virus and infected the aedes aegypti and the aedes albopictus with two genetically engineered clone of the virus, one had mutation and the other did not. The transmission of the mutant virus was much greater than the other virus in aedes albopictus. This proved that E1-A226V is directly responsible for Chikungunya adaptation to the aedes albopictus. The Asian tiger mosquito is spreading in Europe and the United States and interestingly since there is a global warming increment there could be an increase in new geographic locations for the Chikungunya virus to infect.

Connective tissue metabolism in chikungunya patients

This study was done on 75 Chikungunya virus infected patients. Since it causes severe arthritis in human hosts by a large area of necrosis and collagenosis or fibrosis. In this research the connective tissue was the main focus since the virus damages the cartilage and increases the levels of proline, mucopolysaccharide, and hydroxyproline measurements of the infected hosts. These tests are done through urine analyses to check if there is a presence of mucoplysaccharides in the urine. The study had results with moderate to severe cases. Since the connective tissue metabolism was greatly increased due to the infection, there was proline, hydroxyproline and mucopolysaccharides present in the urine. This indicated that the patients that were infected by the virus had damage done to their cartilage and connective tissue which particles had excreted through their urine.

Biomarkers detected for chikungunya virus

This study was performed at Singapore's Tan Tock Seng Hospital. In this study researchers investigated many biological factors such as cytokines and chemokines that were produced in the human blood. Cytokines are proteins, peptides or glycoprotein's that are signaling molecules that like hormones and neurotransmitters are used in cellular communication. Chemokines on the other hand are small cytokines with low molecular weight that are also released by many cells. There are three specific biological factors that were distinguished in patients with the severe form of the disease, interleukin-1 beta, interleukin-6 and RANTES that is a protein. RANTES is an acronym for Regulated on Activation, Normal T Expressed and Secreted.

The study was conducted on blood samples obtained from ten patients who developed the disease during the Singapore's Chikungunya virus outbreak in January 2008. Lisa Ng, Ph.D., principal investigator of the Chikungunya research team at SIgN and co-author of the PLoS one article, said, "This first comprehensive report, which examines the cellular signals produced as part of the human immune response to Chikungunya virus infection, enables us to understand the changes in molecular signals in the body when infection sets in. These biomarkers can potentially lead to the development of therapeutics to reduce the severity of the disease and halt its progression." Severe form of Chikungunya was indicated decrease in the levels RANTES but an increased in the levels of interleukin-1 beta, interleukin-6. This study proves that cytokines could be used as biomarkers in predicting the severity of the disease since they provide immunological information to understand the effect of Chikungunya in the human host.

Three specific biomarkers provide an accurate indication of the severity of Chikungunya fever (CHIKF), which is emerging as a threat in South-East Asia, the Pacific and Europe, according to research conducted in Singapore. Since the biomarkers can be easily detected and measured in blood, this finding could expedite identification and monitoring of patients. The study, the first comprehensive investigation of the many biological factors such as cytokines and chemokines produced in the human body in response to Chikungunya virus infection, was conducted by researchers at A*STAR's Singapore Immunology Network (SIgN) and the Communicable Disease Centre (CDC) at Singapore's Tan Tock Seng Hospital (TTSH). Cytokines are proteins, peptides or glycoproteins that belong to a category of signaling molecules that, like hormones and neurotransmitters, are used extensively in cellular communication. Chemokines are small cytokines of relatively low molecular weight that are released by a variety of cells.

The Singapore scientists found that levels of three specific biological factors, interleukin-1, beta, (IL-1β), interleukin-6 (IL-6) and RANTES, distinguished patients with the severe form of the disease from those in whom the infection was mild. The findings of the study, conducted on blood samples obtained from 10 patients who developed the disease during Singapore's CHIKF outbreak in Jan. 2008, were published online one year later (Jan. 2009) by the *PLoS ONE*. Lisa Ng, Ph.D., principal investigator of the Chikungunya research team at SIgN and co-author of the PLoS ONE article, said, "This first comprehensive report, which examines the cellular signals produced as part of the human immune response to Chikungunya virus infection, enables us to understand the changes in molecular signals in the body when infection sets in. These biomarkers can potentially lead to the development of therapeutics to reduce the severity of the disease and halt its progression." Dr. Ng and her colleagues discovered that an increase in the levels of IL-1β and IL-6, with a

concomitant decrease in RANTES, was an indication of a severe form of CHIKF. This finding would allow for quicker and more accurate prognosis of infected patients. The scientists also determined that the level of RANTES was lower in patients with severe CHIKF, as compared to those with dengue. This result could potentially enable physicians and scientists to distinguish quickly between CHIKF and dengue fever – two diseases that present clinically similar symptoms. SIgN Chairman Philippe Kourilsky, Ph.D., said, "This is indeed a significant breakthrough in the research on Chikungunya fever, which is emerging as a threat in South-East Asia, the Pacific and Europe. The landmark findings are a testament to the successful collaboration between a basic research institute and a hospital, where both parties combine their resources and expertise to achieve clinical relevance. SIgN will continue to work with our partners in the hospitals to better understand the disease and translate such findings into relevant clinical outcomes." In addition to TTSH, SIgN has clinical collaborations with Alexandra Hospital, Singapore General Hospital and National University Hospital, in research areas such as immunology and cancer studies. Associate Professor Leo Yee Sin, M.D., Clinical Director of CDC at TTSH, said, "This study proves that cytokines could be used as biomarkers in predicting the severity of the disease. They provide immunological information for us to understand the causal effect of Chikungunya in the human host. Further research along a similar vein is ongoing with a larger number of cases from later Chikungunya outbreaks that had occurred in Singapore." Research is now underway in Singapore to ascertain the immune and pathogenic mechanisms behind CHIKF, which could guide the development of future therapeutic applications. This research is being conducted through a follow-up on more than 100 cases of CHIKF, to further refine the understanding of CHIKF clinical manifestation over a prolonged period of time. Prof. Leo added, "We are hopeful that our research endeavor can further our understanding of Chikungunya and enable us to apply the knowledge gained to better manage the disease". With outbreaks in the Pacific region in recent years, CHIKF has emerged as a potentially serious international health threat. However, little is known about the disease progression and the immune response in patients. In late 2007, SIgN initiated clinical immunology research on Chikungunya led by Dr. Lisa Ng to study the immunological process of CHIKF. During the 2008 outbreak of CHIKF in Singapore, the CDC team led by Associate Professor Leo Yee Sin, Clinical Director of the CDC, TTSH, responded swiftly to contain it and set up outbreak research through the support of National Healthcare Group Domain-Specific Review Boards. CDC's swift action enabled prospective sample collection at a very early stage of the outbreak, which allowed the research team to study early cytokine response.[20]

Table 2. Zoonotic viral diseases

Arthropod/ (arbovirus)	Mosquito	Bunyaviridae	Arbovirus encephalitis: La Crosse encephalitis. California encephalitis Viral hemorrhagic fever: Rift Valley fever (*RVFV*)
		Flaviviridae	Arbovirus encephalitis: Japanese encephalitis. Australian encephalitis. St. Louis encephalitis. West Nile fever Viral hemorrhagic fever: Dengue fever *other:* Yellow fever. Zika fever
		Togaviridae	Arbovirus encephalitis: Eastern equine encephalomyelitis · Western equine encephalomyelitis. Venezuelan equine encephalomyelitis *other:* **Chikungunya**. O'Nyong-nyong fever. Ross River fever
	Tick	Bunyaviridae	Viral hemorrhagic fever: Crimean-Congo hemorrhagic fever
		Flaviviridae	Arbovirus encephalitis: Tick-borne encephalitis. Powassan encephalitis. Deer tick virus encephalitis Viral hemorrhagic fever: Omsk hemorrhagic fever. Kyasanur forest disease (*Alkhurma virus*)
		Reoviridae	Colorado tick fever
Mammal	Rodent (Robovirus)	Arenaviridae	Viral hemorrhagic fever: Lassa fever. Venezuelan hemorrhagic fever. Argentine hemorrhagic fever. Bolivian hemorrhagic fever. Lujo virus
		Bunyaviridae	*Puumala virus. Andes virus. Sin Nombre virus.* Hantavirus
	Bat	Filoviridae	VHF: Ebola hemorrhagic fever. Marburg hemorrhagic fever
		Rhabdoviridae	*Australian bat lyssavirus. Mokola virus. Duvenhage virus. Lagos bat virus.* Chandipura virus(sandfly)
		Bornaviridae	*Menangle. Henipavirus.* Borna disease (*Borna disease virus*)
	Multiple	Rhabdoviridae	Rabies

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