Loosening Malaria's Deadly Grip:

Increased phagocytosis of *Plasmodium falciparum* infected red blood cells by sickle-cell haemoglobin.

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Malaria is a major global health problem, caused mainly by *Plasmodium falciparum* parasites. Immunity to malaria is only acquired after continued exposure to the parasite, but even then, the immunity is only partial. The individuals living in high endemic areas can experience symptomatic or asymptomatic infections. The latter can be caused by various blood conditions, including having the sickle-cell trait. This is a condition where individuals have one normal haemoglobin and one abnormal sickle haemoglobin gene. This abnormal haemoglobin has inhibitory mechanisms that protect an individual from developing severe malaria, with symptoms like seizures and ending possibly in death. The fact that sickle-cell carriers are protected against malaria is well known, however the mechanisms that cause this protection are not very well understood. This review, therefore, focusses on two main processes with which sickle-cell carriers are protected from severe malaria. The first is the increased sickling of *Plasmodium* infected red blood cells and the second is the decreased adherence of infected red blood cells to endothelial cells. Both mechanisms seem to increase the phagocytosis of infected red blood cells by the spleen and consequently reducing the risk of developing severe malaria. These findings are important to further the understanding of the mechanisms underlying the protection of sickle-cell carriers against malaria.

Introduction

Malaria is a life-threatening disease caused by *Plasmodium* parasites that are transmitted to humans through the bite of mosquitoes. It is a global health problem, causing many deaths each year.[1] There are five *Plasmodium* species, *Plasmodium falciparum* (Pf), *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi*. Of these species, Pf causes most of the morbidity and mortality, mostly in sub-Saharan Africa.[2] Malaria predominantly occurs in the regions around the equator. In 2018 there were 288 million cases of malaria worldwide, with an estimated 405,000 people dying of severe malaria.[3] Malaria patients, when experiencing symptoms, often show signs of fever, headaches and tiredness. The symptoms start mild and are difficult to recognise as malaria. If not treated quickly, Pf malaria can progress to more severe symptoms including jaundice, seizures, coma and eventually death. The first symptoms appear approximately 10 days after the parasite is introduced into the body. With correct medication the patient is usually symptom free within two weeks. If the disease is not properly treated, the symptoms may re-occur later.[2]

Malaria is transmitted through the bite of mosquitoes. The transmission and incidence of malaria depends on factors affecting the Pf parasite and the mosquitoes and varies from place to place. There are areas where the transmission and incidence of disease is high, low or presents as a recurring short epidemic. An area with high incidence of disease is also referred to as a high endemic area. Temperature, relative humidity and rainfall are some of the main factors that affect the transmission of malaria.[4]

In high endemic regions malaria is especially dangerous to pregnant women and children. This is mostly due to their reduced immunity. In pregnant women a malaria infection can lead to complications like low birth weight, prematurity and increased perinatal mortality.[5] It is likely that the earliest a child can get infected with a malaria parasite is around six months after birth. Around this time the maternal antibodies begin to cease, leaving the child with a weaker immune system. Children under five years old have a high risk of developing severe malaria with symptoms like cerebral malaria and anaemia.[2] A pregnant woman, when infected, can also pass the parasite to her unborn child via the placenta, this is called congenital malaria.[6]

There are several medications on the market that prevent malaria and are mostly used by travellers visiting high endemic areas. It is recommended to use artemisinin-combination medication for anyone that visits these areas. When correctly treated, people with malaria can recover completely. However, this is not always possible due to the expensive medication. Especially in high endemic regions, where malaria has a negative impact on the economic development due to increased healthcare costs, lost ability to work and a decrease in tourism.[1,2]

There have been copious studies showing that different people may respond in various ways to an infectious disease. Depending on their genetic makeup, people have a different risk of dying when they encounter a parasitic organism, malaria is no exception here.[7] There are various genetic red blood cell disorders that may provide resistance to malaria, preventing the infected individual from dying from the disease. These genetic disorders include thalassaemia, glucose-6-phosphate dehydrogenase deficiency and sickle-cell trait.[8]

A sickle-cell carrier has inherited the sickle-cell gene from one of their parents. It is a condition that does not induce illness and has been believed to be protective against severe malaria.[9] Sickle-cell carriers, however, when exposed to extreme conditions can experience similar symptoms as patients with sickle-cell anaemia. Although, the protective effect of the sickle-cell gene against malaria is well known, the exact mechanisms of protection are still unclear. There are various biochemical and immune-mediated mechanisms that have been proposed, and it is likely that multiple complex mechanisms are responsible for the observed protection.[10]

Malaria parasites cause ailment by infecting red blood cells. It is therefore not surprising that if the red blood cells are abnormal, like in sickle-cell carriers, this might influence the success rate of the parasite as they induce conditions that make the red blood cells change shape.[7] In individuals with two normal haemoglobins the parasite easily infects the red blood cells and takes over the biology of the host cell. The parasite changes the morphology and modifies it to make the infected red blood cell (iRBC) adhesive. *Plasmodium falciparum* erythrocyte membrane protein 1 has been shown to be an important part in defining the adhesive properties of an iRBC. When infected the cells can adhere to both neighbouring non-infected red blood cells and to the endothelial cells of the blood vessels.[11] The mechanism by which sickle-cell carriers are protected against malaria is only partly understood. It is suggested that the mechanism for the advantage of sickle-cell carriers is not the inability of malaria parasites to invade red blood cells. As sickle-cell carriers present a decreased chance of developing severe malaria, however, they do still get infected. They often have decreased amounts of iRBCs in their blood vessels and are less likely to develop cerebral malaria or malaria with severe anaemia.[12] Other mechanisms that have been suggested to be protective against malaria include increased sickling and decreased cytoadhesion of iRBCs. The protection of sickle-cell carriers seems to be specific to Pf and does not apply to the other types of malaria parasite.[13] However, a better understanding of these mechanisms is needed to improve the current knowledge of the host-parasite relationship, including the role of the host immune system in protection against malaria.

This review explores the main findings from the research on the mechanisms surrounding the resistance of sickle-cell carriers to severe malaria and shows the current gaps in the knowledge of the process of malaria resistance.

Malaria

Malaria is caused by a parasite of the genus *Plasmodium*. Malaria is transmitted through the bite of an infected insect, usually a female *Anopheles* mosquito.[2] The mosquito is infected by the parasite when she feeds on the blood of an infected individual.[14] Of the five *Plasmodium* species, a protozoan parasite, Pf causes the most deaths and is the most prevalent parasite, other parasites like *Plasmodium vivax, Plasmodium ovale,* and *Plasmodium malariae* also cause malaria, however, with much milder symptoms. *Plasmodium knowlesi* hardly causes any illness in humans.[2]

Life cycle

The life cycle of Pf in humans is complex and tightly regulated. The female mosquito transfers a few (10-15) Pf sporozoites from her salivary glands to the person she is feeding on. The sporozoites travel via the draining lymph nodes, to the blood.[15] The sporozoites are also able to go through tissue and travel via the bloodstream to the liver.[2] In the liver the sporozoites infect the hepatocytes, the main parenchymal tissue of the liver, and proliferate, making thousands of merozoites.[2] This is the asymptomatic stage. Following this, the parasites lyse the hepatocytes and enter the bloodstream, the merozoites invade the red blood cells and digest the haemoglobins and oxygen, to use later as energy for replication.[2] When they have used all the haemoglobin in the cell, they lyse the red blood cell and quickly invade another red blood cell, replicating exponentially. In this blood stage, the symptomatic stage, the symptoms start to appear. Although not completely understood, it is known that in this stage female and male gametocytes form. These gametocytes circulating in the bloodstream are then taken up by the next female mosquito while she takes blood (Figure 1).[2]

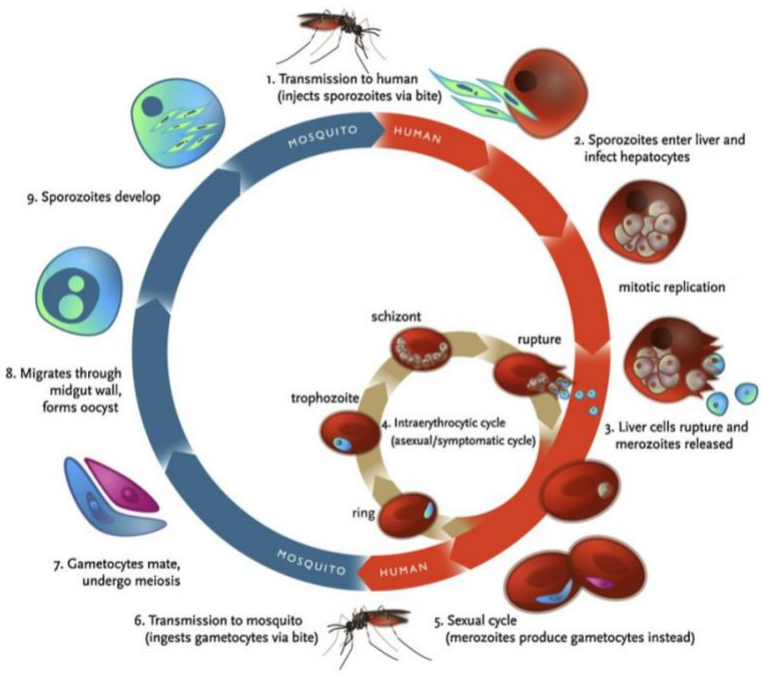

Figure 1. The life cycle of the Pf parasite. Showing the interaction of the parasite with the red blood cells, creating Infected red blood cells (iRBCs).[16]

Transmission

As stated above, malaria is transmitted through the bite of a female mosquitoes, most often between dusk and dawn. They lay their eggs in still standing water, which hatch into larvae and eventually turn into mosquitoes. Each species of mosquito has its own preferred habitat, for example the *Anopheles* prefers shallow fresh water, like puddles or water collected in hoof prints. High endemic areas are often characterised by specific climatic conditions, as that affects the number and survival rate of mosquitoes. These conditions are the temperature, relative humidity and rainfall. Transmission is increased in regions where the lifespan of mosquitoes is longer, so the Pf parasite has time to complete its development inside the mosquito, and where mosquitoes prefer to bite humans rather than other animals.[17]

Maximum and minimum temperatures have an effect on the life cycle of Pf parasites. Below 18°C and above 40°C, the life cycle of Pf in the mosquito body is limited. The time required for the parasite to complete its development in the gut of the mosquito is around 10 days. When the temperature decreases, the number of days necessary to complete the development of the Pf parasite increases. An increasing temperature causes the time of the parasites to complete their development to decrease, with 27°C being the optimum temperature. Malaria transmission in areas colder than 18°C, however, still occur, as the mosquitoes often live in houses which tend to be warmer than the outside temperature.[18,19]

The survival of mosquitoes is greatly influenced by relative humidity. The relative humidity is the amount of air versus the amount of water in the air and the air temperature and the maximum amount of water the air can hold, presented as a percentage. Mosquitoes survive better and become more active under conditions of high humidity greater than 60%. When the humidity is below 60% the lifespan of the mosquitoes is too short to transmit the disease.[18,20]

As the mosquitoes breed in shallow water, the right amount of rainfall is important.[21] Therefore, malaria transmission is often highest following the rainy season. However, there are also regions where less rainfall and drought can favour mosquito breeding and malaria transmission. These areas are often covered in vegetation with flowing streams and rivers. So when there is less or no rain the flow of the streams and rivers is interrupted and stagnant water is left in which the mosquitoes can breed.[22]

Diagnosis

In order to effectively manage malaria, quick and accurate diagnosis is necessary, especially in high endemic areas where diagnostic expertise is often lacking. The diagnosis of a malaria infection is challenging as it depends on various factors. These factors include the different forms of the malaria species, the different stages of infection, immunity and more. These factors influence the identification and interpretation of malaria parasitaemia in a diagnostic test. Clinical diagnosis is least expensive and is based on the patient's signs, symptoms and physical findings. The earliest symptoms are very non-specific and differ per individual. These symptoms often include dizziness, fever and headaches.[23] Because of the non-specific nature of the symptoms it is quite hard to make a diagnosis. The symptoms are similar to other common, and potentially life-threatening. Malaria is, when possible, best diagnosed using microscopic examinations of the blood, blood films or antigen-based rapid diagnostic tests. There are PCR methods to detect the DNA of the parasite, but, due to their complexity and

costs they are rarely used in malaria endemic regions. Quick and convenient rapid diagnostic tests are currently used in many remote settings, however, they are expensive and in need of quality control.[2]

Cytoadhesion

The Pf parasite modifies the red blood cell in order to evade the immune system. In normal occasions, the non-infected elastic erythrocytes pass through the vascular system with the blood flow. Malaria iRBCs on the other hand acquire adhesive properties, become stiff and sequester in the microvasculature to avoid passage through and destruction by the spleen.[24] This cytoadhesive behaviour of iRBCs can be explained by *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) on the surface of the iRBCs.[25] PfEMP1 is a major surface antigen, exposed by the parasite, that mediates antibody-dependent immune response.[26] This response clears the majority of infected cells from the circulation.[27] However, iRBCs use PfEMP1 to bind to various endothelial cell surface receptors, this is called cytoadhesion. The cytoadhesive properties induced by PfEMP1 make sure that iRBCs can sequester in deep tissues by binding to blood vessel walls. This cytoadhesion of iRBCs to endothelial cells is a method of Pf parasites to evade the elimination by the spleen and at the same time complete its cell cycle, multiply and re-invade non-infected red blood cells.[11] One of these endothelial cell receptors is endothelial protein C receptor (EPCR). This receptor is expressed on leukocytes and endothelial cells. The EPCR pathway has anti-inflammatory and anti-thrombotic effects that protect endothelial cells, upholding vascular integrity. The binding of iRBCs to endothelial cells via PfEMP1 to EPCR is linked to severe malaria.[28] The spleen uses macrophages to phagocytose foreign microbes that have invaded the body.[29] The cytoadhesion of iRBCs also causes activation of endothelial cells by proinflammatory mediators like tumour necrosis factor alpha (TNF-α). Various studies have shown that macrophages and dendritic cells release TNF-α after coming into contact with iRBCs aggravating the disease.[29] The cytoadherence of iRBCs can result in blockage of small blood vessels and impaired tissue perfusion.[24] If the iRBCs adhere to endothelial cells in the vessels that provide blood to the brain it can cause cerebral malaria, manifesting as swelling of the brain or brain damages and eventually seizures and coma.[30] In addition, the Pf parasite can switch quickly between antigenic variants of PfEMP1 during infection. This enables the parasite to cytoadhere in order to evade immune attacks and to maintain long-term chronic infections.[26] These complications can be life threatening, however they are lessened in sickle-cell trait carriers.[31]

Prevention

There are various prevention methods that reduce the risk of getting bitten by mosquitoes, using mosquito nets, insect repellents or mosquito control. The latter is mostly focussed on draining standing water, as this can become a breeding ground for mosquitoes.[2] When travelling to a high endemic area, travellers are advised to use antimalaria medication. This medication is often a combination of artemisinin with another antimalarial drug. By combining two active ingredients with different mechanisms of action, combination therapies are the most effective antimalaria medications available at the moment and cause the least resistance. There are various artemisinin-based combination therapies that the WHO recommends using, based on the results of therapeutic efficacy studies against the local strains of Pf. The combination of artemisinin with lumefantrine is the most often recommended.[14] Artemisinin in itself kills parasites by causing damage to necessary proteins

and by inhibiting the proteasome activity of the parasite. This results in the accumulation of proteasome substrates, which activates the stress response of the body and the parasites are killed.[32] Lumefantrine is active against erythrocytic stages of Pf. Lumefantrine also has a longer half-life compared to artemisinin and is believed to clear residual parasites that artemisinin has not been able to get rid of.[33] In high endemic regions it is recommended that a malaria infection is established before the start of the treatment, because of possible drug resistance. The chloroquine-resistant Pf, a Pf parasite that has developed resistance against the cheap antimalarial medication chloroquine, has become a huge problem in parts in Southeast Asia and Africa. Chloroquine has previously been one of the main medications used against malaria.[2]

In high endemic areas there are often multiple forms of malaria resistance. Some of which are unique, and others are very general. For example, the resistance against malaria in S and C alleles from the β-globin chain are caused by different changes at the same codon. On the other hand, there are many changes that modify levels of expression and provide resistance against malaria, like G6PD, α-/β-thalassaemia and sickle-cell trait. The latter is often identified as a major malaria resistance factor. Despite this, the exact mechanisms that cause the resistance are still unknown.[34] Further in this review two main mechanisms of the protection of sickle-cell carriers against malaria will be discussed.

Sickle-cell trait

Haemoglobins are proteins inside red blood cells that bind to the oxygen from the lungs and with the aid of red blood cells carry this oxygen to other parts in the body.[35] Sickle haemoglobins can alter red blood cells into a crescent shape. This distortion can cause red blood cells to be broken down prematurely, leading to anaemia. Anaemia can cause shortness of breath, fatigue and delayed development. Painful episodes can happen when the stiff and inflexible red blood cells get stuck in capillaries, depriving organs of oxygen, leading to organ damage.[36] The sickle-cell trait (HbAS) occurs by the inheritance of one normal haemoglobin (HbA) gene and one abnormal sickle haemoglobin (HbS) gene. Individuals that have two abnormal HbS genes develop sickle-cell anaemia.[35] Individuals carrying one HbS do not normally suffer from the sickling of their red blood cells and are asymptomatic. They have around 40% HbS in their normal biconcave red blood cells.[37] HbS is different from wild-type haemoglobin by a single amino acid substitution of glutamic acid to valine. This substitution occurs in the β-globin chains.[38]

It is estimated that around 300 million people worldwide are sickle-cell carriers. The highest concentration (1 in 3 carriers) is seen in Africa, especially in the high endemic malaria regions.[39] In Western countries the prevalence is much lower, around 1 in 500.[40] This frequency might increase due to the migration of people moving from high endemic regions to Western countries.[41]

Diagnosis

Sickle-cell trait is most often diagnosed with a blood test. In infants the fingertip or heel is pricked, for adults a blood sample is drawn from a vein in the arm. These blood tests are generally done as a routine in new-borns. In the laboratory the presence and amount of HbS in the sample is determined or mutations in the genes are detected in order to diagnose the correct condition. The blood of individuals with a HbS gene is analysed more thoroughly when

requested and can allow intervention before any possibly affected organs can sustain severe damage.[42]

Polymerisation

The shape of the red blood cells of sickle-cell carriers depends on the rate of deoxygenation and the concentration of HbS in the blood. In the presence of oxygen HbS does not polymerise.[43] A HbS polymer is a rope-like fibre that initially grows alongside other HbS fibres, interacting with the surrounding cellular environment of the red blood cells. Upon deoxygenation HbS undergoes polymerisation and the valine of one HbS molecule connects to the alanine, phenylalanine and leucine of another nearby HbS molecule.[44] The amino acid substitution to valine causes a change of the surface of HbA and HbS from hydrophilic to hydrophobic, this results in the HbS to associate with other HbS and HbA molecules rather than with the cellular environment. The connected fibres form one massive fibrous polymer. When a HbS loses an oxygen molecule a hydrophobic patch appears, on both Hb and HbS, that valine interacts with resulting in distortion. Oxygen cannot efficiently bind to the HbS fibres, so the sickled red blood cell carries less oxygen compared to red blood cells with normal HbA.[45] The fibre connections stretch and deform the red blood cells, resulting in a distortion of the now less flexible red blood cells into a crescent or sickled shape and alters their intercellular viscosity. This sickled shape interferes with the function of the cells.[46,47] The rigidness of sickled red blood cells make it difficult to get through capillaries, slowing down or stopping the blood flow. This results in deprived organs and can cause inflammation and damage to major organs, like the brain. Sickled red blood are disposed of by the spleen much quicker than normal red blood cells, which can cause anaemia (Figure 2).[48]

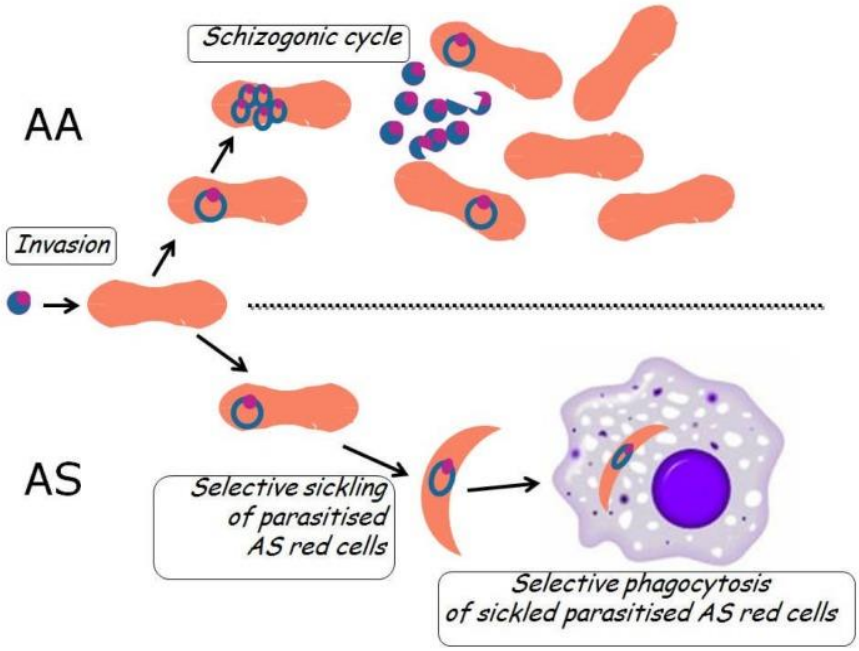

Figure 2. Sickling of iRBCs of normal individuals and sickle-cell carriers. The upper part of the figure shows the malaria infection of red blood cells in a normal person. The lower part of the figure shows the malaria infection of red blood cells in a sickle-cell carrier undergoing sickling. Falling prey to macrophages in the spleen and in other organs. Phagocytosis of iRBCs interrupts the life cycle of the Pf parasite, which keeps the parasitaemia under control.[7]

The sickle-cell trait has been believed to be a benign. Unlike sickle-cell anaemia, sickle-cell trait does not usually cause blockage in small blood vessels and the patients therefore do not

experience the painful bouts that are typical for sickle-cell anaemia.[9] However, sickle-cell carriers can develop the same symptoms as sickle-cell anaemia patients if they are exposed to certain conditions.[41] These conditions, like dehydration, severe tissue hypoxia and hypothermia increase the polymerisation of HbS and consequently the sickling of red blood cells.[49]

Although, the sickle-cell trait can cause complications, the prognosis of individuals carrying one sickle-cell gene is promising. The average life expectancy is the same as the general population, while the survival of individuals with sickle-cell anaemia is reduced or do not survive early childhood.[50]

The effect of HbS on malaria infection

Although sickle-cell carriers have a significant advantage over people with normal Hb in their blood cells they are not resistant to getting infected by the parasite and even have a similar prevalence. When it comes to a malaria infection, individuals with the sickle-cell trait are able to inhibit the parasite from causing the symptoms that are often associated with a malaria infection. This is especially the case with the more severe symptoms, like seizures, coma and death.[7] Asymptomatic individuals are less likely to seek treatment for the infection. This allows the parasites to linger longer in the blood stream and it could be an explanation as to why the prevalence of malaria is equal in people with and without the sickle-cell trait, despite a decrease in severity of the infection in sickle-cell carriers.[51]

There are various mechanisms that might explain why having one altered haemoglobin gene protects sickle-cell carriers from developing severe malaria. The most recent studies mainly focus on two processes, the increased sickling of iRBCs and the impaired cytoadherence of iRBCs. Both these mechanisms decrease the chance of red blood cells and iRBCs clustering and blocking the blood flow in smaller blood vessels that lead to important organs. The reduced manifestation of vascular obstruction also lessens the chances of developing severe malaria.

Increased sickling

After the malaria parasites have entered the red blood cells, they use the oxygen that is stored in the cells. The oxygen consumption becomes, at some stage in the life cycle of the parasite, large enough so that the oxygen tension in the individual iRBCs is decreased to below the critical level.[52] As previously mentioned, hypoxia can lead to sickling of red blood cells that contain HbS.[49] Therefore, this decrease of oxygen tension can lead to sickling of the iRBCs. At this stage the sickling is still reversible, but when the cell is in its sickled state it is highly susceptible to phagocytosis.[52] The sickled cells have an increased expression of opsonins. Opsonins are antibodies that bind to foreign microorganisms or cells making them more susceptible to phagocytosis, an example of an opsonin is IgG.[53] Normally the surface receptors are hidden, so normal red blood cells do not get removed. The exact mechanism by which these receptors are exposed is still not fully understood.[54] They are also present on damaged red blood cells, signalling for them to be destroid.[55] When IgG binds to a sickled iRBC it attracts macrophages and binds to them, thus sticking the iRBC to the macrophage. This gives the macrophage the opportunity to start phagocytosis.[53] As each cell is removed by phagocytosis, the parasite is also disposed of and its life cycle is abruptly terminated.[52]

The sickling of iRBCs, due to the parasitic oxygen consumption, results in an increase in opsonisation and clearance by the spleen. This seems to lead to increased and improved antigen presentation and earlier development of acquired immunity to a malaria infection.[56] The enhanced phagocytosis also results in a decreased parasitaemia compared to that in people without the sickle-cell trait or sickle-cell disease. These individuals do not develop sickled iRBCs and therefore do not have an increased expression of antigens. Subsequently, the macrophages do not get extra signals but remove the iRBCs just like any other damaged red blood cell.[53]

Another study showed that the humoral immune system of sickle-cell carriers is directed at surface receptors of iRBCs specifically, as high levels of IgG only recognised and bound to PfEMP1 and not to any other parasite antigens.[13]

Because the red blood cells of individuals with sickle-cell disease sickle even faster than those of sickle-cell carriers, due to the higher amount of HbS in the red blood cells, this advantage should also apply to them. However, this is not the case, the high number of sickled cells increase the possibility of obstruction in capillaries, like the iRBCs that cytoadhere to endothelial cells. These two events increase the chance of blocking the blood flow to the brain, resulting in cerebral malaria, causing seizures, coma, and possibly death.[57]

Inhibited cytoadherence

The decrease in oxygen tension in iRBCs does not only cause an increase of opsonisation but it also results in a decrease of adherence to endothelial cells. The parasites occupying red blood cells containing HbS intensify the oxidative stress the cells already suffer from.[58] When HbS comes into contact with oxygen it results in the formation of highly reactive oxidants. These oxidants hinder various physiological processes of the red blood cell, including membrane stability and viscoelasticity.[59] The aggravated oxidative stress has a big influence in the formation of membrane protrusions on iRBCs, in which PfEMP1 is presented. These protrusions are called knobs.[60] iRBCs have less but larger knobs and the crescent shape of the cells causes stretched out and stiffened membrane, loss of surface area[61], and decreased cell volume. The membrane of iRBCs containing Hb also stiffens but not to the same extent.[62]

Because iRBCs have less knobs, the amount of PfEMP1 is also decreased. As there is less expression of PfEMP1 on the surface of the iRBCs they have a reduced ability to adhere to the endothelial cells in the blood vessels.[63] Knobs collect adhesin molecules, like PfEMP1, and bring them to the cell surface.[64] Knobs on the surface of iRBCs of sickle-cell carriers have been shown to have an impaired interaction with the membrane of the iRBCs and with the mechanisms that is involved in moving adhesins to the cell surface.[60] The altered knobs on iRBCs cause the cells to roll faster over the endothelial cells resulting in reduced contact time and area. The sickled iRBCs, therefore, do not have enough time to adhere to the endothelial cells. Due to the decreased cytoadherence there is less obstruction of capillaries, reducing the chance of developing cerebral malaria.[63]

The changed cytoadherence behaviour leads to decreased endothelial cell activation. Activated endothelial cells stimulate the expression of surface receptors that bind to opsonins, resulting in cytoadherence, which in turn leads to vascular obstruction. Endothelial cells are activated through cell-to-cell contact[29] and depends on contact time. The iRBCs are

the main activators of endothelial cells, however, now due to their altered shape and knob formation, the cells have less contact with the endothelial cells.[65]

The binding of iRBCs to endothelial cells is not the only way iRBCs can cause obstruction in capillaries. IRBCs also bind to non-infected red blood cells to form cell clusters, this is called rosetting.[66] Rosetting also contributes to vascular blockage. Both cytoadhesion and rosetting, in a lesser way, are regulated by PfEMP1. Comparable to the decreased cytoadherence the formation of rosettes, the iRBCs-red blood cell clusters, is also reduced.[63] Rosette formation is also mediated by rosettins, which are protein ligands on iRBCs that bind to carbohydrate receptors on the non-infected red blood cells.[67] For sickle-cell carriers these receptors are less accessible due to the distortion and stiffening of the iRBCs which decreased binding. The capacity of rosetting is dependent on a high enough oxygen pressure in the red blood cells, which is often lacking in infected sickle-cell carriers.[68]

Conclusion

Malaria, especially severe malaria, is a dangerous infectious disease and can cause a number of different symptoms from fever to seizures and possibly death. There are various blood conditions that show some form of protection against the infection. With a high prevalence in high endemic malaria regions, sickle-cell trait is one of the more researched conditions with regard to malaria. Although the HbS in sickle-cell trait on their own are not something remarkable, when put together with a malaria infection they show traits that inhibit parasites from aggravating the symptoms. Both methods, increasing sickling and decreasing cytoadherence, subsequently increase the phagocytosis of iRBCs by the spleen.

So, these studies have shown that both biochemical and immune processes promote the protection against a malaria infection in sickle-cell carriers. However, these mechanisms are still not fully understood, more research is needed in order to get a step closer to understanding the mechanisms in more detail. This review did not focus on the combination of various blood conditions together, and whether they amplify or counteract each other. One study reported that, when α-thalassaemia and sickle-cell trait were combined, the α-thalassaemia inhibited the protective mechanisms of the sickle-cell haemoglobins.[69] It would be interesting to see if there are other combinations like this. Another interesting concept is the increased risk of pregnant women and children acquiring a malaria infection and in what way they are affected by the infection when they are sickle-cell carriers. Does it only protect the pregnant women, or also the unborn child from the symptoms? Are children that have the sickle-cell trait more protected against developing severe malaria, is this something that is exclusive to adults or are children even better protected? These are all questions that have not been answered yet and can provide more insight in the processes of HbS and malaria parasites.

Although there are still some unanswered questions, these current findings will undeniably improve the knowledge underlying the protection mechanisms of HbS and detailed descriptions can offer new perspectives on treatment options or a possible cure for malaria, a devastating disease that continues to inflict tremendous medical, social and economic burdens to a large proportion of the human population.

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