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REVIEW OPEN ACCESS

Understanding disease pathogenesis and host response of endemic malaria in previously exposed individuals compared to naïve individuals

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Abstract

Plasmodium falciparum and *Plasmodium vivax* are two major species of malaria that can establish a focus of infection in millions of individuals per year. Principally, this occurs in the tropical and subtropical regions of the world where malaria is endemic due to the ubiquity of the disease vector, the *Anopheles* mosquitos. Malaria takes the lives of thousands of infected individuals as the progression of disease symptoms having fatal consequences. This disease mainly affects children and pregnant women which poses a great public health concern. It is also a global economic burden from the millions of international dollars are aliquoted for research yearly. This review looks to discuss the pathogenesis of malaria, various host immune responses, the development of clinical immunity in reinfected individuals, and the effects that the presence of one species may have on the pathogenesis and disease outcome of another malarial species in co-infected individuals. Overall, this manuscript aims to provide an understanding of malarial infection and the differing host immune mechanisms of previously exposed individuals compared to those of naïve individuals in environments where malaria is of high prevalence. These highlights indicate a need for further research in order to better understand host-species and species-species interactions so that proper therapeutics and vaccinations may be developed as to not inhibit the beneficial effects species may have on one another in mixed species interactions as well as to aid in the development of clinical immunity.

Keywords: malaria; immunopathogenesis; anopheles; co-infection; plasmodium

Introduction

Malaria is one of the most prevalent parasitic diseases in the world. The source of this infection is due to the spread of various parasite species that belong to the *Plasmodium* family, which develop a focus of infection in millions of people per year (1). There are five malarial parasite species that can establish pathogenesis in humans, of which *P. falciparum* and *P. vivax* are the most common. These two species present significant morbidity, where the former poses the greatest risk of mortality [1]. Transmission of this parasite is spread to humans via the bite of a female mosquito from the *Anopheles* family. Therefore, malaria is common to many tropical and subtropical regions of the world where mosquito ubiquity of this species is high. This includes African, Southeast Asian, and Eastern Mediterranean regions in which the vast majority of cases are reported each year [2].

Symptoms of malaria are initially non-specific and imitate those of an influenza infection [3]. They are characterized by discomfort, fever, headache, chills, body aches, nausea, vomiting, and diarrhea [1, 3]. However, disease symptoms often swiftly advance to more serious complications resulting in severe clinical infections that can have potentially fatal consequences. Such complications include, but are not limited to, anemia, jaundice, convulsions, organ dysfunction, and coma [1, 3]. Because of this, prompt diagnosis

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is important. The severity of this disease imposes a multitude of detrimental effects on endemic countries.

According to the latest *World Malaria Report* published by the World Health Organization (WHO), there were 228 million reported cases of malaria globally in 2018 [4]. Out of those reported cases, it was recorded that 402,000 infections resulted in death, approximately 67% of which were children under the age of 5 [4]. Next to young children, pregnant women are also stated to be high-risk groups where infection can result in complications such as preterm birth, various neonatal developmental issues, and an increased risk of mortality for both the mother and child, pre- and post-childbirth [4,5]. This disease poses serious public health and social problems as many of the regions with a high prevalence of malaria have few resources for diagnosis, treatment, and prevention [2]. Additionally, malaria presents a large burden on both domestic and international economies. Various public and global health programs continue to invest millions of dollars in funding annually for research towards prevention and elimination methods, with a global target of a large 6 billion dollars per year [6]. However, such efforts have had no considerable prevail as malaria continues to scourge multitudes of individuals yearly, compounding the pre-existing burden.

The ubiquity of malaria in endemic regions is not only the cause for an elevated risk of primary infection, but it also poses a significant increase in the risk of reinfection. This paper looks to discuss the pathogenesis of malaria, various host immune responses, the development of clinical immunity in reinfected individuals, and the effects that the presence of one species may have on the pathogenesis and disease outcome of another malarial species in co-infected individuals in environments where malaria is of high prevalence.

I. Parasite pathogenesis

The transmission of malaria is facilitated by the passage of *Plasmodium* parasites from a female mosquito vector, specifically of the *Anopheles* family, to a human host via bite [7]. Upon blood meal, the infected mosquito releases saliva that contains sporozoites, or free parasite forms, into the skin of the host where they can subsequently enter the host's bloodstream and target the liver [1, 7]. The sporozoites can clear circulation and reach the liver in under one hour. Once in the liver, they begin to infect hepatocytes, where they can remain for 7-10 days [3, 7]. During this period of time, the sporozoites undergo asexual reproduction in order to propagate and transform into schizonts which will eventually divide into daughter cells, or merozoites [3, 8]. This portion of disease progression is the first stage in the establishment of a malarial infection termed the liver stage [7].

The conclusion of the liver stage is characterized by the maturation of schizonts [1, 7]. Mature schizonts rupture within the infected hepatocytes freeing thousands of merozoites into the bloodstream, where they are then referred to as exo-erythrocytic merozoites [1, 8]. Exo-erythrocytic merozoites have one specific function: to invade erythrocytes, or red blood cells, and asexually reproduce to result in the production of differentiated forms of merozoites [1, 3, 8]. These transformed merozoites are subsequently liberated into the bloodstream where they can perpetuate the invasion of red blood cells (RBCs) [1]. This second phase of disease progression, where the parasite continues to multiply and invade RBCs, is termed the blood stage, or erythrocytic stage, of infection [7, 8].

Concurrent to the blood stage, there are a copious amount of parasitic waste products and toxins that are released within the host. The presence of these factors in circulation triggers the activation of the host's innate immune response that results in the secretion of various cytokines, or cell messengers, from immune cells. These cytokines promote an inflammatory response that is responsible for the rise of clinical symptoms within the host [9]. The blood stage of infection is associated with all malarial disease symptoms, whereas the liver stage of infection is known to be asymptomatic, or clinically silent [1, 10]. This is because the liver has immune privilege, meaning it is able to tolerate the presence of foreign material without evoking a significant immune response [11]. Additionally, the parasite load in this stage of infection is low, therefore, the subsequent immune response is accordingly low which explains the absence of overt symptoms [1]. Nonetheless, host immune cells are activated upon parasite entrance into the host and therefore are responsive in both stages of infection [7, 11]. Because of this, malarial species have developed various mechanisms to elude the host's immune response so that the parasite is able to establish a sustainable state of pathogenesis.

II. Parasite evasion mechanisms

In order for the liver stage of infection to be established, the parasite must avoid being phagocytosed by Kupffer cells (KCs), the resident macrophages of the liver [7]. To do so, sporozoites utilize their circumsporozoite protein to bind to low-density lipoprotein receptor-related protein (LRP-1) that is expressed on the surface of Kupffer cells [7, 11]. This interaction alters cytokine functions and is therefore able to subdue phagocytosis by KCs [11]. Additionally, the interaction can alter the expression of secondary messengers in order to regulate

signaling pathways. For example, it increases the levels of cAMP, a secondary messenger, to halt the downstream production of reactive oxygen species, in which typically inflicts cellular damage to kill the parasite [11]. Lastly, sporozoite release of circumsporozoite protein can downregulate the NF-kB pathway, which modulates immune response to infection in order to promote parasite development [11].

Once the parasite successfully establishes liver stage infection, it must evade subsequent host immune defenses as it progresses to the blood stage; these mechanisms may vary between species. For *P. falciparum,* as the parasite propagates within red blood cells, it begins to express membrane protrusions, called Knobs, which can be seen by the host immune system as foreign. In order to avoid immune recognition, *P. falciparum* presents variant surface antigens (VSA) on the surface of the infected red blood cell (iRBC) allowing it to be masked [7]. For example, the expression of VSA multigene family *P. falciparum* erythrocyte membrane protein-1(PfEMP1), which is specific to the *P. falciparum* species. PfEMP1 fosters the binding of iRBCs to host endothelial cells [7, 12, 13]. This allows the iRBCs to sequester in the microvessels of diverse organ tissues so that they can evade circulatory clearance at the spleen. This is referred to as cytoadherence [7, 12]. For *P. vivax,* the parasite propagates in young, immature RBCs known as reticulocytes [14]. For this reason, this species avoids clearance at the spleen during the blood stage of infection through a different mechanism.

P. falciparum is sequestered in various organ tissues in an effort to avoid entrance into the spleen to evade clearance. Conversely, *P. vivax* does enter the spleen in the blood stage of infection where it is then able to induce evasion mechanisms [14]. *P. vivax* iRBCs display invaginations on their surface to allow for greater deformability in the infected reticulocytes [14]. This causes the iRBCs to remain flexible and have a larger propensity for continuous passage through the spleen [15]. Typically, RBCs that circulate through the spleen must have a degree of flexibility in order to fit through the sinusoids, or vessels, of the spleen. If RBCs have reduced deformability, they will not pass through such vessels and will be cleared by the spleen [15]. This occurs in *P. falciparum* infection where the presence of Knobs reduces the deformability of the cells. This is cause for the parasite to induce cytoadherence in order to avoid entering the spleen at all [14, 15]. *P. vivax* infected cells, on the other hand, do not need to implement cytoadherence mechanisms to avoid the spleen as their increased flexibility allows them to circulate freely through the organ. However, *P. vivax* iRBCs do in fact demonstrate cytoadherence once inside the spleen in order to evade removal by macrophages within the organ [15]. The infected reticulocytes will adhere to barrier cells, normally meant to elevate the magnitude of splenic filtration, to mask their detection from other immune cells, specifically macrophages, within the organ [14].

Another form of general cytoadherence that plays a role in parasitic immune evasion at the blood stage of infection is called rosetting. Rosetting is characterized by the binding of iRBCs to uninfected red blood cells via the interaction between parasitic ligands on the iRBC and surface receptors on the uninfected red blood cell [7, 13]. This process allows the parasite to circumvent immune recognition. Additionally, rosetting offers a quick, efficient way for merozoites in the iRBCs to invade the uninfected red blood cell that it is bound to [7]. Therefore, not only does rosetting provide an evasion mechanism but it also aids in parasite propagation, much like the effects of the circumsporozoite protein function in the liver stage of infection. These immune evasion strategies are momentous in regard to the parasite's ability to implement a sustainable infection.

III. Host immune responses to infection: pre-exposed and naïve individuals

Upon activation of the immune system by the recognition of a foreign body, the human host has two major defenses: the innate response and the adaptive response. The former is the first to act against the intruding pathogen, and if it is unable to provide proper defense, the latter develops in an attempt to clear the infection. Additionally, the adaptive response is held accountable for providing the host with future protection against reinfection via the development of pathogen-specific defense mechanisms.

Host sensing of malarial infection begins shortly after entrance of the parasite via the recognition of pathogen-associated molecular patterns (PAMPs), present on the surface of the parasite, by host pathogen-recognition receptors. The host's innate immune cells are then activated by cytokines and chemokines, cell signaling molecules that are produced as a result of various signaling cascades initiated upon detection of PAMPs. Although the parasite avoids detection by Kupffer cells in the liver stage of infection, malaria PAMPs are still recognized by host pathogen-recognition receptors (PRRs) located in the cytosol of infected hepatocytes; this institutes a type one interferon (IFN) response [1]. This response is constituted by the production of IFN-gamma by natural killer cells, mobilization of innate cells, and the secretion of chemokines [1].

As the liver stage of infection is known to be clinically silent due to the host's inability to generate sufficient inflammation or defense responses upon recognition, the consequent induction of IFN-gamma (IFN-γ) has been determined to upregulate antiparasitic mechanisms in the later blood stage of infection [1, 16]. Resulting from the lack of adequate innate defenses at the liver stage, there is a suppressed initiation of antibodies in the adaptive response at this stage. It has been found that this absence of an antibody response corresponds to an equal likelihood of infection at the liver stage in those who were previously exposed compared to naïve individuals, as a protective memory response in pre-exposed individuals cannot form at this stage [17].

However, the type one interferon response stimulated in the liver stage still provides a degree of innate defense mechanisms. As natural killer cells are activated, they free parasitic components by killing infected hepatocytes and expelling their contaminants [1]. Subsequently, activated antigen presenting cells (APCs) can identify these parasite components. This provides the APCs with the potential to regulate immune defenses in the blood stage of infection by showcasing the parasitic antigen to other immune cells. Dendritic cells (DCs) are a type of antigen presenting cell that are also characterized by their capacity to bridge the innate and adaptive immune responses. When activated, dendritic cells are able to secrete proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-alpha) and interleukin-12 (IL-12), as well as activate T cells of the adaptive response [1]. The presence of TNF-alpha with IFN-γ, a signaling molecule stimulated by the type one interferon response, cause the downstream production of toxic radicals, such as reactive oxygen species, that function to kill the parasite in the blood stage of infection via cellular damage [11, 17]. IL-12 is additionally responsible for the innate upregulation of the type one interferon response causing an increase in IFN-γ release from natural killer cells, which then signals both helper and effector T cells to initiate an adaptive response [1].

IV. Acquired host immune responses to infection: Pre-exposed individuals

Dendritic cells are able to revert their function from inducing proinflammatory responses to anti-inflammatory responses [1]. The overproduction of proinflammatory cytokines and the subsequent increase of unregulated inflammation is linked to the advancement of parasite pathogenesis and an increased risk of fatality due to the increased production of detrimental pyrogenic, or fever-inducing, mediators [16, 17]. Therefore, the balance between pro- and anti-inflammatory responses is extremely important to the outcome of infection. It has been reported that reinfected individuals have reduced amounts of natural killer cells producing proinflammatory IFN-γ than naïve individuals in endemic areas; this is cause for belief that this downregulation may be a potential result of acquired immunity [16].

Additionally, it has been discovered that as a result of repeated infections, previously exposed individuals have acquired the ability to upregulate T cell secretion of IL-10, an anti-inflammatory cytokine, where naïve individuals cannot [17]. This obtained immune function results in the proactive regulation of inflammation upon infection which allows reinfected individuals to experience mild symptoms compared to that of naïve individuals who experience more severe malaria [17]. This acquired decrease in both the intensity and presence of overt symptoms among infected individuals is referred to as clinical immunity [18]. By decreasing morbidity, the establishment of clinical immunity subsequently reduces the risk of infection-associated mortality. An individual's ability to obtain clinical immunity is dependent upon the length and frequency of previous infections, as well as the strain type of the parasite population within the host. It was found that numerous, long-lasting infections of various *Plasmodium* species lead to the development of stronger clinical immunity via distinct mechanisms [19].

Parasite proteins that are subject to antigenic variation, such as those involved in cytoadherence, are targets for clinical immunity [18]. This is because memory antibodies, which are specific to each variant antigen, must form in order to have an effective clinical immune response to infection by various strains [18, 19]. The PfEMP1 family of variant surface antigens, expressed by *P. falciparum* iRBCs to induce cytoadherence, is a result of the transcription of the diverse *var* gene [7, 20]. *Plasmodium falciparum* parasites have approximately 60 highly variable copies of this gene [20]. It was discovered that after primary infection, a naïve individual may develop anti-PfEMP1 antibodies against as many as six *P. falciparum* lines that present different PfEMP1 variants [21]. Therefore, it can be concluded that numerous reinfections of *P. falciparum,* containing diverse proteins, allows for the formation of a large repertoire of specific antibodies which will prevent the cytoadherence mechanism of iRBCs [19]. This forces the *P. falciparum* iRBCs to circulate through the spleen for clearance which aids in the prevention of severe disease development by reducing the parasite population within the host [21].

Comparatively, variant surface antigens expressed by *P. vivax* iRBCs are a result of the transcription of the *vir* gene. *Plasmodium vivax* parasites have 346 copies of this gene, the variety of which confers that the translated proteins demonstrate diverse immune evasion functions. It has been theorized that some of these proteins are responsible for the cytoadherence of *P. vivax* iRBCs to barrier cells in the spleen in order to evade being phagocytosed by resident macrophages. It was discovered that previously exposed individuals had developed anti-VIR antibodies in which had the capacity to prevent the iRBCs from binding to splenic barrier cells, promoting parasite clearance by macrophages in which reduces the parasite population within the host [22]. This allows reinfected individuals to experience less severe infection compared to that of naïve individuals who lack these defenses. This also provides an understanding as to why significant reinfection rates are correlated with asymptomatic infections, as individuals are able to expand their antibody repertoire for better clinical immunity [20].

V. Mixed species infections

As the formation of clinical immunity relies on an individual's collective exposure to infection by various *Plasmodium* species, an increase in exposure to diverse species will consequently increase the length of time required to reach clinical immunity [19]. This is due to subjection to one species of the *Plasmodium* family not conferring protection against another species of the same family [23]. Therefore, if a person developed clinical immunity against *P. falciparum* but then became infected with *P. vivax*, they would experience symptoms as the acquired immunity from the former infection cannot act against the latter. Furthermore, this can explain why exposure to various *Plasmodium* species lengthens the amount of time needed to acquire clinical immunity to malaria.

Individuals living in endemic environments are vulnerable to various malarial species infections that circulate among *Anopheles* mosquitos, making co-infection with more than one species via bites from multiple infected mosquitos a high possibility [24]. The rate of mixed species infections in endemic areas has been recorded to exceed 20%, where individuals infected with *P. falciparum* have a 1 in 3 chance of contracting a subsequent illness by *P. vivax* that overlaps the former infection. However, the ubiquity of simultaneous mixed species infections from the bite of a single mosquito is relatively rare, yet possible [25]. The concurrent presence of *P. vivax* has been shown to decrease the morbidity caused by anemia of *P. falciparum* infection in a host. It has been suggested that this is caused by the decrease in *P. falciparum* presence in the blood, as a result of reduced pathogenicity induced by the presence of a fever caused by *P. vivax* at a lower parasitemia. Compared to infection of *P. falciparum* alone, the co-infection of these two species has been clinically shown to present reduced anemia, and therefore a decrease in the severity of overt symptoms [23]. The mechanism of how *P. vivax* is able to suppress the intensity of *P. falciparum* is still under research. However, the concurrent use of *P. vivax* chemotherapies may presumably increase the morbidity of *P. falciparum* because of the beneficial effects this co-infection has on *P. falciparum* outcome [23].

Host immune responses to single species infections have been the target of research in an effort to understand how pathogen density in the blood is regulated in order to perpetuate a level of parasitemia that remains below the threshold in which causes febrile effects [24]. It is assumed that inflammatory molecules, secreted by host innate responses, play a role in balancing this density-dependent prevention of severe disease in co-infections with more than one species of malaria present, as there is a lack of cross-reactive acquired immune responses in previously exposed people [24, 26]. Because of this, host immunity obtained from vaccines specific to one species may increase the potentiation of infection by the other present species, as density-dependent modulation of co-infections would be reduced by host immunity to one of the present species [26]. Therefore, if potential vaccines are not multivalent, individuals living in endemic regions may experience a higher risk of disease pathogenesis upon mixed species infections [26]. Naïve individuals, however, lack acquired clinical immunity entirely and therefore will experience less severe disease upon co-infection as suppression of *P. falciparum* is possible via uninterrupted *P. vivax* infection compared to pre-exposed individuals that may have developed this protective function. This is explained by the discovered phenomenon that the growth of one parasite species to above the threshold in which presents febrile effects is what initiates the inhibition of the second, minority species via regulation of its density present in the blood [26]. In summary, for this species-induced regulation to occur, the growth of the former species cannot be inhibited by host immunity. Consequently, this furthers the assumption that naïve individuals may experience a better outcome of a mixed species infection compared to that of a previously exposed individual. Further research is required to uncover the effects of the interactions between other malarial species in co-infections, as the interaction between *P. falciparum* and *P. vivax* have been the main point of focus due to their high prevalence in endemic regions [25].

Mixed species infections have shown clinical significance as the presence of *P. vivax* has been proven to reduce the severity of *P. falciparum* in co-infected individuals due to the regulation of parasitemia. For this species-induced regulation to occur, the growth of the former species cannot be inhibited by host immunity; therefore, naïve individuals may experience a better outcome of a mixed species infection compared to that of a previously exposed individual whose immune system may prevent pathogenesis of the beneficial species.

Conclusion

In conclusion, this paper reviewed the current understanding of malarial infection, mixed species infections, and the differing host immune mechanisms of previously exposed individuals compared to those of naïve individuals in environments where malaria is of high prevalence. These highlights indicate a need for further research in order to better understand host-species and species-species interactions so that proper therapeutics and vaccinations may be developed.

Conflict of interest

The authors declare no conflicts of interest.

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