MALARIA

A SCIENTIFIC STUDY CONCERNING PHYSIOLOGICAL AND BIOCHEMICAL IMPLICATIONS

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Malaria: A Scientific Study

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Malaria is by far the world's most common cause of parasitic morbidity and mortality. In the year 2017, approximately 219 million global cases of malaria occurred along with 435,000 deaths caused by infection. Malaria is a parasitic disease of red blood cells caused by microorganisms of the Plasmodium species usually contracted and spread by the female Anopheles mosquito: typical symptoms include headache, vomiting, and fever with more serious manifestations resulting in jaundice, seizures, coma and even death. While malarial infection is usually non-fatal, some types are implicated in chronic recurrence, vascular occlusion, and diminished oxygen transport (hypoxia). The type and severity of malarial infection is predicated upon the species of parasite, with each of four types possessing different characteristics and distinct impacts on pathogenesis and mortality. Treatment primarily involves pharmaceuticals targeting various stages of the Plasmodium life cycle by either killing the parasite or preventing its replication. Prevention involves protection from mosquitoes with netting and the recent completion of clinical trials on the first anti-malarial vaccine to be approved by the WHO in 2015. In addition, high-risk populations in historically tropical areas may possess genetic alterations that facilitate resistance to the malarial disease process of which the biochemical consequences of Sickle Cell Disease as well as a condition known as Glucose 6-Phosphate Dehydrogenase Deficiency will be discussed. This review concerns the specific challenges associated with current malarial treatment as well as current efforts to produce a lasting viable alternative to current disease management.

Introduction

Malaria is a parasitic disease that infiltrates red blood cells and is caused by microorganisms of the *Plasmodium* genus[1]. The name *malaria* (from Italian) stems from a proposed causative role of "bad air" from marshy environments as being responsible for infection[1]. It was not until 1880, that the French physician Alphonse Laveran isolated organisms from within red blood cells found to be uniquely present in those infected with malaria. He determined that these isolated organisms were intracellular parasites of the Plasmodium species and, a few years later, Sir Ronald Ross isolated Plasmodia from Anopheles mosquito stomach tissue, indicating a possible vector for malarial transmission[1].

Malaria remains the most common global cause of parasitic morbidity and mortality. In 2017, approximately 219 million global cases of malaria occurred along with 435,000 total deaths caused by this parasitic infection[2].

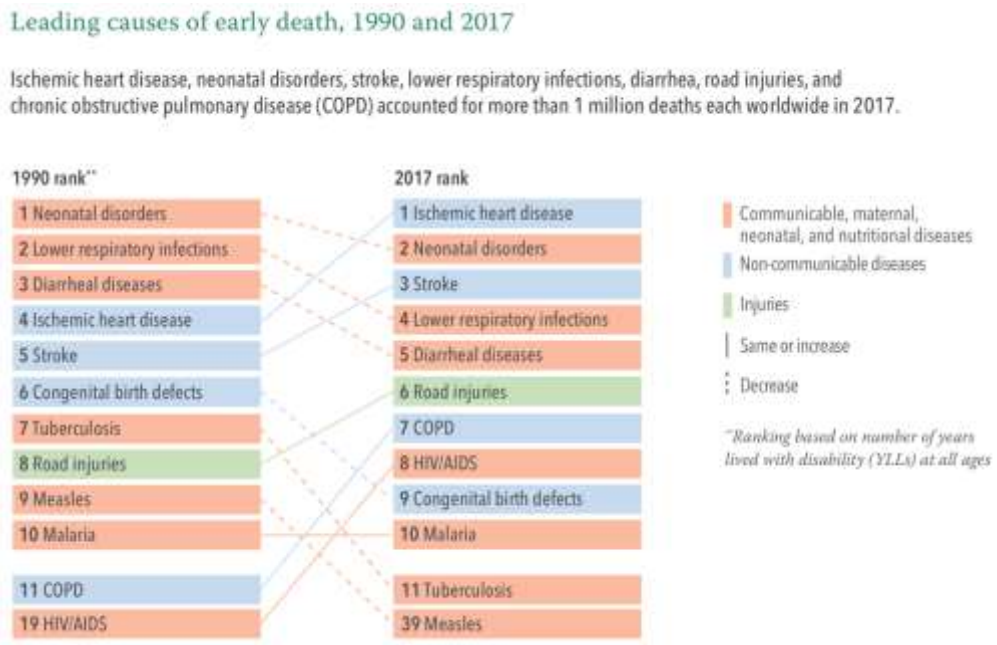

Figure 1: Malaria has remained consistent as a leading cause of early death[3]

Malaria is well-known for its recurrent fevers that cyclically wax and wane depending upon parasite life-cycle stage in those infected. Fever onset typically aligns with the lysis of red blood cells and generally declines in intensity until the next round of hemolysis occurs. Far from a newly discovered phenomenon, Hippocrates, "The Father of Medicine", is believed to have differentiated the intermittent fevers of malaria from those of other febrile illnesses and denoted the periodicity of infection in 400 BC[4]. Many symptoms from malarial infection are variable as well as non-specific and include headache, weakness, abdominal pain, and vomiting[4].

Creative Challenge: How are biochemical and physiological systems affected by Plasmodium pathogenesis? What efforts are taken to reduce the impact of malarial transmission and what novel techniques are being implicated in mitigating disease?

Malaria Types and Pathogenesis

The "type" of malarial infection that occurs is determined by the species of parasite, each possessing different characteristics that uniquely impact pathogenesis and influence mortality. Malaria in humans is acquired from four species of Plasmodium: *P. falciparum*, *P. malariae*, *P. ovale*, and *P. vivax*[4]. *P. falciparum* is the species that causes the most severe complications associated with malaria and is responsible for nearly all malarial mortalities. These severe complications may include pulmonary edema, cerebral malaria, acute renal (kidney) failure, acidosis, hypoglycemia, anemia, and bleeding[4,5].

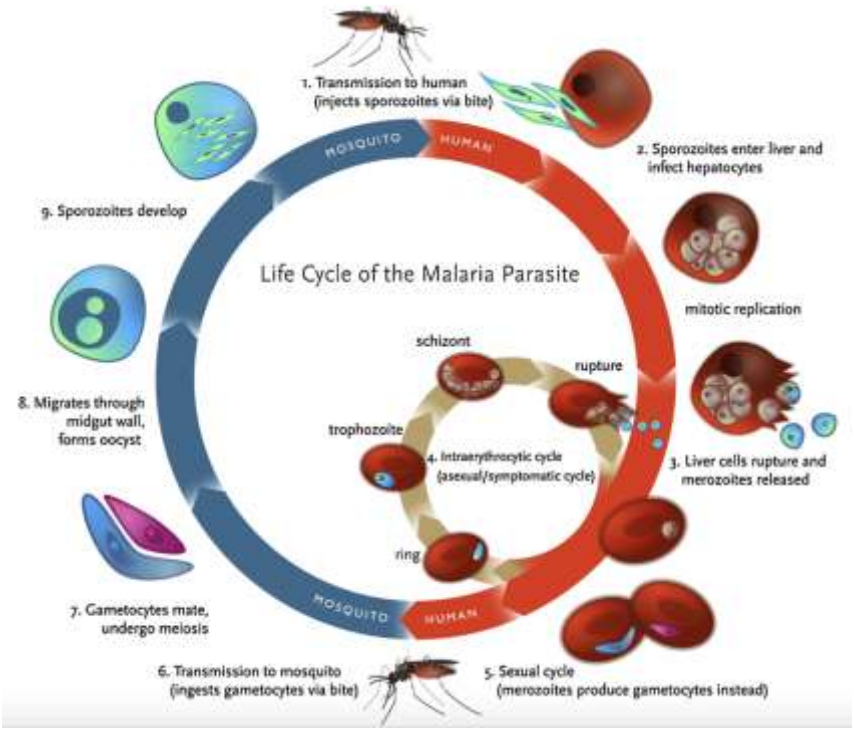

Figure 2: Life-cycle of plasmodium species implicated in malaria[13]

Malarial infection is typically categorized by two major stages: erythrocytic and exoerythrocytic stages[5]. The exoerythrocytic stage refers to parasitic infiltration into sites such as the liver while erythrocytic stage concerns accumulation and completion of life-cycle within circulating red blood cells. Initial infection occurs when an infected female anopheles mosquito feeds on human blood and releases immature plasmodium called sporozoites into the host circulation[5]. These sporozoites enter the liver where they grow and mature into schizonts and later merozoites. Merozoites are released into the blood stream where they encounter red blood cells (RBC)[5]. Upon cell invasion, these plasmodia develop ring-shaped structures within the RBC, an essential diagnostic marker to determine malaria infection. While infection in the blood is often readily apparent, Plasmodia within the liver remain relatively undetectable as patients are asymptomatic and diagnostics are generally based on blood smears. Although all species of Plasmodium transition to the liver immediately upon host introduction, *P. vivax* and *P. ovale* can exist within the liver in a dormant stage, evading host immune processes for months or even years[5]. The cause of this latency is not well-defined.

Severe Malaria

While species such as *P. vivax* and *P. malariae* are restricted to invading young and old red blood cells respectively, *P. falciparum* can invade any red blood cell regardless of age; thus, very high levels of the parasite can be found in blood (parasitemia)[6]. One of the distinctive characteristics of *P. Falciparum* is a consequence of its' more effective host incorporation as well as its' possessing a unique protein called Plasmodium falciparum

erythrocyte membrane protein 1 (PfEMP1) that can lead to vascular occlusion. PfEMP1 is expressed by mature erythrocytic parasites and is anchored to knobs found upon the surface of the infected red blood cell (iRBC)[7]. Once attached to the red blood cell surface, PfEMP1 can mediate connections with either uninfected red blood cells or endothelial tissue aptly known as cytoadhesion. This adhesion allows the iRBC to sequester within tissues, allowing the parasite to complete its life-cycle without being eliminated by the spleen. Increased cytoadhesion with vascular walls may also occlude blood flow, resulting in diminished oxygen transport to tissues and thus tissue death and necrosis[7].

This inadequate blood perfusion may also lead to a severe loss in oxygen and glucose assimilation critical to tissue such as brain[8]. Despite the prevalence of parasites within the brain, many brain related complications, including coma and epilepsy, can often be reversed with treatment and reveal little long-standing tissue necrosis. One proposed mechanism for the reversibility of neurological issues comes from the theory that certain chemicals are responsible for interference with neurotransmission[8]. It has been suggested that nitric oxide (NO), a byproduct of host defense signaling, can readily cross the blood brain barrier and negatively impact brain signaling. Once this chemical is no longer present, as in the case of malarial clearance, the negative impacts to the brain signaling cascade no longer occur. Other metabolites produced by infection such as Quinolinic acid are also suspected to be implicated in seizures related to malarial infection[8].

Laboratory Diagnostics

All plasmodium species share a common ring structure within red blood cells, as seen on microscopy, and are thus often difficult to differentiate by physical attributes alone[9]. A

few key characteristics of the ring structure, of the parasite within the red blood cell, exist that can help enable the differentiation of species. The ring structure of *P. falciparum* is often identified as having delicate cytoplasm and small chromatin dots whereas the other Plasmodia species demonstrate sturdy cytoplasm with large chromatin content when examined microscopically. *P. malariae* and *P. ovale* are virtually indistinguishable save for *P. ovale* containing Schuffner's dots and *P. malariae* often having Ziemann's stippling. Schuffner dots are generally smooth and brown in color, while Ziemann granules are coarse and black. Red blood cells which contain *P. vivax* are generally seen as much larger than cells containing other plasmodium species[9]. Characteristics of both the parasite and its host cell differ depending on life-cycle stage. Due to extensive similarity, accurate species diagnosis may prove difficult by morphology alone and distinctions based on physical illness may be required to determine exact species identity[9].

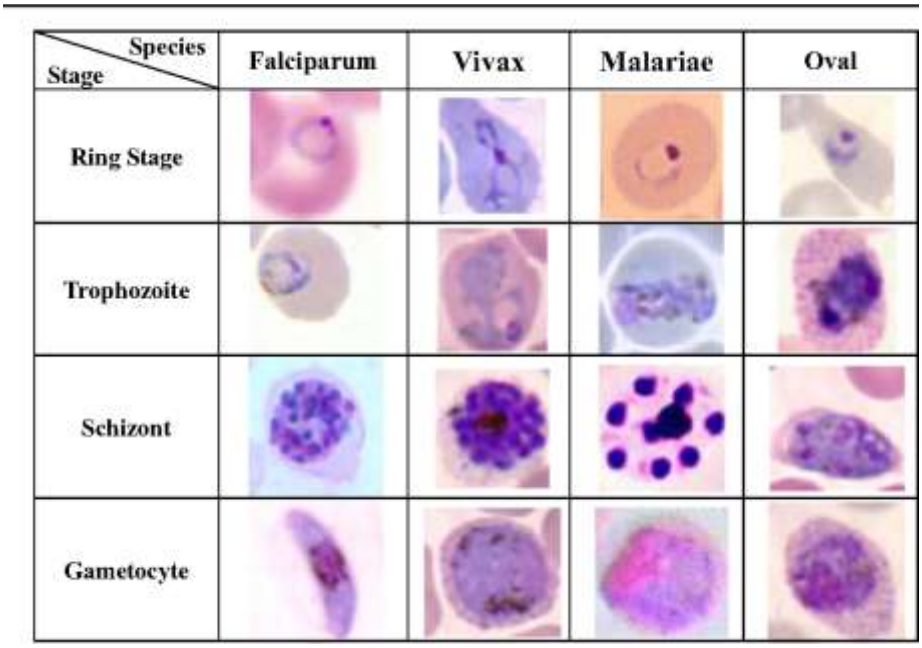

Figure 3: Representative images of life stages of infectious Plasmodia[9]

Samples are prepared by obtaining blood smears from peripheral areas of the patient such as a fingertip. The blood is allowed to dry on a slide and then subjected to Giemsa staining, a stain limited to phosphate groups of DNA. Mature red blood cells do not contain DNA; thus, the stain proves a selective diagnostic marker for identifying plasmodium species. While Giemsa staining has long been the "gold standard" for malaria diagnosis and still remains the first-step in identification, a technique known as Polymerase Chain Reaction (PCR) is a more sensitive and objective diagnostic tool. An amplification of the 18S rRNA gene in each isolated parasite reveals a different segment length specific to each species, providing a means of genetic differentiation on the basis of fragment size[10]. When combined with light-microscopy identification, the PCR method provides an attractive addition to current disease identification and aides in treatment selection. A current limitation with this technology remains its cost as well as the need for skilled technicians. Thus, in areas where malaria is endemic, there are some impediments to its use and overall success[10].

Chemoprophylaxis

An early malarial remedy successfully used in Latin America for hundreds of years was to chew the bark of a cinchona tree. This appeared to be both a protective and a therapeutic measure against infection by the indiginous people[11]. Centuries later, the chemical agent responsible for parasitic prevention in the cinchona bark was isolated by the British and identified as Quinine (Gin and Tonic). Quinine is effective against intra-erythrocytic plasmodium and gametocytocidal for most malaria parasites; however, its' specific anti-malarial mechanism is still unknown. While Quinine and its derivatives remain

relevant in malarial treatment, synthetic anti-malarials such as chloroquine have taken precedence in clinical use[11]. Despite the beneficial impact that chloroquine has had on managing infections, global resistance to chloroquine treatment has sharply risen within the last century, particularly in South America and South-East Asia. *Plasmodium falciparum* in particular has demonstrated remarkable abilities to adapt in becoming resistant to medication use with it also developing resistance to newer pharmaceuticals such as Artemisinin as well.

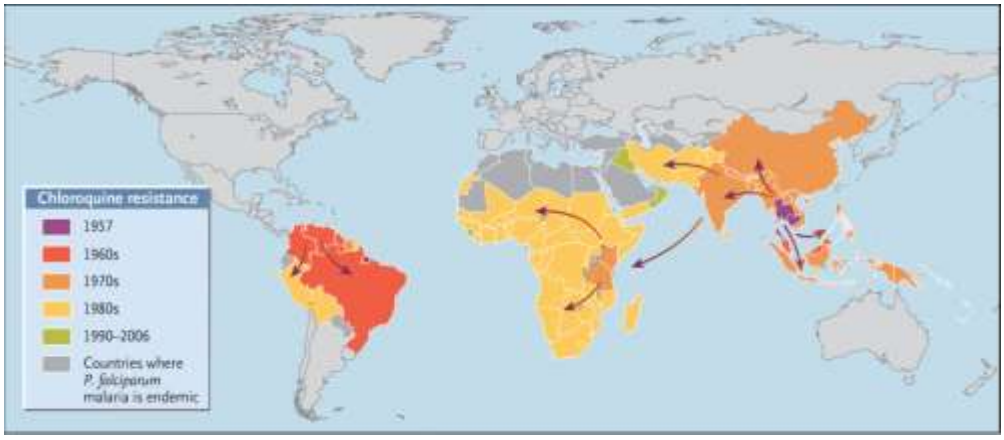

Figure 4: Development of global *P. falciparum* resistance to malaria[12]

Many medicines induce resistance within their targets by vastly increasing the proportion of resistant to non-resistant strains. These resistant strains thus experience less growth competition and are able to persist when non-resistant parasites are eliminated[13]. The spread of resistance from host to greater population is difficult for Plasmodium due as they must successfully produce three-unlikely events. First, the parasite must develop changes in its genome, which facilitate a resistance to the pharmaceutical. Second, the Plasmodium must survive host defenses long enough to produce gametocytes, the reproductive cells of

Plasmodium. Third, the host must experience an event, such as a mosquito bite or blood transfusion, that enables transfer to another organism[13]. Another interesting phenomenon is that resistance spread is attenuated with introduction of the organism to an untreated host. This is partly due to increased competition with non-resistant strains, therefore limiting proliferation and the production of gametocytes.

The anti-malarial Artemisinin shares a common history with Quinine as an herbal remedy for plasmodium infection. The theory of Artemisinin's protective qualities came from a Chinese analysis and screening of hundreds of folk medicines sharing this common ingredient[14]. *In vitro* studies of *Plasmodium falciparum* subjected to Artemisinin treatment, revealed a significant inhibition on activity. Artemisinin is suggested to produce its anti-malarial effect by forming radicals through its peroxide bond. Parasitic consumption of hemoglobin is implicated in iron release, which interacts with artemisinin's bond to produce toxic oxygen species that damage red blood cell membranes[15]. These red blood cells are prone to apoptosis as well as rapid clearance from circulation.

The plasmodia species has a distinct life-cycle progression: schizont to merozoite to trophozoite. A schizont is the obligate intracellular *Plasmodium* form, which causes rupture of host cell and release as a merozoite[5]. Merozoites, the free form of plasmodium within the blood stream, can enter RBCs where they develop into trophozoites[5]. Response to medication largely depends upon the specific stage the *Plasmodium* occupies with different sensitivity related to different tissues. Many current medications are derivatives of quinine and include quinidine, primaquine, mefloquine, and chloroquine. These artificial compounds all share a nitrogen containing aromatic ring, but there are differences in identity of the groups appended to these rings.

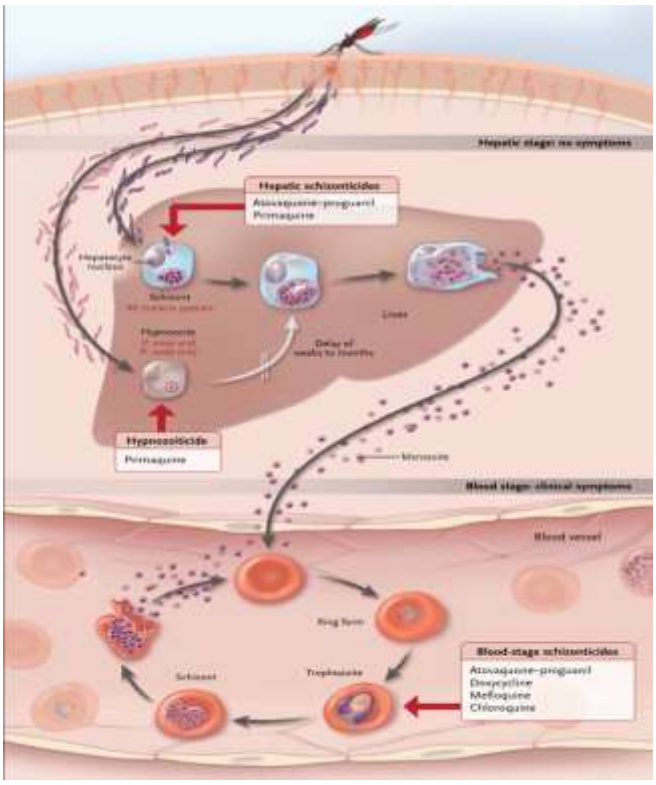

Figure 5: Anti-malarials and their respective targets[28]

The hypnozoite stage is of immense clinical importance as it only exists in *P. vivax* and *P. ovale* infections, the species that can remain dormant within the liver. The drug of choice for these infections is primaquine, a drug that specifically attacks the latent parasitic organism[16]. A typical treatment course involves primaquine supplementation coupled with a schizonticide to prevent possible relapse following care.

Primaquine, also used in *P. falciparum* treatment, is not associated with liver damage and no serious instances of adverse drug reaction under normal dosages are known to occur. Complications may arise if primaquine is given to those with glucose-6-phosphate dehydrogenase deficiency as hemolytic anemia may occur. Thus, the use of primquine is contraindicated in patients that have G6PD deficiency[16].

Folate is a nutrient that serves an essential role in the synthesis, replication, and repair of DNA. If folate levels are low, protozoa such as Plasmodium will cease to replicate. Sulfonamides, often referred to as sulfa drugs, are a broad class of antibiotics that can attenuate folate synthesis within parasites[17]. The method of sulfonamides' folate inhibition is related to mimicry of PABA, an intermediate in folate synthesis. While PABA undergoes further metabolism, enzymes that recognize PABA can bind sulfonamides but produce no active metabolites. Sulfa drugs are well-known for their variety of side-effects, which often include allergy and abnormal hemoglobin synthesis; however, they remain a promising and reasonable alternative to quinine derivations in light of increasing chloroquine resistance[17].

Vaccination

While chemoprophylaxis is a viable means of disease management, inherent disadvantages exist as it is a therapeutic rather than preventative measure in most cases. The costs and resources associated with treating malarial infections far exceed those of an effective vaccine. Vaccines introduce pathogenic material to a subject in a controlled manner, which does not produce symptoms of typical infection[29]. The subject recognizes this substance as foreign and the body begins to develop immune responses concerned with removing the infection, leading to immunity[29].

In the 1960s, the injection of plasmodia attenuated by radioactive treatment revealed a protective effect from later parasitic infection[18]. Later, of the numerous potential candidate proteins to establish effective vaccination, the circumsporozoite protein (CSP) was selected as a possible inductor of immune response, and the gene encoding this surface protein was identified[18]. CSP was chosen in part due to its surface expression on pre-erthyocytic

sporozoites as well as upon infected hepatocytes. Thus, the immune system is induced to recognize plasmodia before symptoms manifest as a result of erythrocyte infiltration.

Vaccines composed of CSP constructs initially yielded no substantial protective benefit however, later coupled with an alternative protein from a Hepatitis B virus; this hybrid vaccine was developed under the name RTS,S[18].

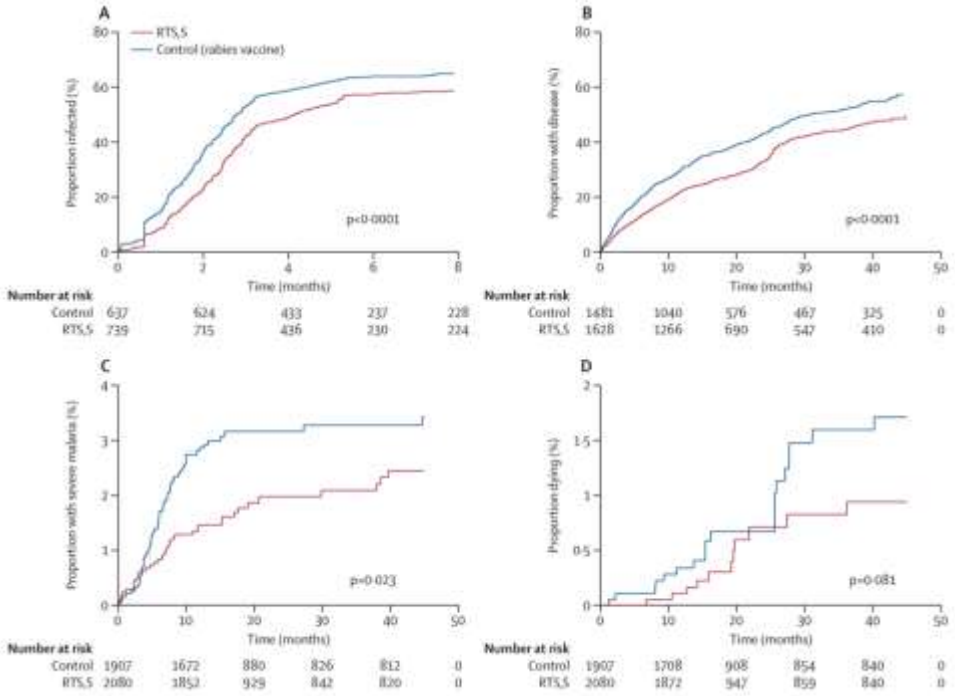

Figure 6: RTS,S is associated with reductions in overall disease and severe disease[19]

The results of multiple vaccine trials indicate that, while this vaccine is reasonably effective, immunity tends to decrease over time and is largely dependent on the number of booster shots received. This suggests vaccination would be best coordinated with birth or infancy, a period where infection is generally much more serious[18].

Genetic resistance to malaria

Unique environmental pressures can select for desirable traits as well as mutations if they enable greater viability for an organism. Populations who live, or have historically lived, within tropical and sub-tropical areas rich in malaria show a *much* greater incidence of genetic alterations which produce a protective effect against infection. Two of the most prevalent biochemical/genetic alterations compromise the stability of Red Blood Cells under stress: Sickle Cell Disease and Glucose 6-Phosphate Dehydrogenase Deficiency.

Glucose 6-Phosphate Dehydrogenase (G6PD) is a metabolic enzyme that facilitates the generation of glutathione, a protective antioxidant responsible for sequestering harmful compounds known as reactive oxygen species[22]. Malaria introduces hydroxyl radicals within RBCs that create oxidative stress. In individuals with normal G6PD, glutathione can mitigate this stress on the RBC cell wall; however, if an individual is deficient in G6PD, this unattenuated oxidative build-up can facilitate a bursting (hemolysis) of infected cells when exposed to further stress such as Plasmodium infection[22]. A destruction of red blood cells containing plasmodium destroys the parasite along with the erythrocyte. Thus, hemolysis is a protective outcome of parasitic infiltration. In regions where malaria is not prevalent or in high altitude environments that cause elevated levels of oxidative stress, G6PD deficiency tends to be a harmful trait as it increases the fragility of red blood cells and causes hemolysis along with disease processes associated with this.

Sickle Cell Disease has similar effects to G6PD Deficiency in its abetment of RBC removal, but the cause of this increased toxicity differs. In Sickle Cell Disease, RBC lysis is accelerated by an atypical hemoglobin morphology rather than the concentration of a

biochemical product[23]. Normal red blood cells have a smooth rounded shape but sickle cells present with an abnormal semi-lunar distortion. The sickle shape is not omnipresent, but manifests only under specific conditions. When oxygen is bound to hemoglobin, the mutated valine is prevented from interacting with residues necessary for altering shape; however, when oxygen is not bound to hemoglobin, interacting residues are left exposed, which cause negative modifications to RBC architecture[23]. Plasmodium infection is thought to interfere with successful oxygen binding to hemoglobin, thus helping to initiate the sickle shape. These sickled cells, containing parasitic organisms are then rapidly removed from the circulation by the spleen and other organs.

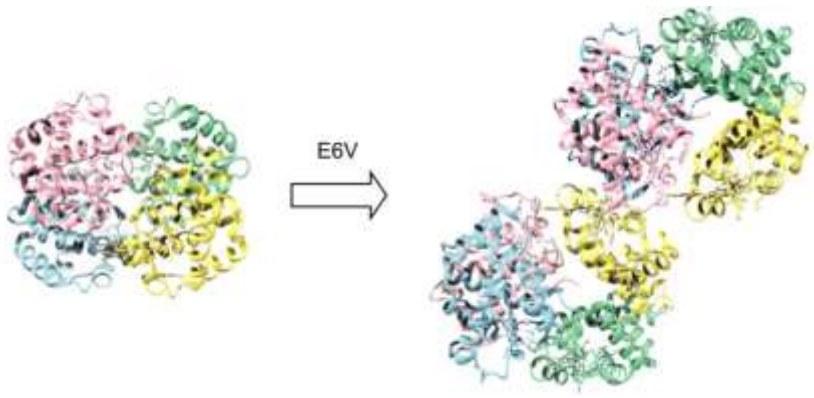

Figure 7: Morphological changes to hemoglobin in response to Sickle Cell mutation[24]

An Ounce of Prevention

Preventative measures such as the use of mosquito bed netting have been met with demonstrable success. A series of 23 experimental trials indicated that the use of insecticide-

treated nets when compared to no net use was responsible for reducing child mortality by 17% with a 44% reduction in cases of severe malaria[25]. However, net acquisition does not necessarily denote compliance, as many recipients report using their nets for alternative purposes and thus, some level of educational reform may be helpful/necessary to impact public awareness[25].

Successful spread of malaria depends on many factors which include a mosquito lifetime, access to blood meal, and range of distribution. Due to all the potential impediments to transmission, Sir Ronald Ross accurately determined that in order to eliminate malarial infection one did not have to eradicate the entire Anopheles population, but only reduce mosquito number to a critical threshold[13]. This idea formed the large-scale eradication movement in the 1950s, which resulted in the large-scale use of insecticides. This application showed tremendous success in regions such as South Asia, but the efforts ultimately failed due to deficient funding as well as rising concerns about the safety of many of the pesticides in use[13].

DDT has also been used extensively in the latter half of the 20th century as a very effective insecticide for mosquitos; however, it's use expanded greatly to other insects and was heavily used in agriculture before becoming restricted in recent years due to a variety of concerns[26]. The most concerning effect of DDT is its implications in both teratogenicity and carcinogenicity as well as its' environmental toxicity, which include damage to surrounding foliage as well as non-specific effects on innocuous insects, and wildlife[26]. Rachel Carson, in her influential book *Silent Spring*, famously targeted DDT as an agent that caused irrevocable harm to nature, arguing for its elimination. Newer pesticides have since been more tightly

regulated in their production and their use, and few have shown efficacy at mosquito elimination comparable to DDT[27].

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