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Arthrogenic Alphaviruses and Inflammatory Myopathies

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1. Introduction

There is increasing evidence to suggest that viruses have aetiological roles in inflammatory myopathies. It has been reported that viruses, by means of direct infection of the skeletal muscle, can cause myalgias, polymyositis, and virus-associated rhabdomyolysis. It has also been found that some viruses can cause myositis through a secondary immune-mediated phenomenon. In addition, there are emerging reports of cases of idiopathic polymyositis suspected to be associated with infectious agents, predominantly viruses.

Arthrogenic alphaviruses such as chikungunya virus (CHIKV), Ross River virus (RRV) and sindbis virus (SINV) are known to cause outbreaks of polyarthritides worldwide (Griffin, 2007). The clinical presentation of alphavirus infection includes fever, rash, arthralgia, and arthritis, however, one of the predominant features of alphaviral disease is myalgia with corresponding myositis. The recent outbreaks of CHIKV in several countries surrounding the Indian Ocean have seen millions of people affected, with case reports showing a high incidence of myalgia and skeletal muscle involvement.

The ability of these viruses to cause long-term disease sequelae persisting for months or even years after the initial infection is of particular interest. Although difficult to substantiate indefinitely, there is significant reason to suspect that this long-term impact, combined with the virus's ability to cause acute muscle damage, could make arthrogenic viruses a potential cause of idiopathic inflammatory myopathies.

The mechanisms by which alphaviruses cause musculoskeletal disease are now being unraveled with clear evidence to suggest that the viral-induced inflammatory response can lead to destruction of striated muscle fibres, providing a possible cause for the symptoms of myalgia. In recent years, the use of animal models of alphavirus-induced myositis has increased our understanding of the inflammatory myopathies caused by these viruses. Using these animal models it has been shown that in the acute phase of infection, skeletal muscle is the major site of viral replication, resulting in striated muscle fibre destruction (Lidbury *et al.*, 2000; Morrison *et al.*, 2006). In the sub-acute phase of infection, the virus triggers an extensive inflammatory immune response, resulting in the influx of inflammatory cells and production of soluble mediators, leading to extensive myositis. The use of animal models has been instrumental in elucidating the role of alphaviruses as triggers of inflammatory myopathies.

This chapter will discuss the role of alphavirus infections as triggers of myositis, including how animal models are being used to dissect the pathobiology of disease and identify potential drug candidates to ameliorate disease.

2. Alphaviruses

Alphaviruses are a group of mosquito-transmitted viruses of the *Togaviridae* family. The virus particle is approximately 60 nm in size and is comprised of a single-strand of ribonucleic acid (RNA) of approximately twelve kilobases, encased in a nucleocapsid with a lipid membrane envelope. The RNA genome is divided into two regions, the non-structural and the structural regions. The non-structural region encodes four nonstructural proteins (nsPs; nsP1 to nsP4), and the structural region encodes the capsid protein (C) and three glycoproteins (E1, E2 and E3). The glycoproteins E1 and E2 protrude from the viral lipid envelope and are the most external and immunodominant epitopes being exposed to a significant amount of immune pressure.

The *Alphavirus* genus can be classified into two subgroups depending on differences in disease aetiologies; the Old World and the New World alphaviruses. The Old World alphaviruses, including RRV, o'nyong-nyong (ONNV), Semliki Forest virus (SFV), SINV, mayaro virus (MAYV), Barmah Forest virus (BFV) and CHIKV, typically cause fever, rash, myalgia, arthralgia and arthritis in humans, with symptoms often persisting for several months to years following infection (Table 1) (Suhrbier & La Linn, 2004). The New World alphaviruses, including Venezuelan equine encephalitis virus (VEEV), Eastern equine encephalitis virus (EEEV) and Western equine encephalitis virus (WEEV), cause severe disease in humans targeting the central nervous system (CNS), often resulting in encephalitis (Johnston & Peters, 1996), and are not associated with myositis. This chapter will focus on Old World alphaviruses.

Old World alphaviruses have been associated with large outbreaks of disease worldwide. These outbreaks are often explosive in nature, affecting many thousands to millions of people such as the 1959-1962 outbreak of ONNV fever in Africa, involving an estimated two million cases (Lanciotti *et al.*, 1998; Posey *et al.*, 2005; Williams *et al.*, 1965), the 1979-1980 outbreak of RRV in the South Pacific, resulting in more than 60,000 reported cases (Harley *et al.*, 2001) and the 2005-2006 outbreak of CHIKV in India with at least 1.5 million people affected (Josseran *et al.*, 2006; Kalantri *et al.*, 2006; Yergolkar *et al.*, 2006). In addition to the larger outbreaks, alphaviruses are also known to cause smaller, sporadic outbreaks such as that of MAYV in Brazil in 1978 and 2008 (Azevedo *et al.*, 2009; Pinheiro *et al.*, 1981), outbreaks of SINV in Finland and Sweden as the causative agent of Pogosta and Ockelbo diseases respectively (Kurkela *et al.*, 2004; Skogh & Espmark, 1982) and the recent 2009 outbreaks of CHIKV in Thailand, Singapore, Malaysia and Indonesia (Hapuarachchi *et al.*, 2010; Higgs & Ziegler, 2010; Ng *et al.*, 2009a; Thavara *et al.*, 2009; Theamboonlers *et al.*, 2009). Although the predominant clinical feature in most alphaviral outbreaks is the typical arthritis/arthralgia, a second important feature of the disease is myalgia.

The course of a typical alphavirus infection first involves the bite of an infected mosquito, resulting in subcutaneous inoculation of the virus. This is then followed by the initial viral replication and systemic viraemic spread. The primary viraemia marks the acute phase of the infection. Infection progresses to secondary sites where the virus can replicate, however, by this stage the body mounts an immune response to counteract the virus. It is at this point that specific clinical signs and symptoms of disease commence, which correlate closely to the immune inflammatory response, and mark the sub-acute phase of infection. The sub-acute phase can last several weeks, and there have been numerous cases documented where disease symptoms have lasted for months to years, thereby marking the chronic stage of alphaviral infection. In all three stages of alphaviral infection; acute, sub-acute and chronic, there is clear evidence of inflammatory response in the muscle and symptoms of myalgia.

Old World alphavirus	Geographic distribution	Cases	Comments
Ross River (RRV)	Australia West Pacific	~5,000 pa in Australia	Reported in travelers and visiting US military personnel
Barmah Forest (BFV)	Australia	~1500-2000 pa in Australia	Probably underdiagnosed due to similarity with RRV
Sindbis group (SINV)	Africa, Asia, Australia	Rare *	Although differently named, probably all the same virus
Karelian fever	Russia	Rare	Outbreaks occur frequently in Europe
Ockelbo	Sweden	30 pa	
Pogosta	Finland	100–200 pa	
Mayaro (MAYV)	South America	Small sporadic epidemics, underdiagnosed due to circulating dengue	Reported in travelers in Europe and the USA
O'nyong-nyong (ONNV)	Central and East Africa	Small sporadic epidemics, >2 million 1959–1962 epidemic	
Igbo Ora	Central Africa	Rare	Serologically related to ONNV and CHIKV
Chikungunya (CHIKV)	South and East Asia, Africa, West Pacific	Recurrent epidemics, anywhere from a thousand to a million cases reported annually since it re-emergence in 2005	Large epidemics in countries around the Indian Ocean from 2005-present. Reported in travelers in Europe and the USA

*Cases believed to be under reported world-wide

These viruses are generally transmitted by mosquitos and constitute over 40 members, several of which cause polyarthritits/arthralgia/myalgia in humans. pa, per annum.

Table 1. Old World viruses of the *Alphavirus* genus.

2.1 Ross River virus (RRV)

RRV was first isolated in 1959 in Queensland, Australia and was identified as an arbovirus causing polyarthritits by the early 1960s. It circulates endemically in Australia and the South Pacific (Harley *et al.*, 2001). Like all alphaviruses, the virus is maintained in transmission cycles between its mosquito vector and vertebrate hosts. In the case of RRV the predominant vectors are the mosquitoes *Culex annulirostris* and *Aedes vigilax* and the vertebrate hosts are commonly found to be native marsupials (Harley *et al.*, 2001; Old & Deane, 2005; Oliveira *et al.*, 2006). In Australia, there are between 5,000 to 8,000 cases of RRV reported annually (Harley *et al.*, 2001), with patients displaying symptoms of arthritis, arthralgia, myalgia, fatigue, febrile illness and rash (Fraser, 1986; Harley *et al.*, 2002; Harley *et al.*, 2001). The characteristic feature of RRV-infection in its acute stage is a febrile illness, which is frequently ignored by patients. Following the non-specific fever, comes the onset of RRV-specific symptoms, such as rash, arthritis, arthralgia and myalgia. It is at this sub-acute stage that patients generally seek medical attention, with infection confirmed by the detection of virus-specific IgM/IgG in the serum. The acute symptoms can last for weeks to months, however chronic disease associated with RRV infection in some cases has been reported to last up to a year or more (Mylonas *et al.*, 2002). A survey conducted in southeast Queensland of 67 adult patients with RRV disease found that RRV disease was often severe at onset, with patients presenting predominantly with polyarthralgia. Around one third to one half also experienced rash, fever, myalgia, and/or fatigue. In all but 2% of cases, symptoms resolved over an average 3 to 6-month period. In the 2% of cases, symptoms appeared to last longer than 1 year (Mylonas *et al.*, 2002). These clinical features are common in RRV-infected patients.

2.1.1 Models of RRV disease

A small animal model of RRV-disease has been developed in 20-24 day old C57BL/6 mice (Lidbury *et al.*, 2008; Morrison *et al.*, 2007; Morrison *et al.*, 2006), subcutaneously infected with RRV. The infection progresses with virus titre peaking in the first 24-48 hours post-infection (p.i.), with virus detected in the serum, skeletal muscles and joints, indicative of productive infection. Disease onset is observed by 4-5 days p.i., with clinical disease signs that include ruffled fur and weight loss. By 7-12 days p.i., peak disease is reached, with severe hind limb dysfunction observed to the point of paralysis, loss of grip strength and severe weight loss; at this point viraemia is no longer detectable. The infection elicits an inflammatory response, detectable primarily within the muscle tissues, with severe myositis observed in the skeletal muscle (Figure 1). By 15-21 days p.i., the disease signs resolve, with mice regaining hind limb function and beginning to gain weight. By 30 days p.i. mice show no signs of disease, although myositis is still evident (Figure 1F). Interestingly, histology of the myofibres shows the presence of centralized nuclei indicative of muscle fibre regeneration (Morrison *et al.*, 2006).

2.2 Chikungunya virus (CHIKV)

CHIKV was first isolated during an outbreak of dengue-like fever in Tanzania in 1952 (Robinson, 1955). Due to the similarities in febrile illness, it is possible that CHIKV may have been assumed as dengue fever for hundreds of years (Carey, 1971). The word ‘chikungunya’ comes from the Makonde language of Tanzania, and means ‘that which bends up’, which describes the clinical signs of disease frequently observed in CHIKV-infected patients (Griffin, 2007). The current geographical distribution of CHIKV covers the continents of Africa and Asia including India and the islands of the Indian Ocean. The original vector of CHIKV is *Aedes aegypti* with transmission cycles existing between vector and monkeys, however *Aedes albopictus* has been implicated as the vector responsible for a number of the recent outbreaks and the resulting spread of CHIKV into new global regions.

Since its identification in 1952 (Ross, 1956), laboratory-confirmed outbreaks of CHIKV have occurred annually in south and central Africa and South East Asia (Higgs & Ziegler, 2010). Recently, the re-emergence of CHIKV in the French island of La Réunion saw a third of the population infected (more than 250,000 people) (Renault *et al.*, 2007). The virus then rapidly spread to the Indian Ocean, India and South East Asia, with estimates as high as five million reported cases since 2006 (Josseran *et al.*, 2006; Kalantri *et al.*, 2006; Yergolkar *et al.*, 2006). CHIKV cases have also been reported among travellers in Europe and the USA (Liumbruno *et al.*, 2008) with a localised outbreak occurring in Italy (Enserink, 2007). Currently the virus continues to circulate and cause sporadic outbreaks in the Asia Pacific region, the most recent being the 2009 outbreaks in South East Asia affecting more than 100,000 people (Centers-for-Disease-Control-and-Prevention, 15 August 2010; Thavara *et al.*, 2009; Theamboonlers *et al.*, 2009).

Typical symptoms of CHIKV infection are abrupt febrile illness, headache, arthralgia, myalgia and in some cases maculopapular rash. Similar to other alphaviruses the incubation period for CHIKV ranges from 3 to 7 days, and as few as 5% of the CHIKV cases are asymptomatic. The acute signs and symptoms usually resolve in less than 2 weeks, but arthralgia and myalgia may linger for weeks, months or even years (Couderc & Lecuit, 2009; Jaffar-Bandjee *et al.*, 2009). This acute phase of infection is frequently more severe in both newborns and elderly patients who often have an extremely high viral load (Jaffar-Bandjee *et al.*, 2009). As with other alphaviruses the sub-acute phase, defined by the presence of

arthralgia and myalgia, appears to be largely modulated by the immune system's response to the invading pathogen. The chronic phase of CHIKV infection has been studied more extensively than that of other alphaviruses and appears to be associated with the persistence of specific IgM and chronic arthralgia/myalgia (Jaffar-Bandjee *et al.*, 2009). It is postulated that the ongoing symptoms may be linked to the virus's capacity to persist in tissues by mechanisms that currently remain ill-characterised.

Fig. 1. RRV induces inflammation in hind limb skeletal muscle tissue of C57BL/6 mice. Mice were infected with RRV or mock-infected with diluent (PBS). At 3, 5, 7, 10, and 30 days post-infection (p.i.), mice were sacrificed, quadriceps removed, 5 μ m sections generated then H&E stained. (A) Mock infection, (B) RRV 3 days p.i., (C) RRV 5 days p.i., (D) RRV 7 days p.i., (E) RRV 10 days p.i. and (F) RRV 30 days p.i. (magnification, x200). Copyright © 2006, American Society for Microbiology. All Rights Reserved. Journal of Virology, Jan. 2006, p.737–749 Vol. 80, No. 2 0022-538X/06/\$08.00_0doi:10.1128/JVI.80.2. 737– 749.2006.

2.2.1 Models of CHIKV disease

Currently three different mouse models of CHIKV infection have been developed. Neonatal C57BL/6 inbred mice and CD-1 outbred mice were found to be susceptible to CHIKV infection, showing an age-dependent disease severity with a model established in 14-day-old mice (Couderc *et al.*, 2008; Morrison *et al.*, 2011; Ziegler *et al.*, 2008). These models show extensive myositis in the skeletal muscle at 7–10 days p.i. during the stage of sub-acute infection (Morrison *et al.*, 2011). Subcutaneous infection of adult C57BL/6 mice leads to a self-limiting disease characterised by arthritis, tenosynovitis and myositis. Histological studies revealed a pronounced infiltration of monocytes, macrophages and natural killer (NK) cells in muscle tissues and synovial membranes indicative of myositis and arthritis (Gardner *et al.*, 2010). Finally, adult mice partially deficient in the type I interferon (IFN) receptor (IFN- α/β R+/-) developed mild CHIKV infection which closely resembles the course of human infection, with virus recovered from muscles, joints and skin (Couderc *et al.*, 2008).

In addition to the use of small animal models, some studies have utilized non-human primates to investigate the mechanisms of CHIKV disease. A model was developed in cynomolgus macaques (*Macaca fascicularis*) to closely mimic the human disease and to test the effectiveness of immunological interventions (Labadie *et al.*, 2010). In this model, viraemia peaked at two days p.i. and subsided by 15 days p.i., with extensive mononuclear cell infiltration in the lymphoid tissues and liver of infected macaques. Long-term infection was also observed, with the virus persisting in the lymphoid organs, liver, joints and muscles. However, the major limitation with this study was the lack of pathology observed in muscle and joint tissues, which is not consistent with observations in humans.

2.3 Sindbis virus (SINV)

SINV is found world-wide and is the most widely distributed of all known arboviruses (Tesh, 1982). It was first identified in 1952, being isolated from *Culex* mosquitoes collected in the village of Sindbis near Cairo (Taylor *et al.*, 1955). The first description of clinical symptoms of SINV infection came from a single case in Uganda in 1961 and included fever, malaise, pains in the joints, muscles and tendons, and rash (Malherbe *et al.*, 1963).

In Europe, SINV is the causative agent of Ockelbo, Pogosta, and Karelian fever, all of which exhibit significant morbidity. These fevers are named according to the regions where they circulate, despite being similar in clinical presentations. The major symptoms in addition to joint and muscle inflammation are fever, fatigue, headache and rash (Laine *et al.*, 2004). The diagnosis is based on the clinical picture and serology. The musculoskeletal symptoms of SINV infection may also continue long-term with chronic SINV-associated disease symptoms being identified in a number of cases 2.5 years after the onset (Laine *et al.*, 2000).

SINV outbreaks occur in Europe in common cycles. In 1981 Sweden reported 54 cases (Ockelbo), Russia 200 cases (Karelian) and Finland 300 cases (Pogosta) of clinically and serologically diagnosed SINV infection (Brummer-Korvenkontio *et al.*, 2002; Espmark & Niklasson, 1984; L'Vov D *et al.*, 1982). Outbreaks of Pogosta disease have thus far emerged every seven years since the first outbreak was noted in 1974, including a large outbreak in 2002 (Sane *et al.*, 2010).

2.3.1 Models of SINV disease

Several mouse models of SINV infection have been developed; one of particular relevance involves the subcutaneous injection of neonatal outbred mice with SINV. In this model, primary replication of the virus occurs in the skin, fibroblasts and connective tissues, followed by systemic viraemia (Johnson, 1965). The SINV-induced inflammatory response is characterised by extensive virus replication in extraneural tissues and induction of high levels of pro-inflammatory cytokines and corticosterone, with mice frequently dying of inflammatory disease by day 5 p.i. (Klimstra *et al.*, 1999; Trgovcich *et al.*, 1996; Trgovcich *et al.*, 1997). Although these studies show the onset of myositis in skeletal muscle, the major limitation of this model is the rapid mortality.

2.4 Other alphaviruses

Barmah Forest virus (BFV) is a closely related alphavirus and shares a similar distribution to RRV. BFV infection is believed to be largely under-diagnosed due to the similarity to RRV in disease presentation and geographic distribution (McGill, 1995). However, the clinical disease presentation of BFV infection has been documented to involve arthralgia and

myalgia, suggesting that this is another alphavirus with the potential for causing an inflammatory myopathy (Jacups *et al.*, 2008).

O'nyong-nyong virus (ONNV) is an alphavirus antigenically related to CHIKV. It was first isolated in East Africa and continues to circulate endemically in Africa producing a similar disease presentation to CHIKV-infection. Between 1959 and 1962 there was a large outbreak of ONNV in East Africa involving an estimated 2 million people in which 71% of infected patients suffered from myalgia (Posey *et al.*, 2005; Williams *et al.*, 1965). In 1996-1997 ONNV re-emerged in south-central Uganda (Kiwanuka *et al.*, 1999) and is another alphavirus with the potential to cause global outbreaks of disease.

Mayaro virus (MAYV) was first isolated in 1954 and is endemic to South America where it causes sporadic cases and smaller outbreaks of disease that manifest with fever, headaches, arthralgia and myalgia (Anderson *et al.*, 1957). Cases of MAYV infection and outbreaks have been documented in Trinidad, Surinam, Brazil, Bolivia, French Guinea and Peru (Tesh *et al.*, 1999). Moreover, imported cases of MAYV infection have been recently reported in two European countries demonstrating a potential for this virus to spread to non-endemic areas (Hassing *et al.*, 2010; Receveur *et al.*, 2010). A study of cases of MAYV in Peru by Tesh *et al.* found myalgia to be a predominant symptom with a prevalence of 77.3%. The strong level of muscle involvement in MAYV infection, coupled with the incidences of MAYV spread into other regions makes this an important virus causing myositis.

Semliki Forest virus (SFV) was first isolated in 1942 in Uganda from *Aedes abnormalis* mosquitoes in the Semliki Forest. Despite SFV being the most extensively studied member of the alphavirus genus, the virus has rarely been documented as causing disease in humans. The few reported cases of human disease have involved headache, arthralgia and myalgia, presentations that are commonly associated with other alphavirus disease manifestation. In 1987 an outbreak of SFV was recorded in the Central African Republic with severe and prolonged myalgia as a major clinical symptom (Mathiot *et al.*, 1990).

3. Tissue tropism and myositis in acute alphavirus infection

During the acute phase of a viral infection the cells and tissues that are infected can affect both the progression and clinical attributes of the disease. In CHIKV infection, skeletal muscle is the primary site of virus infection and replication, resulting in muscle fibre destruction and subsequent myositis. One of the difficulties in studying infection with CHIKV and other alphaviruses is that the incubation period and acute phase of infection, at the onset of primary viraemia, often produces non-specific symptoms that patients do not report and therefore clinicians are rarely able to document the early stages of infection. Furthermore, since alphaviruses produce non-fatal diseases, histopathological studies in humans are generally uncommon.

There have been numerous studies on alphaviral tropism *in vitro*, but these studies can only provide limited information on the mechanisms of the acute phase of infection, as they may not fully relate to the natural course of virus infection *in vivo*. The establishment of animal models of disease, in mice and macaques, has helped in progressing the understanding of alphavirus pathogenesis. In particular, cells derived from muscle tissues have been identified as sites of infection. Infected muscle cells undergo cell death, leading to muscle tissue damage and myositis, which commences at the sub-acute phase of infection.

3.1 Models of acute alphavirus infection *in vivo*

In animal models of RRV and CHIKV infection, virus initially replicates at the site of inoculation before being disseminated through the blood stream to other tissues such as skeletal muscle and joint tissues (Gardner *et al.*, 2010; Labadie *et al.*, 2010; Morrison *et al.*, 2011; Morrison *et al.*, 2006). Replication in skeletal muscle results in severe necrotic myositis with extensive myofibre destruction (Couderc & Lecuit, 2009). The damage to the skeletal muscle was clearly demonstrated using Evan's Blue dye (EBD) staining of affected tissues. EBD is commonly used to identify disrupted tissues as the tissues become permeable to EBD uptake. Following viral infection and replication, muscle tissues exhibit extensive tissue destruction, shown by prominent EBD staining (Figure 2). The occurrence of tissue pathology following viral infection and replication may correspond to the onset of myositis.

Fig. 2. RRV-infection causes skeletal tissue damage in mice. C57BL/6 mice were mock-infected (A) or infected with RRV (B). At 10 days p.i. mice were injected with 1% Evans Blue dye (EBD) and six hours later mice were sacrificed, quadriceps muscle removed and 10 μ m cryosections generated. The uptake of EBD (red) and DAPI (blue; for nuclei staining) was visualised by fluorescence microscopy (magnification, x200).

Within the skeletal muscle, the cells targeted by the virus have been identified as fibroblasts and muscle satellite cells. In CHIKV-infected IFN- α/β receptor deficient mice, fibroblasts within the skeletal muscles and joint connective tissues were found to be the main target for infection (Couderc *et al.*, 2008). Analysis of CHIKV-infected human muscle, joint and skin biopsy samples confirmed these observations, with fibroblasts and muscle satellite cells identified as major targets in peripheral tissues (Couderc & Lecuit, 2009; Ozden *et al.*, 2007).

3.2 Cells susceptible to alphavirus infection *in vitro*

There are a number of *in vitro* studies demonstrating the permissibility of various cells to alphavirus infection. Although these studies have limitations, they provide insight into the cell types that may be the target of infection *in vivo*. CHIKV and RRV are known to infect cells of epithelial and fibroblast origin and some immune cells (Sourisseau *et al.*, 2007).

Myogenic progenitor cells have been shown to be permissive to CHIKV infection (Ozden *et al.*, 2007). A study by Sourisseau *et al.*, characterised permissibility to CHIKV infection in a number of human cell types. The study demonstrated that several cells that can be found within muscle tissues such as primary fibroblasts, endothelial cells and immune cells such as monocyte-derived macrophages were susceptible to CHIKV infection (Sourisseau *et al.*,

2007). Virus infection and replication in these cells *in vitro* results in rapid shut down of host cell transcription and translation resulting in cytopathic effect (CPE) and cell death. Studies of CHIKV in epithelial (HeLa) cells *in vitro* have shown infection can lead to apoptosis which may help explain the destruction of muscle fibres seen *in vivo* (Sourisseau et al., 2007).

4. Sub-acute alphavirus infection and myositis

Severe forms of alphavirus disease result in chronic incapacitating myalgia, arthralgia and arthritis. One of the major reasons for the lack of information on the mechanisms of how these viruses cause musculoskeletal disease is due to the absence of studies in humans. Patients often only report on the symptoms they feel, and in most cases the acute phase of infection often go unreported, and the full extent of the disease not properly documented. One of the key questions is whether the symptoms of arthralgia and myalgia are a result of myositis and if so, how is the inflammatory process triggered. In recent years, the use of animal models of alphaviral-induced disease has assisted in unraveling the pathobiology of infection. By combining the clinical findings from human cases with findings from animal models, a clearer understanding of how alphaviruses cause myalgia and myositis is emerging (see Figure 3 for an overview).

4.1 Clinical observations for sub-acute alphavirus induced myopathies

There have been several studies on the clinical presentation of RRV disease, however most of these studies have focused largely on clinical reports based on patient feedback and limited biochemical and immunological analyses of patient's blood. There have been a limited number of studies on human synovial tissues. These studies have focused on the synovial fluid to determine the nature of the cellular infiltrates (Fraser, 1986; Fraser *et al.*, 1981). Unfortunately, these studies did not extend to muscle tissues. Similarly, studies on human SINV infection with Pogosta disease mainly involved analysis of human serum for antibody and viral antigen detection.

Human CHIKV infection has been clinically well documented (Chow *et al.*, 2011; Pialoux *et al.*, 2007; Powers & Logue, 2007; Simon *et al.*, 2007; Sissoko *et al.*, 2010; Taubitz *et al.*, 2007). The serology profile during the course of infection has been documented by numerous studies, showing that viraemia lasts 5-7 days (acute infection), with a subsequent IgM+/IgG- sub-acute infection progressing for a further week until IgG antibodies are detected and in some cases IgM was still detectable (Staikowsky *et al.*, 2009). Interestingly, it has been reported that levels of IgM have remained high for months and even years in many cases of alphavirus infection (Chopra *et al.*, 2008; Kurkela *et al.*, 2005; Niklasson *et al.*, 1988). In addition, it has been suggested that the presence of IgM may serve as a marker for viral persistence, whereby latent infection may be linked to the long term disease sequelae and chronic pathologies associated with alphavirus infection, including the on-going inflammatory myopathies (Jaffar-Bandjee *et al.*, 2009).

A study by Ozden et al., analysed muscle biopsies of CHIKV-infected patients providing new insight into CHIKV pathogenesis in muscle tissues (Ozden *et al.*, 2007). Patients were either IgM+ or IgG+ without detectable viraemia and were therefore classified as being in the sub-acute or recurring stage of infection. In a CHIKV patient presenting with recurrent symptoms including fever, arthralgia and myalgia, histological analysis of muscle biopsies demonstrated atrophy and necrosis (Figure 4A and 4D) and vacuolization of muscle fibres (Figure 4B) and interstitial mixed acute and chronic inflammation (Figure 4C). Viral antigens were detected in the periphery of muscle fibres in single cells (Figure 5B, 5C) or in groups of

cells (Figure 5A, 5D). Viral antigen was also detected (to lower levels) in the muscle biopsy of a patient with recurrent infection (Figure 5F), indicating that muscle satellite cells may harbour persistent alphaviral infection.

*Modeled on CHIKV infection (Labadie et al., 2010)

Fig. 3. Comparison of symptoms resulting from naturally human alphavirus infection, laboratory-infected nonhuman primate model of CHIKV, and laboratory-infected alphavirus mouse-models. Infection is broken down into three stages: acute, sub-acute, and chronic. The acute phase is defined by the presence of viraemia, the sub-acute phase is defined by the presence of IgM+ antibodies and the chronic phase is defined by IgG+ conversion. The chronic phase in humans includes persistent and recurrent myalgia and arthralgia that can last for months to years and may possibly result in symptoms synonymous with idiopathic inflammatory myopathies. Aspartate transaminase (AST) and alanine transaminase (ALT), monocyte chemoattractant protein (MCP)-1, tumour necrosis factor (TNF), interferon (IFN), Interleukin (IL), macrophage inflammatory protein (MIP), RANTES (CCL5) and reactive nitrogen intermediates (RNI).

Fig. 4. Time-course and histopathological data concerning CHIKV-infected patients. Patient #1, quadriceps biopsy was obtained during the CHIKV epidemic outbreak in the Reunion Island, presented a classical clinical picture of CHIKV infection. In addition, signs of rhabdomyolysis were reported. The muscle biopsy was performed on the quadriceps muscle 10 days post disease onset (illustrated by the red star). Patient #2 complained, in January 2006, of headaches, arthralgia and a rash; around three months later, she was admitted to hospital with a classical clinical picture of CHIKV infection, including myalgia. A biopsy was performed in the quadriceps muscle during this recurrent phase of the disease (red star). (A) and (B): Sections from the muscle biopsy of patient #1 H&E stained. (A) at x60 magnification showing lack of cellular infiltrates. Atrophy and necrosis of muscle fibres could be seen, as well as central nuclei (arrow). (B): at x140 magnification showing vacuolization of muscle fibres. (C) and (D): Sections from the muscle of patient #2 showing an important mononuclear infiltration in H&E stained at x140 magnification (C) and fibrosis in Masson's trichrome stained sections at x80 magnification (D). © 2007 Ozden et al., 2007.

Fig. 5. Detection of CHIKV antigens from muscle biopsies of CHIKV infected patients. Detection of CHIKV antigens by immunofluorescence (IF) and immunoperoxidase (IP). (A-D): Detection of CHIKV in muscle biopsys from patient #1 located at the periphery of the myotubes, as multiple cells per microscopic field (A, D), or as single cell per field (B, C). Some immunoreactive cells (arrows) were detected at the periphery of muscle fibres with central nuclei (C, arrowhead). (E, F): Detection of CHIKV by IF in muscle biopsy sections from patient #1 (E) and #2 (F). Magnification: $\times 300$ (A,C,D,E,F); $\times 400$ (B). © 2007 Ozden et al.

4.2 Cellular factors in alphavirus induced myopathies

Histopathological analysis of patients with a range of myopathies has demonstrated inflammatory infiltrates in the muscle tissues, primarily macrophages (Hewer & Goebel, 2008) and mononuclear cells. Infiltration of these cells is characteristic of idiopathic inflammatory myopathies. However, due to the lack of suitable animal models the mechanism by which inflammatory myopathies occur and the role that macrophages and mononuclear cells play in tissue pathology is poorly understood.

In animal models of alphavirus induced disease, the inflammatory response seen in the joint, bone and skeletal muscle tissue following an acute infection is predominantly regulated by the innate immune system (Morrison *et al.*, 2007; Morrison *et al.*, 2006). The inflammatory infiltrates consist of macrophages, NK cells, CD4⁺ and CD8⁺ T cells. The key role of macrophages and monocytes in the pathogenesis of alphavirus disease was first identified using the mouse model of RRV infection (Lidbury *et al.*, 2000). The selective depletion of macrophages by treatment of mice with macrophage-toxic agents such as silica or carrageenan, was found to almost entirely abrogate disease and the clearance of macrophages from damaged tissue correlated with recovery (Lidbury *et al.*, 2000). The high degree of macrophage and monocyte infiltration into infected tissues and joints has also been confirmed in the models of CHIKV infection (Gardner *et al.*, 2010; Morrison *et al.*, 2011).

The exact role that macrophages play in mediating disease is an area of ongoing research. It has been shown that infiltrating macrophages are a key mediator of muscle tissue destruction by mechanisms which involve the production of soluble factors such as macrophage inflammatory protein (MIP)-1, IFN- γ , interleukin (IL)-1 β , reactive nitrogen intermediates (RNI), tumour necrosis factor (TNF)- α , and monocyte chemoattractant protein (MCP)-1 (Lidbury *et al.*, 2008). In the macaque model of CHIKV infection, an up-regulation of IFN- α/β , IFN- γ , MCPs, IL-6 and TNF- α was detected, all synonymous with a strong, macrophage-induced inflammatory response (Labadie *et al.*, 2010). IL-1 β , RNI and TNF- α are factors known to trigger apoptosis (Bhaumik & Khar, 1998; Griffin & Hardwick, 1997; Roulston *et al.*, 1999) while MCP-1 and MIP-1 are involved in amplifying the inflammatory response (Carr *et al.*, 1994; Lidbury *et al.*, 2008; Ren *et al.*, 2010; Wolpe *et al.*, 1988).

Macrophages have also been implicated as a cellular reservoir for alphavirus persistence and therefore a contributing factor in the development of chronic disease symptoms. There have been numerous studies showing long-term persistence of RRV in macrophages *in vitro* (Linn *et al.*, 1996; Linn *et al.*, 1998; Way *et al.*, 2002) however how this pertains to human infection is largely unknown. Recently, the study by Labadie *et al.*, has shown macrophages act as a cellular reservoir for the persistence of CHIKV during the late stages of infection in macaques. This is the first *in vivo* study to show that CHIKV persistence in macrophages may be responsible for the long-lasting symptoms observed in humans. A study of viral persistence in macrophages during human infection has demonstrated evidence of CHIKV antigens in perivascular synovial macrophages in a CHIKV patient 18 months after the initial infection (Hoarau *et al.*, 2010). Thus, it is reasonable to speculate that viral persistence in macrophages may trigger low levels of macrophage activation long term, which may contribute to chronic disease symptoms.

To investigate the role of both T cells and the adaptive immune system in the development of RRV disease, studies were done on RAG-1 deficient mice, which lack functional T and B lymphocytes. Morrison *et al.*, infected RAG-1 deficient with RRV and monitored for clinical signs of disease (Morrison *et al.*, 2006). RAG-1 deficient mice developed disease signs similar to wild-type mice. Histological analysis revealed extensive myositis in the skeletal muscle with the inflammatory infiltrates identified as macrophages and NK cells, similar to observations in wild-type mice. Taken together, the results show that the adaptive immune response does not play a critical role in the development of RRV disease (Morrison *et al.*, 2006). Furthermore, the population of infiltrating CD4⁺ and CD8⁺ T cells found in the skeletal muscles of wild-type mice does not appear to play a role in the development of myositis as the absence of T and/or B cell functions does not affect disease development or outcome. Similar studies have been done using SINV infection in immunodeficient SCID or RAG-1 deficient mice. The studies found the clinical outcomes of SINV infection in immunodeficient neonatal mice to be comparable to that in immunocompetent neonatal mice (Burdeinick-Kerr *et al.*, 2009; Levine & Griffin, 1993). Results of these studies suggest that the adaptive immune response does not play a role in the development of RRV-induced myositis and is not essential in the clinical outcome of alphaviral disease.

4.3 Soluble factors in alphavirus induced myopathies

There have been limited studies on the measurement of pro-inflammatory factors in serum and synovial aspirates taken from confirmed cases of RRV infection. The levels of complement factor C3a, MCP-1, TNF- α , IFN- γ and RNI were all elevated in RRV patients

(Lidbury *et al.*, 2008; Morrison *et al.*, 2007). Furthermore, cells obtained from synovial fluid have been found to be mononuclear in nature being mainly monocytes and activated macrophages (Clarris *et al.*, 1975; Fraser *et al.*, 1981). In terms of RRV-induced myopathies, the limitations encountered in studying the pathogenesis in humans, particularly in investigating the role of soluble factors, have been achieved using a mouse model of RRV-disease.

Animal models have been used to determine the role of various cytokines and chemokines in the development of alphaviral-induced myopathies. Studies have shown that IFN- γ , TNF- α , IL-1 β , IL-6, macrophage inflammatory protein (MIP)-1 α , MCP-1, MCP-2, and MCP-3 are up-regulated in response to RRV infection (Lidbury *et al.*, 2008; Rulli *et al.*, 2009). The high levels of these factors in diseased tissues implicate their role in RRV-induced myositis (Lidbury *et al.*, 2008; Rulli *et al.*, 2009). Recently, a study by Rulli *et al.*, showed that the MCPs inhibitor, bindarit, reduced RRV disease symptoms by decreasing myositis and muscle tissue destruction (Rulli *et al.*, 2009). Bindarit down-regulated the production of chemokines MCP-1, -2 and -3, resulting in the reduction of macrophage recruitment into muscle tissues. These findings suggest a potential application of bindarit in the treatment of alphaviral-induced musculoskeletal disease such as myositis (Rulli *et al.*, 2009).

Several additional soluble factors have also been shown to play a role in the myositis that develops during the sub-acute phase of alphaviral infection. Studies using the RRV mouse model have shown that the complement component 3 (C3) contributes to the destructive phase of the inflammatory disease and promotes the development of severe myositis (Morrison *et al.*, 2008). The main function of the complement system is to eliminate invading organisms. It is activated through a number of different pathways and is centered around the action of the protein C3. In the mouse model of RRV disease, C3 activation products were detected at the sites of RRV-induced inflammation and C3 was found to be critical for myositis and the tissue destruction phase of RRV-induced inflammatory myopathies (Morrison *et al.*, 2007). Furthermore, mice deficient in functional receptor for C3 (CR3; CD11b deficient mice) showed significantly reduced disease symptoms and tissue destruction following RRV infection (Morrison *et al.*, 2008), confirming the critical role of complement in the regulation of inflammatory processes at the site of alphaviral-induced myositis. These studies show complement to be a potential target for anti-viral therapies in treating alphavirus induced myositis.

Similar to studies with RRV, analysis of serum samples from CHIKV-infected patients has also shown elevated levels of cytokines and chemokines. Of particular interest is the up-regulation of IL-6, TNF- α , IL-1 β , IFN- α , interferon-inducible protein-10 (IP-10) and monokine induced by IFN- γ (MIG) (Hoarau *et al.*, 2010; Ng *et al.*, 2009b). There has been an association between high levels of viraemia in acute CHIKV-infected patients and a higher production of pro-inflammatory cytokines such as IL-6 and TNF- α (Chow *et al.*, 2011). Presentation of chronic disease symptoms has also been associated with elevated levels of IL-6 and granulocyte macrophage colony-stimulating factor (GM-CSF) (Chow *et al.*, 2011). Whereas in patients who have fully recovered from disease, high levels of eotaxin and hepatocyte growth factor have been detected (Chow *et al.*, 2011).

Analysis of pro-inflammatory mediators in an adult C57BL/6 mouse model of CHIKV disease revealed an up-regulation in the expression of TNF- α , MCP-1, IFN- γ , IL-6 and IFN- α/β in the sera of infected mice (Gardner *et al.*, 2010). These results were consistent with findings using the macaque model of CHIKV disease where IFNs were shown to play an

important role in CHIKV pathogenesis, with IFN- α/β and IFN- γ , along with IL-6 and MCP-1, up-regulated at 2-10 days p.i. (Labadie *et al.*, 2010).

In patients with idiopathic inflammatory myopathies, the levels of several cytokines including MCP-1 and TNF- α are elevated (Bartoli *et al.*, 2001). A large number of studies have suggested a role for IL-1 β , IFN- α and TNF- α as mediators of inflammatory myopathies (Lundberg, 2000; Lundberg & Dastmalchi, 2002; Salomonsson & Lundberg, 2006). TNF has been extensively implicated in idiopathic inflammatory myopathies and is a potential therapeutic target. Enbrel® is an anti-TNF- α drug used to treat rheumatoid arthritis; has also given promising results in the treatment of myositis (Sprott *et al.*, 2004). Although TNF is similarly increased in alphavirus disease and myositis, Enbrel® treatment has been shown to both increase virus titre and alphavirus myositis (Zaid *et al.*, 2011). Therefore the use of anti-TNF therapy is not recommended in people presenting with myositis in which alphaviruses have not been ruled out as a potential causative agent.

4.4 Muscle enzymes in alphavirus induced myopathies

In addition to the roles of cytokines and chemokines in mediating alphavirus-induced myositis, muscle enzymes are also implicated in disease. Studies from the Reunion outbreak have found myalgia to be a major symptom of CHIKV infection (Paquet *et al.*, 2006). Elevated levels of creatine phosphokinase (CPK), a muscle enzyme marker for idiopathic inflammatory myopathies, and rhabdomyolysis have been reported in cases of CHIKV-infection (Lundberg, 2001). Ozden *et al.*, analysed muscle biopsies of CHIKV-infected patients and in one case, a CHIKV-infected patient in the sub-acute phase presented with rhabdomyolysis with elevated CPK (41600 IU/mL) in addition to symptoms of myalgia and elevated myoglobin. Demonstrating a similarity between idiopathic inflammatory myopathies and alphavirus-induced myositis (Ozden *et al.*, 2007).

5. Alphaviruses and idiopathic inflammatory myopathies (IIM)

The term idiopathic inflammatory myopathies (IIM) is used to describe myopathy arising from an unknown cause which involves inflammation of the muscles. The term encompasses a group of three related muscles disorders (Polymyositis, Dermatomyositis and Body Inculsion Myositis), which can be distinguished by clinical, histopathological, and immunological features (Lundberg & Dastmalchi, 2002).

Identification of possible triggers for IIM has been the topic of numerous studies with a focus on investigating the potential role of infectious agents in the aetiology of IIM. It has long been suspected that infectious agents may have a direct role in IIM with bacteria and viruses (notably retroviruses and entroviruses) being frequently implicated (Ytterberg, 1996). As muscle inflammation is one of the primary symptoms of the acute and sub-acute stages of viral infection, it is thought that the onset of an idiopathic inflammatory myopathy could be triggered by a chronic underlying viral infection.

The ability of alphaviruses to cause persistent and chronic infections has been recently studied with the demonstration of the presence of viral antigens in the muscle and synovial tissue of infected patients (Hoarau *et al.*, 2010; Ozden *et al.*, 2007). Invading pathogens can cause muscle tissue damage in both direct (by means of infection), and indirect (involving immune mediated damage) mechanisms. The question that remains is whether, in cases of IIM where virus antigen is no-longer detected, is it possible that virus persistence at an earlier stage triggers an immune response which continues for prolonged periods? There

have been numerous studies on the potential long-term disease sequelae due to alphavirus infection, suggesting that long-term chronic illness, lasting for years, may occur (Fraser, 1986; Harley *et al.*, 2002; Laine *et al.*, 2000; Mylonas *et al.*, 2002; Niklasson & Espmark, 1986; Niklasson *et al.*, 1988). However these studies have been unable to conclusively demonstrate alphaviruses as the cause of the symptoms presented. A lack of differential diagnosis, combined with potential underdiagnosis of other conditions that may exist concurrently in patients, and the lack of experimental long-term disease models, has led to the uncertainty regarding the level of long-term alphavirus disease.

Current medical practice requires cultures and/or other direct diagnostic tests to confirm the causative agents of infectious myositis. In the absence of such diagnosis the clinical manifestations observed in a patient are termed idiopathic. It is suspected that the time between the primary infection and the related immunological clinical representation is so great that clinicians are ill-equipped to make a proper diagnosis using current medical practices. Therefore what is in reality an infectious myositis becomes inaccurately diagnosed as an idiopathic inflammatory myopathy.

6. Conclusion

The increasing frequency and severity of alphavirus epidemics highlights the importance of these viruses in the incidence of inflammatory myopathies. At present, knowledge is lacking in the clinical aspects of human myositis following alphavirus infections with speculation that virus persistence in the muscle and macrophages may explain the recurrent and chronic symptoms of myalgia. In addition the correlation between a previous exposure to an alphavirus infection and the development of idiopathic inflammatory myopathies is ill-defined, with current diagnostic tests unable to isolate alphaviruses or detect alphavirus antigen in the presenting patient. It is evident that further research is needed to fully understand the aetiology and pathobiology of alphavirus-induced inflammatory myopathies. The establishment of animal models of chronic alphavirus disease would be of immense benefit in bridging the gap in our understanding of the role of viral infections in chronic myositis. Such a model will also be of value in dissecting disease mechanisms as well as investigate new treatment modalities.

7. References

- Anderson, C. R., Downs, W. G., Wattley, G. H., Ahin, N. W. & Reese, A. A. (1957). Mayaro virus: a new human disease agent. II. Isolation from blood of patients in Trinidad, B.W.I. *Am J Trop Med Hyg* 6, 1012-1016.
- Azevedo, R. S., Silva, E. V., Carvalho, V. L., Rodrigues, S. G., Nunes-Neto, J. P., Monteiro, H., Peixoto, V. S., Chiang, J. O., Nunes, M. R. & Vasconcelos, P. F. (2009). Mayaro fever virus, Brazilian Amazon. *Emerg Infect Dis* 15, 1830-1832.
- Bartoli, C., Civatte, M., Pellissier, J. F. & Figarella-Branger, D. (2001). CCR2A and CCR2B, the two isoforms of the monocyte chemoattractant protein-1 receptor are up-regulated and expressed by different cell subsets in idiopathic inflammatory myopathies. *Acta Neuropathol* 102, 385-392.
- Bhaumik & Khar (1998). Cytokine-induced production of NO by macrophages induces apoptosis and immunological rejection of AK-5 histiocytic tumor. *Apoptosis* 3, 361-368.

- Brummer-Korvenkontio, M., Vapalahti, O., Kuusisto, P., Saikku, P., Manni, T., Koskela, P., Nygren, T., Brummer-Korvenkontio, H. & Vaheri, A. (2002). Epidemiology of Sindbis virus infections in Finland 1981-96: possible factors explaining a peculiar disease pattern. *Epidemiol Infect* 129, 335-345.
- Burdeinick-Kerr, R., Govindarajan, D. & Griffin, D. E. (2009). Noncytolytic clearance of sindbis virus infection from neurons by gamma interferon is dependent on Jak/STAT signaling. *J Virol* 83, 3429-3435.
- Carey, D. E. (1971). Chikungunya and dengue: a case of mistaken identity? *J Hist Med Allied Sci* 26, 243-262.
- Carr, Roth, Luther, Rose & Springer (1994). Monocyte chemoattractant protein1 acts as a T-lymphocyte chemoattractant. *Proc Natl Acad Sci* 91, 3652-3656.
- Centers-for-Disease-Control-and-Prevention (15 August 2010). Chikungunya fever in Asia and the Indian Ocean. In *Outbreak Notice*.
- Chopra, A., Anuradha, V., Lagoo-Joshi, V., Kunjir, V., Salvi, S. & Saluja, M. (2008). Chikungunya virus aches and pains: an emerging challenge. *Arthritis Rheum* 58, 2921-2922.
- Chow, A., Her, Z., Ong, E. K., Chen, J. M., Dimatatac, F., Kwek, D. J., Barkham, T., Yang, H., Renia, L., Leo, Y. S. & Ng, L. F. (2011). Persistent arthralgia induced by Chikungunya virus infection is associated with interleukin-6 and granulocyte macrophage colony-stimulating factor. *J Infect Dis* 203, 149-157.
- Clarriss, B. J., Doherty, R. L., Fraser, J. R., French, E. L. & Muirden, K. D. (1975). Epidemic polyarthrititis: a cytological, virological and immunochemical study. *Aust N Z J Med* 5, 450-457.
- Couderc, T., Chretien, F., Schilte, C., Disson, O., Brigitte, M., Guivel-Benhassine, F., Touret, Y., Barau, G., Cayet, N., Schuffenecker, I., Despres, P., Arenzana-Seisdedos, F., Michault, A., Albert, M. L. & Lecuit, M. (2008). A mouse model for Chikungunya: young age and inefficient type-I interferon signaling are risk factors for severe disease. *PLoS Pathog* 4, e29.
- Couderc, T. & Lecuit, M. (2009). Focus on Chikungunya pathophysiology in human and animal models. *Microbes Infect* 11, 1197-1205.
- Enserink, M. (2007). Epidemiology. Tropical disease follows mosquitoes to Europe. *Science* 317, 1485.
- Espmark, A. & Niklasson, B. (1984). Ockelbo disease in Sweden: epidemiological, clinical, and virological data from the 1982 outbreak. *Am J Trop Med Hyg* 33, 1203-1211.
- Fraser, J. R. (1986). Epidemic polyarthrititis and Ross River virus disease. *Clin Rheum Dis* 12, 369-388.
- Fraser, J. R., Cunningham, A. L., Clarriss, B. J., Aaskov, J. G. & Leach, R. (1981). Cytology of synovial effusions in epidemic polyarthrititis. *Aust N Z J Med* 11, 168-173.
- Gardner, J., Anraku, I., Le, T. T., Larcher, T., Major, L., Roques, P., Schroder, W. A., Higgs, S. & Suhrbier, A. (2010). Chikungunya virus arthritis in adult wild-type mice. *J Virol* 84, 8021-8032.
- Griffin, D. E. (2007). Alphaviruses. In *Fields virology*, 5th edn, pp. 1023-1059. Edited by D. M. Knipe: Lippincott Williams & Wilkins.
- Griffin, D. E. & Hardwick, J. M. (1997). Regulators of apoptosis on the road to persistent alphavirus infection. *Annu Rev Microbiol* 51, 565-592.
- Hapuarachchi, H. C., Bandara, K. B., Sumanadasa, S. D., Hapugoda, M. D., Lai, Y. L., Lee, K. S., Tan, L. K., Lin, R. T., Ng, L. F., Bucht, G., Abeyewickreme, W. & Ng, L. C. (2010). Re-emergence of Chikungunya virus in South-east Asia: virological evidence from Sri Lanka and Singapore. *J Gen Virol* 91, 1067-1076.

- Harley, D., Bossingham, D., Purdie, D. M., Pandeya, N. & Sleigh, A. C. (2002). Ross River virus disease in tropical Queensland: evolution of rheumatic manifestations in an inception cohort followed for six months. *Med J Aust* 177, 352-355.
- Harley, D., Sleigh, A. & Ritchie, S. (2001). Ross River virus transmission, infection, and disease: a cross-disciplinary review. *Clin Microbiol Rev* 14, 909-932, table of contents.
- Hassing, R. J., Leparac-Goffart, I., Blank, S. N., Thevarayan, S., Tolou, H., van Doornum, G. & van Genderen, P. J. (2010). Imported Mayaro virus infection in the Netherlands. *J Infect* 61, 343-345.
- Hewer, E. & Goebel, H. H. (2008). Myopathology of non-infectious inflammatory myopathies - the current status. *Pathol Res Pract* 204, 609-623.
- Higgs, S. & Ziegler, S. A. (2010). A nonhuman primate model of chikungunya disease. *J Clin Invest* 120, 657-660.
- Hoarau, J. J., Jaffar Bandjee, M. C., Krejbich Trotot, P., Das, T., Li-Pat-Yuen, G., Dassa, B., Denizot, M., Guichard, E., Ribera, A., Henni, T., Tallet, F., Moiton, M. P., Gauzere, B. A., Bruniquet, S., Jaffar Bandjee, Z., Morbidelli, P., Martigny, G., Jolivet, M., Gay, F., Grandadam, M., Tolou, H., Vieillard, V., Debre, P., Autran, B. & Gasque, P. (2010). Persistent chronic inflammation and infection by Chikungunya arthritogenic alphavirus in spite of a robust host immune response. *J Immunol* 184, 5914-5927.
- Jacups, S. P., Whelan, P. I. & Currie, B. J. (2008). Ross River virus and Barmah Forest virus infections: a review of history, ecology, and predictive models, with implications for tropical northern Australia. *Vector Borne Zoonotic Dis* 8, 283-297.
- Jaffar-Bandjee, M. C., Das, T., Hoarau, J. J., Krejbich Trotot, P., Denizot, M., Ribera, A., Roques, P. & Gasque, P. (2009). Chikungunya virus takes centre stage in virally induced arthritis: possible cellular and molecular mechanisms to pathogenesis. *Microbes Infect* 11, 1206-1218.
- Johnson, R. T. (1965). Virus invasion of the central nervous system: a study of sindbis virus infection in the mouse using fluorescent antibody. *Am J Pathol* 46, 929-943.
- Johnston, R. E. & Peters, C. J. (1996). *Alphaviruses*. Philadelphia, Pa: Lippincott-Raven Publishers.
- Josseran, L., Paquet, C., Zehgnoun, A., Caillere, N., Le Tertre, A., Solet, J. L. & Ledrans, M. (2006). Chikungunya disease outbreak, Reunion Island. *Emerg Infect Dis* 12, 1994-1995.
- Kalantri, S. P., Joshi, R. & Riley, L. W. (2006). Chikungunya epidemic: an Indian perspective. *Natl Med J India* 19, 315-322.
- Kiwanuka, N., Sanders, E. J., Rwaguma, E. B., Kawamata, J., Ssengooba, F. P., Najjemba, R., Were, W. A., Lamunu, M., Bagambisa, G., Burkot, T. R., Dunster, L., Lutwama, J. J., Martin, D. A., Cropp, C. B., Karabatsos, N., Lanciotti, R. S., Tsai, T. F. & Campbell, G. L. (1999). O'nyong-nyong fever in south-central Uganda, 1996-1997: clinical features and validation of a clinical case definition for surveillance purposes. *Clin Infect Dis* 29, 1243-1250.
- Klimstra, W. B., Ryman, K. D., Bernard, K. A., Nguyen, K. B., Biron, C. A. & Johnston, R. E. (1999). Infection of neonatal mice with sindbis virus results in a systemic inflammatory response syndrome. *J Virol* 73, 10387-10398.
- Kurkela, S., Manni, T., Myllynen, J., Vaheri, A. & Vapalahti, O. (2005). Clinical and laboratory manifestations of Sindbis virus infection: prospective study, Finland, 2002-2003. *J Infect Dis* 191, 1820-1829.
- Kurkela, S., Manni, T., Vaheri, A. & Vapalahti, O. (2004). Causative agent of Pogosta disease isolated from blood and skin lesions. *Emerg Infect Dis* 10, 889-894.

- L'Vov D, K., Skvortsova, T. M., Kondrashina, N. G., Vershinskii, B. V. & Lesnikov, A. L. (1982). [Etiology of Karelia fever--a new arbovirus infection]. *Vopr Virusol* 27, 690-692.
- Labadie, K., Larcher, T., Joubert, C., Mannioui, A., Delache, B., Brochard, P., Guigand, L., Dubreil, L., Lebon, P., Verrier, B., de Lamballerie, X., Suhrbier, A., Cherel, Y., Le Grand, R. & Roques, P. (2010). Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages. *J Clin Invest* 120, 894-906.
- Laine, M., Luukkainen, R., Jalava, J., Ilonen, J., Kuusisto, P. & Toivanen, A. (2000). Prolonged arthritis associated with sindbis-related (Pogosta) virus infection. *Rheumatology (Oxford)* 39, 1272-1274.
- Laine, M., Luukkainen, R. & Toivanen, A. (2004). Sindbis viruses and other alphaviruses as cause of human arthritic disease. *J Intern Med* 256, 457-471.
- Lanciotti, R. S., Ludwig, M. L., Rwaguma, E. B., Lutwama, J. J., Kram, T. M., Karabatsos, N., Cropp, B. C. & Miller, B. R. (1998). Emergence of epidemic O'nyong-nyong fever in Uganda after a 35-year absence: genetic characterization of the virus. *Virology* 252, 258-268.
- Levine, B. & Griffin, D. E. (1993). Molecular analysis of neurovirulent strains of Sindbis virus that evolve during persistent infection of scid mice. *J Virol* 67, 6872-6875.
- Lidbury, B. A., Rulli, N. E., Suhrbier, A., Smith, P. N., McColl, S. R., Cunningham, A. L., Tarkowski, A., van Rooijen, N., Fraser, R. J. & Mahalingam, S. (2008). Macrophage-derived proinflammatory factors contribute to the development of arthritis and myositis after infection with an arthrogenic alphavirus. *J Infect Dis* 197, 1585-1593.
- Lidbury, B. A., Simeonovic, C., Maxwell, G. E., Marshall, I. D. & Hapel, A. J. (2000). Macrophage-induced muscle pathology results in morbidity and mortality for Ross River virus-infected mice. *J Infect Dis* 181, 27-34.
- Linn, M. L., Aaskov, J. G. & Suhrbier, A. (1996). Antibody-dependent enhancement and persistence in macrophages of an arbovirus associated with arthritis. *J Gen Virol* 77, 407-411.
- Linn, M. L., Mateo, L., Gardner, J. & Suhrbier, A. (1998). Alphavirus-specific cytotoxic T lymphocytes recognize a cross-reactive epitope from the capsid protein and can eliminate virus from persistently infected macrophages. *J Virol* 72, 5146-5153.
- Liumbruno, G. M., Calteri, D., Petropulacos, K., Mattivi, A., Po, C., Macini, P., Tomasini, I., Zucchelli, P., Silvestri, A. R., Sambri, V., Pupella, S., Catalano, L., Piccinini, V., Calizzani, G. & Grazzini, G. (2008). The Chikungunya epidemic in Italy and its repercussion on the blood system. *Blood Transfus* 6, 199-210.
- Lundberg, I. E. (2000). The role of cytokines, chemokines, and adhesion molecules in the pathogenesis of idiopathic inflammatory myopathies. *Curr Rheumatol Rep* 2, 216-224.
- Lundberg, I. E. (2001). The physiology of inflammatory myopathies: an overview. *Acta Physiol Scand* 171, 207-213.
- Lundberg, I. E. & Dastmalchi, M. (2002). Possible pathogenic mechanisms in inflammatory myopathies. *Rheum Dis Clin North Am* 28, 799-822.
- Malherbe, H., Strickland-Cholmley, M. & Jackson, A. L. (1963). Sindbis virus infection in man. Report of a case with recovery of virus from skin lesions. *S Afr Med J* 37, 547-552.
- Mathiot, C. C., Grimaud, G., Garry, P., Bouquety, J. C., Mada, A., Daguisy, A. M. & Georges, A. J. (1990). An outbreak of human Semliki Forest virus infections in Central African Republic. *Am J Trop Med Hyg* 42, 386-393.

- McGill, P. E. (1995). Viral infections: alpha-viral arthropathy. *Baillieres Clin Rheumatol* 9, 145-150.
- Morrison, T. E., Fraser, R. J., Smith, P. N., Mahalingam, S. & Heise, M. T. (2007). Complement contributes to inflammatory tissue destruction in a mouse model of Ross River virus-induced disease. *J Virol* 81, 5132-5143.
- Morrison, T. E., Oko, L., Montgomery, S. A., Whitmore, A. C., Lotstein, A. R., Gunn, B. M., Elmore, S. A. & Heise, M. T. (2011). A mouse model of chikungunya virus-induced musculoskeletal inflammatory disease: evidence of arthritis, tenosynovitis, myositis, and persistence. *Am J Pathol* 178, 32-40.
- Morrison, T. E., Simmons, J. D. & Heise, M. T. (2008). Complement receptor 3 promotes severe ross river virus-induced disease. *J Virol* 82, 11263-11272.
- Morrison, T. E., Whitmore, A. C., Shabman, R. S., Lidbury, B. A., Mahalingam, S. & Heise, M. T. (2006). Characterization of Ross River virus tropism and virus-induced inflammation in a mouse model of viral arthritis and myositis. *J Virol* 80, 737-749.
- Mylonas, A. D., Brown, A. M., Carthew, T. L., McGrath, B., Purdie, D. M., Pandeya, N., Vecchio, P. C., Collins, L. G., Gardner, I. D., de Looze, F. J., Reymond, E. J. & Suhrbier, A. (2002). Natural history of Ross River virus-induced epidemic polyarthritides. *Med J Aust* 177, 356-360.
- Ng, L. C., Tan, L. K., Tan, C. H., Tan, S. S., Hapuarachchi, H. C., Pok, K. Y., Lai, Y. L., Lam-Phua, S. G., Bucht, G., Lin, R. T., Leo, Y. S., Tan, B. H., Han, H. K., Ooi, P. L., James, L. & Khoo, S. P. (2009a). Entomologic and virologic investigation of Chikungunya, Singapore. *Emerg Infect Dis* 15, 1243-1249.
- Ng, L. F., Chow, A., Sun, Y. J., Kwek, D. J., Lim, P. L., Dimatatac, F., Ng, L. C., Ooi, E. E., Choo, K. H., Her, Z., Kourilsky, P. & Leo, Y. S. (2009b). IL-1beta, IL-6, and RANTES as biomarkers of Chikungunya severity. *PLoS One* 4, e4261.
- Niklasson, B. & Espmark, A. (1986). Ockelbo disease: arthralgia 3-4 years after infection with a Sindbis virus related agent. *Lancet* 1, 1039-1040.
- Niklasson, B., Espmark, A. & Lundstrom, J. (1988). Occurrence of arthralgia and specific IgM antibodies three to four years after Ockelbo disease. *J Infect Dis* 157, 832-835.
- Old, J. M. & Deane, E. M. (2005). Antibodies to the Ross River virus in captive marsupials in urban areas of eastern New South Wales, Australia. *J Wildl Dis* 41, 611-614.
- Oliveira, N. M., Broom, A. K., Mackenzie, J. S., Smith, D. W., Lindsay, M. D., Kay, B. H. & Hall, R. A. (2006). Epitope-blocking enzyme-linked immunosorbent assay for detection of antibodies to Ross River virus in vertebrate sera. *Clin Vaccine Immunol* 13, 814-817.
- Ozden, S., Huerre, M., Riviere, J. P., Coffey, L. L., Afonso, P. V., Mouly, V., de Monredon, J., Roger, J. C., El Amrani, M., Yvin, J. L., Jaffar, M. C., Frenkiel, M. P., Sourisseau, M., Schwartz, O., Butler-Browne, G., Despres, P., Gessain, A. & Ceccaldi, P. E. (2007). Human muscle satellite cells as targets of Chikungunya virus infection. *PLoS One* 2, e527.
- Paquet, C., Quatresous, I., Solet, J. L., Sissoko, D., Renault, P., Pierre, V., Cordel, H., Lassalle, C., Thiria, J., Zeller, H. & Schuffnecker, I. (2006). Chikungunya outbreak in Reunion: epidemiology and surveillance, 2005 to early January 2006. *Euro Surveill* 11, E060202 060203.
- Pialoux, G., Gauzere, B. A., Jaureguierry, S. & Strobel, M. (2007). Chikungunya, an epidemic arbovirolosis. *Lancet Infect Dis* 7, 319-327.
- Pinheiro, F. P., Freitas, R. B., Travassos da Rosa, J. F., Gabbay, Y. B., Mello, W. A. & LeDuc, J. W. (1981). An outbreak of Mayaro virus disease in Belterra, Brazil. I. Clinical and virological findings. *Am J Trop Med Hyg* 30, 674-681.

- Posey, D. L., O'Rourke, T., Roehrig, J. T., Lanciotti, R. S., Weinberg, M. & Maloney, S. (2005). O'Nyong-nyong fever in West Africa. *Am J Trop Med Hyg* 73, 32.
- Powers, A. M. & Logue, C. H. (2007). Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus. *J Gen Virol* 88, 2363-2377.
- Receveur, M., Ezzedine, K., Pistone, T. & Malvy, D. (2010). Chikungunya infection in a French traveller returning from the Maldives, October, 2009. *Euro Surveill* 15, 19494.
- Ren, Guo, Guo, Lenz, Qian, Koenen & al, e. (2010). Polymerization of MIP-1 chemokine (CCL3 and CCL4) and clearance of MIP-1 by insulin-degrading enzyme. *The EMBO Journal* 29, 3952-3966.
- Renault, P., Solet, J. L., Sissoko, D., Balleydier, E., Larrieu, S., Filleul, L., Lassalle, C., Thiria, J., Rachou, E., de Valk, H., Ilief, D., Ledrans, M., Quatresous, I., Quenel, P. & Pierre, V. (2007). A major epidemic of chikungunya virus infection on Reunion Island, France, 2005-2006. *Am J Trop Med Hyg* 77, 727-731.
- Robinson, M. C. (1955). An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952-53. I. Clinical features. *Trans R Soc Trop Med Hyg* 49, 28-32.
- Ross, R. W. (1956). The Newala epidemic. III. The virus: isolation, pathogenic properties and relationship to the epidemic. *J Hyg (Lond)* 54, 177-191.
- Roulston, A., Marcellus, R. C. & Branton, P. E. (1999). Viruses and apoptosis. *Annu Rev Microbiol* 53, 577-628.
- Rulli, N. E., Guglielmotti, A., Mangano, G., Rolph, M. S., Apicella, C., Zaid, A., Suhrbier, A. & Mahalingam, S. (2009). Amelioration of alphavirus-induced arthritis and myositis in a mouse model by treatment with bindarit, an inhibitor of monocyte chemotactic proteins. *Arthritis Rheum* 60, 2513-2523.
- Salomonsson, S. & Lundberg, I. E. (2006). Cytokines in idiopathic inflammatory myopathies. *Autoimmunity* 39, 177-190.
- Sane, J., Guedes, S., Kurkela, S., Lyytikainen, O. & Vapalahti, O. (2010). Epidemiological analysis of mosquito-borne Pogosta disease in Finland, 2009. *Euro Surveill* 15.
- Simon, F., Parola, P., Grandadam, M., Fourcade, S., Oliver, M., Brouqui, P., Hance, P., Kraemer, P., Ali Mohamed, A., de Lamballerie, X., Charrel, R. & Tolou, H. (2007). Chikungunya infection: an emerging rheumatism among travelers returned from Indian Ocean islands. Report of 47 cases. *Medicine (Baltimore)* 86, 123-137.
- Sissoko, D., Ezzedine, K., Moendandze, A., Giry, C., Renault, P. & Malvy, D. (2010). Field evaluation of clinical features during chikungunya outbreak in Mayotte, 2005-2006. *Trop Med Int Health* 15, 600-607.
- Skogh, M. & Espmark, A. (1982). Ockelbo disease: epidemic arthritis-exanthema syndrome in Sweden caused by Sindbis-virus like agent. *Lancet* 1, 795-796.
- Sourisseau, M., Schilte, C., Casartelli, N., Trouillet, C., Guivel-Benhassine, F., Rudnicka, D., Sol-Foulon, N., Le Roux, K., Prevost, M. C., Fsihi, H., Frenkiel, M. P., Blanchet, F., Afonso, P. V., Ceccaldi, P. E., Ozden, S., Gessain, A., Schuffenecker, I., Verhasselt, B., Zamborlini, A., Saib, A., Rey, F. A., Arenzana-Seisdedos, F., Despres, P., Michault, A., Albert, M. L. & Schwartz, O. (2007). Characterization of reemerging chikungunya virus. *PLoS Pathog* 3, e89.
- Sprott, H., Glatzel, M. & Michel, B. A. (2004). Treatment of myositis with etanercept (Enbrel), a recombinant human soluble fusion protein of TNF-alpha type II receptor and IgG1. *Rheumatology (Oxford)* 43, 524-526.
- Staikowsky, F., Talarmin, F., Grivard, P., Souab, A., Schuffenecker, I., Le Roux, K., Lecuit, M. & Michault, A. (2009). Prospective study of Chikungunya virus acute infection in the Island of La Reunion during the 2005-2006 outbreak. *PLoS One* 4, e7603.

- Suhrbier, A. & La Linn, M. (2004). Clinical and pathologic aspects of arthritis due to Ross River virus and other alphaviruses. *Curr Opin Rheumatol* 16, 374-379.
- Taubitz, W., Cramer, J. P., Kapaun, A., Pfeffer, M., Drosten, C., Dobler, G., Burchard, G. D. & Loscher, T. (2007). Chikungunya fever in travelers: clinical presentation and course. *Clin Infect Dis* 45, e1-4.
- Taylor, R. M., Hurlbut, H. S., Work, T. H., Kingston, J. R. & Frothingham, T. E. (1955). Sindbis virus: a newly recognized arthropodtransmitted virus. *Am J Trop Med Hyg* 4, 844-862.
- Tesh, R. B. (1982). Arthritides caused by mosquito-borne viruses. *Annu Rev Med* 33, 31-40.
- Tesh, R. B., Watts, D. M., Russell, K. L., Damodaran, C., Calampa, C., Cabezas, C., Ramirez, G., Vasquez, B., Hayes, C. G., Rossi, C. A., Powers, A. M., Hice, C. L., Chandler, L. J., Cropp, B. C., Karabatsos, N., Roehrig, J. T. & Gubler, D. J. (1999). Mayaro virus disease: an emerging mosquito-borne zoonosis in tropical South America. *Clin Infect Dis* 28, 67-73.
- Thavara, U., Tawatsin, A., Pengsakul, T., Bhakdeenuan, P., Chanama, S., Anantapreecha, S., Molito, C., Chompoosri, J., Thammapalo, S., Sawanpanyalert, P. & Siriyasatien, P. (2009). Outbreak of chikungunya fever in Thailand and virus detection in field population of vector mosquitoes, *Aedes aegypti* (L.) and *Aedes albopictus* Skuse (Diptera: Culicidae). *Southeast Asian J Trop Med Public Health* 40, 951-962.
- Theamboonlers, A., Rianthavorn, P., Praianantathavorn, K., Wuttirattanakowit, N. & Poovorawan, Y. (2009). Clinical and molecular characterization of chikungunya virus in South Thailand. *Jpn J Infect Dis* 62, 303-305.
- Trgovcich, J., Aronson, J. F. & Johnston, R. E. (1996). Fatal Sindbis virus infection of neonatal mice in the absence of encephalitis. *Virology* 224, 73-83.
- Trgovcich, J., Ryman, K., Extrom, P., Eldridge, J. C., Aronson, J. F. & Johnston, R. E. (1997). Sindbis virus infection of neonatal mice results in a severe stress response. *Virology* 227, 234-238.
- Way, S. J., Lidbury, B. A. & Banyer, J. L. (2002). Persistent Ross River virus infection of murine macrophages: an in vitro model for the study of viral relapse and immune modulation during long-term infection. *Virology* 301, 281-292.
- Williams, M. C., Woodall, J. P., Corbet, P. S. & Gillett, J. D. (1965). O'nyong-nyong Fever: An epidemic virus disease in East Africa. 8. Virus isolations from *Anopheles* mosquitoes. *Trans R Soc Trop Med Hyg* 59, 300-306.
- Wolpe, S. D., Davatelis, G., Sherry, B., Beutler, B., Hesse, D. G., Nguyen, H. T., Moldawer, L. L., Nathan, C. F., Lowry, S. F. & Cerami, A. (1988). Macrophages secrete a novel heparin-binding protein with inflammatory and neutrophil chemokinetic properties. *J Exp Med* 167, 570-581.
- Yergolkar, P. N., Tandale, B. V., Arankalle, V. A., Sathe, P. S., Sudeep, A. B., Gandhe, S. S., Gokhle, M. D., Jacob, G. P., Hundekar, S. L. & Mishra, A. C. (2006). Chikungunya outbreaks caused by African genotype, India. *Emerg Infect Dis* 12, 1580-1583.
- Ytterberg, S. R. (1996). Infectious agents associated with myopathies. *Curr Opin Rheumatol* 8, 507-513.
- Zaid, A., Rulli, N. E., Rolph, M. S., Suhrbier, A. & Mahalingam, S. (2011). Disease exacerbation by etanercept in a mouse model of alphaviral arthritis and myositis. *Arthritis Rheum* 63, 488-491.
- Ziegler, S. A., Lu, L., da Rosa, A. P., Xiao, S. Y. & Tesh, R. B. (2008). An animal model for studying the pathogenesis of chikungunya virus infection. *Am J Trop Med Hyg* 79, 133-139.

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The term "myositis" covers a variety of disorders often designated "idiopathic inflammatory myopathies". Although they are rather rare compared to other rheumatic diseases, they often cause severe disability and not infrequently increased mortality. The additional involvement of important internal organs such as the heart and lungs, is not uncommon. Thus, there is a great need for a better understanding of the etiopathogenesis of myositis, which may lead to improved treatment and care for these patients. Major advances regarding research and medical treatment have been made during recent years. Of particular importance is the discovery of the Myositis specific autoantibodies, linking immunological and pathological profiles to distinct clinical disease entities. A wide range of aspects of myopathies is covered in the book presented by highly qualified authors, all internationally known for their expertise on inflammatory muscle diseases. The book covers diagnostic, pathological, immunological and therapeutic aspects of myositis.

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