Microreview

Complement receptor 1 and malaria

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Summary

*Plasmodium falciparum* malaria is an intracellular parasite that is transmitted by *Anopheles* mosquitoes. It is responsible for approximately 1 million deaths per year. Most deaths occur as a result of complications such as severe anaemia or cerebral malaria (coma). The complement receptor 1 is a key complement regulator found on the surface of red cells and most leucocytes. A growing body of evidence suggests that this molecule plays a critical role in the pathogenesis of *P. falciparum* malaria. Initial reports showed that CR1 enables the binding of infected red cells to uninfected red cells to form rosettes, which can potentially obstruct small capillaries. However, further evidence suggests that CR1 is also important in the control of complement activation and immune complex formation during malaria infection. Most recently, CR1 has also been shown to be a receptor for the invasion of red cells by the parasite. Its polymorphic nature almost certainly has allowed the selection of variants that have helped human-kind survive the scourge of malaria. The finding of conflicting reports about the exact role of these variants in severe disease underlies the complexity of the parasite–host interactions and highlights the need for further studies.

Introduction

*Plasmodium falciparum* is a unicellular organism transmitted by *Anopheles* mosquitoes. It is responsible for close to 1 million deaths per year. Most deaths occur in children in sub-Saharan Africa as a result of complications such as severe malarial anaemia (SMA) or cerebral malaria (CM).

The life cycle of the parasite is complex. Sporozoites are inoculated by the infected female *Anopheles* mosquito and they travel to the liver where they invade hepatocytes. Inside the hepatocytes the sporozoites divide into thousands of merozoites, which burst out into the circulation 2–3 weeks later. Merozoites are able to invade red cells and multiply asexually into more merozoites, which are then able to infect more red cells constituting the blood stage cycle, which is responsible for all the morbidity and mortality from the disease. Some newly infected red cells develop into gametocytes, which when taken up by the mosquito undergo sexual reproduction in its midgut leading to production of more sporozoites, which migrate to the salivary gland completing the life cycle.

The pathogenesis of severe malaria is poorly understood. Mounting evidence suggests that the complement receptor 1 (CR1) plays a critical role in the pathogenesis of SMA and CM. However, the exact mechanisms remain to be determined. In addition, recent studies suggest that CR1 is an alternate receptor for the invasion of red cells by *P. falciparum*. In this review, I present the data supporting the role of CR1 in the pathogenesis of malaria and discuss its potential mechanisms.

CR1 and the complement system

The complement system is part of the innate immune response and consists of a complex cascade of molecular steps that have as their central event the activation of complement factor 3 (C3) into C3b (Zipfel and Skerka, 2009). C3b contains a thioester group that can react with any membrane or bacterial surface (Gros *et al.*, 2008). Coating of a cell with C3b, also referred to as opsonization, places that cell at risk for destruction when it encounters macrophages while circulating through the spleen or liver. There are three widely recognized pathways for complement activation (Gros *et al.*, 2008): The classical pathway that depends on the binding of C1q to closely apposed antibodies; the mannose binding lectin pathway that depends on the binding of mannose binding lectin to mannose containing surfaces; and the alternative pathway which depends on the hydrolysis of C3 which is stabilized by activated factor B. Each of these pathways leads to the formation of C3 convertases that cleave C3 into C3b and C3a. In addition to binding to surfaces, C3b...
can couple to C3 convertases leading to the formation of C5 convertases, which cleave C5 into C5a and C5b. The latter initiates the cascade of polymerization that gives rise to formation of the terminal complement complex (TCC). When inserted into a cell membrane, the TCC can lead to lysis of the target cell.

Host cells have a number of surface proteins that prevent the activation of C3 and formation of the TCC. These include decay accelerating factor (DAF/CD55), membrane cofactor protein (MCP/CD46), membrane inhibitor of reactive lysis (MIRL/CD59), and the complement receptor 1 (CR1/CD35) (Zipfel and Skerka, 2009). CR1, CD55 and MCP have similar organization and together they are known as the regulators of complement activation. Their genes reside in a single cluster on chromosome 1 (Rey-Campos et al., 1988). They accelerate the decay of C3 and C5 convertases (CR1 and DAF) (Iida and Nussenzweig, 1981), and serve as cofactors in the Factor I-mediated inactivation of C4b and C3b to C4bi and C3bi (CR1 and MCP) (Medof et al., 1982; Medof and Nussenzweig, 1984). MIRL is found on chromosome 11 and has a unique structure (Bickmore et al., 1993). It is the only terminal regulator working to prevent the assembly of the TCC by blocking the addition of C9.

CR1 has a number of features that are unique as a complement regulator. It has a molecular weight of average MW 200 kD (Fearon, 1985). The prototypical molecule consists of 30 short consensus repeats (SCRs) organized in four long homologous regions (LHRs) A–D, each composed of seven SCRs. There are two distinct functional domains each composed of three SCRs (vertical hatched boxes): site 1 in LHR-A (SCRs 1–3) binds mainly C4b and has convertase decay accelerating activity, and two virtually identical copies of site 2 in LHR-B (SCRs 8–10) and LHR-C (SCRs 15–17) that bind C3b and C4b, as well as P1MP-1, and possess Factor I cofactor activity. Functional differences in sites 1 and 2 are determined by amino acid sequence differences. Boxes with the same hatching pattern reflect near amino acid identity of the SCRs. SCR 25 (stippled box) carries the Knops blood group antigens McC, Sl and Knp. The SCR repeats are followed by a transmembrane domain (TM) and a cytoplasmic tail (CYT).

CR1 polymorphisms

CR1 has size, quantitative and sequence polymorphisms. First described were size polymorphisms that are due to deletions or duplications of LHRs that perhaps take place during unequal crossover events (Holers et al., 1987; Vik and Wong, 1993). Four different size variants (CR1*1—4) have been identified ranging in MW from 190 to 250 kD (Nickells et al., 1986; Moulds et al., 1998). The number of molecules present on a red cell varies and is regulated by putative co-dominant alleles L (low expression) and H (high expression), which give rise to low expressors (LL), intermediate expressors (HL) and high expressors (HH) (Wilson et al., 1982). In Caucasians, but not in Africans, the quantitative polymorphism has been linked to the presence of a Hind III restriction site in intron 27 (Wilson et al., 1986; Wong et al., 1989; Herrera et al., 1998; Rowe et al., 2002), which in turn has been linked to several other substitutions in the coding nucleotide sequence (Table 1) (Xiang et al., 1999).
CR1 carries the antigens for the Knops blood group system Knops (Kn<sup>ab</sup>), McCoy (McC<sup>ab</sup>), Swain-Langley (Sl<sup>1/2</sup>) and York (Yk<sup>+</sup>) (Moulds et al., 1991; Rao et al., 1991). The McC, Sl and Knp antigens were later localized to SCR 25 of LHR-D and linked to single nucleotide substitutions resulting in amino acid substitutions K1590E, R1601G and V1561M respectively (Moulds et al., 2001; 2004) (Table 1) (Fig. 1). The McC<sup>ab</sup> and Sl<sup>2</sup> antigens are common among Africans but uncommon among Caucasians (Moulds et al., 2000), suggesting that they are under some selection pressure. Further work determined that there was a second polymorphic site where some anti-Sl antibodies bound that resulted from a S1610T amino acid substitution further downstream from the original site in SCR 25 (Moulds et al., 2002). The presence of R1601 and S1610 gives rise to the conformational epitope Sl<sup>3</sup> (Moulds et al., 2002). A new antigen in the Knops system was recognized by the discovery of an antibody against I1615 produced by a person, who was homozygous for 1615V and was named KAM+. (Moulds, 2005). Most recently, the Yk antigen was localized to SCR22/23 and linked to a T1408M amino acid substitution (Veldhuisen et al., 2011).

In addition, a number of other polymorphisms have been identified within and outside complement binding sites. Polymorphisms within complement binding sites include I643T and a Q981H substitutions in SCR 10 and 16 respectively (Birmingham et al., 2003) and R483C substitutions in SCR 8 (Birmingham et al., 2007). The Q981H substitution was noted to result in increased binding for C4b (Birmingham et al., 2003). Interestingly, although the Q981H substitution is uncommon in Africans and Caucasians, it is very common in Asians (Thomas et al., 2005), again suggesting that selection pressures are at play here. The R483C substitution has a carrier frequency of 6.3% among African Americans compared with 2.6% carrier frequency among Caucasians and shows lower affinity for C3b and C4b (Table 1) (Birmingham et al., 2007).

### CR1 and severe malarial anaemia

Severe malarial anaemia is one of the most dreaded complications of *P. falciparum* infection. It is usually seen in children less than 5 years of age living under the most intense transmission conditions in Africa (Snow et al., 1997; Marsh and Snow, 1999). Several observations suggest that the destruction of red cells cannot simply be accounted by the direct effect of the parasite. A mathematical model has shown that an average of 8.5 uninfected red cells are destroyed for every parasitized red cell (Jakeman et al., 1999). In a prospective study the proportion of red cell mass lost attributable to the parasite was calculated as 7.9% of the total lost (Price et al., 2001). Additionally, patients treated for *P. falciparum* malaria continue to experience red cell destruction after treatment (Camacho et al., 1998). These data indicate that there are alternative mechanisms for the destruction of red cells that are not directly related to the parasite.

### Table 1 Most commonly studied CR1 polymorphisms.

| Nucleotide substitution | Amino acid substitution | Location | Phenotype | References |
|-------------------------|-------------------------|----------|-----------|------------|
| A/T                     | None                    | Intron 27| H/L       | Wilson et al. (1986); Wong et al. (1989) |
| C1597T                  | R483C                   | SCR 8    | Reduced binding of C3b and C4b | Birmingham et al. (2007) |
| G3093T*                 | Q981H                   | SCR 16, Exon 19 | H/L, Increased binding of C4b | Xiang et al. (1999); Birmingham et al. (2003) |
| T2078C*                 | I643T                   | SCR 10/11, Exon 13 | H/L | Xiang et al. (1999); Birmingham et al. (2003) |
| C5507G*                 | P1827R                  | SCR 28, Exon 33 | H/L | Herrera et al. (1998); Xiang et al. (1999) |
| A3650G*                 | H1208R                  | SCR 19, Exon 22 | H/L | Xiang et al. (1999) |
| A4870G*                 | I1615V                  | SCR 25, Exon 29 | H/L, KAM± | Xiang et al. (1999); Moulds (2005) |
| C5654T                  | T1876I                  | SCR 29, Exon 34 | Unknown | Xiang et al. (1999) |
| A1360G†                 | T445A                   | SCR 7, Exon 9 | H/L partial | Xiang et al. (1999) |
| A4795G                  | K1590E                  | SCR 25, Exon 29 | McC<sup>ab</sup> | Moulds et al. (2001) |
| A4828G                  | R1601G                  | SCR 25, Exon 29 | Sl<sup>1/2</sup>, Sl<sup>2</sup> reduced rosetting | Moulds et al. (2001) |
| A4708G                  | V1561M                  | SCR 25, Exon 29 | Kn<sup>ab</sup> | Moulds et al. (2004) |
| A4828A/A4855            | R1601/S1610             | SCR 25, Exon 29 | Sl<sup>3</sup> | Moulds et al. (2002) |
| C4223T                  | T1408M                  | SCR 22/23 | Yk<sup>ab</sup> | Veldhuisen et al. (2011) |

*These alleles have good genotypic concordance with the Hind III polymorphism in intron 27 in both Caucasians and Africans but only good phenotypic concordance in Caucasians.
†These alleles have partial concordance with the Hind III polymorphism in intron 27 among Caucasians and poor concordance among individuals of African descent.
H/L, high or low expressor among Caucasians but not among individuals of African descent.
Given that there is sufficient evidence to suggest that uninfected red cells are destroyed, researchers have sought for potential mechanisms. Knowing that C3b and IgG deposition leading to erythrophagocytosis are important mechanisms of red cell destruction during normal cell senescence (Lutz et al., 1990; Bratosin et al., 1998), researchers have looked for evidence of the role of these molecules in red cell destruction during malaria infection. Work by Facer and collaborators documented deposition of C3d, a breakdown product of C3b, and IgG on red cells of patients with malaria although there wasn’t a good correlation with the level of anaemia (Facer, 1980a, b, 1979). More recent work has confirmed that C3 and IgG deposition are commonly found on red cells of children with SMA (Waitumbi et al., 2000; Goka et al., 2001; Helegbe et al., 2007; Owuor et al., 2007).

In order to explain complement deposition on red cells of children with SMA the integrity of the complement regulatory proteins on these red cells has been examined. Using cytofluorometry, a number of studies have documented that red cells of children with SMA have lower levels of CR1 and CD55 than those of children with uncomplicated malaria or who are uninfected (Waitumbi et al., 2000, 2004; Stoute et al., 2003). Furthermore, this partial deficiency is not found in other types of severe malaria such as CM (Owuor et al., 2007). The deficiency in complement regulatory proteins does not appear to have a genetic basis since long-term follow-up of these patients after treatment showed normalization of CR1 and CD55 levels (Stoute et al., 2003). Deficiency in red cell CR1 in patients with malaria has been found by other groups (Ansar et al., 2009; Sinha et al., 2009). However, in one study there was no correlation between the CR1 level and severe disease, perhaps because there were few patients with SMA (Sinha et al., 2009).

Since children with SMA are generally younger than children with CM, other studies explored the possibility that there is age-dependent fluctuation in the expression of red cell complement regulatory proteins. Cross-sectional studies showed a biphasic distribution in the levels of red cell CR1 and CD55 with age, being high at birth and reaching a nadir between 6 and 24 months, the age period of greatest susceptibility to malaria due to absence of immunity and during which most cases of SMA are seen (Waitumbi et al., 2004). A subsequent study showed that C3 deposition on red cells peaked at the age when red cell complement regulatory proteins are at its lowest and that children with malaria in this age range had the highest level of C3 deposition on their red cells (Odhiambo et al., 2008).

The relationship between red cell CR1 level and SMA has been investigated in several studies by means of genotyping. In The Gambia, the Hind III polymorphism in intron 27 was used as a surrogate marker for CR1 level based on a previous report that it correlated with the CR1 level (Wilson et al., 1986). No relationship was found between it and SMA (Bellamy et al., 1998), which is not surprising given that we now know that that polymorphism does not correlate with red cell CR1 levels in Africans (Herrera et al., 1998; Rowe et al., 2002). In Papua New Guinea the A3650G polymorphism in exon 22 (Xiang et al., 1999) was used to study the relationship between CR1 expression level and severe malaria (Cockburn et al., 2004). Paradoxically, only the HL genotype was associated with protection against SMA. The reason for this association is unclear since most individuals in the study region had very low CR1 levels and there was no statistical difference in CR1 levels between HH and HL genotypes. Since the polymorphism studied is known to be in linkage disequilibrium with other polymorphisms that lead to amino acid substitutions, it is likely that the findings reflect more of a functional than a quantitative difference in CR1 level.

Most recently, Sinha and colleagues studied the association between red cell CR1 levels and disease in endemic and non-endemic regions of India using both direct measurement of CR1 and polymorphisms in intron 27 and exon 22 (Sinha et al., 2009). Overall, CR1 levels were very low and uninfected controls in the malaria endemic region had even lower CR1. This result was similar to that of a previous study comparing red cell CR1 levels in an endemic and non-endemic area of Kenya (Waitumbi et al., 2004). These results suggest that chronic exposure to malaria can result in lower CR1. However, alternative or contributing factors include differences in the genetic background or nutritional status of the populations. In the same study (Sinha et al., 2009) individuals with severe malaria in the non-endemic region tended to have low red cell CR1 levels whereas in the endemic region there was no difference in the CR1 levels between severe and non-severe cases and yet, uninfected controls had significantly lower CR1. The lack of stratification of the types of severe malaria in this study limits the interpretation of these findings. Nonetheless, the fact that there were a higher proportion of SMA cases in the non-endemic area is consistent with the postulate that low red cell CR1 levels are a risk factor for this complication.

The mechanism for the loss of CR1 and CD55 during malaria infection is unclear. However, it is well known that malaria infection results in the formation of ICs (Jhaveri et al., 1997; Stoute et al., 2003; Mibe et al., 2005; 2008). It is likely that CR1 is lost as part of its role in the IC removal mechanism (Sherwood and Virella, 1986; Davies et al., 1990; Reinagel et al., 1997; Taylor et al., 1997). It has been proposed that individuals with low red cell CR1 as a result of repeated malaria infections or who have low CR1 due to physiologic changes in expression with age
are at high risk for SMA due to IC formation and complement activation during malaria infection (Stoute et al., 2003; Stoute, 2005). In the setting of increased ICs, red cells with low CR1 may be more susceptible to C3b deposition and removal of erythrocytes in the liver leading to severe anaemia.

In addition to exploring the relationship between CR1 levels and SMA, evidence for the role of CR1 in the pathogenesis of SMA has been sought by studying the association between several polymorphisms and the risk of severe disease. Two studies, one in The Gambia and one in Kenya, explored the relationship between Knops Sl and McC polymorphisms that occur in LHR-D (Fig. 1) and severe malaria (Zimmerman et al., 2003; Thathy et al., 2005). Neither study found an association between these polymorphisms and SMA. However, they differed in their findings with respect to CM (see below).

**CR1 and cerebral malaria**

CM is another complication of *P. falciparum* infection that claims many lives, especially in Africa and Southeast Asia. Epidemiologically and clinically, CM is distinct from SMA. In Africa, CM is most common in children who are older than children with SMA and it tends to occur in areas where transmission is unstable (Snow et al., 1997; Marsh and Snow, 1999). For instance, in one study in western Kenya children with SMA had a mean age of 16.9 months
compared with 32.2 months for children with CM (Owuor et al., 2007). In Southeast Asia, where immunity develops slowly, if ever, the risk of CM increases with age (Luxemburger et al., 1997). The pathognomonic mark of CM is the finding of cerebral capillaries obstructed by sequestered infected red cells that are adhered to endothelial cells. However, about one-third of individuals who die of CM lack this finding perhaps due to misdiagnosis (Dorovini-Zis et al., 2011). Two schools of thought prevail, one that considers sequestration the primary event in the pathogenesis of CM and another that considers CM the result of the production of pro-inflammatory cytokines and immune mediators that can interfere with neurotransmission (Clark and Alleva, 2009). In this review, I will concentrate on the potential role of CR1 in the process of capillary obstruction.

One of the virulent factors of *P. falciparum* that is easily measurable is its ability to form rosettes, which are formed by the adherence of late stage trophozoites to uninfected red cells. Rosetting has been associated with most forms of severe malaria (Carlson et al., 1990; Rowe et al., 1995; Doumbo et al., 2009), but mechanistically its potential role in the development of CM is easiest to understand. It is important to point out that despite the interest that rosetting has received, to this date, there is no evidence that it actually occurs *in vivo* outside the laboratory and in some studies there has been no association between rosetting and severe malaria (Al Yaman et al., 1995).

The first indication that CR1 may be involved in the pathogenesis of severe malaria came as a result of the observations that it is the rosetting receptor on uninfected red cells and that red cells of the Knops *S*/*2* phenotype rosette less (Rowe et al., 1997). Since *S*/*2* and *McC* alleles are much more common in Africans than in Caucasians, it was hypothesized that these alleles may have arisen as a result of selection pressures by the malaria parasite. Working in The Gambia, Zimmerman and co-workers found no association between these polymorphisms and SMA or CM (Zimmerman et al., 2003). On the other hand, a study in Kenya revealed an association between *S*/*2*/*2* genotype and protection against CM (Thathy et al., 2005). The contradictory findings between these two studies may have been due to differences in study design or true differences in the epidemiology or genetic make-up of the populations under investigation. Nonetheless, based on the role of CR1 in rosetting and the protection conferred by the *S*/*2* allele it is possible that red cells with high CR1 can plug brain capillaries. ICs carried by these red cells could also interact with monocytes and endothelial cells leading to production of local inflammatory mediators, which can cross the blood brain barrier (Stoute, 2005) (Fig. 2).

Other groups have studied the association between CR1 expression level and CM. In Thailand, the LL genotype based on the intron 27 Hind III polymorphism was associated with severe malaria (Nagayasu et al., 2001). This is counterintuitive since lower levels of CR1 should result in lower risk of CM based on the rosetting hypothesis. A recent study in Thailand of 17 different polymorphisms identified one in the promoter region of CR1 that was associated with protection against CM and increased red cell CR1 (Teeranaipong et al., 2008). Thus, in Thailand it seems unlikely that rosetting plays a role in the mechanism of CM. If increased CR1 level is protective and low levels of CR1 are not, this seems to point to a complement and/or IC-mediated phenomenon having a role in CM.

In India, the association between CR1 and CM seems to be totally different from Thailand. In the hyperendemic region of Orissa increased red cell CR1 level, as determined by genotyping of the intron 27 Hind III allele, the exon 22 A3650G, and the exon 33 C5507G, was associated with CM (Rout et al., 2011). As a whole, these studies demonstrate the complexity of the interactions between CR1 and malaria and underlie the need for further studies.

**CR1 and red cell invasion**

The invasion of red cells by *P. falciparum* is a complex multistep process that involves multiple ligands on the merozoite surface and multiple red cell receptors. Through inhibition studies and use of mutant red cells it has been shown that glycophorins and their sialic acid residues are the major receptors for the invasion of red cells by *P. falciparum* (Deas and Lee, 1981; Pasvol and Jungery, 1983; Friedman et al., 1984; Perkins, 1984; Hadley et al., 1987). However, treatment of red cells with neuraminidase, which removes sialic acid greatly, reduces the invasion of some strains by 80% or more while others are able to invade albeit with diminished efficiency (Mitchell et al., 1986). Strains that are greatly affected by removal of sialic acid are referred to as sialic acid-dependent whereas strains that are not are referred to as sialic acid-independent. Dolan and co-workers postulated the existence of a sialic acid-independent receptor that is trypsin sensitive and referred to it as the ‘X’ receptor (Dolan et al., 1994). Further studies showed that the sialic acid-independent ligand expressed by the parasite is the *P. falciparum* reticulocyte binding homologue 4 (PFH4) (Stubbs et al., 2005; Gaur et al., 2006; Tham et al., 2009). Based on similarities of protease sensitivity patterns between CR1 and the ‘X’ receptor Stoute and colleagues demonstrated that CR1 is the ‘X’ receptor, initially with the use of laboratory strains (Spadafora et al., 2010) and later with wild isolates (Awandare et al., 2011). Their finding was further confirmed by the demonstration...
that PfRh4 interacts with CR1 (Tham et al., 2010). A recent study also proposed that binding site for PfRh4 resides in SCR1-3 (Tham et al., 2011).

Despite the above findings the role of CR1 in invasion is not very clear. Attempts at inhibiting CR1-mediated invasion in intact red cells with antibodies against CR1 and soluble CR1 (sCR1) only result in 20–30% inhibition (Awandare et al., 2011). In addition, very high concentrations of antibody against PfRh4 are required to inhibit sialic acid-independent invasion (Tham et al., 2009). It has been postulated that the use of ligands by the parasites proceeds in a hierarchical manner (Baum et al., 2005) and hence, certain receptor-ligand pairs such as CR1-PfRh4 would only be utilized if the host is making antibodies that block other pathways or if other receptors are missing due to mutations (Persson et al., 2008). However, a number of studies have documented the lack of association between red cell CR1 level and \textit{P. falciparum} infection (Soares et al., 2008; Lin et al., 2010). Further studies are needed to understand the role of CR1 in the invasion of red cells by \textit{P. falciparum}. For now, we can add CR1 to the repertoire of receptors that is known to mediate red cell invasion (Fig. 2).

Conclusion

CR1 is a highly versatile molecule that appears to play a critical role at different levels in the interaction between \textit{P. falciparum} and its host. The finding of conflicting data between studies on different populations reflects the complexity of parasite–host interactions and underlies the need for more studies to elucidate the role that this important molecule plays in the pathogenesis of malaria.

Acknowledgements

Work in my laboratory has been supported by the US Military Infectious Diseases Research Program and a grant from the National Institute of Heart, Lung, and Blood (RO1 HL 7502–04).

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