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Review Article

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Balancing functions of regulatory T cells in mosquito-borne viral infections

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ABSTRACT

Mosquito-borne viral infections are on the rise worldwide and can lead to severe symptoms such as haemorrhage, encephalitis, arthritis or microcephaly. A protective immune response following mosquito-borne viral infections requires the generation of a controlled and balanced immune response leading to viral clearance without immunopathology. Here, regulatory T cells play a central role in restoring immune homeostasis. In current review, we aim to provide an overview and summary of the phenotypes of FOXP3^{+} Tregs in various mosquito-borne arboviral disease, their association with disease severity and their functional characteristics. Furthermore, we discuss the role of cytokines and Tregs in the immunopathogenesis of mosquito-borne infections. Lastly, we discuss possible novel lines of research which could provide additional insight into the role of Tregs in mosquito-borne viral infections in order to develop novel therapeutic approaches or vaccination strategies.

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Introduction

Vector-borne diseases are a major health problem worldwide accounting for more than one million deaths every year. In particular, viral diseases transmitted by mosquitos and ticks are globally distributed and result in nearly half a billion infections in humans every year [1]. Clinically significant arboviruses such as dengue (DENV), yellow fever (YFV), chikungunya (CHIKV), and Zika (ZIKV) viruses are all transmitted by *Aedes* species mosquitoes, whereas other clinically significant arboviruses such as West Nile virus (WNV) and Japanese encephalitis virus (JEV) are transmitted by *Culex* species [1,2]. These mosquitos thrive well in tropical and subtropical areas. Current climate change and rapid urbanization creates new ecological niches favourable of mosquito proliferation, thereby increasing the population at risk of arbovirus infection [3].

Most infections in humans with these viruses are asymptomatic or result in mild symptoms. Clinical signs mostly appear 3–6 days after the bite of an infected mosquito. DENV, ZIKV, CHIKV and YFV infection all elicit similar symptoms during the acute phase of disease, which include fever, headache, skin rash, muscle and joint pain. However, less than 5% of symptomatic DENV and YFV infected individuals develop critical illness, which can be life threatening. Severe disease is characterized by a cytokine storm, vascular leakage syndrome, haemorrhage and shock [4,5]. ZIKV infection in pregnant women can cause fetal brain damage, often resulting in miscarriage, stillbirth, or microcephaly in newborns [6]. In about 40% of cases, CHIKV infection can cause chronic arthritis that is clinically mistaken with seronegative rheumatoid arthritis, an autoimmune disease marked by symmetrical joint pain, swelling, and morning stiffness [7]. After JEV infection, only around 1% of infected people develop encephalitis, but the case fatality rate among clinical cases is high, and up to half of the survivors develop long-term neurological or psychological complications [8]. WNV infections can lead to encephalitis or meningitis in less than 1% of the symptomatic cases [9]. Until now, there is no treatment for most of these diseases available targeting viral replication or modulating the immune response. Unspecific treatment is focused on relieving severe symptoms and on supporting the patient to overcome the infection. For YFV, JEV and DENV vaccines are available with variable efficacy, however, no approved vaccines exist for many other mosquito-borne viral infections [1].

The mechanisms that lead to severe disease in a small subset of individuals after infection remain to be fully understood and are disease-specific. However, one common theme is that it appears that disease is

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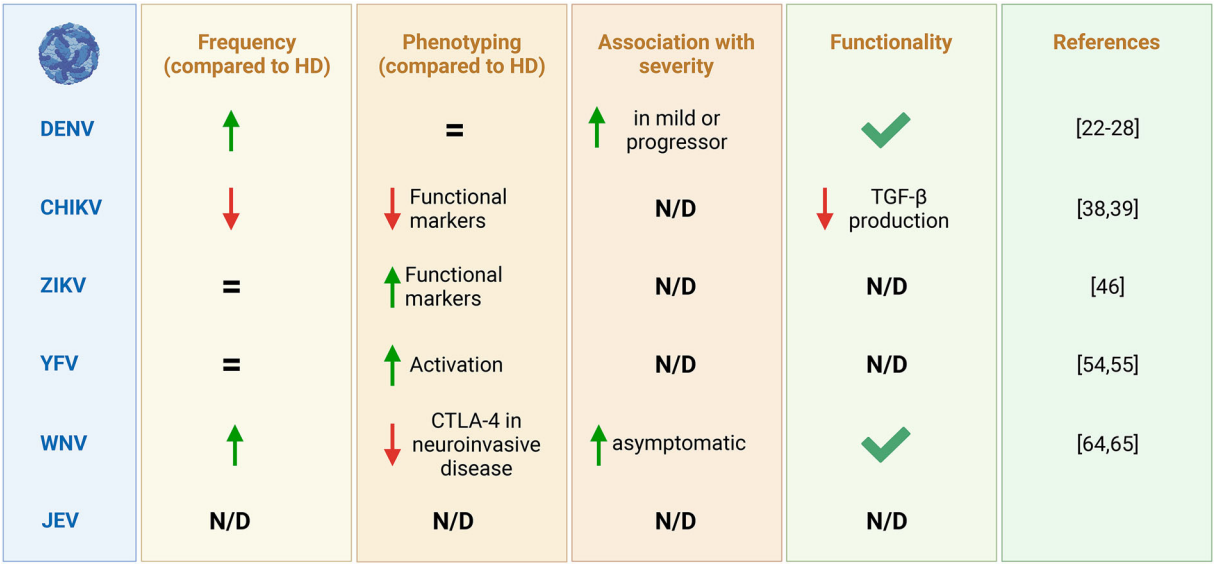

Figure 1. Summary of characteristics and functionalities of Tregs in humans after mosquito-borne viral infections (or vaccination in case of YFV). N/D: not determined, HD: healthy donor. Created with Biorender Text.

caused, at least in part, by an immune response to the virus that is both excessive and poorly regulated [1,10]. This highlights the importance of proper regulatory and feedback mechanisms after infection. Several cell types with regulatory functions are described, including $CD4^+CD25^+$ forkhead box protein P3 $(FOXP)^+$ regulatory T cells (Tregs), type 1 regulatory T cells, invariant natural killer T cells, double negative $CD3^+$ T helper cells, regulatory $CD8^+FOXP3^+$ T cells, regulatory B cells, and myeloid-derived suppressor cells [11]. Among them, Tregs are the most-studied and well-characterized immune regulatory cell type, and their paramount importance for immune homeostasis has been amply demonstrated.

Tregs are essential in maintaining immune homeostasis and peripheral tolerance [12,13]. They are characterized by the expression of the transcription factor *FOXP3* and interleukin (IL)−2 receptor α-chain (CD25) and low expression of the IL-7 receptor α-chain (CD127) [12,13]. Tregs have the ability to suppress and neutralize responses of both the innate and adaptive immune system by various mechanisms. Tregs secrete anti-inflammatory cytokines such as IL-10, transforming growth factor-β (TGF-β), and IL-35 [13,14]. Moreover, Tregs suppress immune responses via cell–cell contact dependent mechanisms involving cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1) or CD39 [15]. Dysfunctional Tregs are linked to autoimmune disorders such as multiple sclerosis and chronic infections and display a pro-inflammatory phenotype [12,13,16]. Importantly, Tregs are also known to play a critical role in viral infections [17].

In current review, we aim to provide an overview and summary of the phenotypes and functions of $FOXP3^+$ Tregs in various mosquito-borne arboviral disease. We also discuss their association with disease severity and their functional characteristics (Figure 1, Table 1).

Tregs in dengue virus infection

DENV consists of four serotypes which co-circulate in hyperendemic regions. Primary infection with any of the four DENV serotypes induces the production of antibodies with potent protective capacity against homotypic re-infection. On the other hand, secondary infection with a different serotype is a major risk factor to develop severe disease [4]. A mismatch between the infecting serotype and the memory adaptive immune response is hypothesized to lead to exacerbated immune reactions resulting in severe dengue [4]. Antibodies developed during primary infection are believed to contribute to severe disease during secondary infection via the proposed mechanism of antibody-dependent enhancement [4]. In addition, a secondary infection can reactivate cross-reactive memory T cells, leading to the expansion of a memory T cell pool with low specificity for the secondary infecting serotype which can result in ineffective viral clearance and enhanced cytokine production [18]. In contrast, increasing number of recent studies point towards a protective function of T cells during DENV infections [19]. For example, human leukocyte antigen alleles associated with protection from severe dengue disease are also associated with strong and multifunctional T cell responses, such as cytokine production and cytotoxic activity [19,20]. During mild or asymptomatic acute infection, T cells are more activated compared to severe disease [21].

Frequencies of Tregs in the circulation of dengue-infected patients are increased compared to healthy

Table 1. Summarizing table of the phenotypes of Tregs observed in human arbovirus infection and mouse models and their association with disease severity.

	Treg phenotypes in healthy controls versus patients	Ref	Treg phenotypes and their association to severity	Ref
DENV	Naïve Tregs expanded in acute dengue patients (CD45RA, CLTA-4, CD95)	[22]	$PD1^+$ $CTLA4^+$ Tregs associated to disease progression	[28]
CHIKV	Decreased expression of functional markers (CD39, PD-1, CD73, CTLA-4) in acute CHIKV infected patients	[39]	Not investigated	
ZIKV	Increased expression of functional markers (CD39, CD73, CTLA-4, PD-1) in acute ZIKV infected patients	[46]	Not investigated	
YFV vaccination	Increased expression of activation markers (Ki-67, CD38, CD25) after vaccination. Increased memory Tregs ($CD45RA^-$) after vaccination	[54] [55]	Not investigated	
WNV	Not investigated		Reduced frequencies of $CLTA\text{-}4^+$ Tregs is associated with neurological symptoms in humans Mice with symptomatic disease have reduced frequencies of $CLTA\text{-}4^+$ and $CD44^+$ Tregs Decrease of $CXCR3^+$ Tregs and increase of $CD73^+$ Tregs in spleen at time of symptoms in mice	[65] [66] [66]

controls in the acute phase of infection but return to baseline during the recovery phase [22,23]. Two studies found that Tregs are expanded in the blood of patients with mild dengue compared to severe dengue patients [23,24]. In contrast, Estrada-Jimenez *et al* show decreased gene expression of *FOXP3* in mild dengue patients compared to severe patients [25], and frequencies of Tregs did not differ in mild and severe dengue patients in a cohort from Sri Lanka [22]. Phenotypically, the Tregs observed in dengue patients are predominantly $CD45RA^+$, indicating a more naïve-like phenotype. These cells expressed CTLA-4, an immune checkpoint molecule which inhibits T cell activation when it binds to its ligands CD80/CD86, and CD95, which triggers apoptosis when it binds to its ligand, FasL [22]. A recent study investigated both the B- and T cell compartment in a cohort of Singaporean patients. The study revealed that proliferating plasmablasts ($Ki67^+$) during acute disease correlate positively with frequencies of Tregs, whereas PD-1 expressing plasmablasts correlate negatively with $CD38^+CCR7^-$ Tregs (effector memory-like Tregs with high suppressive function). These data indicate a potential regulatory role for Tregs on plasmablast formation [27]. Interestingly, by utilizing mass cytometry on a cohort of Columbian dengue patients, Robinson et al observed both the frequencies of Tregs and plasmablasts are increased in acute dengue-infected patients who progress to severe dengue compared to patients who do not develop severe complications [28]. Moreover, Tregs express higher levels of checkpoint inhibitors such as PD-1 and CTLA-4 in patients progressing to severe dengue [28]. Human challenge models are useful to understand better the dynamics of the immune response to DENV infection. For example, in a human challenge model with recombinant attenuated DENV-2 frequencies of Tregs decline during the acute stage of infection based on transcriptomic analysis. Unfortunately, these results are not confirmed by flow cytometry analysis, maybe due to the lack of anti-FOXP3 antibodies in the staining panels [26].

The function of Tregs from dengue patients has been assessed as well by in vitro co-culture experiments. Tregs are able to suppress the proliferation of responder T cells (Tresp) to a similar degree in the acute phase of DENV infection and after recovery. Moreover, Tregs isolated during the acute phase are able to suppress interferon gamma (IFN-γ) production in Tresp as well as the production of tumour necrosis factor alpha (TNF-α) in monocytes in vitro co-culture experiments [23].

Liver is the most affected organ in patients with severe dengue (Figure 2). 60–90% of severe dengue patients has hepatocellular injury [29]. In liver biopsies of severe dengue patients, FOXP3 is noticeably absent while pro-inflammatory markers such as toll-like receptor (TLR) 2 and TLR3, inducible nitric oxide synthase, IL-6, IL-18, TGF-β, and granzyme B are highly expressed [30]. The authors hypothesize that the inflammatory milieu in the liver can suppress the generation or influx of $FOXP3^+$ Tregs [30].

Mouse lacking type I IFN receptors are susceptible to DENV infection with mouse-adapted DENV strains. A classical mouse model of dengue utilizes IFN-α/$\beta R^{-/-}$ mice, where mice develop dengue-like disease when infected with a sufficiently high DENV challenge dose [31]. In this mouse model, a lack of Treg expansion is observed in the spleen in response to DENV infection [32]. However, it has been shown that type I IFN are necessary to maintain FOXP3 expression and Treg function under inflammatory conditions in mice [33]. Therefore, type I IFN receptor deficient mice are probably not a good model to assess Treg development and function. In contrast, wild type C57BL/6 mice rapidly clear DENV infection due to a strong type I IFN response. Here, DENV induces the proliferation of Tregs via the TLR2/MyD88 pathway [34].

Taken together, the frequency of Tregs in the blood of dengue patients increases after DENV infection. In some studies, higher Treg frequencies are linked to milder disease, while in other studies, higher Treg frequencies are linked to more severe disease. A further understanding on the Treg subsets that circulate or

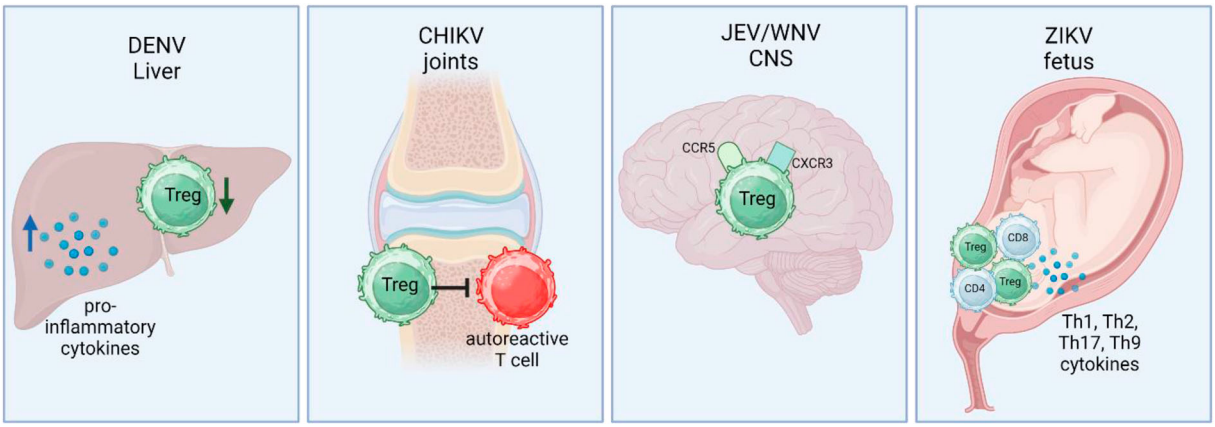

Figure 2. Presence and function of Tregs in the target organs of mosquito-borne viral infections. In DENV, a major target of infection is the liver. In liver biopsies of severe dengue patients, FOXP3$^+$ Treg frequencies are reduced while there was an increase in the production of pro-inflammatory cytokines (30). In CHIKV-infected mice, Treg suppressive mechanisms can prevent the accumulation of T cells in the joints leading to less joint inflammation [40,41]. In WNV and JEV-infected mice, Tregs are shown to express CCR5 and CXCR3, indicating that these might migrate to the CNS to restrict immunopathology [67,72]. In cases of fatal microcephaly, the brain is infiltrated with increased frequencies of FOXP3$^+$ cells, along with increases in total CD4$^+$ and CD8$^+$ T cells and increases in Th1, Th2, Th17 and Th9 cytokines [49]. Created with Biorender.

migrate to the inflamed tissues, such as the liver, is warranted. However, due to the lack of suitable mouse models these studies remain challenging (Table 1).

Tregs in chikungunya virus infection

CHIKV, an alphavirus, causes arthralgia and myalgia, as well as other symptoms such as rash, fever and headache. Depending on the CHIKV genotype and patient characteristics, 20-50% of patients develop chronic arthritis [7]. The mechanisms of induction of chronic arthritis are not well characterized, but symptoms are similar to those of rheumatoid arthritis, an autoimmune disease with altered Treg/effector T cell ratio [7].

The T cell response to CHIKV infection has a dual role in protection and pathogenesis, which is dependent of the stage of disease [35]. The contribution of cellular immunity to viral clearance after CHIKV infection seems limited despite the expansion of CHIKV-specific T cells [35]. CD8$^+$ T cells predominate in the early stages of infection and express activation markers such as CD69 and HLA-DR [36]. CD4$^+$ T cells are activated during the chronic phase of infection and can induce or perpetuate inflammation by producing pro-inflammatory cytokines [35]. Interestingly, during the early acute phase of infection, CD95-mediated apoptosis of CD4$^+$ T cells is observed, indicating that CHIKV infection could induce CD4$^+$ T cell apoptosis via CD95-CD95L interactions [36]. Even though the exact mechanism remains to be determined, large numbers of CD4$^+$ T cells migrate to the joint and produce high levels of IFN-γ [37].

In two studies, phenotyping of the Treg compartment in acute and chronic chikungunya infection has been performed. A first study by Kulkarni et al, in 2017, shows that the frequencies of Tregs were lower in acute and chronic chikungunya patients compared to recovered individuals and healthy controls. The ratio of Treg/effector T cells is decreased in the acute phase of infection. This decrease in Treg/effector T cell ratio can modify the amplitude of the effector T cell response [38]. Along the same line, Treg frequencies are reduced during the acute and chronic phase of disease in a Brazilian cohort compared to healthy donors. Moreover, the expression of Treg functional markers (CD39, PD-1, CD73 and CTLA-4) is decreased in Tregs from chikungunya patients compared to healthy donors during the acute phase of infection. In parallel, TGF-β production by Tregs is reduced [39]. Taken together, both studies confirm reduced frequency of Tregs in the blood of acute chikungunya patients whith a reduced expression of functional markers.

In a mouse model of CHIKV, it has been shown that expansion of Tregs leads to a reduction of joint inflammation. In this model, the JES6-1 anti-IL2 antibody, which selectively expands mouse Tregs by forming a complex with IL-2, was co-administered to C57BL/6 mice inoculated with CHIKV [40]. Mice treated with the JES6-1 antibody were protected from CHIKV-induced pathology. In another study, C57BL/6 mice are treated with CLTA-4 Ig, which mimics the action of Tregs by inhibiting T cell activation and proliferation. CTLA-4 Ig treatment results in the reduction of T cell accumulation in the joints without affecting viral infection in CHIKV infected mice [41]. Thus, these two studies indicate that Treg suppressive mechanisms can prevent the accumulation of T cells in the joints leading to less joint inflammation (Figure 2).

Taken together, human studies show that the frequency and functionality of Tregs is reduced during the acute phase of chikungunya infection. Studies in rodents indicate that the restoration of Treg suppressive mechanisms could prevent the accumulation of T cells in the joints leading to less inflammation.

Tregs in Zika virus infection

ZIKV infection usually results in a mild, self-limiting disease [42]. ZIKV is considered a major public health issue since a large outbreak of ZIKV lead to a cluster of microcephaly cases and an increase in patients with neurological disorders in Brazil in 2015 [43].

Together with antibodies, $CD8^+$ T cells are thought to play a protective role in ZIKV infection. ZIKV-specific $CD8^+$ T cell responses are characterized by the release of IFN-γ, TNF-α and granzyme B [44]. In the $CD4^+$ T cell compartment, a reduction of IFN-γ-producing $CD4^+$ T cells is observed during acute ZIKV infection [45].

One study assesses the phenotype of Tregs during the acute phase of infection in a Brazilian patient cohort [46]. Even though the frequency of circulating Tregs is not different in controls compared to acute-infected patients, Tregs from acute ZIKV infected patients express increased amounts of CD39, CD73, perforin, granzyme, PD-1 and CTLA-4, all markers involved in Treg function. These markers could be upregulated in an attempt to control the strong inflammatory responses during the acute infection.

The most important hallmark of ZIKV infection is the potential effect on unborn fetuses. ZIKV infection in pregnant women can result in abortions, stillbirths, fatalities, and congenital abnormalities such as microcephaly [6,42]. During normal pregnancy, Tregs play a crucial role in the decidua (a specialized layer of endometrium forming the maternal interface with the embryo) where they sustain an anti-inflammatory environment. The decidua is an immunologically complex environment where immune tolerance to parental antigens is maintained while also protecting the fetus against vertical transmission of maternal pathogens, such as ZIKV [47]. In a study in non-human primates, decidual T lymphocytes from ZIKV-infected female macaques show decreased expression of granzyme B, HLA-DR and Ki-67 in memory $CD4^+$ and $CD8^+$ T cells and decreased frequency of $CXCR3^+$ cells compared to decidual T cells from non-infected macaques [48]. Even though no direct differences in the frequencies of Tregs (defined as $CD25^+CD127^-$ cells) or the phenotype of Tregs are observed, the study suggests that ZIKV can induce local immunosuppression in the decidua. If this immunosuppression is mediated by bona fide Tregs or other regulatory cell types remains to be further investigated. In the brain tissue of fatal microcephaly cases, increased frequencies of infiltrating Tregs in the brain compared to control fetuses is observed. This is accompanied by increases in total $CD4^+$ and $CD8^+$ T cells frequencies and elevated concentrations of Th1, Th2, Th17 and Th9 cytokines [49] (Figure 2).

Taken together these studies suggest that Tregs upregulate functional markers during acute ZIKV infection, probably in an attempt to control the strong inflammatory responses. However, the role of Tregs in the target organs of infection, such as the brain or decidua, the role of Tregs is less clear.

Tregs in yellow fever virus vaccination

Yellow fever is a mosquito-borne viral disease which is endemic in South America and Africa. The illness ranges from a fever with aches and pains to severe liver disease with haemorrhage and development of jaundice. A safe and effective vaccine is available and provides life-long protection in most individuals [50]. As this is a live-attenuated virus inoculation (strain YF-17D) inducing viremia in vaccinees, vaccination has been used as a model to study the immune responses to YFV. Vaccination induces seroconversion and memory T cell responses in more than 90% of individuals in most of the studies, with neutralizing antibodies at protective levels and long-lived memory T cells for more than 10 years [50]. However, as vaccination with this live-attenuated virus does not cause disease, immune responses in severe yellow fever cases have not been studied. A protective effect of the T cell response to YFV has been demonstrated both in humans and mice models. For example, a polyfunctional $CD4^+$ T cell response after YF-17D immunization complements neutralizing antibodies for protection against a virulent YFV challenge in mice [51]. Moreover, cytokine-producing human $CD4^+$ T cells expressing CD40L, a molecule important for stimulation of B cell responses, are associated with the levels of neutralizing antibodies after YF-17D immunization in humans [52] and with vaccine-induced protection in mice [53].

The frequency and phenotype of Tregs has been studied in the context of YFV vaccination. A study by Blom et al shows that the frequency of circulating Tregs was not altered after vaccination, but Tregs were transiently activated at day 10 post-vaccination as demonstrated by co-expression of Ki-67 and CD38 and decreased expression of PD-1 at this time-point [54]. A follow-up study showed a shift from naïve-like $CD45RA^+$ to $CD45RA^-$ memory-like Tregs within 7 days post vaccination accompanied by an increase in CD25 in both $CD45RA^+$ and $CD45RA^-$ Tregs. CD31 expression increased on $CD45RA^+$ Tregs, whereas HLA-DR expression increased on $CD45RA^-$ Tregs, indicating the activation of both naïve and memory-like Tregs. All

changes were transient and returned to baseline at day 14 after vaccination [55].

Taken together, these two studies show a transient increased activation of the Treg compartment after YFV vaccination. However, the functional implications of these phenotypic changes in Treg subsets and their possible contribution to protective immune response cannot be investigated using YFV vaccination as model of natural infection. Therefore, studies of Tregs after natural YFV infection in mild and severe patients should be performed.

Tregs in West Nile virus infection

Most WNV infections remain asymptomatic, but about 0.6% of cases can develop acute neuroinvasive disease leading to meningitis and encephalitis [9]. $CD4^+$ T cells and B cells are critical for protection against WNV infection. Neutralizing antibodies, which are directed against the viral envelope glycoprotein, are considered a correlate of protection [59]. $CD4^+$ T cells also play a critical role in the defence against WNV, both by promoting protective antibody responses and by direct killing of infected cells [60]. The role of $CD8^+$ T cells after WNV infection is less clear, since they could also play a pathogenic role especially inducing pathology in the central nervous system (CNS) [61].

Tregs aid in the removal of neurotropic viruses from the CNS by inducing apoptosis of virus-infected macrophages and by supporting effector T cell trafficking to the infection site. On the other hand, they limit proliferation and inflammatory cytokine secretion of T cells in the CNS [62,63]. A longitudinal study found that the Treg compartment in the blood expands up to 2–3 months post WNV-infection. Moreover, higher frequencies of Tregs are detected in asymptomatic WNV-infected patients compared to symptomatic WNV-infected patients [64] and patients with neuroinvasive WNV-infections have a reduced frequency of CTLA-4^+ Tregs in the blood [65]. Functionally, Tregs from WNV-infected individuals were able to suppress T cell proliferation as well as Tregs obtained from healthy donors in an in vitro suppression assay [64]. Hence, the data obtained from the study of human cases indicates that circulating Tregs are associated with protection from disease.

These observations in humans have been confirmed in mice models, where lower frequencies of CTLA-4^+ and $CD44^{hi}$ Tregs were observed in symptomatic compared to asymptomatic WNV-infected mice [66]. Moreover, mice lacking Tregs had increased mortality rates after WNV infection [64]. A week following WNV infection, a decreased frequency of $CXCR3^+$ Tregs and increased frequency of $CD73^+$ Tregs in the spleen coincided with symptomatic disease and neuroinvation [66]. It is possible that Tregs migrate to the CNS to limit immunopathology, as CXCR3 has been shown to mediate T cell trafficking to the brain during WNV infection [67]. CD73, togethether with CD39 generatates immuno-suppressive adenosine, which could result in a reduced antiviral response and associated immunopathology [68].

In conclusion, these studies suggest that lower frequencies of peripheral Treg after infection are associated with symptomatic WNV infection and neurological symptoms, probably due to trafficking of these cells to the inflamed CNS.

Tregs in Japanese encephalitis virus infection

Another mosquito-borne viral infection that can lead to CNS inflammation is JEV. It is the most common cause of viral encephalitis in Asia. Twenty-five to 30% of cases of JEV infections are fatal, with 50% of infections resulting in long-term neuropsychiatric problems [8]. Observations from animal models suggest that T cells only play a secondary role in protection from JEV infection, next to neutralizing antibodies. However, they could also contribute to immunopathology or protection [69]. Adoptive transfer of primed T cells can protect animals from intracerebral JEV challenge [70]. In addition, a polyfunctional, IFN-γ dominated $CD4^+$ T cell response correlates with better clinical outcome in convalescent JEV-infected patients [71].

The role of Tregs in JEV infection is mainly studied in mice models. To our knowledge, no studies have been reported yet that characterize the phenotypic or functional profile of Tregs in human JEV infected individuals.

CCR5 is a chemokine receptor involved in the migration of effector T cells, including activated Tregs, into inflamed tissue. CCR5-deficient mice show worsened encephalitic symptoms and mortality compared to their WT counterparts, and this is associated with reduced Treg responses and increased Th17 frequencies in the brain. Adoptive transfer of $CCR5^+$ Tregs into CCR5-deficient mice can ameliorate JE progression, indicating the importance of CCR5-mediated Treg migration to the inflamed CNS [72]. One in vitro study shows that JEV-infected dendritic cells expanded Tregs in allogenic mixed lymphocyte reactions by increasing the expression of PD-L1 on their surface [73]. Confirming these in vitro results, dendritic cell-depleted mice exhibit a decreased frequency and absolute number of $CD4^+$ $FOXP3^+$ Tregs in the spleen and brain after JEV infection [74]. In contrast to the effects of wild type JEV, in vitro infection of dendritic cells with the attenuated JEV vaccine strain impairs the expansion of Tregs. In the context of vaccination, this could be beneficial as this could aid in the expansion of an effector T cell population providing protective immunity [75].

In conclusion, Treg expansion and migration of Tregs to the inflamed CNS seems to protect mice

from JEV-induces encephalitis. However, the phenotype of Tregs in JEV infection in humans and their association to severe disease remains to be determined.

Cytokines and Tregs in severe mosquito-borne viral infections

A common feature of patients with severe mosquito-borne viral disease such as dengue, chikungunya or yellow fever is an exacerbated and overreaching inflammatory response or so-called cytokine storm. High amounts of pro-inflammatory cytokines such as IL-2, IL-6, IFN-γ and TNF-α can be detected in plasma of patients with severe disease [21,76,77] (Figure 3).

Notably, a strongly pro-inflammatory environment and increased levels of various effector cytokines, such as IL-6 and IL-12 can overcome the suppressive capacity of Tregs and modify Treg phenotype [14,78]. Indeed, IL-6 activates the transcription factor STAT3, which could downregulate FOXP3 while promoting the expression of RORγt, a Th17 cell-associated transcription factor [79]. Moreover, high amounts of IL-6 or IL-12 can increase the ratio of effector T cells over Treg which can lead to the inability of Tregs to suppress T effector cells. Taken together, the high amounts of cytokines observed in patients with severe mosquito-borne viral infections can alter Treg biology and function which might contribute or sustain immunopathology and severe disease.

Interestingly, cytokine storms with increased concentrations of pro-inflammatory cytokines such as IL-6 have been observed in severe COVID-19 patients. IL-6 signalling blockade by tocilizumab, a monoclonal antibody against IL-6 receptor, which is an approved treatment for several autoimmune diseases [81], is tested for treatment of severe COVID-19 [82]. Taken together results from several clinical studies, it appears that patients with severe COVID-19, are likely to benefit from treatment with tocilizumab, when given at the right time after onset of severe disease [82]. It remains to be investigated if tocilizumab could be considered a treatment option for severe disease following arbovirus infections where high levels of IL-6 are associated with severe disease.

Perspectives

Under steady state conditions, Tregs are essential in maintaining immune homeostasis and peripheral tolerance. However, during viral infections, they might

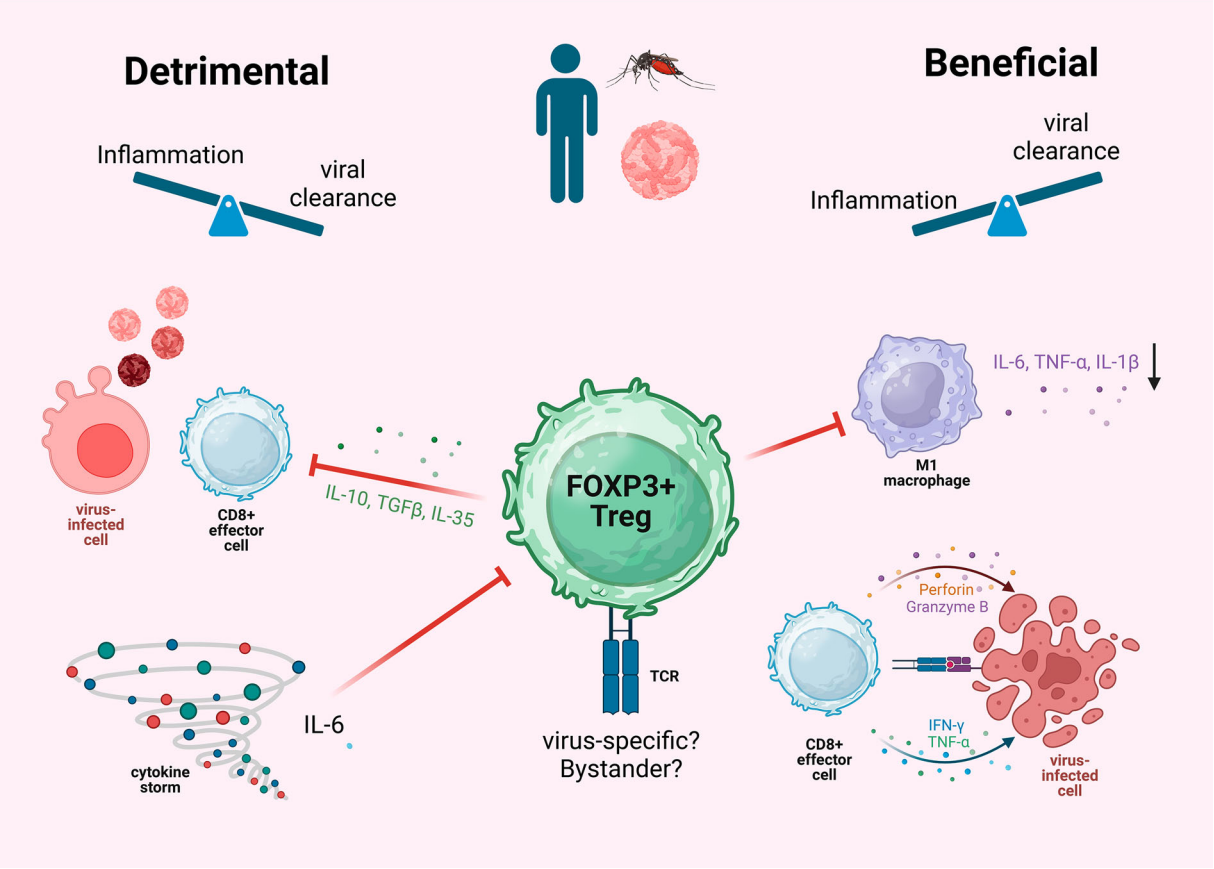

Figure 3. Summary of the possible beneficial and detrimental roles of Tregs after mosquito-borne viral infections. Tregs could inhibit the formation or activation of virus-specific $CD8^+$ T cells resulting in less viral clearance. However, Treg function is needed to dampen excessive immune inflammation by for example M1 macrophages and Treg function is needed in the resolution phase of the infection. Excessive cytokine production as observed in some patients with severe arbovirus infection might impair the generation and function of inducible Tregs. Created with Biorender.

inhibit pathogen-specific immune responses such as the generation or maintenance of virus-specific effector T cells, thereby interfering with efficient and timely viral clearance (Figure 3). In the context of arboviral infection by flaviviruses or alphaviruses, a rather limited amount of information is available on the phenotype, function and location of Tregs.

With recent advances in understanding the phenotypes and functionalities of different Treg populations, such as Th1-like Tregs, or inducible Tregs in viral infections [17], more detailed phenotypic analysis is warranted in patients with different arbovirus infections. Moreover, evidence for a protective or pathogenic role of Tregs in several arbovirus infections is lacking. Therefore, studies in patients associating different Treg phenotypes with different disease outcomes will further elucidate the role of Tregs in protection or disease. Large flow cytometry panels, including lineage and functional markers such as Helios, activation markers, chemokine receptors, adhesion molecules, checkpoint inhibitors and metabolic markers or single-cell RNA sequencing approaches are now available to further describe Treg subsets in different human conditions. However, next to more in depth phenotypic analysis, in vitro analysis of the suppressive function of Tregs isolated from patients in the acute phase of infection is warranted. Adoptive transfer studies in mouse models of arbovirus infections can provide more mechanistic understanding of the function of Tregs in infection and pathology, even though animal models of arboviral disease do not always recapitulate mechanisms and phenotype of human disease (for example, a lack of pre-existing immunity, a more rapid viral replication, dependency on type I IFN deficient models …).

Phenotype and function of antigen-specificity of virus-specific $CD4^+$ and $CD8^+$ T cells remains an active field of research. While for DENV for example the major protective $CD4^+$ and $CD8^+$ T cell epitopes have been identified [19], T cell epitope mapping for CHIKV remains largely to be performed. Recent evidence has shown that virus-specific Tregs can be activated and expanded upon acute infection with vaccinia virus or influenza virus [84,85]. Antigen-specific Tregs are hypothesized to be more effective in regulating the ongoing immune response in a disease-specific manner. With the development of peptide-based T cell vaccines for arboviruses [86], more information on the antigen-specificity of arbovirus-specific Tregs is warranted to further optimize and adapt this vaccination strategy.

As exemplified by the role of IL-6 in severe COVID-19 as mentioned above, further research about the effect of pro-inflammatory cytokines on Treg function in the context of severe arbovirus infections can help to provide a rationale for the treatment of severe forms of arbovirus diseases with cytokine inhibitors such as anti-IL6 receptor blockade (tocilizumab).

Conclusions

Understanding of the mechanisms by which Tregs modulate disease pathogenesis after viral infection is limited, particularly in the context of mosquito-borne viral infections, which mostly affect individuals in tropical and subtropical regions of the world. Additional insight into the phenotype and function of Tregs and their and association with disease severity after mosquito-borne viral infection is warranted to further advance vaccination and treatment strategies.

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Author contribution

SS drafted the manuscript and figures; TC and MK supervised the work. All authors approve the final version of the manuscript.

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