Chapter

Regulation of T-reg/Th-17 Balance: One Step Closer Towards Immunotherapy Against Malaria Infection

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Abstract

According to World Malaria Report 2020, the rate of decline in malaria case incidence and deaths caused by malaria has ceased in latter half of the past decade. Though Artemisinin Combination Therapy (ACT) is still the major therapeutic approach globally to treat malaria patients, increased resistance of *Plasmodium* sp. to artemisinin can be looked upon as a major factor responsible for the rate of decline. In the present world, immunotherapeutic approaches are in the limelight to treat several infections, autoimmune disorders, cancers but application of such therapeutic measures in case of malaria are yet not available. Among different immune cells, T-regulatory cells (T-reg) and Th-17 cells and the balance between them, helps in determining the outcome of the immune response in host during both lethal and non-lethal malaria. TGF\(\beta\) and IL-6 are two major cytokines that play important role in fine tuning the Treg/Th-17 balance by modulating dendritic cell responses, specially by regulating the ratio between myeloid DC and plasmacytoid DC (mDC/pDC). Studies in rodent malaria models have revealed that neutralization of IL-6 by using anti IL-6 monoclonal antibodies *in-vivo* has been found effective in declining the parasitemia, malaria induced deaths and also in reverting back the altered T-reg/Th-17 balance to normal levels. Apart from these, autophagy is one of the major factors which also contributes to regulate the T-reg/Th-17 balance. In malaria infected mice, autophagy induction has been found to normalise the dysregulated T-reg/Th-17 ratio and promote anti-inflammatory Th-2 pathway by supressing pro-inflammatory Th-1 pathway. So, Treg/Th-17 balance and its associated regulators can be important immunotherapeutic targets for malaria prevention in near future.

**Keywords:** Malaria, drug resistance, immunotherapy, T regulatory cells, Th-17, IL-6, TGF\(\beta\), dendritic cells, autophagy

1. Introduction

World Malaria Report 2020 published by World Health Organization estimated 229 million cases of malaria infection around the world in 2019 among which 94%
of the cases were reported from the WHO African region. The number of estimated cases globally in 2019 was 1 million more than that of the previous year. But in the context of last 20 years, the number of the existing malaria cases has declined from 238 million in 2000. Besides, the total number of estimated cases globally, another parameter that has been in the centre of studying the impact of this disease is, malaria case incidence (cases per 1000 population at risk). Malaria case incidence reduced from 80 in 2000 to 57 in 2019 globally but the rate of decline has ceased in the latter half of the past decade. The deaccelerating rate of decline has also been found in case of malaria mortality rate (i.e. deaths per 100000 population at risk). Despite the steady reduction in number of malaria induced deaths in the past two decades, more than 400 thousand malaria deaths have been reported in 2019. Children aged below 5 years account for 67% of the total malaria deaths, which is a major concern [1].

Among various Plasmodium strains that can infect human beings, cerebral malaria causing Plasmodium falciparum bring about majority of malaria deaths in Africa and parts of Asia. Apart from Plasmodium falciparum, another strain, Plasmodium vivax also cause malaria deaths in various other parts of the world [2, 3]. Among several available therapeutic and controlling measures, Artemisinin based Combination Therapy (ACT) is being used worldwide and has been of great success in combating this disease [4–6]. But in recent times, the use of ACT got a major setback due to emergence of Artemisinin resistant Plasmodium strains [7, 8]. It may be one of the plausible causes behind the diminishing rate of decline in the rate of malaria case incidence and malaria mortality rate since 2015. Researchers worldwide are putting up constant efforts on making ACT more effective and finding other therapeutic strategies to combat this disease in order to eradicate it in near future. Among other therapeutic measures, immunotherapy has been the prime focus of study over the past decade. Nowadays immunotherapy is being used for various infectious diseases and cancer therapy and the success rate of such therapies are quite promising [9–11]. In case of malaria, immunotherapeutic strategies are not yet available for use. This compels researchers worldwide to find various molecules or cells that can be targeted for effective therapeutic measures in malaria infection [12].

In malaria different stages of the parasitic life cycle can trigger both the innate and adaptive immune response within the host. It is quite difficult to study whether the immune cells play protective or pathogenic or dual roles, especially in human [13]. Still, long-term research reveals specific roles of antibodies and B cells in protection of the host body against the malaria parasite. Besides, several other immune cells like inflammatory cytokines (TNF α, TGFβ, IFN-γ etc.), different subsets of T cells (T-helper cells and Cytotoxic T cells), NK cells and Macrophages also play their part in protection or pathogenesis or both depending on the type of malaria parasite and the stage of life cycle they are in [14]. During life cycle of Plasmodium sp. within the host, several major organs and the immune environment within those organs show changes due to presence of parasite factors. Spleen, being a major lymphoid organ and the main blood filtration unit, harbours most of these immune cells [15, 16]. In presence of Plasmodium sp. in host body, the immune environment changes rapidly in a day specific manner post infection. Investigation of the changes and regulatory mechanisms within splenic compartment during infections in humans is difficult for several reasons. Most of the study is restricted to observations of clinical symptoms and analysis of tissue sections that are available only after post-mortem. So, there is always lack of enough samples available to investigate the changes and their associated mechanisms in spleen and other lymphoid organs properly [17, 18]. To overcome this, researchers worldwide have focused on studying the major changes in
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DOI: http://dx.doi.org/10.5772/intechopen.97045

rodent models of malaria. Murine malaria models are very much in use for their ready availability. Various rodent specific parasite strains like Plasmodium berghei ANKA, Plasmodium yoelii, Plasmodium chabaudi are constantly used in laboratories and they almost resemble different parameters (i.e. anaemia, body temperature changes, loss of weight, and occasional death) shown by human during malaria infection. Apart from these basic parameters, several immune parameters like changes in T helper cell and Cytotoxic T cell percentages in lymphoid organs, activities of B cell, concentration of antibodies, disruption of blood brain barrier and migration of immune cells in the brain during cerebral malaria infection also show resemblance to that of human malaria infections. Plasmodium berghei ANKA and Plasmodium chabaudi infections show similar symptoms, immunological changes as discussed with that of Plasmodium falciparum infection in human which might be due to similarities in infective strategies. Both these rodent and human strains can disrupt the blood brain barrier in a similar manner and immune cells (majorly T cells) infiltrate in the brain which can be lethal to the respective hosts. Another rodent specific non-lethal strain Plasmodium yoelii has similar effect on the host immune system to that of Plasmodium vivax infection in humans [19]. Working with these rodent strains of Plasmodium sp. has been found effective in inferring how the immune system is being regulated during malaria and the elaborated regulatory mechanisms that controls the inflammatory balance that occurs. The balance between pro-inflammatory and regulatory immune responses determines the outcome of malaria infection [20]. The balance is maintained by various cytokines, chemokines, several immune cells (macrophages, dendritic cells) and processes like autophagy. The role of CD4+ T helper cells and CD8+ cytotoxic T cells has been found important in regulating the immune response during malaria infection using both rodent models and human samples. The focus has now been shifted to find out the exact role of different subsets of CD4+ T helper cells and how the balance between them defines the outcome of malaria infection. Among these subsets, Th1/Th2 balance and the cytokines regulating this balance has been found crucial for monitoring the immune homeostasis [21, 22]. But recently, balance between two other subsets of T helper cells was found to be important in regulation of immune responses in various infections, autoimmunity and also cancer immunology. These are termed as T regulatory cells that regulates immune-tolerance by secretion of IL-10 and Th17 cells which inflicts inflammatory responses by secreting IL-17, IL-22, IL-23. Naïve CD4+ T cells differentiate into T-regulatory cells (T-reg) in presence of TGFβ and into Th-17 in presence of TGFβ and IL-6. Majority of functions executed by these cells are regulated by their major transcription factors FOXP3 and RORγT for T-reg and Th17 cells respectively [23–25]. As discussed, the differentiation of Treg and Th17 cells is reciprocally regulated by shared and different cytokines and recent studies even show the plasticity of these cells which states that each subset can convert itself to the other one under different inflammatory stimuli [26–28]. These stimuli modulates the cytokine environment of the host and also changes the homeostatic balance between pro-inflammatory and anti-inflammatory cytokines that culminates into Treg/Th17 disbalance. So, T-reg/Th17 balance and regulation of factors that influence this balance has been found to be pivotal in several viral, bacterial and parasitic infections. In case of several autoimmune disorders like rheumatoid arthritis (RA), psoriasis, inflammatory bowel disease (IBD), multiple sclerosis (MS), Th-17 is the major role player and the T-reg/Th-17 balance skews towards pro-inflammatory Th-17 mediated response. Therapeutic approaches which target Th-17 cells and its functional transcription factor RORγT has been successful in reverting the T-reg/Th-17 cell ratio to normal levels [29]. Monoclonal antibodies designed against the human IL-6R, and drugs like sarilumab and tocilizumab
can reduce Th-17 cells and increase T-reg cells that helps in amelioration of RA in humans [30]. In malaria, the T-reg cells has been found to help the malaria parasite to evade the immune response [31]. Apart from T-reg, Th-17 cells have been also known to play an important role in blood brain barrier disruption, which is a prime reason behind deaths due to cerebral malaria. This article summarizes the recent advancements on understanding Treg/Th-17 balance with respect to malaria [32].

2. Differential role of T-regulatory cells and Th-17 cells in malaria

During malaria, failure in development of an effective pro-inflammatory and anti-inflammatory balance has been found to contribute towards unrestricted replication of parasite and severe immunopathology [31, 33]. Several subsets of T cells (Th-1, Th-2, NKT cells) are involved in controlling the lethal and non-lethal malaria infection [34]. T-reg cells have been primarily found to control the immune evading mechanism of the Plasmodium sp. in both mouse and human [35]. A number of other studies have also reported that T-reg may play an important part in facilitating parasite clearance and enhance parasite burden [36, 37]. However, in a separate study, depletion of Foxp3+ T-reg failed to provide protection against experimental cerebral malaria (ECM), which questions the actual role of T-reg in lethal and non-lethal malaria [38]. Augmented generation of Th-17 cells and quick death due to high inflammation in several organs in adult healthy mice upon ablation of T-reg cells, point towards a counter regulatory pathway that might control the pathogenic Th-17 pathway [39]. Th-17 cell itself and cytokines associated with its differentiation from naive CD4+ T cells has been found to play a role in blood brain barrier (BBB) disruption and cooperate with each other to allow migration of T cells into the brain [40]. As BBB disruption is a salient feature of lethal cerebral malaria, Th-17 pathway and its probable counter regulatory pathway controlled by T-regulatory cells is thought to be important in depicting the probable outcome of the immune response elicited by the host against the malaria parasite. In malaria, the balance between pro-inflammatory and anti-inflammatory factors was found to be important when we reported differential expressions of anti-inflammatory TGFβ and pro-inflammatory TNFα and their role in regulation of splenocyte apoptosis [41]. Keeping the outcome of evaluation of TGFβ and TNFα in context to splenocyte apoptosis and shared requirements of TGFβ during differentiation of T-reg and Th-17 cells, we checked whether the balance between anti-inflammatory T regulatory cells and pro-inflammatory Th-17 cells (T-reg/Th-17) is important in malaria immunology in both spleen and brain. T regulatory cells were found to increase in spleen of non-lethal P. yoelii infection at 8 days post infection (dpi) in a day specific manner but in case of lethal P. berghei ANKA infection, it decreased with an increase in the infection and the percentage of T-reg in spleen was lowest at 8 dpi. Not only Tregs but the transcription factors, specially FOXP3 also showed similar trend in spleen of lethal and non-lethal malaria infection. In contrast to the T-regulatory cells, Th-17 cells increased significantly at 8 dpi in lethal P. berghei ANKA infection but decreased optimally at 8 dpi after an initial surge at 2 dpi. The major transcription factor of Th-17 cells shows the similar trend in both lethal and non-lethal malaria infection as does Th-17 cells [42]. Not only in spleen but also in cerebral cortex and cerebellum of the P. berghei ANKA infected mice, differential expression of FOXP3 and RORγT has been found to be critical in regulating the glial cell mediated neuro-inflammation and neuronal cell death [43]. So, the contrasting behaviour shown
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DOI: http://dx.doi.org/10.5772/intechopen.97045

by these two cells and their transcription factors highlights the importance of T-reg/Th-17 balance and their regulators in malaria.

3. Role of cytokines (TGFβ and IL-6) in regulation of T-reg/Th-17 balance in malaria

TGFβ and IL-6 are cytokines that play major roles in the regulation of innate and adaptive immune responses in different viral (viz. influenza A, Respiratory Syncytial virus etc.), bacterial (viz. Streptococcus, Mycobacterium etc.), parasitic (viz. Leishmania, Trypanosoma, Toxoplasma etc.) infections, cancers and autoimmune disorders [44–47]. In malaria, IL-6 is found in circulation of patients infected with Plasmodium vivax and Plasmodium falciparum and it plays a major role in host response [48–50]. There are reports stating that decreased IL-6 levels upon treatment with anti-malarial compounds is associated with decreased parasitaemia [51–53]. However, several reports raise question on actual involvement of IL-6 in the pathogenesis of cerebral malaria [54–56]. In case of TGFβ, we have found that low concentration of TGFβ was found to be pro-inflammatory whereas high concentration of TGFβ have anti-inflammatory effects [41]. So, as factors responsible for disease outcome in malaria, both of these cytokines and their regulatory effect on T-reg/Th-17 balance seem to be important. We neutralized TGFβ and IL-6 by administration of neutralizing antibodies in-vivo at specific concentration. Parasitaemia was highest in TGFβ neutralized group than any other groups whereas parasitaemia was lowest in IL-6 neutralized group. This has been supported by the results of survival percentages of mice, where TGFβ neutralized group showed lowest survival percentage and IL-6 neutralized group showed the highest survival percentage of mice. Thus, it is quite evident that TGFβ and IL-6 directly affects the outcome of the immune response elicited by the host in malaria. Focusing on the effect of these two cytokines on the T-reg/Th-17 balance, it is found that neutralization of TGFβ results in significant induction of Th-17 cells at 8 dpi than control and infected ones. Whereas neutralization of IL-6 causes reduction in percentage and number of Th-17 cells than Plasmodium berghei ANKA infected group. Analysis of percentage and number of T regulatory cells in spleen show the reverse phenomenon to that of Th-17 cells upon neutralization of TGFβ and IL-6. Thus T-reg/Th-17 balance, which is skewed towards Th-17 in Plasmodium berghei ANKA infection is dependent on fine tuning maintained by TGFβ and IL-6. IL-6 neutralization reverts the dysregulated T-reg/Th-17 balance to homeostatic levels by inhibiting Th17 induction, but neutralization of TGFβ has opposing effect and causes the balance to skew more towards Th17. These changes in T-reg/Th17 balance by regulatory effects of TGFβ and IL-6 is mainly maintained by expression of STAT3 and STAT5, which are the major signalling molecules that take part in the signalling mechanism of these two cytokines [57]. Neutralization of TGFβ and IL-6 not only have its impact on splenic T-reg/Th-17, but also in that of cerebral cortex and cerebellum. In Anti-IL-6 treated Plasmodium berghei ANKA infected mice, glial cell mediated neuroinflammation is reduced whereas the anti-TGFβ treated mice upon infection show similar level of neuroinflammation as that of only infected mice. Consistent to that, astrocyte and microglia activation levels show similar changes in IL-6 and TGFβ neutralized groups. Regarding T-reg/Th-17, the major transcription factor of T-reg cells, FOXP3 expression was significantly higher in Anti-IL-6 treated infected group and significantly lower in Anti-TGFβ treated infected mice. The expression of IL-17, a major cytokine secreted by Th-17 cells, show the opposite result to that of FOXP3 in both the groups than the only
Plasmodium berghei ANKA infected ones [43]. But the actual percentages of the T-reg and Th-17 in cerebral cortex and cerebellum and their changes upon neutralization of these two cytokines is not yet investigated. Though there are few reports that cerebral malaria development is independent of IL-17 [58], several other reports shows that significant amount of IL-17 is found in circulation of malaria infected mice and human patients [59–61]. Genetic variants of IL-17 and its receptor IL-17RA increase the risk of malaria as investigated in African population [62]. Protective role of IL-17 during malaria pathogenesis has been found by working with IL-17RA deficient mice, in which IL-17 doesn't function in a proper way. These IL-17RA deficient mice show increased parasitemia, earlier onset of malaria, increased mortality during acute stage than the wild type mice [63]. So, it can be summarised that IL-17 itself and IL-17 expressing CD4+ T helper cells (Th17 cells) is of pivotal importance during malaria but the actual outcome of the immune response against the malaria parasite is dependent on the Treg/Th-17 balance, which is maintained majorly by TGFβ and IL-6.

4. Role of plasmacytoid dendritic cells (pDC) and myeloid dendritic cells (mDC) in regulation of Treg/Th-17 balance in malaria

Dendritic cells (DC), a professional antigen presenting cell, function as a bridge between innate and adaptive immune responses. In various infections, including malaria, different subsets of dendritic cells and co-stimulatory molecules (CD40, CD80, CD86, MHC-II etc.) expressed by them show significant changes which indicates that dendritic cells play a major role in the regulation of T cell differentiation and function [64]. Among different subsets, plasmacytoid DC (pDC), specially the tolerogenic pDCs induces and regulates the function of T regulatory cells [65]. Myeloid DC (mDC), on the other hand mainly secretes factors which are important for differentiation of Th-17 cells from naïve CD4+ T cells in several inflammatory disorders. Regulation of mDC function by several microRNA or other factors has its effect on Th-17 induction and function [66, 67]. In malaria, it has already been reported that mDC/pDC ratio has an impact on host immune response against Plasmodium sp. and disease pathogenesis [68, 69]. Analysis of splenic mDC/pDC ratio in Plasmodium berghei ANKA infection has shown that the ratio is increased significantly and the result is consistent with Th-17 mediated response against the murine cerebral malaria. This increased mDC/pDC ratio has been shown to revert back to homeostatic levels upon neutralization of IL-6, which also has its impact on Th-17 cells and functions in controlling the disease progression as discussed earlier [57]. Thus mDC/pDC ratio may be crucial in serving as a mediator that regulates the T-reg/Th-17 ratio in malaria. However, further investigation is still required to actually find out how exactly mDC/pDC ratio regulates the T-reg/Th-17 balance and how it influences the outcome of the immune response against malaria parasite.

5. Role of autophagy in the regulation of T-reg/Th17 balance in malaria infection

Autophagy is a well-known process which plays a beneficial role against infectious disease not only by degrading pathogens but also by activating host immune system. Autophagy plays an important role in multiple aspects of immune system like cytokine balance, modulation of immune cells, innate and adaptive immunity and antigen presentation [70]. In our study we have found increased expression of
all five major markers of autophagy pathway viz. BECLIN1, ATG3, ATG5, ATG7, p62 with the progression of disease and the expressions were highest at 8 dpi *Plasmodium berghei* ANKA infection. An increase in the expression of LC3B has also been found. Simultaneously, the ratio of LC3B:LC3A increased at 8 dpi *Plasmodium berghei* ANKA infection which indicates the conversion of LC3A to LC3B and an upregulation of autophagic flux [71]. It has been reported that pDC harbours live *Plasmodium* parasite which have the ability to cause malaria symptoms when transferred to naïve mice [72]. Rapamycin (known autophagy inducer) treatment reduces the plasmodium load in splenic pDC. Autophagic induction increases the expression of CD205 and MHC I on pDC which stimulates antigen processing and antigen presentation respectively as compared to non-treated PbA infected group. Relative downregulation of proinflammatory cytokines like IL-6 and TNFα and positive induction of anti-inflammatory cytokines like IL10 was observed in autophagy induced mice. A tilt towards low Treg/Th-17 and high mDC/pDC ratio have been observed during malaria infection which induce Th1 pathway mediated immune regulation and poor prognosis for host. But autophagy induction can shift the Treg/Th17 balance towards increased T-reg population along with increased pDC population which can alter the mDC/pDC ratio, suppress the proinflammatory response and promote Th2 pathway [73]. Autophagic regulation of splenic red pulp macrophages show similar results in context to Treg/Th-17 balance [74]. Upregulation of proinflammatory cytokines production and alteration of Treg/Th-17 balance towards increased population of Th17 is a major cause for poor prognosis of malaria. Autophagy induction can revert the imbalance and help in betterment of host immune response.

6. Conclusion and future perspectives

Despite of continuous efforts towards invention of a proper and effective vaccines for malaria prevention, very few of them have their impact on reducing the number of malaria cases and malaria induced mortality. ACT still is the major therapeutic strategy in combating this disease, although emergence of Artemisinin resistance has been a major worry for the effectiveness of ACT during treatment of malaria patients. Immunotherapeutic strategies have been quite promising in several inflammatory disorders, cancers, autoimmune disorders and other infections. In case of malaria, although immunomodulation is very effective in murine studies, causing declination of parasitemia and increasing the survival percentages, application of those immunotherapeutic strategies in human is still awaiting. The balance between two T helper cell subsets i.e. T regulatory cells and Th-17 cells has been found to be important in both lethal and non-lethal malaria and factors which regulate this balance seems to play a pivotal role in disease manifestation. Studies using murine models has been quite effective in determining the factors and how they influence the disease outcome by regulating the Treg/Th-17 balance. Among those factors, TGFβ and IL-6 directly regulate the percentage of cells, expression of their characteristic transcription factors and functional cytokines secreted by Treg and Th-17 cells. Neutralization of IL-6 has direct effect on parasitaemia and survival percentages of mice infected with *Plasmodium* sp. It also reverses the dysregulated Treg/Th-17 ratio to optimal levels and can be a target for future therapeutic interventions against malaria infection. mDC/pDC ratio also play the role of a regulator and as a bridge to control Treg/Th-17 ratio. IL-6 neutralization can also bring the altered mDC/pDC ratio to normal levels. Apart from these, autophagic regulation of dendritic cells and macrophages in the spleen has its effect on Treg/Th-17 balance. Though, use of T-regulatory cells and drugs that directly
regulate the altered ratio is regarded as a potentially attractive therapeutic strategy in autoimmune disorders, application of these approaches in malaria and other parasitic infections needs more attention and caution. Further investigations are still required to achieve the goal of a malaria free world.

Acknowledgements

The authors are thankful to the Department of Zoology, University of Calcutta for their support and research scholars of the Immunology Laboratory for their generous help for completing this research work. We also like to acknowledge Department of Science and Technology, Govt. of India (SB/SO/HS-106/2013, dated November 21, 2014), Department of Atomic Energy -BRNS: (37(1)/14/54/2014-BRNS/1740 dated October 28, 2014), West Bengal Department of Biotechnology (22(Sanc)/BT (Estt)/RD-20/2013 dated January 07, 2015) for their financial support to carry out these works. Fellowship support from Council of Scientific and Industrial Research (CSIR), India (for SM, SG) and University Grants Commission (UGC), India is also hereby acknowledged.

Conflict of interest

The authors declare no conflict of interest.

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References

[1] World malaria report 2020, World Health Organization.

[2] Institute of Medicine (US) Committee on the Economics of Antimalarial Drugs; Arrow KJ, Panosian C, Gelband H, editors. Saving Lives, Buying Time: Economics of Malaria Drugs in an Age of Resistance. Washington (DC): National Academies Press (US); 2004. 6, The Parasite, the Mosquito, and the Disease. Available from: https://www.ncbi.nlm.nih.gov/books/NBK215619/

[3] Kwiatkowski DP. How malaria has affected the human genome and what human genetics can teach us about malaria. Am J Hum Genet. 2005 Aug;77(2):171-92. doi: 10.1086/432519. Epub 2005 Jul 6. PMID: 16001361; PMCID: PMC1224522.

[4] Eastman RT, Fidock DA. Artemisinin-based combination therapies: a vital tool in efforts to eliminate malaria. Nat Rev Microbiol. 2009 Dec;7(12):864-74. doi: 10.1038/nrmicro2239. Epub 2009 Nov 2. PMID: 19881520; PMCID: PMC2901398.

[5] Laloo DG, Shingadia D, Bell DJ, Beeching NJ, Whitty CJM, Chiodini PL; PHE Advisory Committee on Malaria Prevention in UK Travellers. UK malaria treatment guidelines 2016. J Infect. 2016 Jun;72(6):635-649. doi: 10.1016/j.jinf.2016.02.001. Epub 2016 Feb 12. PMID: 26880088; PMCID: PMC7132403.

[6] Tse EG, Korsik M, Todd MH. The past, present and future of antimalarial medicines. Malar J. 2019 Mar 22;18(1):93. doi: 10.1186/s12936-019-2724-z. PMID: 30902052; PMCID: PMC6431062.

[7] Talman AM, Clain J, Duval R, Ménard R, Ariey F. Artemisinin Bioactivity and Resistance in Malaria Parasites. Trends Parasitol. 2019 Dec;35(12):953-963. doi: 10.1016/j.pt.2019.09.005. Epub 2019 Nov 4. PMID: 31699532.

[8] Lu F, He XL, Richard C, Cao J. A brief history of artemisinin: Modes of action and mechanisms of resistance. Chin J Nat Med. 2019 May 20;17(5):331-336. doi: 10.1016/S1875-5364(19)30038-X. PMID: 31171267.

[9] Rosenberg SA. Decade in review-cancer immunotherapy: entering the mainstream of cancer treatment. Nat Rev Clin Oncol. 2014 Nov;11(11):630-2. doi: 10.1038/nrclinonc.2014.174. Epub 2014 Oct 14. PMID: 25311350; PMCID: PMC6310157.

[10] Ramamurthy D, Nundalall T, Cingo S, Mungra N, Kanaan M, Naran K, Barth S. Recent advances in immunotherapies against infectious diseases. Immunotherapy Advances. 2021 Jan;1(1):ltaa007.

[11] Naran K, Nundalall T, Chetty S, Barth S. Principles of immunotherapy: implications for treatment strategies in cancer and infectious diseases. Frontiers in microbiology. 2018 Dec 21;9:3158.

[12] Cabral-Miranda G, Heath MD, GomesAC, Mohsen MO, Montoya-Diaz E, Salman AM, Atcheson E, Skinner MA, Kramer MF, Reyes-Sandoval A, Bachmann MF. Microcrystalline Tyrosine (MCT®): A Depot Adjuvant in Licensed Allergy Immunotherapy Offers New Opportunities in Malaria. Vaccines. 2017; 5(4):32. https://doi.org/10.3390/vaccines5040032

[13] Belachew EB. Immune Response and Evasion Mechanisms of Plasmodium falciparum Parasites. J Immunol Res. 2018 Mar 25;2018:6529681. doi: 10.1155/2018/6529681. PMID: 29765991; PMCID: PMCS889876.
[14] Long CA, Zavala F. Immune Responses in Malaria. Cold Spring Harb Perspect Med. 2017 Aug 1;7(8):a025577. doi: 10.1101/cshperspect.a025577. PMID: 28389518; PMCID: PMC5538407.

[15] Del Portillo HA, Ferrer M, Brugat T, Martin-Jaular L, Langhorne J, Lacerda MV. The role of the spleen in malaria. Cell Microbiol. 2012 Mar;14(3):343-355. doi: 10.1111/j.1462-5822.2011.01741.x. Epub 2012 Feb 2. PMID: 22188297.

[16] Henry B, Roussel C, Carucci M, Brousse V, Ndour PA, Buffet P. The Human Spleen in Malaria: Filter or Shelter? Trends Parasitol. 2020 May;36(5):435-446. doi: 10.1016/j.pt.2020.03.001. Epub 2020 Mar 30. PMID: 32298631.

[17] Dinis-Oliveira RJ, Vieira DN, Magalhães T. Guidelines for Collection of Biological Samples for Clinical and Forensic Toxicological Analysis. Forensic Sci Res. 2017 Jan 16;1(1):42-51. doi: 10.1080/20961790.2016.1271098. PMID: 30483610; PMCID: PMC6197137.

[18] Tashjian RS, Williams RR, Vinters HV, Yong WH. Autopsy Biobanking: Biospecimen Procurement, Integrity, Storage, and Utilization. Methods Mol Biol. 2019;1897:77-87. doi: 10.1007/978-1-4939-8935-5_8. PMID: 30539436; PMCID: PMC6777723.

[19] White NJ, Turner GD, Medana IM, Dondorp AM, Day NP. The murine cerebral malaria phenomenon. Trends Parasitol. 2010 Jan;26(1):11-5. doi: 10.1016/j.pt.2009.10.007. Epub 2009 Nov 22. PMID: 19932638; PMCID: PMC2807032.

[20] Gonçalves RM, Lima NF, Ferreira MU. Parasite virulence, co-infections and cytokine balance in malaria. Pathog Glob Health. 2014 Jun;108(4):173-8. doi: 10.1179/2047773214Y.00000000139. Epub 2014 May 23. PMID: 24854175; PMCID: PMC4069333.

[21] Kurup SP, Butler NS, Harty JT. T cell-mediated immunity to malaria. Nat Rev Immunol. 2019 Jul;19(7):457-471. doi: 10.1038/s41577-019-0158-z. PMID: 30940932; PMCID: PMC6599480.

[22] Perez-Mazliah D, Langhorne J, CD4 T-cell subsets in malaria: TH1/TH2 revisited. Frontiers in immunology. 2015 Jan 12;5:671.

[23] Lee GR. The Balance of Th17 versus Treg Cells in Autoimmunity. Int J Mol Sci. 2018 Mar 3;19(3):730. doi: 10.3390/ijms1903730. PMID: 29510522; PMCID: PMC5877591.

[24] Eisenstein, E., Williams, C. The Treg/Th17 Cell Balance: A New Paradigm for Autoimmunity. Pediatr Res 65, 26-31 (2009). https://doi.org/10.1203/PDR.0b013e31819e76c7

[25] Knochelmann, H.M., Dwyer, C.J., Bailey, S.R. et al. When worlds collide: Th17 and Treg cells in cancer and autoimmunity. Cell Mol Immunol 15, 458-469 (2018). https://doi.org/10.1038/s41423-018-0004-4

[26] Omenetti S, Pizarro TT. The Treg/Th17 Axis: A Dynamic Balance Regulated by the Gut Microbiome. Front Immunol. 2015 Dec 17;6:639. doi: 10.3389/fimmu.2015.00639. PMID: 26734006; PMCID: PMC4681807.

[27] Kleinewietfeld M, Hafler DA. The plasticity of human Treg and Th17 cells and its role in autoimmunity. Semin Immunol. 2013 Nov 15;25(4):305-12. doi: 10.1016/j.smim.2013.10.009. Epub 2013 Nov 5. PMID: 24211039; PMCID: PMC3905679.

[28] Lee YK, Mukasa R, Hatton RD, Weaver CT. Developmental plasticity of Th17 and Treg cells. Current opinion in immunology. 2009 Jun 1;21(3):274-280.
[29] Fasching P, Stradner M, Graninger W, Dejaco C, Fessler J. Therapeutic Potential of Targeting the Th17/Treg Axis in Autoimmune Disorders. Molecules. 2017 Jan 14;22(1):134. doi: 10.3390/molecules22010134. PMID: 28098832; PMCID: PMC6155880.

[30] Raimondo MG, Biggioggero M, Crotti C, Becciolini A, Favalli EG. Profile of sarilumab and its potential in the treatment of rheumatoid arthritis. Drug Des Devel Ther. 2017 May 24;11:1593-1603. doi: 10.2147/DDDT.S100030. PMID: 28579757; PMCID: PMC5447699.

[31] Hansen DS, Schofield L. Natural regulatory T cells in malaria: host or parasite allies? PLoS Pathog. 2010 Apr 29;6(4):e1000771. doi: 10.1371/journal.ppat.1000771. PMID: 20442856; PMCID: PMC2861684.

[32] Balasa R, Barcutean L, Balasa A, Motataianu A, Roman-Filip C, Manu D. The action of TH17 cells on blood brain barrier in multiple sclerosis and experimental autoimmune encephalomyelitis. Human immunology. 2020 May 1;81(5):237-243.

[33] Couper KN, Blount DG, Wilson MS, Hafalla JC, Belkaid Y, Kamanaka M, Flavell RA, de Souza JB, Riley EM. IL-10 from CD4CD25Foxp3CD127 adaptive regulatory T cells modulates parasite clearance and pathology during malaria infection. PLoS Pathog. 2008 Feb 29;4(2):e1000022. doi: 10.1371/journal.ppat.1000022. PMID: 18401464; PMCID: PMC2291447.

[34] Rouse BT, Suvas S. Regulatory cells and infectious agents: detenues cordiale and contraire. J Immunol. 2004 Aug 15;173(4):2211-2215. doi: 10.4049/jimmunol.173.4.2211. PMID: 15294929.

[35] Walther M, Jeffries D, Finney OC, Njie M, Ebonyi A, Deininger S, Lawrence E, Ngwa-Amambua A, Jayasooriya S, Cheeseman IH, Gomez-Escobar N, Okebe J, Conway DJ, Riley EM. Distinct roles for FOXP3 and FOXP3 CD4 T cells in regulating cellular immunity to uncomplicated and severe Plasmodium falciparum malaria. PLoS Pathog. 2009 Apr;5(4):e1000364. doi: 10.1371/journal.ppat.1000364. Epub 2009 Apr 3. PMID: 19343213; PMCID: PMC2658808.

[36] Feng H, Zhu XT, Qi ZM, Wang QH, Wang GG, Pan YY, Li Y, Zheng L, Jiang YJ, Shang H, Cui L, Cao YM. Transient attenuated Foxp3 expression on CD4+ T cells treated with 7D4 mAb contributes to the control of parasite burden in DBA/2 mice infected with lethal Plasmodium chabaudi chabaudi AS. Scand J Immunol. 2012 Jan;75(1):46-53. doi: 10.1111/j.1365-3083.2011.02622.x. PMID: 21916916.

[37] Haque A, Best SE, Amante FH, Mustafah S, Desbarries L, de Labastida F, Sparwasser T, Hill GR, Engwerda CR. CD4+ natural regulatory T cells prevent experimental cerebral malaria via CTLA-4 when expanded in vivo. PLoS Pathog. 2010 Dec 9;6(12):e1001221. doi: 10.1371/journal.ppat.1001221. PMID: 21170302; PMCID: PMC3000360.

[38] Steeg C, Adler G, Sparwasser T, Fleischer B, Jacobs T. Limited role of CD4+Foxp3+ regulatory T cells in the control of experimental cerebral malaria. J Immunol. 2009 Dec 1;183(11):7014-7022. doi: 10.4049/jimmunol.0901422. Epub 2009 Nov 4. PMID: 19890049.

[39] Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity. 2006 Feb;24(2):179-189. doi: 10.1016/j.immuni.2006.01.001. PMID: 16473830.

[40] Cipollini V, Anrather J, Orzi F, Iadecola C. Th17 and Cognitive
Plasmodium Species and Drug Resistance

Impairment: Possible Mechanisms of Action. Front Neuroanat. 2019 Nov 19;13:95. doi: 10.3389/fnana.2019.00095. PMID: 31803028; PMCID: PMC6877481.

[41] Keswani T, Bhattacharyya A. Splenocyte apoptosis in Plasmodium berghei ANKA infection: possible role of TNF-α and TGF-β. Parasite Immunol. 2013 Feb;35(2):73-90. doi: 10.1111/pim.12005. PMID: 23009201.

[42] Keswani T, Bhattacharyya A. Differential role of T regulatory and Th17 in Swiss mice infected with Plasmodium berghei ANKA and Plasmodium yoelii. Exp Parasitol. 2014 Jun;141:82-92. doi: 10.1016/j.exppara.2014.03.003. Epub 2014 Mar 24. PMID: 24675415.

[43] Sarkar S, Keswani T, Sengupta A, Mitra S, Bhattacharyya A. Differential modulation of glial cell mediated neuroinflammation in Plasmodium berghei ANKA infection by TGF β and IL 6. Cytokine. 2017 Nov;99:249-259. doi: 10.1016/j.cyto.2017.07.026. Epub 2017 Aug 10. PMID: 28803696.

[44] Gough NR. Enhancing and Inhibiting TGF-β Signaling in Infection. Science Signaling. 2015 Jan 13;8(359):ec9-.

[45] Massagué J. TGFbeta in Cancer. Cell. 2008 Jul 25;134(2):215-30. doi: 10.1016/j.cell.2008.07.001. PMID: 18662538; PMCID: PMC3512574.

[46] Kumari N, Dwarakanath BS, Das A, Bhatt AN. Role of interleukin-6 in cancer progression and therapeutic resistance. Tumour Biol. 2016 Sep;37(9):11553-11572. doi: 10.1007/s13277-016-0987-7. Epub 2016 Jun 3. PMID: 27260630.

[47] Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol. 2014 Sep 4;6(10):a016295. doi: 10.1101/cshperspect.a016295. PMID: 25190079; PMCID: PMC4176007.

[48] J. Scheller, A. Chalaris, D. Schmidt-Arras, S. Rose-John, The pro- and antiinflammatory properties of the cytokine interleukin-6, Biochim. Biophys. Acta 1813 (2011) 878-888.

[49] L.J. Robinson, M.C. D’Ombra, D.I. Stanisic, J. Taraika, N. Bernard, J.S. Richards, et al., Cellular tumor necrosis factor, gamma interferon, and interleukin-6 responses as correlates of immunity and risk of clinical Plasmodium falciparum malaria in children from Papua New Guinea, Infect. Immunol. 77 (2009) 3033-3043.

[50] J. Jason, L.K. Archibald, O.C. Nwanyanwu, M. Bell, I. Buchanan, J. Larned, et al., Cytokines and malaria parasitemia, Clin. Immunol. 100 (2001) 208-218.

[51] E. Hugosson, S.M. Montgomery, Z. Premji, M. Troye-Blomberg, A. Björkman, Relationship between antipyretic effects and cytokine levels in uncomplicated falciparum malaria during different treatment regimes, Acta Trop. 99 (2006) 75-82.

[52] J.Y. Seoh, M. Khan, S.H. Park, H.K. Park, M.H. Shin, E.H. Ha, et al., Serum cytokine profiles in patients with Plasmodium vivax malaria: a comparison between those who presented with and without hyperpyrexia, Am. J. Trop. Med. Hyg. 68 (2003) 102-106.

[53] J.L. Sarthou, G. Angel, G. Aribot, C. Rogier, A. Dieye, A. Toure Balde, et al., Prognostic value of anti-Plasmodium falciparum-specific immunoglobulin G3, cytokines, and their soluble receptors in West African patients with severe malaria, Infect. Immun. 65 (1997) 3271-3276.

[54] I.M. Medana, N.H. Hunt, G. Chaudhri, Tumor necrosis factor alpha
expression in the brain during fatal murine cerebral malaria: evidence for production by microglia and astrocytes. Am. J. Pathol. 150 (1997) 1473-1486.

[55] G.E. Grau, S. de Kossodo, Cerebral malaria: mediators, mechanical obstruction or more?, Parasitol Today 10 (1994) 408-409.

[56] G.E. Grau, G. Bieler, P. Poinaire, S. De Kossodo, F. Tacchini-Cotier, P. Vassalli, et al., Significance of cytokine production and adhesion molecules in malarial immunopathology, Immunol. Lett. 25 (1990) 189-194.

[57] Keswani T, Sarkar S, Sengupta A, Bhattacharyya A. Role of TGF-β and IL-6 in dendritic cells, Treg and Th17 mediated immune response during experimental cerebral malaria. Cytokine. 2016 Dec;88:154-166. doi: 10.1016/j.cytoby.2016.08.034. Epub 2016 Sep 12. PMID: 27632786.

[58] Ishida H, Matsuzaki-Moriya C, Imai T, Yanagisawa K, Nojima Y, Suzue K, Hirai M, Iwakura Y, Yoshimura A, Hamano S, Shimokawa C. Development of experimental cerebral malaria is independent of IL-23 and IL-17. Biochemical and biophysical research communications. 2010 Nov 26;402(4):790-795.

[59] Helegbe GK, Huy NT, Yanagi T, Shuaibu MN, Kikuchi M, Cherif MS, Hirayama K. Elevated IL-17 levels in semi-immune anaemic mice infected with Plasmodium berghei ANKA. Malar J. 2018 Apr 17;19(1):34. doi: 10.1186/s12936-018-2257-x. PMID: 29665817; PMCID: PMC5905139.

[60] Raballah E, Kempaiah P, Karim Z, Orinda GO, Otiengo MF, Perkins DJ, Ong'echa JM. CD4 T-cell expression of IFN-γ and IL-17 in pediatric malarial anemia. PloS one. 2017 Apr 20;12(4):e0175864.

[61] Bueno LL, Morais CG, Lacerda MV, Fujiwara RT, Braga EM. Interleukin-17 producing T helper cells are increased during natural Plasmodium vivax infection. Acta Trop. 2012 Jul;123(1):53-57. doi: 10.1016/j.actatropica.2012.02.071. Epub 2012 Mar 27. PMID: 22476130.

[62] Marquet S, Conte I, Poudiougou B, Argiro L, Cabantous S, Dessein H, Burté F, Oumar AA, Brown BJ, Traore A, Afolabi NK. The IL17F and IL17RA genetic variants increase risk of cerebral malaria in two African populations. Infection and immunity. 2016 Feb 1;84(2):590-597.

[63] Ghosh D, Brown SL, Stumhofer JS. IL-17 Promotes Differentiation of Splenic LSK+ Lymphoid Progenitors into B Cells following Plasmodium yoelii Infection. J Immunol. 2017 Sep 1;199(5):1783-1795. doi: 10.4049/jimmunol.1601972. Epub 2017 Jul 21. PMID: 28733485; PMCID: PMC5585076.

[64] Yap XZ, Lundie RJ, Beeson JG, O’Keeffe M. Dendritic Cell Responses and Function in Malaria. Front Immunol. 2019 Mar 4;10:357. doi: 10.3389/fimmu.2019.00357. PMID: 30886619; PMCID: PMC6409297.

[65] Matta BM, Castellaneta A, Thomson AW. Tolerogenic plasmacytoid DC. Eur J Immunol. 2010 Oct;40(10):2667-76. doi: 10.1002/eji.201040839. PMID: 20821731; PMCID: PMC3974856.

[66] Terhune J, Berk E, Czerniecki BJ. Dendritic Cell-Induced Th1 and Th17 Cell Differentiation for Cancer Therapy. Vaccines (Basel). 2013 Nov 21;1(4):527-49. doi: 10.3390/vaccines1040527. PMID: 26344346; PMCID: PMC4494209.

[67] Iférgan I, Chen S, Zhang B, Miller SD. Cutting Edge: MicroRNA-223 Regulates Myeloid Dendritic Cell-Driven Th17 Responses in Experimental Autoimmune Encephalomyelitis. J Immunol. 2016 Feb 15;196(4):1455-1459. doi: 10.4049/jimmunol.1501965. Epub
Turner, T.C., Arama, C., Ongoiba, A. et al. Dendritic cell responses to *Plasmodium falciparum* in a malaria-endemic setting. Malar J 20, 9 (2021).

Keswani T, Sengupta A, Sarkar S, Bhattacharyya A. Dendritic cells subsets mediated immune response during *Plasmodium berghei ANKA* and *Plasmodium yoelii* infection. Cytokine. 2015 Jun;73(2):198-206. doi: 10.1016/j.cyto.2015.02.023. Epub 2015 Mar 16. PMID: 25792277.

Deretic V, Saitoh T, Akira S. Autophagy in infection, inflammation and immunity. Nature Reviews Immunology. 2013 Oct;13(10):722-737.

Sengupta A, Mukherjee S, Ghosh S, Keswani T, Sarkar S, Majumdar G, Das M, Bhattacharyya A. Partial impairment of late-stage autophagic flux in murine splenocytes leads to sqstm1/p62 mediated nrf2-keap1 antioxidant pathway activation and induced proteasome-mediated degradation in malaria. Microb Pathog. 2020 Oct;147:104289. doi: 10.1016/j.micpath.2020.104289. Epub 2020 Jul 18. PMID: 32693118.

Wykes MN, Kay JG, Manderson A, Liu XQ, Brown DL, Richard DJ, et al. Rodent blood-stage *Plasmodium* survive in dendritic cells that infect naive mice. Proc Natl Acad Sci U S A 2011;108:11205e10.

Sengupta A, Keswani T, Sarkar S, Ghosh S, Mukherjee S, Bhattacharyya A. Autophagic induction modulates splenic plasmacytoid dendritic cell mediated immune response in cerebral malarial infection model. Microbes Infect. 2019 Dec;21(10):475-484. doi: 10.1016/j.micinf.2019.05.004. Epub 2019 Jun 8. PMID: 31185303.

Sengupta A, Sarkar S, Keswani T, Mukherjee S, Ghosh S, Bhattacharyya A.