Review Article

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.org

doi: 10.4103/2221−1691.276315

Impact Factor: 1.59

Mayaro fever: A brief review on the immune profile

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ABSTRACT

Mayaro virus is an emergent alphavirus that infects humans, leading to Mayaro fever. Approximately fifty percent of infected patients develop arthritis symptoms in the recovery phase, a phase that can last up to a year. The literature about Mayaro virus infection and its immune response is scarce, which may hamper the development of treatment strategies. We summarize changes in cytokines and chemokines in the acute and recovery phase in Mayaro virus infected patients, and relate this molecular characterization with the immune response. VEGF and IL-12/p70 show pronounced changes in patients in the acute phase, suggesting the development of cellular immunity and Th1 response. IL-6, IL-7, CXCL8/IL-8, IL-13, IL-17, and IFN-γ are elevated in patients with arthritis symptoms in the long-term recovery phase, which may be related to the continuous inflammatory process, a possible Th2 inhibiting and promoting Th17 process. Although few studies discuss the issue, with a small number of patients and different backgrounds, inflammatory and immune response and manifestations seem to be closely linked. This information may help to develop the appropriate treatment strategies in Mayaro virus infection. Therefore, we analyzed and summarized data available in literature.

KEYWORDS: Emergent virus; Mayaro virus; Cytokines; Chemokine; Molecular characterization

1. Introduction

Mayaro virus (MAYV) is an arbovirus that belongs to the *Alphavirus* genus and Togaviridae family. It occurs in the peri-Amazonian region and is considered as a potential emergent virus[1−3]. MAYV infects humans through the bite of mosquitoes, which induces Mayaro fever disease and can lead to hemorrhagic phenomena and persistent incapacitating arthritis[4−7].

The main clinical manifestation of Mayaro fever is incapacitating arthritis, which can be manifested in the long-term recovery phase as a chronic form, occurring in up to 50% of patients and persisting for at least one year[8−10]. Because of the scarce literature about MAYV, the specific mechanisms involved in infection, pathogenesis and chronic manifestations are not clear[11,12], hampering the development of therapeutic strategies of chronic arthritis caused by this virus[13].

Analysis of patients with Mayaro fever revealed that chronic arthritis in the long-term recovery phase occurs independently of the immune response mediated by anti-MAYV neutralizing antibodies[9], excluding the adaptive immunity participation in this process and indicating the major participation of innate immunity response. Following being established for alphaviruses infections, the persistence of the symptoms of chronic arthritis may be related to the maintenance of the virus or their products in target cells, with subsequent accumulation of immune-inflammatory mediators such as cytokine interleukin 6 (IL-6) and granulocyte-macrophage colony-stimulating factor (GM-CSF)[14].

Cytokine and chemokine characterizations of MAYV infection can be an important tool to develop the adequate treatment and the therapeutic practices against the Mayaro fever disease[13], similar

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How to cite this article: Ferreira JM, Campos-Ferreira DS, Figueiredo EVMDS, Lima Filho JLD. Mayaro fever: A brief review on the immune profile. Asian Pac J Trop Biomed 2020; 10(3): 95-100.

Article history: Received 26 September 2019; Revision 15 November 2019; Accepted 15 January 2020; Available online 7 February 2020

to the treatment of rheumatic diseases, since the immunological and molecular profile helps in the implementation of therapeutic practices, improving the quality life of the patients[15,16]. We summarize the immune profile of chronic arthritis caused by MAYV infection in the acute phase and long-term recovery phase.

2. Background: MAYV and Mayaro fever

MAYV belongs to the Semliki Forest antigenic serocomplex[1,2] and was originally isolated in 1954 from the blood of five febrile rural workers near the town of Mayaro, Trinidad, and Tobago, naming virus taxa[1]. Information about this virus morphology is scarce, and information in the literature shows that MAYV has the basic morphology of *Alphavirus*[17]. The virus capsid presents 240 copies of capsid protein (C) and glycoproteins E1 and E2[18,19].

Viral glycoproteins of MAYV have crucial biological holes. E2 is responsible for virus receptor binding and E1 plays a complementary role in virus entry and fusion activity[20,21]. The proteins C, E1, and E2 have assembled in a 1:1:1 stoichiometry, creating two nested T=4 icosahedral shells that sandwich a host-derived lipid bilayer[22].

The genome of MAYV consists of a positive single-stranded RNA with 11.5-12 kb and about two-thirds are directly read as an mRNA to encode four nonstructural proteins (nsP1–4) for transcription and replication of viral RNA[23,24]. The structural proteins [C (capsid), E3, E2, 6K, and E1] are encoded by a subgenomic positive-strand RNA referred to as 26S RNA, transcribed from 3' one-third of the genomic RNA. The organization of the genome present on flank 5' -7-methylguanylate (m7G) cap and a 3-poly-A tail, and can be summarized as 5'm7G-nsP1-nsP2-nsP3-nsP4-(junction)-C-E3-E2–6K-E1-A-3' genes[24,25].

Genotypes and phylogeny of MAYV may be based on *E1–E2* genes sequences and full-genome. Three genotypes of MAYV are currently recognized: D (dispersed), L (limited) and N (new)[2,5,6,24,26]. Dispersed genotype was found in a vast area of South America such as Trinidad and Tobago, Brazil, Peru, Bolivia, and Venezuela, while limited and new genotypes were found only in Brazil and Peru, respectively[27].

MAYV causes acute febrile illness (Mayaro fever), which can be mildly to moderately severe, ranging from 3-7 d with an average of 5-d duration. Viremia is observed during the acute phase (lasts for 2-3 d), while the uneventful recovery may occur in a short period after the acute phase. Thus, the incubation period can range from 3-11 d[5,23,24,28–30].

Mayaro fever is a disease with generic symptoms such as fever, fatigue, headache, retroorbital pain, skin rashes, with eruption occurring in more than 40% of cases, which are manifested before, simultaneously or after symptoms of arthralgia, lasting 7-10 d. In addition, myalgia, joint edema, and arthritis and arthralgia stiffness (including fingers, foot, hands, joints, and ankles in general) can be observed in Mayaro fever and similar to the symptoms observed in other *Alphavirus*. Arthritis can indicate that MAYV present a tropism for synovial tissue, developing pathologies[14,31–33].

3. Cytokines and chemokines associated with MAYV in the acute phase

Few studies in the literature evaluated cytokines and chemokines in MAYV infected patients with arthralgic symptoms[9,34,35]. Among the cytokines and chemokines available and analyzed in the literature are: interleukins (IL) IL-1Ra, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-13, IL-17, IL-12p70, RANTES/CCL5, vascular endothelial growth factor (VEGF), eotaxin/CCL11, IP-10/CXCL10 (interferon-γ–induced protein 10), macrophage inflammatory protein (MIP)-1α and MIP-1β, GM-CSF, basic fibroblast growth factor (bFGF), granulocyte colony-stimulating factor (G-CSF), monocyte chemoattractant protein-1 (MCP-1/CCL2), platelet-derived growth factor B (PDGFB), TNF-α and IFN-γ. Cytokines and chemokines of patients from Germany, Netherlands, Switzerland, and Peru were evaluated.

Differences in cytokine levels may be observed during the infection in different clinical phases in patients. Clinical data about the first week of MAYV infection (≤30 days after symptom onset) in European patients indicates a significant increase in the concentration of IL-10, IL-12p70, RANTES/CCL5, and VEGF, and a significant decrease in eotaxin/CCL11 when compared with serum samples of healthy donors[34]. TNF was decreased in comparison to healthy donors but did not differ statistically[34]. In contrast, the levels of TNF-α were increased a few hours post-infection in *in vitro* assays performed by Cavalheiro and co-workers[32].

Santiago *et al*.[9] observed a significant decrease of MIP-1β in the acute phase (the first week after infection) in Peruvian patients as well as increases in cytokines and chemokines including IL-1Ra, IL-6, IL-7, CXCL8/IL-8, IL-13, IL-17, G-CSF, IFN-γ, PDGF-BB, TNF-α, VEGF, and IL-12p70 in comparison to healthy donor controls. An increase of MCP-1/CCL2 was also observed, remaining on high levels during the convalescent phase (6 months post-infection).

VEGF and IL-12p70 have been reported as common immune mediators present in all patients in the acute phase of Mayaro fever, which can suggest cell production of the immune system and Th1 induced response (inflammatory response) against the infection. VEGF may also act in hematopoiesis and as a key regulator in angiogenesis and its activity is mediated by the binding and stimulation of two transmembrane endothelial receptor tyrosine kinases, VEGF receptors (VEGFR) types 1 and 2[36–38].

Th1 response for T cell generation occurs by stimuli of the IL-12 family of cytokines. IL-12 is composed of two covalently linked subunits, IL-12p35, and IL-12p40. Since IL-12p35 and IL-12p40 are expressed together, the bioactive IL-12p70 is formed. IL-12 has been linked with innate immunity and in the development of adaptive immunity characterized by the induction of IFN-γ production. IL-12 and IFN-γ induce the activity and proliferation of MΦs, NK cells, and T cells, which also secrete IL-12[39,40].

4. Alteration in levels of immune mediators in the long–term recovery phase with chronic polyarthritis

Clinical cases of European patients characterized by Tappe and collaborators[34] as 'post-acute-phase' (>30 days after symptom onset, with arthralgia) showed that the concentrations of IL-5, IL-6, IL-7, CXLC8/IL-8, IL-9, IL-10, IL-13, IL-17, IP-10/CXCL10, RANTES/CCL5, MIP-1α, MIP-1β, GM-CSF, and IFN-γ were higher compared with the serum samples from healthy blood donors. The cytokine levels measured in the acute phase in the same study did not differ significantly from those measured in the recovery phase. Moreover, there were no significant changes in IL-1b, IL-2, IL-4, bFGF, G-CSF, MCP-1, and PDGFB in either phase.

In accordance with classification by Tappe and co-workers, the case report of Theilacker and co-workers[35] may be labeled as 'post-acute'. In this case report, a woman with prolonged arthralgia showed, on 83 days post initial symptoms, CXLC8/IL-8 was 15-fold higher than normal, while the chemokines CCL5/RANTES, CXCL9/MIG, MCP-1/CCL2, and CXCL10/IP-10 and cytokines IL-2, IL-4, IL-5, IL-10, TNF, and IFN-γ were not different from the healthy control. The woman was medicated with non-steroidal anti-inflammatory drugs and two posterior analyses were conducted. Each subsequent analysis was performed 106 and 167 days after initial arthralgic symptoms. There were no differences found in serum between the woman and health control, including the ratio level of CXCL8/IL-8. Non-steroidal anti-inflammatory drug treatment may suppress CXCL8/IL-8, since this anti-inflammatory compound shares a pathway dependent on the suppression of nuclear factor kappa B activity, decreasing the transcription of growth factor, chemokines, and proteases such as COX-2, VEGF, CXCL8/IL-8, MCP-1/CCL2 and MIP-1α/CCL3[41].

Santiago *et al*.[9] found elevated levels of immune mediators including G-CSF, IL-1Ra, IL-6, IL-7, CXCL8/IL-8, IL-13, IL-17, IFN-γ, CCL2/MCP-1, PDGF-BB, VEGF and TNF-α in Peruvian patients with persistent arthralgia, and many of these cytokines and chemokines remained significantly elevated along with the persistent arthralgia. IL-13 remained markedly increased in subjects with persistent arthralgia compared with those who fully recovered. It was suggested that IL-13 could potentially serve as a predictor of severe outcomes after MAYV infection.

Increased IL-6, IL-7, CXCL8/IL-8, IL-13, IL-17, and IFN-γ were observed in patients with arthralgic symptoms in the recovery phase[9,34]. These cytokines can indicate a major inflammatory response (a continuous response even after the acute phase with Th1 process transformation), concomitantly with maturation, activation, and recruitment of immunity cells. These findings may suggest that during arthralgic symptoms in the recovery phase may occur the inhibition of Th2 response, due to the presence of IFN-γ, as well as a trigger to the Th17 process. Mayaro infection course-related symptoms and immune response patterns are compiled in Figure 1.

IL-6 is a pleiotropic cytokine[42] that can be produced by a panoply of cells, including synovial cells. Interferon regulatory factor 1 acts in the activation of *IL-6* gene whose expression plays a major role in local inflammation, by stimuli of CXCL8/IL-8 production and expression of adhesion molecules, resulting in the recruitment of leukocytes[43,44]. Added to that, IL-6 is an essential compound that together with IL-1, IL-21, IL-23, and TGF-β induces helper T cell expression, in the Th0 stage, the Th17 phenotype, and thus promotes the production of IL17[45], an interleukin present in Mayaro infection.

CXCL8/IL-8 has been considered a promising biomarker for several inflammatory diseases. This chemokine is secreted by monocytes, activated macrophages, and endothelial cells. CXCL8/IL-8 attracts and activates neutrophils[42], nevertheless, its level is commonly increased in many inflammatory conditions, which requires careful interpretation of its increased level and its possible correlation with

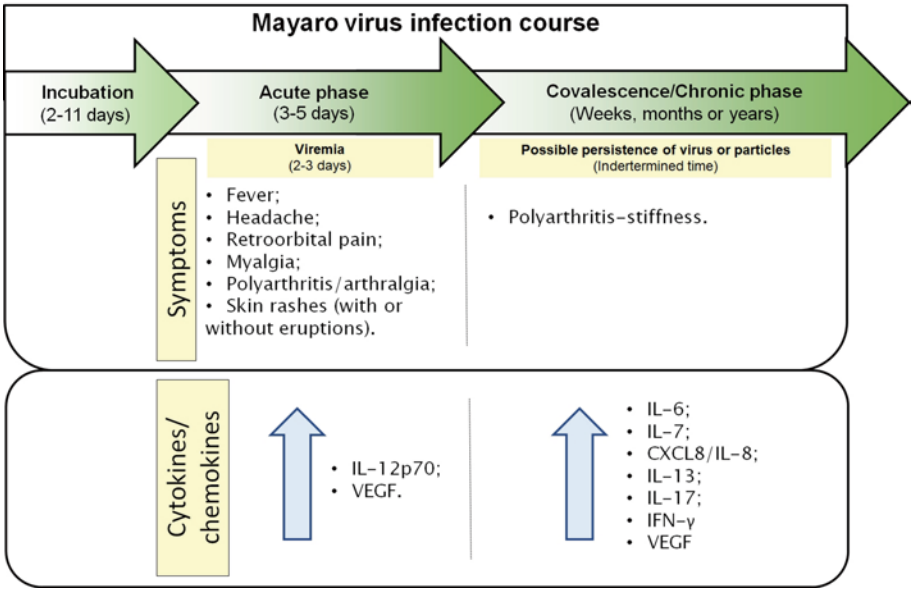

Figure 1. Symptoms, time and altered cytokines and chemokines in the acute and convalescent phases of Mayaro virus infection in patients.

clinical condition diagnosis or prognosis of diseases[46].

IL-7 is mainly produced by stromal cells in the bone marrow and thymus, as well as by B cells, monocytes and macrophages[47]. IL-7 strongly stimulates the proliferation of lymphoid progenitor cell lines (pre-pro B cells and precursors of T cells), whereas, in monocytes, IL-7 may induce the expression of important cytokine receptors in pre-T cells, stimulates the lytic activities and induces the secretion of cytokines such as IL-6[42]. In the recovery phase of MAYV infection, a high level of IL-7 was reported, which may lead to an increased level of IL-6 and then CXCL8/IL-8.

In patients without persistent arthritis, IL-7 and VEGF were increased only during the convalescent phase and 3 and 12 months after infection, respectively. IL-7 and VEGF were both significantly elevated in the acute and recovery phase in subjects with persistent arthralgia and were suggested as possible biomarkers for the severity of MAYV infection[9].

IL-13 is an immunoregulatory cytokine that is secreted by the activated Th2 cells. Eosinophil granulocytes also secrete IL-13 under the influence of IL-5 and GM-CSF (also secreted by Th2-type response). Innate helper type 2 cells also may secrete IL-13 upon stimulus (from IL-25 and IL-33), and all these factors help regulate the function of human B cells and monocytes. In general, the activity of IL-13 resembles that of IL-4 and can activate monocyte cell lines and inhibit the production of inflammatory cytokines (IL-1α, IL-1β, IL-6, IL-8, G-CSF, and IFN-α)[42].

IL-17, a molecule also associated with the recovery phase of MAYV infection, is a proinflammatory cytokine that can cause neutrophilia. This cytokine mobilizes granulocytes by granulopoiesis, causes migration with CXC chemokines and prolongs their lives in target tissues[42]. It also stimulates the production of COX-2 and nitric oxide, a compound that presents antiviral properties, acts on stromal cells such as keratinocytes, fibroblasts, epithelial cells, and endothelial cells and induces the secretion of proinflammatory cytokines such as IL-6, IL-8, and G-CSF. High levels of IL-17 have been found in chronic inflammation[42].

IFN-γ (IFN type Ⅱ) is a pleiotropic cytokine that induces antiviral, antiproliferative, and immunomodulatory effects in numerous target cells[48]. IFN-γ is mainly produced by T cells and natural killer cells[49] and induces the activation of macrophages, resulting in the production of inflammatory cytokines such as TNF-α, IL-1, IL-12 and IL-18[47]. IFN-γ belongs to the Th1 cytokine response, a group of cytokines essential to promote immune cell activation, which can contribute to the generation of tissue injuries[50]. Considering the ability of molecules of the Th1 cytokine group to cause tissue injuries, the increased levels of molecules of this group may be related to synovial cell injuries observed in MAYV infection.

5. Other immune contributions *in vitro* and *in vivo*

Camini *et al*.[51] observed the increase of reactive oxygen species (ROS) in HepG2 cells (human liver cancer) after MAYV infection and proposed that MAYV infection may induce oxidative stress in infected cells[51]. In order to compare the effect of infection in different cell lines, J774 cell (murine macrophage phagocytic cell), a cell line related to the pathogenic arthritogenic symptoms of *Alphavirus*[14,28], was tested. The increase of ROS level was noted, which was similar to that observed by Camini *et al*., showing the relevance of the ROS level in MAYV infection[32].

In the *in vitro* study of Cavalheiro *et al*.[32], RAW 264.7 (mouse leukemic macrophage) and J774 cell lines were infected with MAYV. The increase of ROS production was found 6 hours after MAYV infection in RAW 264.7, a period that coincided with the peak of virus replication and preceded TNF (proinflammatory cytokine Tumor Necrosis Factor) initial secretion. In contrast, infected cells treated with antioxidants *N*-acetyl-*L*-cysteine and apocynin abolished the secretion of TNF, reinforcing the role of ROS in inflammation observed during MAYV infection.

As described in the study of Cavalheiro *et al*.[32], the increase of TNF levels and macrophage cell death (15 hours post-infection) occurred concomitantly, MAYV infection did not induce the secretion of IL-6, RANTES/CCL5 and KC (the CXCL8/IL-8 homolog in mice) in RAW 264.7 cell line and cell death observed 15 hours after infection was related to apoptosis (evaluation of caspase 3/7)[32]. ROS and cytokines could be naturally produced by active macrophages to control the viral replication[52]; however, before the increase of TNF and apoptosis, MAYV infection induced early ROS production (6 hours after infection) and provided conditions that led to increased MAYV replication *in vitro*[32]. In addition, the comparison between the findings of Camini *et al*.[51] and Cavalheiro *et al*.[32] suggests that the estimated time for viral replication and increase in ROS level is different.

Recent *in vivo* studies proposed the role of interferon type Ⅰ (IFN-Ⅰ) response in the restriction of MAYV infection since mice with deficient genes of IFN-Ⅰ showed substantial virus replication and lethality, as well as, beyond tissue inflammation, mice showed high TNF, IL-6, KC, IL-1β, MCP-1, and RANTES expressions[53]. Figueiredo *et al*. suggested that the adaptive response is crucial for MAYV-induced inflammation and lesions, which might be reinforced by the findings of Santos *et al*.[54] where the levels of proinflammatory mediators, such as TNF-α, IL-6, IFN-γ, and MCP-1, were elevated, indicating a Th1 response[54]. Santos *et al*. also reported that the level of cytokines TNF and IFN-γ remained high 10 days after MAYV infection, with subsequent decrease of these cytokines after this period, and increase of an anti-inflammatory cytokine IL-10. Santos *et al*. also suggested that the increased level of IL-4 and TNF cytokines observed 30 days after the infection can be induced due to the persistence of the virus in organisms and the stimuli to antibody production, *i.e.*, TNF response maintained may indicate an extended inflammatory response. Furthermore, cytokines IL-9, IL-13, IL-12p70, and IL-17 did not differ from the control in the *in vivo* assays. Differences among these cytokines were observed only in case report studies[54].

6. Conclusion

Increased VEGF and IL-12p70 were observed in the acute phase of MAYV infection, which can lead to hematopoiesis and transformation of Th0 to of Th1 response, whereas increased IL-6, IL-7, CXCL8/IL-8, IL-13, IL-17, and IFN-γ were observed in the recovery phase in patients with arthritis and infected by MAYV. The data about the recovery phase can imply a Th17 process and Th2 inhibition. The immune response to MAYV is largely unknown. Since MAYV fever is an emerging disease, the data presented in this paper may help understand the immunophysiopathology of this disease. There are a lack of studies discussing this theme, and the studies performed present a small number of patients. Most of the patients studied are from different origins/countries, and due to the lack of information and heterogenity of studied patients, we reinforce that all inferences need be carefully performed, and generalizations should be avoided.

Conflict of interest statement

We declare that there is no conflict of interest.

Acknowledgments

We are grateful to Dr. Jean Carlos Vencioneck Dutra for proofreading the manuscript.

Funding

This work was supported by grants from Fundação de Amparo a Ciência e Tecnologia do Estado de Pernambuco (FACEPE) (grant 09/2017).

Authors' contributions

JMF and JLLF designed the paper; JMF, DSCF and EVMSF analyzed the data; JMF and EVMSF wrote the paper.

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