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Review

Malaria Parasites in the Asymptomatic: Looking for the Hay in the Haystack

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With malaria elimination back on the international agenda, programs face the challenge of targeting all *Plasmodium* infections, not only symptomatic cases. As asymptomatic individuals are unlikely to seek treatment, they are missed by passive surveillance while remaining infectious to mosquitoes, thus acting as silent reservoirs of transmission. To estimate the risk of asymptomatic infections in various phases of malaria elimination, we need a deeper understanding of the underlying mechanisms favoring carriage over disease, which may involve both pathogen and host factors. Here we review our current knowledge on the determinants leading to *Plasmodium falciparum* symptomless infections. Understanding the host–pathogen interactions that are most likely to affect transitions between malaria disease states could guide the development of tools to tackle asymptomatic carriers in elimination settings.

Being Asymptomatic

Peaceful coexistence with the infected host, rarely causing clinical symptoms, is a common but poorly understood phenomenon that occurs for many human pathogens [1]. Because such asymptomatic cases of infection occur without eventual overt symptoms, they do not come to clinical attention, thus representing a large hidden reservoir of active infection that permits their persistence and eventual spread to other human hosts. This is the case for many malaria infections in semi-immune individuals from endemic areas, which commonly cause a mild febrile illness or no apparent symptoms at all, while keeping parasite numbers at low densities [2] (Box 1).

Carriage of **asymptomatic malaria** (see Glossary) infections, being at the same time difficult to detect and to manage, has considerable implications for the design and use of **malaria elimination** strategies. Symptomless infections that persist during the dry season in areas of seasonal transmission have been suggested to seed the malaria outbreaks after annual rains when mosquitoes reappear [3]. Similarly, asymptomatic parasitemia may potentially contribute to persistence of transmission in low-transmission settings [4]. However, the precise contribution of asymptomatic malaria to transmission in areas that have achieved substantial reductions in the malaria burden remains a matter of debate [5], as is the assumption that detecting and targeting these individuals for radical treatment, as opposed to using **mass drug administration** approaches, would be an efficient and effective tactic. Moreover, eradication in Europe, North America, and other parts of the world using no better diagnostics than microscopy [6] suggest that key determinants of success might not be finding the last parasite. From a practical point of view, the proportion of asymptomatic infections in certain situations and phases of malaria elimination may impose the need for modifications of detection methods (**active** versus **passive case detection**) [7] if, for example, symptom-based **surveillance** at health facilities is insufficient and mass blood surveys are necessary to inform the design of elimination interventions.

Trends

Understanding the determinants of asymptomatic malaria infections, as a silent driver of transmission, has become essential for the success of elimination campaigns.

Transmission intensity, parasite virulence, immune resistance and tolerance to infection, host genetic factors, pregnancy, or comorbidities can potentially affect the transition between symptomatic and asymptomatic infection and therefore constitute candidate areas for further research.

Research is needed to understand how the risk of asymptomatic malaria changes when transmission is abruptly interrupted, as well as the timeframes needed to lose host defenses that maintain infections at subclinical levels.

Elimination strategies could be guided by the surveillance of molecular and immunological biomarkers that mirror the dynamics of asymptomatic infections and allow prediction of the risk of asymptomatic infections in target populations.

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Box 1. Asymptomatic Malaria

Malaria is characterized by acute periodic febrile episodes triggered by the rupture of *Plasmodium* schizont stage-infected erythrocytes and the release of merozoites into the bloodstream [11]. This cyclical nature of the emergence of clinical symptoms is what led to the original nomenclature given to the fevers associated with malaria infections ('tertian' or 'quartan' fever) well before its life cycle was understood. However, asymptomatic malaria infections are common in humans living in endemic areas [2], even in low-transmission settings [72], with prevalences four to five times higher than clinically patent infections. Although asymptomatic cases generally correlate with lower-density infections [73] and lower rates of infecting mosquitoes [74], the extended longevity of asymptomatic parasitemia expands the risk of transmission during much of this time and overall it constitutes a high proportion of transmission [74], possibly greater than that derived from clinical malaria cases [75]. Estimates of the burden of asymptomatic malaria depend on the method used to detect infecting parasites and the criteria for the definition of an asymptomatic episode [42]. Disease episodes are commonly defined by the presence of fever [26], although various additional, nonspecific symptoms such as headache, myalgia, arthralgia, and shivering have been also used [42]. However, little is known about the silent or long-term health consequences of asymptomatic infections. Compared with uninfected individuals, individuals with asymptomatic infections in Sumba (Indonesia) showed higher levels of inflammation markers, von Willebrand factor, and platelet factor-4 and lower platelet counts and hemoglobin levels [76]. Asymptomatic plasmodial infection has been also associated with anemia among pregnant women [57] and children [77], as well as with chronic malnutrition [77] and cognitive impairment among children [78]. Studies that have based estimates of asymptomatic malaria at a particular time point [42] may misclassify as asymptomatic certain infections that are at an early stage of progression toward symptomatic disease or in the process of being cleared after antimalarial medication [79]. The few studies that have looked at such progression over time report a shift between states in only a small proportion of infections [80] and up to 90% of asymptomatic infections [81], probably reflecting different epidemiological scenarios. Long-term carriage of asymptomatic infection is suggested by observations in seasonal transmission settings, where asymptomatic infections can last several months until the restart of the following transmission season [82,83]. Asymptomatic *Plasmodium falciparum* infections among migrants who moved from endemic areas to settle in malaria-free countries can persist for as long as 8 years [84]. Although mathematical models suggest that *P. falciparum* can persist for up to 4 years [85], recent estimates suggest that very short infections, of the order of days rather than weeks, may be more common than previously thought [86].

Deciphering the parasite and host factors contributing to the asymptomatic carriage of malaria infection can help to predict where and when asymptomatic carriage is most likely to occur. With a growing need to better understand the silent reservoir of malaria transmission, we here review what is currently known regarding the host and parasite determinants of asymptomatic infections. Through the lens of malaria elimination, we aim to highlight potential biological pathways affecting the transition between malaria disease states that could be exploited for the development of tools to tackle asymptomatics in elimination settings.

Determinants of Asymptomatic Malaria

Why some individuals develop clinical manifestations during malaria infection while others remain asymptomatic is poorly understood. The general state of health and physiological condition of the host, particularly subtle immunity variations, together with host genetic predisposition and parasite factors involved in the virulence of the infection might influence the progression of malaria infection toward an asymptomatic outcome (Figure 1).

Malaria Parasite Factors

The abilities of malaria parasites to multiply, adhere to host tissues, produce toxic substances, or elicit deleterious immune responses have been considered attributes of virulence. Differences noted between *Plasmodium falciparum* strains and isolates in their maximum parasitemia [8], **pyrogenic thresholds** [9], **multiplication rates** [10], and **cytoadhesion** leading to sequestration in vital organs [11] suggest that there might be parasite factors that contribute to the development of asymptomatic infection.

Pyrogenic Threshold

All of the clinical symptoms associated with malaria are caused by the asexual erythrocytic or blood-stage parasites. Lysis of infected erythrocytes releases to the bloodstream not only merozoites but also parasite byproducts such as **hemozoin**, **glycosylphosphatidylinositol**

Glossary

Active case detection: detection by health workers of malaria cases at community and household level in population groups that are considered to be at high risk. Active case detection can be conducted as fever screening followed by parasitological examination of all febrile patients or as parasitological examination of the target population without prior fever screening.

Adaptive immunity: also known as acquired immunity; one of the two main immune strategies in vertebrates (the other being the innate immune system), characterized by its antigen-specific nature and capacity to develop immunological memory after an initial response to a pathogen. Adaptive immune responses can be of two types: humoral, mediated by antibodies produced by B lymphocytes; and cellular, mediated by T lymphocytes.

Antibody-dependent cellular inhibition (ADCI): occurs by monocyte-derived mediators such as TNFα that are released when FcγRII-binding antibodies crosslink monocytes and merozoites. This mechanism was suggested to reversibly inhibit the development of the parasite leading to chronic carriage of low-density infections in asymptomatic individuals.

Asymptomatic malaria: *Plasmodium* infections that do not lead to clinical symptoms and therefore remain undetected by fever-based surveillance systems. These infections can still contribute to the transmission of the parasite in the population, which makes them particularly relevant in the context of malaria elimination.

Chronic carriage: long-term presence of parasitemia in blood that is not causing acute or obvious illness.

Cytoadhesion: ability of erythrocytes infected by mature stages of *Plasmodium falciparum* to bind to host receptors in the microvasculature, to platelets (platelet-mediated clumping), and to uninfected erythrocytes (rosettes) leading to parasite accumulation in vital organs.

Endemicity: degree of malaria transmission in an area defined by spleen rate or parasite rate in children aged 2–9 years: hypoendemic (0–10%); mesoendemic (10–50%);

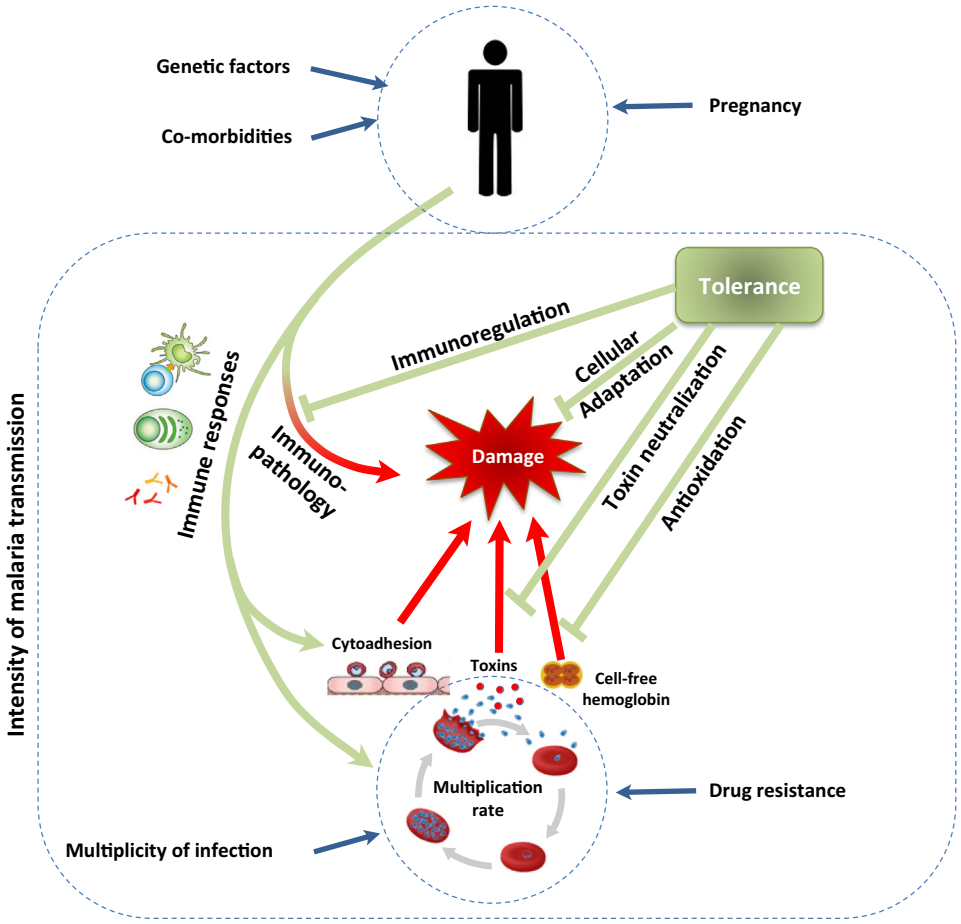

Figure 1. Host and Malaria Parasite Interactions Resulting in Damage and Disease. Disease is the clinical manifestation of body damage that results from host–parasite interactions. Damage may be caused directly by malaria parasites through erythrocyte destruction or sequestration in vital organs or by parasite byproducts (toxins or oxidant agents such as glycosylphosphatidylinositol or hemozoin). However, damage may also arise as a result of the host immune response against infection, leading to inflammation. Host mechanisms of resistance to infection mediated by the innate and adaptive immune system can reduce pathogen burden and ameliorate symptoms. Also, damage caused directly by the pathogen or indirectly by the host immune response can be minimized by immunoregulation, cellular adaptation, or parasite toxin neutralization irrespective of pathogen burden (i.e., tolerance to malaria infection). Such mechanisms may be modulated by previous exposure to malaria parasites, leading to adaptive responses, host genetic predisposition to combat disease, and comorbidities. Identification of host and pathogen factors responsible for influencing the amount of damage caused during an infection might be used to better characterize the infection outcome at a community level. Surveillance tools based on these factors might guide the selection of the most effective elimination strategies to target the asymptomatic reservoir of malaria transmission.

(GPI), and other toxic factors. Such products trigger production of pyrogenic inflammatory mediators and cytokines by macrophages and other innate immune cells that in turn stimulate thermoregulatory regions of the brain to increase body temperature [12]. As parasite density increases, so does the amount of toxins and pyrogenic mediators, and with them the body temperature and the risk of fever. The parasite load threshold associated with the onset of fever in human hosts has been considered a potential indicator of the risk of symptomatic malaria in a parasite carrier [13]. The ability to trigger a fever may be important for the parasite to regulate its growth [12], thereby establishing a longer infection that enhances the probability of transmission. The pyrogenic threshold of *P. falciparum* malaria infections in non-immune individuals has

hyperendemic (constantly over 50%); and holoendemic (constantly over 75%).

Glycosylphosphatidylinositol (GPI): molecules that anchor a diverse range of proteins to the surface of malaria parasites and are thought to function as a toxin that contributes to severe malarial pathogenesis.

Hemozoin: insoluble and chemically inert β-hematin crystals resulting from the detoxification of free heme produced by the parasite metabolism in infected erythrocytes.

Immune resistance to infection: the capacity of the host's innate and adaptive immune system to reduce parasite density levels.

Immunopathology: tissue damage caused by an excessive response of the immune system against an infection.

Innate immunity: the nonspecific immune system of the organism that responds to a pathogen infection in a generic way.

Malaria elimination: permanent interruption of local mosquito-borne malaria transmission in a defined geographical area.

Mass drug administration: strategy that aims to drastically interrupt malaria transmission intensity through the administration of an antimalarial drug to a specific target population regardless of their infection status.

Parasite multiplication rate: rate of parasite growth and proliferation during an infection.

Passive case detection: detection of malaria cases among patients who on their own initiative went to a health post for treatment, usually because of febrile disease.

Pathogen-associated molecular patterns (PAMPs): small molecular motifs conserved within groups of pathogens that are recognized by cells of the innate immune system through their TLRs and other PRRs.

Pattern recognition receptor (PRR): proteins expressed by innate immune cells for the initial detection of PAMPs as well as damage-associated molecular patterns.

***Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1):** a parasite protein that binds to several host receptors to mediate the sequestration of infected erythrocytes.

***Plasmodium falciparum* sirtuin 2a (PfSir2a):** a transcriptional regulatory

been shown to cover a wide spectrum of parasite densities ranging from 10 to 200 000 parasites/μl [9], varying as a function of **transmission intensity**, age, or host ethnicity [9]. In high-transmission areas, clinical illness is not often associated with parasitemia of *P. falciparum* below 10 000 parasites/μl [14]. In general, a relationship between increasing *P. falciparum* parasite density and fever among children has been reported in high-transmission areas [14]. However, this has not been demonstrated in other areas with intense seasonality or in low-transmission settings [15], questioning the reliability of parasite densities in distinguishing malaria attacks from other causes of fever. Moreover, significant differences have been observed between the pyrogenic thresholds for different *P. falciparum* strains [9], suggesting that some strains may induce a febrile response in the host at lower parasite densities compared with other strains. Possible explanations for these differences include variations in the virulence of the strains in inducing an inflammatory response, in their potential for cytoadhesion and sequestration, in the degree of synchronization of parasite populations, or in the length of the replication cycle.

Multiplication Rate

The parasite multiplication rate, which has been linked to the severity of disease in *P. falciparum* malaria [16], depends on the balance between the intrinsic susceptibility of the host erythrocytes, the level of antimalarial immunity, and the ability of the parasites to multiply at high densities [17]. As in other eukaryotes, the synthesis of rRNA determines ribosome production and the potential for parasite growth and proliferation [18]. One candidate molecule to regulate *Plasmodium* rDNA transcription is the highly conserved NAD-dependent deacetylase Sir2 [*P. falciparum* sirtuin 2a (**PfSir2a**)], the first identified epigenetic factor shown to be involved in transcriptional control of antigenic variation in *P. falciparum* [19]. Metabolic changes in NAD^+/NADH and high temperatures found in malaria-induced febrile illness have been suggested to modulate PfSir2a activity [12], balancing the malaria parasite's energy status with rRNA synthesis and parasite proliferation. Therefore, the epigenetic environment may play an important role in the establishment of asymptomatic infections and modulate the switch between **chronic** and acute infection in response to the host or as part of the parasite developmental program [20]. Moreover, regulation of genes involved in DNA replication and the unfolded protein response could potentially lead to dormancy phenotypes in *P. falciparum* malaria that have been suggested to contribute to artemisinin resistance [21].

Parasite Cytoadhesion

The virulence of *P. falciparum* has been linked to the expression of ***P. falciparum* erythrocyte membrane protein 1 (PfEMP1)** on the surface of infected erythrocytes. PfEMP1s bind to several host receptors to mediate the sequestration of infected erythrocytes in vital organs [11] and drive inflammation by blocking the cytoprotective function of the endothelial protein C receptor [22]. PfEMP1s are encoded by approximately 60 ***var* genes** per parasite genome, which are expressed in a mutually exclusive manner to avoid simultaneous recognition by the immune system [11]. Host antibodies against PfEMP1 variants have been suggested to structure the switch between *var* genes in a hierarchical process [23], with parasites expressing conserved and virulent PfEMP1s eventually surpassed by less virulent and more diverse variants that dominate infections in semi-immune individuals. Despite having extreme inter- and intragenome variability, PfEMP1s can be subdivided into three major groups labeled A, B, and C based on motifs in noncoding sequences and locus position [24]. Group A *var* genes have been shown to be preferentially expressed by parasites associated with severe disease syndromes while greater transcription of *var* group C has been found in older children with asymptomatic malaria [25,26]. This differential expression suggests that once the initial *var* gene repertoire is exhausted by increasing immunity, *var* group C, or an alternative, unknown group of *var* gene variants that do not mediate cytoadherence in vital organs, might prevail in asymptomatic infections. Such chronic and asymptomatic malaria infections may survive their encounters with

protein that epigenetically regulates *var* gene switching.
Premunition: exposure-dependent acquisition of immunity in which partially effective responses protect against illness and high numbers of parasites in the blood without completely eliminating the infection.
Pyrogenic threshold: a population-based statistical measure that defines a parasite density above which fever may occur and below which most cases are afebrile.
Submicroscopic infection: malaria infections with parasitemia levels that are below the microscopic threshold for detection.
Surveillance: systematic monitoring of malaria cases and deaths with the purpose of planning and evaluating malaria control program as well as generating an immediate action in response to case identification.
Tolerance to infection: host defense mechanisms that minimize the damage caused directly by the pathogen or indirectly by the host's immune response irrespective of pathogen burden.
Toll-like receptor (TLR): proteins with variable extracellular leucine-rich repeats mainly present at the membrane of innate immune cells; stimulate a proinflammatory response when they recognize structurally conserved molecules derived from microbes.
Transmission intensity: rate at which people living in malaria-endemic areas are bitten by anopheline mosquitoes carrying human malaria sporozoites. Transmission intensity is often expressed as the entomological inoculation rate, which is the average number of inoculations with malaria parasites received by one person during a period of time.
***var* genes:** parasite genes with extreme inter- and intragenome variability that encode the PfEMP1 family of proteins in *P. falciparum*.
Waning of immunity: loss of the acquired immunological capacity to control an infection.

the host immune system by generating novel antigenic variants via mitotic recombination at a very high rate [27].

Multiplicity of Infection and Drug Resistance

Although evidence is far from conclusive, additional parasite-related factors such as drug resistance or multiplicity of infection have been suggested to differ between malaria disease states. One study found a significantly higher prevalence of drug-resistant parasite strains among asymptomatic children compared with febrile children [28]. This reduced virulence among drug-resistant parasites was attributed to a possible fitness loss associated with the acquisition of mutations mediating antimalarial resistance [29]. However, such an interpretation contradicts epidemiological evidence from areas of seasonal transmission where asymptomatic infections persisting during the dry season mostly comprise wild-type parasites [3]. Another study found that the proportion of mixed-genotype infections and the number of genotypes per person was higher among asymptomatic than symptomatic infections and that this diversity of infecting strains might enhance protection against clinical malaria [30]. Additional evidence has further suggested that multiplicity of genotypes could also favor the parasite by extending the duration of infections through the selection of genotypes that ensure parasite survival and transmissibility [31]. Nevertheless, more research is needed to conclusively affirm an association of these factors with asymptomatic infections.

Host Defenses

The processes that determine the acquisition of host defenses that allow asymptomatic carriage of malaria parasites are poorly understood. Hosts can employ two non-mutually exclusive defense strategies against pathogens. One relies on the capacity of the host's immune system to reduce pathogen burden (i.e., resistance to infection, which can be measured as the inverse of the pathogen burden) whereas the other minimizes the damage caused directly by the pathogen or indirectly by the host's immune response irrespective of pathogen burden (i.e., **tolerance to infection**, defined as the slope of host fitness against infection intensity) [32].

Immune Resistance To Malaria

Mechanisms of **innate** and **adaptive immunity** can protect the infected host by reducing malaria parasite load. **Immune resistance** to sporozoites transmitted by *Anopheles* mosquitoes and exoerythrocytic liver stages is rarely if ever achieved, probably because of the low inoculum of parasites in the mosquitoes' saliva and a lack of robust immunity to these stages in individuals naturally exposed to *P. falciparum* [33]. By contrast, immunity against blood-stage parasites determines the course of disease in humans [33] and is acquired at a rate that depends on the intensity of malaria transmission (Box 2).

Innate immune cells such as monocytes/macrophages, natural killer cells, and γδ T cells can sense early blood-stage infection, inhibit parasite multiplication through the production of pro-inflammatory cytokines and chemokines, and direct the quality of adaptive immune responses [34]. This initial detection of parasites is mediated by **pattern recognition receptors (PRRs)** expressed by immune cells and **pathogen-associated molecular pattern (PAMP)** molecules in *P. falciparum* such as GPI, hemozoin, and AT-rich and CpG-containing DNA motifs bound to hemozoin [33]. Adaptive immune responses against malaria parasites have been suggested to rely on the exposure-dependent acquisition of antibodies that reduce parasite growth [17]. This can be achieved through the blocking of critical invasion ligands in the merozoite, by inhibiting secondary proteolytic processing of such parasite ligands or their surface redistribution on merozoites or by blocking their interaction with host receptors. Antibodies can also contribute to opsonin-mediated phagocytosis of infected erythrocytes and **antibody-dependent cellular inhibition (ADCI)**. Finally, antibodies may also target PfEMP1s

Box 2. Transmission Intensity, **Submicroscopic Infections**, and Asymptomatic Carriage

The malaria infection history of a host, mainly determined by the intensity of transmission, affects the outcome of subsequent infections [33]. It has been generally assumed that, in areas of reduced malaria transmission, populations with little exposure to parasites and thus limited potential for the acquisition of antimalarial immunity will develop high-density symptomatic parasitemia when infected and seek antimalarial treatment [87]. Conversely, more asymptomatic infections would be expected in areas of high malaria transmission where individuals can rapidly acquire immunity that partly restricts parasite growth. Such a correlation between high **endemicity**, and thus transmission intensity, and high asymptomatic prevalence has been reported in several African countries, ranging from 20% to 97% in areas of high transmission compared with less than 10% in areas of low transmission [2,42]. At a microscale, several studies also show a higher prevalence of asymptomatic carriers in villages of high compared with low malaria incidence [88]. This is in line with studies showing a reduction in parasite densities with increasing transmission intensity at village level [89] or with increasing proximity to vector breeding sites [90]. Similarly, the performance of rapid tests has been shown to decline with increasing transmission intensity [89], suggesting that people living in high-transmission areas may acquire immunity and control infections to densities below the detection threshold of standard diagnostic techniques. However, the relationship between transmission and parasite density seen on smaller scales is not observed over larger geographical areas, where lower endemicity has been related to a larger proportion of submicroscopic infections [73]. Such a high carriage of infections below the threshold of microscopic detection in areas of low transmission could be explained by the persistence of residual immunity in areas of low transmission, which keeps parasite density at submicroscopic levels [5], or fewer infections to overwhelm the immune response. Moreover, reduced parasite diversity in areas of low transmission may increase the rate of acquisition of immunity, which, combined with fewer opportunities for super-infection, may allow persistence and thus detection of low-density infections for long periods of time [73]. Finally, genetically homogeneous infections in areas of low transmission could reduce within-host competition, eventually selecting for lower levels of virulence [91].

involved in parasite cytoadherence and rosetting, eventually preventing their sequestration in vital organs and leading to their clearance in the spleen [11,33,35].

Despite the apparent importance of antibodies, the role of antibody-mediated immunity for the transition between clinical disease and asymptomatic infection remains unclear. Several reports have shown antibody responses against *Plasmodium* merozoite antigens that predict a reduced risk of symptomatic malaria and high-density *P. falciparum* infections, including those against members of the serine-enriched repeat antigen (SERA) family [36], merozoite protein 4 (MSP-4) [36,37], MSP-1_{19} [38], and apical merozoite antigen 1 (AMA-1) [39]. Antibodies against PfEMP1 have also been suggested as important contributors to the prolonged chronicity characteristic of asymptomatic infections, with disease occurring as a result of holes in the antigenic repertoire recognized by the host [40]. Field studies indicate that cumulative exposure to a variety of diverse PfEMP1s is required for the transition from symptomatic to asymptomatic malaria [35]. Moreover, carriage of antibodies against PfEMP1 among children with ongoing asymptomatic infection is associated with protection from future clinical malaria episodes [41]. Overall, these studies suggest that protection from the onset of febrile malaria may require the acquisition of broadly reactive IgG antibodies at levels that exceed a protective threshold rather than antibody titers for individual antigens [38,41]. Although mainly mediated by IgG, other isotypes such as IgE [42] and polyclonal antibodies [43] have been associated with a reduced risk of developing clinical malaria, indicating a putative role for these immunoglobulins in pathogenesis. However, there remains a lack of specific and validated correlates of immune protection contributing to asymptomatic malaria.

Similarly to antibodies, it is not fully understood which immune cells mediate clinical immunity [33]. Research suggests a protective effect for T cell cytokine responses [particularly interferon gamma (IFN-γ)] on the control of parasite replication [33]. Appropriate dendritic cell maturation and T regulatory cell activation has been described in asymptomatic *Plasmodium* infection, in contrast to reduced maturation markers of dendritic cells and an increased proportion of activated T regulatory cells in acute malaria [44]. Malaria-induced tumor necrosis factor alpha (TNFα) might also increase T cell regulatory activity and therefore more effectively prevent inflammation [45]. Asymptomatic infection has been inversely associated with the frequency of

TNFα-producing $CD4^+$ T cells [46], suggesting that production of this inflammatory cytokine may decrease with increasing cumulative malaria exposure, enabling a transition to asymptomatic infection. Overall, these data provide evidence of appropriate immune activation in asymptomatic infection and immune dysfunction in acute clinical malaria. However, the specific cellular immune responses involved in the transition to and maintenance of asymptomatic infection remain unknown.

Both poor induction of immune responses by the parasite and rapid **waning of immunity** have been proposed to explain the persistence of chronic low-density malaria infections [33]. There is mounting evidence that *Plasmodium* evades humoral immunity through deregulation of T and B cell function [33]. However, controlled human experimental infection has shown to induce long-lasting cellular immune responses to malaria parasites, mainly carried within the αβ T cell and γδ T cell compartment [47]. Moreover, several studies have now shown that *P. falciparum*-specific memory B cells are generated in response to infection, albeit inefficiently, as their prevalence appears to be relatively low among adults (~30–50%) [48]. Once acquired, however, memory B cells are long lived [48] and persist longer than antibodies in the absence of ongoing *P. falciparum* exposure [49]. However, it is unknown whether asymptomatic parasitemia can drive an increase in memory B cells. Overall, direct evidence for the presence or absence of immune memory to malaria, and the implications for the asymptomatic carriage of infection, remains limited.

Tolerance to Malaria Infection

In human malaria, most individuals fail to develop true sterilizing immunity and remain vulnerable to chronic carriage of low-density asymptomatic infections into adulthood [33]. Such a phenomenon, known as **premunition**, is suggested to result in tolerance to malaria infection. Infections may remain asymptomatic if major disruptions of physiological functions are prevented by tolerance mechanisms. Such tolerance developed by the host is likely to be multifactorial and includes the neutralization of parasite toxins and other virulence factors and immunoregulatory mechanisms that reduce the damage triggered by excessive immune responses of the host (i.e., **immunopathology**) as well as cellular and systemic adaptive responses that limit the deleterious effects associated with stress imposed by pathogens and/or host immunity (Box 3).

Tolerance to malaria parasites may be acquired after a single infection, as the pyrogenic threshold during an infection in non-immune hosts increases between the first and second fever episodes [9]. Moreover, a larger proportion of patients remained asymptomatic during the reinfection (11.5%) compared with the initial infection (1.8%) [9], suggesting the persistence of tolerance mechanisms after the infection ends. Malarial tolerance is also thought to be most efficient in childhood and then decline with age. This is because the threshold of parasitemia associated with fever appears to be higher in children than in adults from the same malaria-endemic regions [14]. Thus, children with high parasite densities tend to be more frequently asymptomatic compared with adults with a similar parasite burden (i.e., can tolerate higher parasite burdens), while the latter are more efficient in suppressing peripheral parasitemia and controlling clinical attacks [50]. Taken together, these observations suggest that tolerance mechanisms contributing to asymptomatic malaria in populations intensely exposed to *P. falciparum* can be developed after few infections, persist once the infection is cleared, and be more effectively inducible in children but, once acquired, more evident in adults.

Host Genetic Factors

Host genetic background can determine the susceptibility to different clinical presentations of malaria [51]. Genome-wide association studies have indicated a few genetic variants, including numerous immune system polymorphisms, hemoglobinopathies (HbS, HbC, HbE, thalassemias, Southeast Asian ovalocytosis), and other gene variants linked to the erythrocyte system

Box 3. Mechanisms of Tolerance to *Plasmodium* Infection

The mechanisms contributing to disease tolerance and the control of tissue damage remain poorly understood. However, several adaptive responses have been suggested to operate in the context of tolerance to malaria infections.

Neutralization of Parasite Exoantigens

Parasite toxins such as GPI and hemozoin are released at schizont rupture, enhance inflammatory cytokine production by human monocytes, and lead to fever onset [92]. Antibodies against *Plasmodium falciparum* GPI ameliorate some toxic manifestations of murine malaria [93] and can inhibit TNFα production by mononuclear cells [94]. However, solid evidence for a clinical effect in humans remains lacking [95].

Production of Anti-inflammatory Molecules

Nitric oxide (NO), which has been shown to be produced at higher levels by mononuclear cells in asymptomatic children [96], can suppress the pathogenesis of severe malaria through induction of heme oxygenase-1 (HO-1), CO production, and inhibition of T cell activation [97]. However, conflicting results have questioned the role for NO in mediating tolerance [98].

Cellular downregulation of P. falciparum-inducible inflammation

Repeated malaria exposure can lead to attenuation of the cell-mediated proinflammatory responses through either dysfunction and/or exhaustion of immune cell subsets that produce proinflammatory mediators (e.g., $V\delta2^{+}$ γδ T cells [99]) or the expansion of immunoregulatory cells that control inflammation (e.g., *P. falciparum*-specific $CD4^{+}Foxp3^{-}$ T cells [100]). It has also been suggested that, similar to endotoxin tolerance, regulation of the **Toll-like receptor (TLR)** signaling pathway, which recognizes highly conserved PAMPs, could also induce refractoriness of immune cells to produce inflammatory cytokines after repeated malaria stimulations [33].

Induction of Antioxidant Mechanisms

Cell-free hemoglobin released during the blood stage of *Plasmodium* infection promotes tissue oxidation and organ failure [101]. Animal models have shown that HO-1, encoded by the stress-inducible gene Hmox1, can protect the tissues from the heme toxic effect independently of pathogen load [102]. Such a mechanism could explain the protection mediated by sickle cell anemia [101].

Metabolic Adaptation to Tissue Iron (Fe) Overload

Infected mammals restrict Fe availability to pathogens by sequestering intracellular Fe within various tissues [103], which can in turn promote tissue damage and exacerbate disease severity. Expression of the Fe-sequestering protein ferritin H chain (FtH) is induced in response to *Plasmodium* infection in humans [104] and has been associated with reduced tissue damage, probably through ferroxidase activity [105], which limits the production of free radicals and can confer tolerance to malaria infections.

(such as blood group, glucose 6-phosphate dehydrogenase deficiency, or ATP2B4 calcium transporters) [52]. Significantly fewer studies have directly addressed the role of host genetic variants in asymptomatic infections, reporting inconclusive results probably due to the low power to detect modest effects [42].

Pregnancy

Pregnant women have been identified as a possible large source of chronic asymptomatic carriage of parasites compared with men and non-pregnant women [53]. In areas with stable and intense transmission, malaria in pregnancy has been identified to be commonly asymptomatic [53], even in the presence of infected erythrocytes sequestered in the placenta [54]. Recrudescence of placental parasites can occur up to 187 days after the initial infection [55] and can persist asymptomatically for several months post-delivery [56]. However, such asymptomatic infections have been associated with maternal anemia [57], suggesting a silent health impact of these infections (Box 1). Additionally, the high prevalence of gametocytes reported in Malawian pregnant women [58], although not confirmed in other studies, suggests their potential relevance as a reservoir of malaria parasites. Further studies are needed to confirm the contribution of pregnant women with asymptomatic malaria to the persistence of malaria transmission.

Comorbidities

The health state of the host is modified by other, coinfecting organisms thereby complicating our understanding of the clinical outcome of malaria. *Plasmodium*-infected individuals with hepatitis B virus infection were more likely to be asymptomatic and to have lower levels of parasitemia, possibly through an increase in the production of IFN-γ levels induced by viral infection that can contribute to *Plasmodium* clearance in the liver [59]. Coinfection with soil-transmitted helminths may alter susceptibility to clinical malaria by activating T_H2 cytokines and immunoregulatory pathways that downregulate effector functions involved in resistance to malaria [60]. HIV can also impair lymphocyte functions against malaria infection [61]. Similarly, malnutrition can lead to atrophy of the thymus, leucopenia, and diminished functional T cells that hamper protective immunity to malaria [62].

Disease Patterns During Changes of Malaria Transmission

Understanding the determinants and clinical consequences of malaria declines and resurgences, as well as the timescales for changing disease presentations, has become a priority in the context of the current goal to eradicate malaria. Several studies have shown a shift in the peak of acute malaria incidence toward older ages as malaria exposure wanes [14,63], which may precede changes in the number of episodes and disease spectrum in an area with declining transmission intensities [64]. These observations illustrate that sustained reduction in exposure to malaria infection leads to changes in mean age and presentation of disease [65]. Moreover, increases in parasite density and in the susceptibility to severe disease have been observed in children [66,67] and pregnant women [68] residing in areas where malaria has declined substantially. Overall, these studies suggest that, in highly endemic areas, measures that led to reductions of parasite transmission, and thus immunity, may lead to a change in both the burden and the clinical spectrum of disease.

Information on the burden and significance of asymptomatic malaria infections in areas influenced by intense elimination efforts is very incomplete. Data from various countries and stages of eradication programs conducted in the 1950s showed asymptomatic infection rates ranging from 91% in Thailand to 0% after an epidemic investigation of 77 new malaria clinical cases in Sicily [69,70]. The divergences in the observed proportions suggest a close relation with the epidemiological conditions in the different regions. In areas where malaria was recently hyperendemic and where some residual transmission was still ongoing, the proportion of asymptomatic infections was comparatively higher. In areas where malaria was not highly endemic or where transmission had been interrupted a long time ago (more than 6 or 7 years), the percentage of asymptomatic infections was low. This would indicate some degree of persistence of the immunity originally acquired under hyperendemic conditions as the chief factor in the occurrence of asymptomatic infections (Figure 2). However, the speed at which immunity wanes

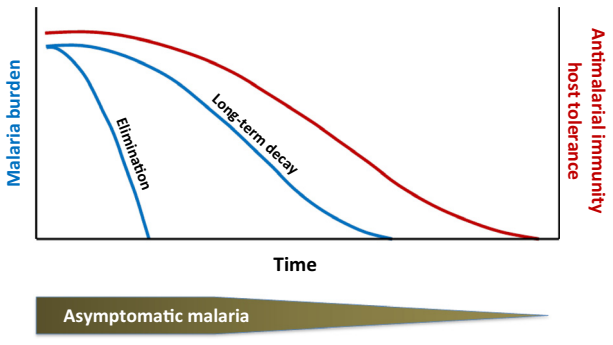

Figure 2. Drops in Transmission, Loss of Immunity and Risk of Asymptomatic Malaria. The proportion of asymptomatic infections may depend on the speed at which malaria transmission decreases, with a significant reservoir of asymptomatic carriers expected when the decrease in transmission is more rapid than the loss of immunity in a population. By contrast, the asymptomatic reservoir would be minimal when transmission has decreased over many years and people have lost all antimalarial immunity.

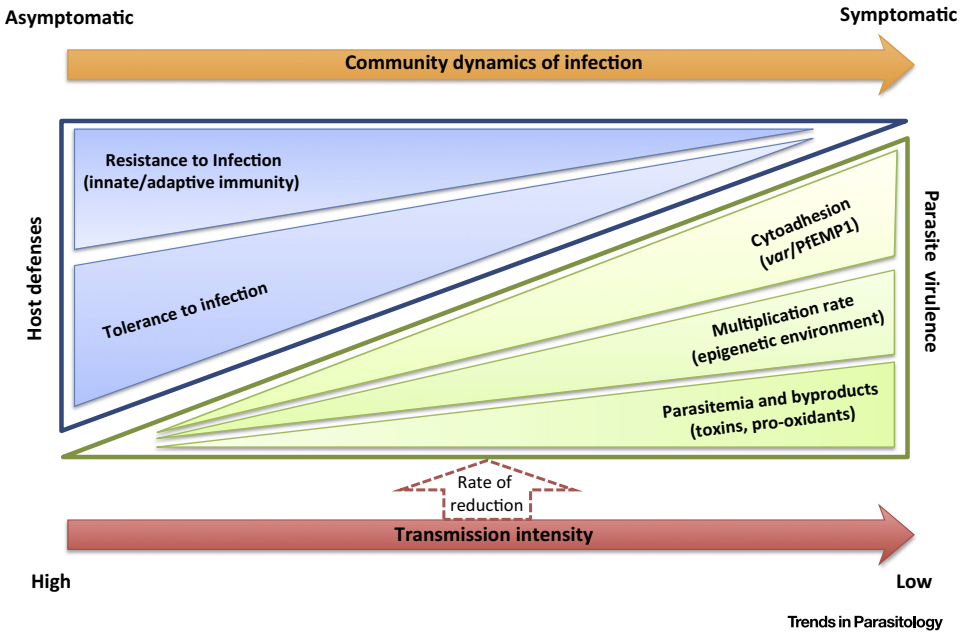

Figure 3. The Parasite, the Host, Asymptomatic Infections, and Transmission Intensity. Evidence has suggested that the carriage of asymptomatic infections may depend on a combination of host and parasite factors mediating immunity and tolerance mechanisms as well as pathogen virulence. A gradual reduction in transmission intensity reduces the probability of pathogen–host encounters, leading to a progressive decrease of exposure-dependent defense mechanisms to resist and tolerate an infection. As host protection weakens, the damage caused by the parasite through its multiplication in erythrocytes, toxin release, and cytoadhesion is expected to increase. Such dynamics support a transition to a symptom-based surveillance system when transmission is sufficiently low. The speed at which malaria transmission decreases may affect the rate at which host defenses are lost and the emergence of more virulent host–parasite interactions when no immunity restricts parasite multiplication. To ensure that this argument still holds during malaria elimination campaigns that seek to rapidly reduce transmission, interventions should ensure that all parasite reservoirs are tackled to avoid the selection of low-virulence parasite populations that may disrupt such dynamics.

in the absence of exposure remains unknown, with some studies suggesting that immunity to febrile malaria can be rapidly lost whereas others indicate long-lasting immunity, particularly against the most severe forms of disease [33]. Moreover, other factors cannot be entirely excluded, such as inherited factors involved in the immune response and the possibility of selecting asymptomatic strains of parasites in the process of eradication. Information on these factors is, however, scanty at this stage, and current elimination efforts, particularly those based on mass drug administration, should be helpful in testing such hypotheses. Knowledge of the host–pathogen interactions that are most likely to result in asymptomatic malaria, especially in the face of transitioning epidemiological conditions after effective reductions in malaria transmission (Figure 3), could eventually provide information about the strategies with the greatest temporal and cost efficiencies in achieving desired elimination outcomes.

Concluding Remarks

Malaria elimination programs must include tactics to target asymptomatic reservoirs that can sustain transmission. As the relative contribution of asymptomatic infections to the overall pool of infections may change during the course of elimination activities, surveillance and response strategies should be adapted to the different epidemiological scenarios [71]. However, our understanding of how malaria transmission, host defenses, and parasite factors interact to trigger the clinical spectrum of malaria infections remains limited, probably because of the complexity of factors influencing the clinical outcome of the infection.

Outstanding Questions

What is the natural progression of asymptomatic malaria infections over time and their potential for evolving into acute disease?

Does the pace at which malaria transmission declines and immunity is lost affect the asymptomatic pool of infection observed in a community?

What is the level of malaria transmission required to sustain host responses capable of keeping infections at asymptomatic levels?

Does clinical immunity develop more rapidly in low-transmission settings than in high-transmission regions, where acquired immunity requires years of intense transmission to develop?

How long does it take to acquire and loose immunological resistance and tolerance to malaria infections?

What is the relative contribution of immune resistance and tolerance to the risk of being asymptomatic? Is it possible to formally disentangle tolerance and resistance in human malaria?

To what extent is the heterogeneity in clinical malaria the result of polymorphisms in the host genetic background?

What are the key parasite factors associated with asymptomatic carriage of malaria infections?

Does the immune-structured expression of *var* genes allow the identification of PfEMP1 types that can be used as surrogates of asymptomatic *Plasmodium falciparum* malaria?

What epigenetic factors modulate parasite population dynamics and its pathogenic potential?

What is the contribution of pregnancy and particular comorbidities (HIV infection, helminthiases, malnutrition) to the overall burden of asymptomatic infections?

Can malaria elimination interventions select for parasite strains that are less virulent or induce higher tolerance by the host, allowing them to escape symptom-based detection methods?

The renewed transition of focus from malaria control to elimination has triggered a new set of research questions that were previously not prioritized (see Outstanding Questions). In this context, prospective and reductionist studies to disentangle host responses operating directly on the parasite and those by which malaria tolerance may occur [32] could provide clues about the strategies developed by the human host to reduce the clinical consequences of malaria infections. On the pathogen side, identification of factors modulating parasite population dynamics and its pathogenic potential could provide insight into the mechanisms favoring asymptomatic carriage over disease. Importantly, such studies should take into account the selective pressure of elimination interventions on the parasite population, potentially leading toward less virulent infection that might remain undetected in the form of asymptomatic infections. Such knowledge can not only improve the management of different diseases outcomes but also increase the success of malaria elimination efforts.

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