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INVITED REVIEW

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Pathogenic Th1 responses in CHIKV-induced inflammation and their modulation upon *Plasmodium* parasites co-infection

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Abstract

The induction of polyarthritis and polyarthralgia is a hallmark of arthritogenic alphavirus infections, with an exceptionally higher morbidity observed with chikungunya virus (CHIKV). While the mechanisms underlying these incapacitating acute symptoms remain partially understood, the progression to chronic conditions in some cases remains unanswered. The highly pro-inflammatory nature of alphavirus disease has suggested the involvement of virus-specific, joint-infiltrating Th1 cells as one of the main pathogenic mediators of CHIKV-induced joint pathologies. This review summarizes the role of cell-mediated immune responses in CHIKV pathogenesis, with a specific focus on pro-inflammatory Th1 responses in the development of CHIKV joint inflammation. Furthermore, due to the explosive nature of arthritogenic alphavirus outbreaks and their recent expansion across the world, co-infections with other highly prevalent pathogens such as malaria are likely to occur but the pathological outcomes of such interactions in humans are unknown. This review will also discuss the potential impact of malaria co-infections on CHIKV pathogenesis and their relevance in alphavirus control programs in endemic areas.

KEYWORDS

$CD4^+$ T cell, chikungunya virus, co-infection, malaria

1 | INTRODUCTION

Arthritogenic alphaviruses are a group of clinically relevant enveloped positive sense, single-stranded RNA viruses that belong to the family *Togaviridae*.[1] These viruses have been linked to the development of acute and persistent arthritic conditions in human populations.[2,3] They are mainly transmitted by *Aedes* and *Culex* mosquitoes which confer them wide global distributions.[4,5] Arthritogenic alphaviruses are typically referred as "Old World alphaviruses" and comprise of chikungunya virus (CHIKV, widely distributed in the tropics), O'nyong-nyong virus (ONNV, restricted to Africa), Mayaro virus (MAYV, endemic to Central and South America), Barmah Forest virus (BFV, confined to Australia), Ross River virus (RRV, reported in Australia, Papua New Guinea, and islands of the South Pacific region), and Sindbis virus (SINV, distributed in Africa, Middle East, Europe, and Australasia).[5]

In humans, arthritogenic alphavirus infection typically causes a febrile illness characterized by high viremia, maculopapular skin rash, muscle pain, hallmark debilitating polyarthralgia, polyarthritis with or without effusions, and in some cases lymphadenopathy.[3,6] The virus incubation period prior to the clinical manifestations depends on the alphavirus species. Typically, it is relatively short with an average of 7-9 days.[2] The disease is self-limiting and usually resolves

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within 2 weeks, but chronic pathologies such as polyarthritis may develop, which could last from months to years.[7] Neurological complications are rare, but recent reports have suggested that serious clinical forms of CHIKV disease could compromise brain tissues leading to permanent neurological damage.[8-11]

Among the arthritogenic alphaviruses, research on CHIKV was the most extensive owing to the global epidemics since 2005.[12] The availability of mouse models that captures major features of human disease have generated a wealth of information.[13,14] These studies have yielded important evidence on the involvement of host immune responses in the development of alphavirus arthritides. CHIKV infections trigger a strong immune response characterized by the release of pro-inflammatory cytokines and chemokines,[15-17] followed by the activation and trafficking of myeloid and lymphoid cells to affected tissues,[18,19] leading to joint swelling. While these immune signatures have been identified, the interplay between these factors underlying the development of acute and chronic forms of arthritis remains elusive.

The striking similarities between CHIKV arthritic disease and rheumatoid arthritis (RA) at the transcriptomic and cytokine/chemokine levels suggested the potential involvement of common causative agents.[20] In fact, two $CD4^+$ effector T cell subsets: Th1 and Th17, have been implicated in the development of RA.[21-24] Th1 cells typically orchestrate cell-mediated responses against intracellular pathogens through the release of signature cytokines such as IFNγ and IL-2,[25-27] whereas IL-17-secreting Th17 cells have been linked to autoimmunity and neutrophil recruitment to the site of infection.[28,29] This prompted the hypothesis that CHIKV-induced inflammation could be also mediated by pathogenic $CD4^+$ T cell responses.

2 | ROLE OF CELL-MEDIATED IMMUNITY IN THE DEVELOPMENT OF CHIKV-INDUCED INFLAMMATION

2.1 | Pro-inflammatory immune mediators induced upon CHIKV infection

Inflammatory cytokines such as IFNγ, IFNα, IL-2, IL-2R, IL-6, IL-7, IL-12, IL-15, IL-17, and IL-18 have been shown to be upregulated during acute CHIKF.[17] Moreover, high levels of IL-15 (a T-cell growth factor),[30] IL-2R (produced upon T cell activation),[31] CXCL9 and CXCL10 (chemokines that bind to CXCR3 primarily expressed on activated T lymphocytes)[32] suggested the involvement of T cell responses during the acute phase of disease. Transcriptomics analysis in CHIKV mouse models revealed overlapping pro-inflammatory gene expression signatures with RA patients.[20] Similarly, canonical pathways analysis showed shared patterns involving monocyte/macrophages, NK cell, B cell, and T cell signaling.[20] Among T cells, $CD4^+$ helper T cells have been associated with acute CHIKF and RA. It has been shown that CHIKV infection triggers strong IFNγ-producing $CD4^+$ T cell responses (Th1).[13] This subset was also reported in the synovium of a patient displaying chronic CHIKV-induced inflammation.[18] Similarly, Th1-polarized cells have been shown to preferentially accumulate in RA joints.[21] Collectively, these observations supported the idea that CHIKV-induced joint swelling and RA could be mediated by pathogenic host immune responses in a similar fashion.

2.2 | Infiltration of innate immune cells into swollen joints

Patrolling monocytes and tissue-resident macrophages are part of the first line of defense upon viral infection. These specialized phagocytic cells play the role of first responders against a wide range of pathogens and, upon activation, release immune modulators such as TNFα, IL-1β, and IL-6 which trigger localized inflammation.[33] Macrophages and monocytes are one of the first immune subsets identified in the synovial tissue cellular infiltrate of CHIKV-infected patients.[18,34] In line with this observation, mouse studies of CHIKV and RRV suggested that the monocytes/macrophages subset represents an important fraction of the cellular infiltrate in mouse swollen joints.[13,35-37] One of the first clues on the functional role of macrophages in CHIKV-induced inflammation was reported by Rulli et al.[38] In this study, treatment with Bindarit, an anti-inflammatory small molecule that modulates the NFκB pathway and inhibits, among others, the synthesis of monocyte chemotactic protein (MCP)-1,[39] ameliorated CHIKV-induced joint swelling in mice. In a parallel study, targeted depletion of macrophages by clodronate liposomes treatment in CHIKV-infected mice yielded a similar outcome.[13] Although these results indicate that monocytes and macrophages might contribute to the CHIKV inflammatory pathology, a separate study suggested that infection in mice deficient of CCR2, a receptor involved in monocyte chemotaxis, resulted in aggravated and prolonged swelling manifestation due to an increased and persistent neutrophil infiltration.[40] Taken together, these observations suggest a model in which monocytes and macrophages could act as a double-edged sword by mediating localized inflammation while restricting excessive infiltration of neutrophils in infected joints.[41]

Neutrophils and NK cells have been also identified in inflamed tissues of CHIKF patients and in the swollen footpads of CHIKV-infected mice.[13,18,19] Increased levels of powerful neutrophil chemoattractants such as CXCL1 and CXCL2, and MPO (myeloperoxidase) have been observed in mouse infected joints at the peak of inflammation.[19,40] Supporting these observations, massive neutrophil infiltration has been linked to an exacerbated swelling pathology in mouse models.[19,40] In a similar fashion, NK cells presence in inflamed tissues is believed to play an important role in CHIKV pathology by exerting direct antiviral activity through cytotoxic mechanisms and by producing IFNγ to enhance both innate and adaptive immune responses.[4,42,43] Upregulation of IL-12, a potent NK cell stimulator, has been reported in joints of virus-infected mice[20] suggesting the involvement of activated NK cells. Moreover, a rapid expansion of NK cells and increased cytotoxic activity during the acute phase of CHIKF have been observed in a patient cohort.[43,44] Some insights on the function of NK cells in

CHIKV-induced swelling have also been observed in one of our studies.[16] Particularly, the more severe early acute joint swelling induced by La Réunion LR2006 OPY1 isolate as compared to the Caribbean CNR20235 isolate was associated with a higher and intensive NK cell activity during the early stages of the immune response in mouse joints. Importantly, the functional role of NK cells in early acute CHIKV-induced swelling was demonstrated by depletion experiments in LR2006 OPY1 infection.[16] These results suggest that NK cells are important mediators of CHIKV-induced joint swelling likely through the activation of myeloid cells which release inflammatory mediators such us IL-6,[45] leading to vascular leakage and edema.

2.3 | Involvement of T cells in CHIKV-induced inflammation

The hypothetical function of T cells during CHIKV infection was initially postulated based on their proliferation and activation profiles in CHIKV-infected patients. Activated peripheral $CD4^+$ and $CD8^+$ T cell levels were found to be elevated in CHIKF patients from the 2006 to 2007 outbreaks in La Reunion Island.[18] Moreover, functional characterization of these cells further revealed the ability to recognize CHIKV-derived peptides and to produce high levels of IFNγ thus suggesting the engagement of Th1-biased immune responses upon CHIKV infection.[18]

In further support of these observations, immune profiling of peripheral blood of patients from the Gabonese CHIKV outbreak of 2007[46] revealed a strong early activation and proliferation of $CD8^+$ T cells followed by the engagement of $CD4^+$ T cell responses at a later point. These results supported a model in which the early expansion of CD8 T+ cells was probably required for clearance of virus-infected cells, whereas the subsequent proliferation of helper T cells was needed to support the proper development of antiviral humoral responses. In support of this model, studies in SINV and RVV previously showed that $CD8^+$ T cells were involved in limiting SINV replication in the central nervous system[47] and eliminating RRV-infected macrophages in vitro.[48]

2.4 | $CD4^+$ but not $CD8^+$ T cells are pathogenic mediators of CHIKV-induced joint swelling

The definitive roles of different T cell subsets were shown through depletion studies in mouse models. Subset specific depletion of $CD4^+$ T cells but not $CD8^+$ T cells abolished the major peak of joint swelling during CHIKV infection with no impact on viral tropism, demonstrating the pathogenic role of $CD4^+$ T cells.[13] In addition, adoptive transfer of $CD4^+$ T cells isolated from CHIKV-infected donors hastened and aggravated peak joint swelling in CHIKV-infected $TCR^{-/-}$ mice as compared to naive donors, demonstrating that this process is mediated by virus-specific $CD4^+$ T cells.[49] CHIKV proteome-wide screening assays identified nsP1-P4-2 (145-162 aa, non-structural protein 1) and E2EP3 (2800-2818 aa, E2 glycoprotein) as dominant mouse $CD4^+$ T cell epitopes.[50] The transfer of primary $CD4^+$ T cell line specific for nsP1-P4-2 and E2EP3 generated through a prime/boost strategy, that predominantly express IFNγ, partially recapitulated joint swelling in $TCR^{-/-}$ mice, further supporting the pathogenic role of Th1 responses.[49]

On the other hand, the protective role of $CD8^+$ T cells was ruled out based on experimental findings. Firstly, infection of CD8 deficient mice or $CD8^+$ T cell-depleted mice did not alter joint pathology or reduce viral load in the blood and tissues.[51] Next, adoptively transferred CHIKV-specific $CD8^+$ T cells induced upon live-attenuated CHIKV vaccination failed to control CHIKV infection in mice.[52] Although $CD8^+$ T cells have been linked to protection against RRV[48] and SINV,[47] findings in CHIKV suggest that $CD8^+$ T cells play different roles in combating alphavirus infections.

2.5 | Th1 cytokines and $CD4^+$ T cells interplay during CHIKV infection

IFNγ is the main effector cytokine produced by polarized Th1 $CD4^+$ T cells and is needed for the control of intracellular infections caused by viruses, bacteria, and protozoa.[26,27,53-55] It is also believed to play a central role on cell-mediated immunity by upregulating phagocytic and microbial killing capabilities in monocytes/macrophages[56-59] as wells as orchestrating, together with TNFα, the recruitment of mononuclear cells to the site of the infection.[60,61]

CHIKV infection in the absence of IFNγ has provided inconclusive results. In Teo et al[49,51] infection of IFNγ-deficient ($IFN\gamma^{-/-}$) mice resulted in a slight increase in viremia, footpad viral load, and footpad swelling, suggesting a possible antiviral role for this cytokine as previously observed in other alphaviruses.[62] This is partially corroborated by a recent work[63] where CHIKV infection of $IFN\gamma^{-/-}$ mice led to higher viremia but a marginal reduction in footpad swelling. Conversely, Nakaya et al[20] reported that CHIKV-infected $IFN\gamma^{-/-}$ animals displayed markedly reduced joint swelling with little effect on viremia. These discrepancies might be due to differential pathogenicity derived from the use of distinct CHIKV isolates: SGP11 in Ref. [49,51] and LR2006-OPY1 in Ref. [20,63].

The contribution of IFNγ to CHIKV-induced inflammation remains unclear, however, recent studies have suggested that $CD4^+$ T cells might mediate CHIKV arthritic disease through the secretion of proteins other than IFNγ. RNA-Seq analysis of CHIKV-infected mouse footpads at the peak of swelling revealed highly induction of Granzyme A,[63] a serine protease produced by NK cells, $CD8^+$ T cells, and Th1 $CD4^+$ T cells.[64-66] Furthermore, deficiency of Granzyme A ($GzmA^{-/-}$) or treatment with Serpinb6b—a Granzyme A inhibitor[67]—in mice abolished CHIKV-induced joint swelling without affecting viremia. Collectively, these results suggest that Th1 cells could be mediating virus-induced swelling through Granzyme A but not IFNγ secretion. Further adoptive transfer experiments using $CD4^+$ T cells from Granzyme A deficient mice into CHIKV-infected $TCR^{-/-}$ recipients are needed to

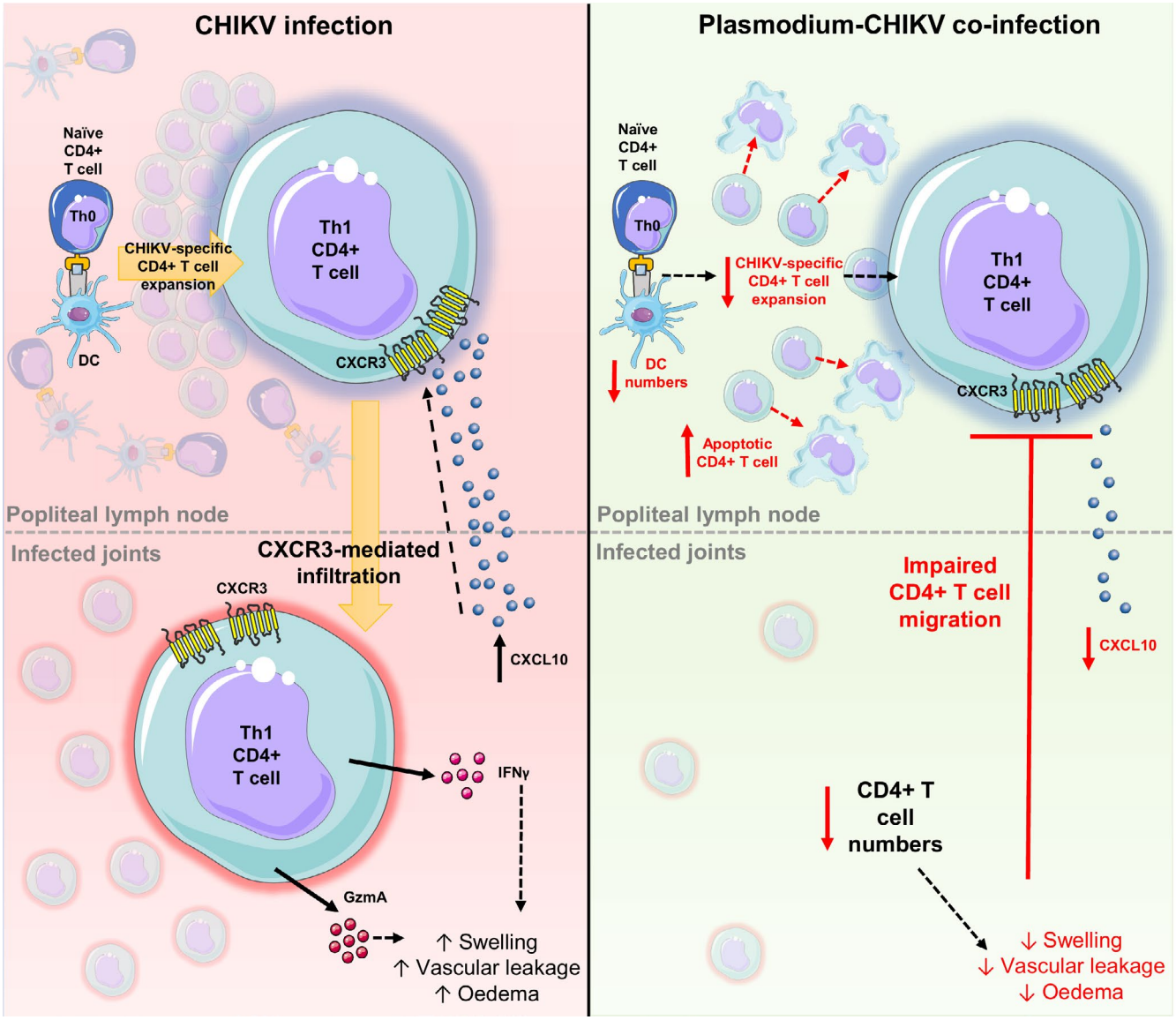

FIGURE 1 Malaria co-infection impairs CHIKV-specific $CD4^+$ T cell responses in the pLN-footpad axis. In CHIKV-infected mice, virus-specific $CD4^+$ T cells multiply in the popliteal lymph node (pLN) and acquire a Th1 phenotype. Th1 cells migrate to infected tissues through a CXCR3-mediated mechanism and potentially drive footpad swelling by releasing cytokines such as INFγ and GzmA. Upon co-infection, a drop in the numbers of conventional dendritic cells (DCs) in the pLN is associated with diminished expansion of pathogenic $CD4^+$ T cells. Moreover, malaria infections supress CHIKV-specific $CD4^+$ T cell responses by inducing early apoptosis of pLN $CD4^+$ T cells and affecting CXCR3-mediated joint infiltration thus leading to suppression of joint swelling. CXCL10: C-X-C motif chemokine 10, INFγ: interferon gamma, GzmA: granzyme A. This figure contain modified images from Servier Medical Art, licensed under a Creative Common Attribution 3.0 Generic License. http://smart.servier.com/

confirm that infiltrating CHIKV-specific Th1 cells mediate swelling through Granzyme A (Figure 1).

3 | CO-INFECTION WITH *PLASMODIUM* PARASITES MODULATES CHIKV PATHOLOGIES

The CHIKV outbreaks in Asia, the Indian Ocean islands and its recent introduction to the Americas and the Caribbean made clear the ability of arthritogenic alphaviruses to rapidly spread and cause epidemics on a global scale. This has been fueled by genomic mutations favoring virus ecological fit to new mosquito vectors[68-71] and an exponential increase in international travelers to and from developing economies.[12,72-75] Importantly, the expansion of CHIKV distribution and the establishment of local transmission hotspots in newly colonized areas pose a high risk of co-infections with other highly prevalent tropical diseases. A number of epidemiological studies have reported co-infections of CHIKV with pathogens such as Zika virus (ZIKV), dengue virus (DENV), and malaria parasites in humans[76-85] although the

pathological outcomes of these co-infections have not been clearly defined. Particularly, different clinical reports from African malaria cohorts[86-89] suggested the presence of CHIKV infections. Furthermore, the highly pro-inflammatory profiles of both diseases—including the detrimental contribution of T cell-mediated immune responses to the pathologies—and the immunosuppressive nature of malaria infections speculate that immune modulation might occur.

3.1 | *Plasmodium*-Chikungunya co-infections do occur in endemic areas

Although CHIKV-malaria co-infections in humans have been shown to occur in the African continent, there was a lack of evidence of such infections in Southeast Asia. However, anti-CHIKV IgM and IgG antibodies detected in 36.5% and 88.5%, respectively,

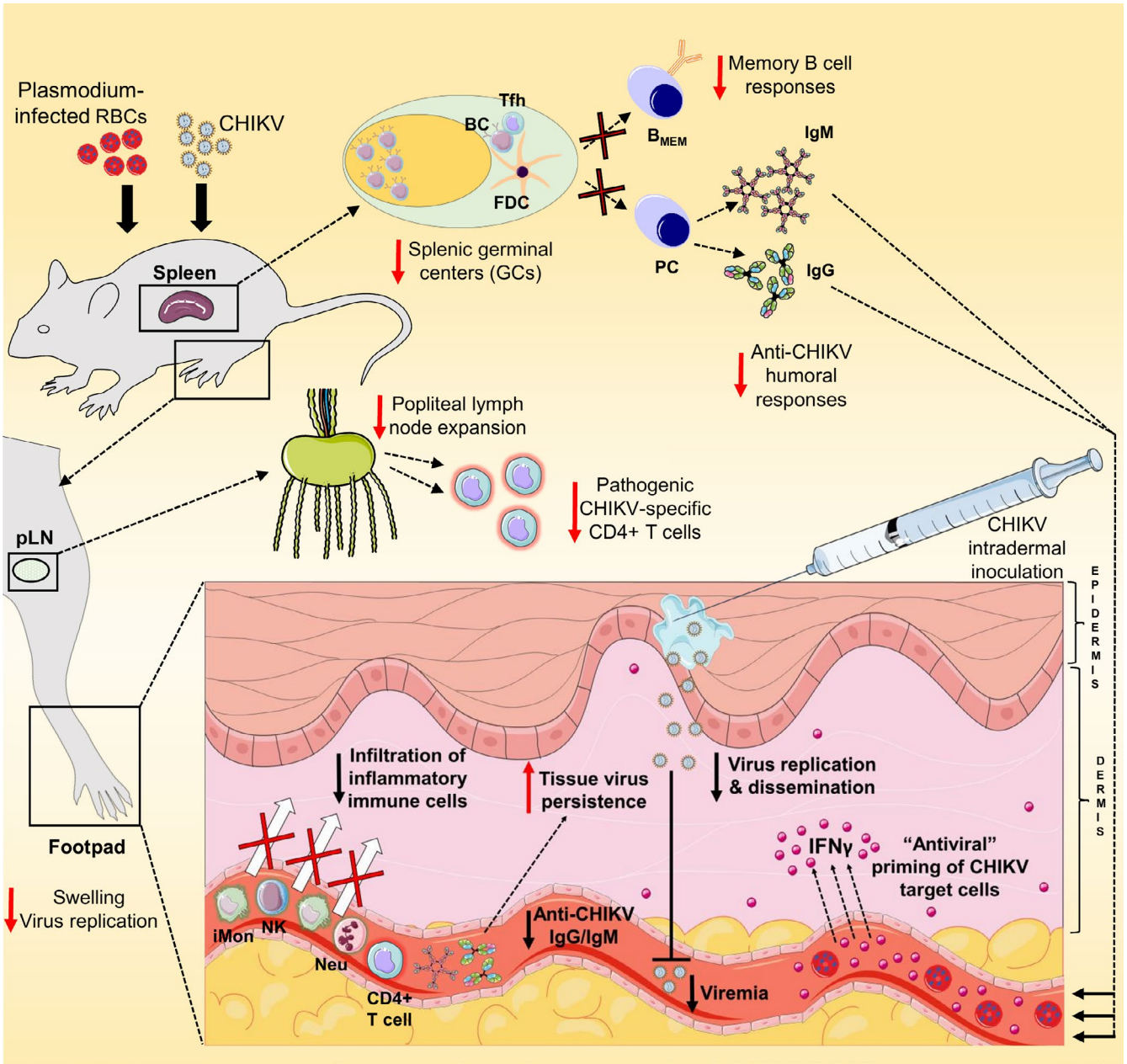

FIGURE 2 Regulation of CHIKV immune responses by malaria infections. Murine malaria infections lead to the dysregulation of splenic germinal center (GC) responses resulting in impaired generation of memory B cells and reduced CHIKV-specific IgG/IgM serum titers associated with delayed viral clearance in infected tissues. The expansion of popliteal lymph nodes is also affected in co-infected animals (see Figure 1). Locally in the footpads, the antiviral affects of INFγ induced by pre-existing malaria infections limit CHIKV replication and dissemination resulting in reduced viremia. Moreover, suppression of CHIKV-induced swelling is associated with diminished infiltration of inflammatory innate subsets and $CD4^+$ T cells into infected tissues. BC, B cell; Tfh, follicular helper T cell; FDC, follicular dendritic cell; BMEM, memory B cell; PC, plasma cell; IgG, immunoglobulin G; IgM, immunoglobulin M; iMon, inflammatory monocytes; NK, natural killer cells; Neu, neutrophils; INFγ, interferon gamma. This figure contain modified images from Servier Medical Art, licensed under a Creative Common Attribution 3.0 Generic License. http://smart.servier.com/

in patients diagnosed with acute *P. vivax* from Thailand suggested both pre-exposure and ongoing CHIKV infection.[90] CHIKV-*Plasmodium* co-infections are therefore not only restricted to Africa and are likely to occur in areas of co-circulation in Asia and Latin America. Moreover, although CHIKV and *Plasmodium* parasites are known to be vectored by *Aedes* and *Anopheles* mosquitoes, respectively, entomological studies have reported the existence of CHIKV-infected *Anopheles* populations that might contribute to viral transmission.[91,92] These reports suggest an increasing likelihood of concurrent co-infections by CHIKV and *Plasmodium* in endemic areas.

There are well-established rodent malaria models such as *Plasmodium berghei* ANKA (PbA) and *P. yoelii* 17X (Py17x). In the lethal experimental cerebral malaria (ECM)[93-95] model, PbA-infected animals succumb during the first week postinfection due to neurological complications. The *P. yoelii* 17X (Py17x) mouse model is a non-lethal, self-resolving infection used in studies of acquired immunity against the parasite.[96] These experimental models would be ideal to study co-infections with CHIKV.

3.2 | Immune modulation of CHIKV innate responses by *Plasmodium* co-infections

Immunosuppression has been well-documented for blood-stage *Plasmodium* infections in both human[97-103] and animal models.[104-113] Furthermore, collective evidence has suggested that *Plasmodium*-associated immunosuppression increases the host susceptibility to secondary infections potentially leading to more complicated pathologies.[114-118] Pre-infection with murine malaria strains for 4 days followed by subsequent inoculation with CHIKV (early sequential co-infection) or concurrent CHIKV-*Plasmodium* co-infection markedly reduced the development of virus-induced joint swelling.[119] Moreover, sequential co-infection but not concurrent co-infection abolished viremia in infected animals. Although one might intuitively think that *Plasmodium*-CHIKV co-infections would worsen the pathological outcome, our observations suggested a protective effect of malaria on CHIKV-induced pathologies. Moreover, the precise timing of pathogen inoculation is essential to determine the immunomodulation outcome.

The reduction of CHIKV-induced footpad swelling in co-infected animals was associated with lesser numbers of joint-infiltrating innate pathogenic subsets such as neutrophils, NK cells, and inflammatory monocytes.[90,119] Reduced edema, muscle necrosis, and vascular leakage were also observed suggesting generalized suppression of signature inflammatory responses induced by CHIKV[95,122] (Figure 2). Of note, blood-stage malaria has been linked to dysregulation in the motility of different inflammatory immune subsets. For example, *P. vivax* controlled human malaria infection has been shown to induce the depletion of neutrophil populations coupled to the downregulation of chemokine receptors CRXCR1, CXCR2, CCR3, and the growth factor receptor CSF3R responsible of neutrophil maturation and survival.[120] Furthermore, an in vitro study reported that parasite-derived antigens such as *P. falciparum* merozoite surface protein 1 (PfMSP-1) impairs neutrophil chemotaxis through the blockade of NFκB signaling.[121] Reduced expression of cytoadhesion molecules such as CD11b, CD11c, and CD18 and diminished diapedesis and responsiveness to chemotactic factors (ie, TNFα or MCP-1) have also been reported in monocytes upon *Plasmodium* infection.[122,123] Complementary studies are needed to demonstrate whether the observed reduction of the innate cellular infiltrate in joints of co-infected animals is due to malaria-associated impaired chemotaxis and dysregulation of the chemokine network in infected tissues.

The abrogation of viremia only in animals pre-infected with malaria (4 days prior CHIKV inoculation) suggested that immune mediators produced upon parasite infection could be responsible for the control of CHIKV dissemination. Human and mouse studies have reported early induction of IFNγ upon *Plasmodium* infections[124-126] and an antiviral role of this cytokine has been also proposed during CHIKV infections.[51,63] In the context of sequential *Plasmodium*-CHIKV co-infection, the abrogation of viremia was reverted in mice lacking IFNγ and in wildtype mice treated with IFNγ neutralizing antibodies.[119] The induced IFNγ by pre-existing *Plasmodium* infection could exert an antiviral activity through the priming of primary CHIKV targets such as fibroblasts, myocytes, endothelial cells, and macrophages during the early stages of infection[13,127,128] (Figure 2). Particularly, endothelial cells and fibroblasts have been shown to display antiviral ability upon IFNγ stimulation by interfering with virus gene transcription[129] and by increasing their responsiveness to viral nucleic acids.[130] Transcriptomic studies would be valuable to identify the antiviral pathways triggered by IFNγ exposure in different CHIKV-target subsets at the site of infection.

Plasmodium inoculation during an ongoing CHIKV infection modified the outcome of murine malaria. Co-infection in mice pre-infected with CHIKV for 4 days with either PbA or Py17x did not affect the development of footpad swelling or viremia. In contrast, ongoing CHIKV infection exacerbated *Plasmodium*-induced pathology by increasing the parasite load in the blood at the later stage of the disease. The availability of type I interferon induced by a pre-existing CHIKV infection[16,17] might be responsible of the increase in parasitemia. Supporting this hypothesis, a recent mouse study reported that blockade of type I interferon signaling resulted in a better parasite control due to the establishment of robust antimalarial humoral responses reflected in increased Tfh numbers, GC reactions, and *Plasmodium*-specific antibody titers.[131,132] Finally, the re-challenge of recovered animals previously infected with either malaria or CHIKV with their respective heterologous pathogen did not affect the normal development of any of their individual pathologies.[119]

3.3 | Immune modulation of CHIKV T cell responses by *Plasmodium* co-infections

Interestingly, the marked reduction of footpad swelling in co-infected animals at 6 days post-CHIKV infection (dpi) revealed diminished

infiltration of virus-specific $CD4^+$ T cells in infected joints.[119] Furthermore, co-infection in $LT\alpha^{-/-}$ animals (devoid of lymph nodes (LNs)), but not splenectomized mice, recovered the major peak of footpad inflammation suggesting that LN is the main secondary lymphoid organ of immune regulation.[90,119]

Immune profiling of the popliteal LN (pLN), the nearest draining LN to the site of infection, from the concurrent co-infected animals revealed decreased numbers of total and CHIKV-specific $CD4^+$ T cells.[119] Concordantly, increased apoptotic $CD4^+$ T cells in the pLN at 5 and 6 dpi were detected. This was supported by previous findings where apoptosis in lymph nodes and secondary lymphoid organs upon malaria infection was described.[133,134] In addition, it is also known that blood-stage malaria infections suppress dendritic cell (DC) responses by affecting DC maturation,[142-144] reducing surface expression of MHC-II[142,145-147] and co-stimulatory molecule CD86,[146] inducing DC apoptosis[148,149] and impairing their ability to process and present parasite-derived antigens to T cells.[150,151] In line with this, reduced numbers of conventional DCs in the pLN of co-infected mice were also observed, suggesting that reduced expansion of $CD4^+$ T cells might be due in part to the drop in DCs numbers associated with malaria.[135-138] Further research is needed to determine whether DCs ability to process and present CHIKV-derived antigens (signal 1) and to co-stimulate (signal 2) naive $CD4^+$ T cell in the pLN is affected upon co-infection. Similarly, whether *Plasmodium* infection alter the phagocytic and migratory potential of DCs, and other APCs, at the site of CHIKV inoculation remains to be explored.

Impaired expansion and early apoptosis of $CD4^+$ T cells in draining lymph nodes in co-infected mice could only partially explain the suppression of joint swelling. Virus-specific $CD4^+$ T cells in the pLN was only suppressed by ~50%, but $CD4^+$ T cells detection in the co-infected animals' footpad was almost completely abolished.[119] Using adoptive transfer of CSFE-labeled $CD4^+$ T cells isolated from CHIKV-infected mice, it was shown that the migratory potential of $CD4^+$ T cells into the infected footpad was also abolished by co-infection. This blockage of migration was mediated by the suppression of Th1-associated chemokines such as RANTES, MIP-1α, MIP-1β, and CXCL10 in the footpad. Furthermore, in vivo blockade experiments showed that Th1 chemokine receptor CXCR3 and not CCR5 is functionally important for $CD4^+$ T cell-mediated CHIKV inflammation and that CXCR3-mediated chemotaxis was impaired during co-infection.[119]

Taken together, these results suggest that the expansion, survival, and trafficking of pathogenic CHIKV-specific $CD4^+$ T cells in the pLN/footpad axis are hampered by co-infections with *Plasmodium* parasites (Figure 1). Additionally, the contribution of T cell anergy[139,140] and exhaustion[141-144] extensively reported upon severe malaria infections remain to be explored.

3.4 | Immune modulation of CHIKV B cell responses by *Plasmodium* co-infections

B cell immune responses are altered during malaria. This has been proposed as a mechanism to avoid the establishment of efficient and long-lasting humoral responses.[145] Notably, the development of naturally acquired immunity against *Plasmodium* is very slow and might take several years[146-149] and is associated with a very low frequency of *Plasmodium*-specific memory B cells detected in populations from malaria endemic areas.[150,151] Studies using murine and non-human primate models[107,132,146] have suggested impaired induction and maturation of germinal centers (GCs) thus leading to the production of low-affinity, short-lived antibody responses and the development of limited B cell memory. Such immune regulation could have important implications on the generation of antibodies against heterologous pathogens upon co-infection.

CHIKV-neutralizing antibody responses are known to play a pivotal role in the control of CHIKV infection. Lum et al[152] reported that CHIKV infection in mice lacking functional B cells resulted in high-level viremia that lasted for more than a year. Notably, co-infection experiments (in both sequential and concurrent settings) revealed delayed virus clearance from footpad tissues.[119] In further support of this, circulating levels of CHIKV-specific IgM and IgG and their virus-neutralizing capacities were found to be reduced at 15 dpi upon concurrent co-infection. Complementary experiments allowed us to identify the spleen as the main site of B cell dysregulation as co-infection in splenectomized mice led to similar CHIKV-specific antibody levels and tissue virus clearance at the later time-points of the disease than non-splenectomized animals.[119] This could be due to delay in the induction of GC reactions and a reduced number of GC-dependent $CD73^+$ memory B cells in the co-infected animals[119] (Figure 2). Although the precise mechanisms governing impairment of CHIKV antibody responses upon malaria co-infection are still unknown, the answer might lay on the impact of *Plasmodium* infections on T cell-dependent enhancement of B cell responses. Millington et al[138] reported that helper T cells activated by hemozoin-loaded DCs displayed reduced migration to B cell compartments in lymphoid-organ follicles leading to defective B cell expansion and antibody production in mice. Similarly, severe malaria infections in mouse models were linked to impaired T follicular helper cells (Tfh) maturation and dysregulation of GC responses.[153]

4 | CONCLUDING REMARKS

The unique protective effects exerted by *Plasmodium* infections against CHIKV pathologies might have relevant implications in the control of both illnesses in endemic areas. Specific interventions aimed at replicating disease-protective mechanisms, will provide new therapeutic approaches and/or adjunct therapies to antiviral or antimalarial treatment.

At the epidemiological level, co-infections by CHIKV and *Plasmodium* parasites can be underestimated in human populations due to the immunosuppressive nature of malaria which can result in the development of asymptomatic CHIKV infections without compromised joints. The absence of alphavirus screening in confirmed malaria cases might also contribute to a higher burden of asymptomatic CHIKV carriers.[87] On the other hand,

reduced viremia upon co-infection could impact CHIKV transmission dynamics by reducing the number of successfully infected mosquitoes after a blood meal. These observations highlight the need for the incorporation of alphavirus diagnosis in malaria intervention programs.

Lastly, O'nyong-nyong virus (ONNV), an emerging arthritogenic alphavirus known to cause similar clinical manifestations than CHIKV,[154,155] is transmitted in the African sub-continent by two main malaria vectors, *Anopheles gambiae* and *A. funestus*, favoring a higher likelihood of co-infections than CHIKV. A similar situation could arise in Latin America, where emerging Mayaro Virus (MAYV) has been recently shown to infect *Anopheles* mosquitoes suggesting that co-infections with malaria could happen.[156] It is therefore crucial to understand the transmission dynamics, pathological implications and burden of alphavirus-*Plasmodium* co-infections for the future development of improved diagnostics and treatment strategies.

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CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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