Review Article

Complement receptor 1 and the molecular pathogenesis of malaria

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Malaria is a pathogenic infection caused by protozoa of the genus plasmodium. It is mainly confined to sub-Saharan Africa, Asia and South America. This disease claims the life of over 1.5 to 2.7 million people per year. Owing to such a high incidence of malarial infections, there is an urgent need for the development of suitable vaccines. For the development of ideal vaccines, it is essential to understand the molecular mechanisms of malarial pathogenesis and the factors that lead to malaria infection. Genetic factors have been proposed to play an important role in malarial pathogenesis. Complement receptor 1 (CR1) is an important host red blood cell protein involved in interaction with malarial parasite. Various polymorphic forms of CR1 have been found to be involved in conferring protection or increasing susceptibility to malaria infections. Low-density allele (L) of CR1 gave contradictory results in different set of studies. In addition, Knops polymorphic forms Sl (a^+) and McC (a) have been found to contribute more towards the occurrence of cerebral malaria in malaria endemic regions compared to individuals with Sl (a^-) / McC (a/b) genotype. This article reviews the research currently going on in this area and throws light on as yet unresolved mysteries of the role of CR1 in malarial pathogenesis.

Key words: Complement receptor 1, Knops polymorphism, *Plasmodium falciparum* erythrocyte membrane protein 1

Introduction

Malaria caused by protozoa of the genus *Plasmodium* is the most important parasitic disease in humans. This disease claims the life of over 1.5 to 2.7 million people per year.[1] Owing to such a high incidence of malarial infections, there is an urgent need for the development of suitable vaccines. Development of vaccines is only possible through the identification of major molecular pathways of pathogenesis and immunity for malarial parasite. Parasite virulence phenotype and host genetic factors are the two major foci of research to understand malarial pathogenesis. *Plasmodium falciparum* virulence phenotype termed as rosetting, causes clumps of erythrocytes and brings about vascular obstruction and impaired tissue perfusion.[2] This property has been associated with severe malaria.[3,4] *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) has been found to be the protein involved in rosetting[5.6] and CR1 is the associated host counterpart.[7]

PfEMP1 is an adhesion protein encoded by the large and diverse var gene family that is involved in clonal antigenic variation and plays a central role in *P. falciparum* pathogenesis.[8,9] The extra-cellular portion of this protein contains several distinct domains, by virtue of which, it interacts with several types of surface molecules like intercellular adhesion molecule (ICAM-1), type A and Type B blood groups, thrombospondin, E-Selectin, chondroitin sulfate, CR1 and CD36. Previously it has been shown that antibodies to PfEMP1 can confer protection to placental malaria.[10] Bull *et al*. showed the existence of rare and prevalent isolates that express a subset of variant surface antigens (PfEMP1) and it was suggested that antibodies to the strain specific PfEMP1 can prevent re-infection with previously encountered isolates.[11.12] Based on these observations, it has been suggested that certain regions of PfEMP1 that are involved in binding with CD36/ circumsporozoite antigen (CSA)/ CR1 may be potential vaccine targets.

CR1 has been shown to play a very important role in malaria pathogenesis. Various polymorphic forms of CR1 have been found to have protective or susceptibility effect in malaria. CR1 exhibits three types of polymorphisms: Density polymorphism (determined by alleles H for high and L for low expression on erythrocyte surface),[13.14] Structural polymorphism (differences in molecular weight ranging from 260-250 Kda, determined by alleles A, B, C

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and D)[13.15.16] and Knops blood group polymorphism.[17]

There have been suggestions that differential expression of this protein on erythrocytes might determine susceptibility of an individual to development of cerebral malaria and severe malaria-associated anemia. Low-density allele of CR1 gave contradictory results in different set of studies.[18.19] Knops polymorphic forms Sl (a^-) and McC (b^+) have been found to be present at increased frequency in malaria endemic regions. In particular, individuals with Sl (a^-) / McC(a/b) genotype were less likely to have cerebral malaria than individuals with Sl (a^+)/ McC(a/a).[20] Because PfEMP1 binds to the active site of CR1, it might inhibit CR1 functions. On the other hand modulating the interaction between CR1 and PfEMP1 somehow, can reduce rosetting- associated severity of malaria. It has been shown that monoclonal antibodies against CR1 can block as well as reverse rosette formation.[21] In order to be able to use CR1- PfEMP1 interaction as a therapeutic target, it is essential to understand the role of CR1 and its polymorphic forms in the patho-physiology of malaria. This article reviews the role of various polymorphic forms of CR1 in malarial pathogenesis.

Etiology of malaria

Malaria is an acute and/or chronic infection caused by protozoans of the genus *Plasmodium.* Four species cause disease in humans namely, *Plasmodium falciparum, Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*. Out of the four species, majority of the infections are caused by the former two species i.e. *P. falciparum* and *P. vivax*.

Characteristics of Plasmodium infection

Plasmodium falciparum causes the most deadly and severe infections.[22,23] It infects all ages of RBCs, leading to a higher parasitemia. Mature trophozoites and schizonts are sequestered in the microvascular system leading to tissue ischemia.[22] A schematic representation of the life cycle of malarial parasite is depicted in Figure 1. The problem of spread of infections becomes all the more acute because of widespread drug resistance in *P. falciparum* infections.[24,25]

Plasmodium vivax usually does not cause life-threatening infections. It only infects reticulocytes and produces hypnozoites, which are latent in the liver. Relapses can occur up to 5 years after infection.[26] The

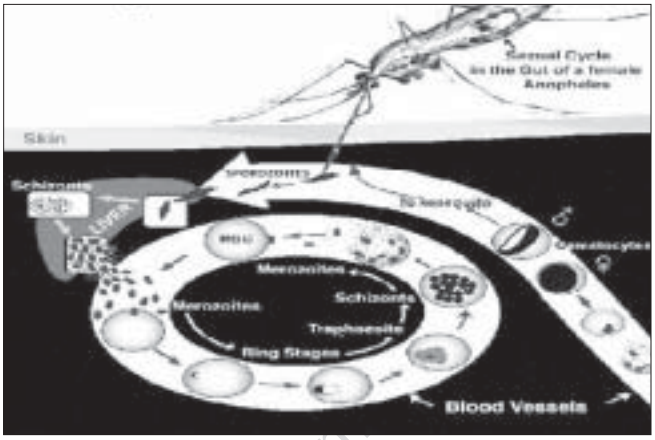

Figure 1: Life cycle of malarial parasite

parasite uses the Duffy blood receptor to enter RBCs; hence Duffy negative individuals are not infected by this species.[27]

Epidemiology of malaria

Malaria was first documented in 1700 B.C.[28] 40% of the world's population lives in areas where there is a risk of acquiring this disease. Worldwide, an estimated 300-500 million people contract malaria each year, resulting in 1.5-2.7 million deaths annually.[29,30] About 90% of these cases are from tropical Africa; remaining cases are reported from Brazil, India and Sri Lanka. India alone contributes 80% of the victims.[31] 0.1 million of these are children below the age of 5 years.[32] In endemic areas, infected children show symptoms between 4 to 8 months of age. Symptoms include fever, irritability, poor feeding, vomiting, diarrhea and convulsions. In most of the cases the infection is limited to recurring episodes of fever, chills and shakes and can be controlled with proper medication.

However in some of the individuals severe malaria can precipitate due to variety of factors including genetic, environmental, socio-economic status of individuals. It has now been realized that treatment or prevention of malaria is only possible through identification of these factors. In particular, greater emphasis is now being laid on identifying the host genetic factors involved in malaria pathogenesis.

Genetic factors in malaria pathogenesis: Host genes involved in malaria

Polymorphic forms of a number of host genes involved in immunity have been associated with protection or

susceptibility to malaria. The most plausible candidates include hemoglobin variants, Duffy blood group antigen variants, glucose-6-phosphate dehydrogenase, histocompatibilty genes, tumor necrosis factor-α, ICAM-1, nitric oxide synthase promoter region (NOS 2).[33] Other candidate genes include CR1, which is involved in erythrocyte rosetting and thrombospondin receptor CD36, which mediates parasite binding to endothelium and also possibly to dendritic cells.[33.34] The candidate genes and their plausible role in malaria pathogenesis are listed in Table 1.

Rosetting of P. falciparum infected erythrocytes

Plasmodium falciparum virulence phenotype is a major area of research these days. This phenotype termed as rosetting[2] causes clumps of cells and brings about

Table 1: The host genes involved in the pathogenesis of malaria

Candidate gene	Plausible affect	Reference	Chromosomal position	Distribution
Duffy antigen/ chemokine receptor (DARC) promoter region	Expression of DARC inhibited on erythrocytes, thereby affecting rosetting and preventing development of severe malaria	57	1q21-q22	Malaria endemic regions particularly Africa
Hemoglobin S	Reduced parasite replication within erythrocytes, enhanced clearance of parasitized erythrocytes and protection against severe malaria	58	11p15.5	Parts of sub-Saharan Africa and Indian subcontinent
Hemoglobin C	Impairment of merozoite release and reduced parasite growth mediated protection against severe malaria	59, 60	11p15.5	West Africa
Hemoglobin E	Preferential phagocytosis of infected cells and parasite growth inhibition to confer protection	61, 62	11p15.5	Southeast Asia
Glucose-6-phosphate dehydrogenase	Deficiency, reduced parasite replication in affected erythrocytes	63	Xq28	Worldwide
HLA-Class I B53 locus	MHC I dependent cytotoxic T cell mechanisms afford immunological protection	64	6p21.3	West Africa
HLA-Class II DQB1 0501 locus	Protection from severe malaria	65	6p21.3	Gabon
HLA-Class II DRB1 1302 locus	Protection from severe malaria	66	6p21.3	Gambia
TNF 308A allele	Increased susceptibility to cerebral malaria due to up-regulated expression of the gene in affected individuals	67, 68	6p21.3	Gambia and Sri Lanka
TNF-376A	Increased susceptibility to cerebral malaria due to up regulated gene expression	69	6p21.3	Gambia and Kenya
iNOS2 -969 promoter	Susceptibility to severe malaria	70	17cen-q11.2	Gambia
NOS2-69546	Protection from re-infection and severe malaria	70	17cen-q11.2	Gabon and Gambia
ICAM-1 (Kilifi allele)	Increased susceptibility	71	19p13.3-p13.2	Kenya
CD36 deficiency allele	Mediates parasite binding to endothelium and, acts to suppress the immune response to parasite antigens, susceptibility to severe malaria	72	7q11.2	Kenya, Gambia
CD36 nonsense mutation	Protection from severe malaria, due to non expression of CD36	73	7q11.2	Kenya

vascular obstruction and impaired tissue perfusion. This property has been associated with severe malaria in many studies in Africa.[3.4] Rosetting is mediated by a protein present on the surface of infected RBCs, PfEMP1.[5.6] *Plasmodium falciparum* secretes protein PfEMP1, which has dual purposes of antigenic variation and cytoadherence.[9] It binds to endothelial receptors (CD36, ICAM-1, CSA on placenta) causing microvascular sequestration, thereby leading to abnormal function of affected tissues [Figure 2].[35,36] Peripheral sequestration also protects *Plasmodium falciparum* from being sequestered in the spleen.

It has been found that rosetting of erythrocytes is associated with the occurrence of severe malaria, subsequently; a role for PfEMP1 in rosetting was suggested.[5.6] CR1 has been found to be the associated host counterpart for mediating the clumping of uninfected erythrocytes.[7]

Complement receptor 1 and rosetting

CR1 is a 200-kDa single chain membrane bound glycoprotein and a member of the regulators of complement activation (RCA) gene cluster. CR1 possesses complex tri and tetra N-linked oligosaccharides in its mature form and the gene for this protein is located on the q32 arm of chromosome 1.[37,38] It is composed of a number of repeated domains called short consensus repeats (SCRs) each of which is composed of 60 amino acids containing four invariant cysteines.[39] The extra-cellular domain of the CR1 is composed of 30 SCRs, the first 28 of which are arranged in tandem repeats in homologous groups of 7, with each group known as long homologous repeat (LHR).[38] SCRs 8-12 and SCRs 15-18 preferentially bind to C3b and SCRs 1-4 preferentially bind to C4b.[38.39]

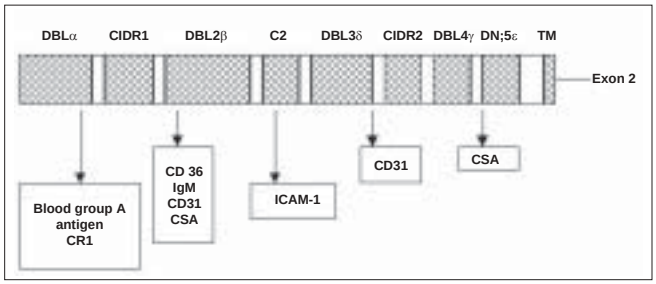

Figure 2: Binding sites for different antigens located on the surface of pFEMP1

DBL - Duffy Binding like, ICAM - Intercellular cell adhesion molecule, CSA - Circumsporozoite antigen, CIDR - Cysteine rich interdomain region, TM - Transmembrane

The region of CR1 that interacts with infected erythrocytes to form rosettes has been mapped to LHR-B and first three SCRs of LHR-C, SCR 10 and 17 have been particularly found to play an important role in this interaction.[37]

Effect of differential CR1 expression on malarial pathogenesis

Erythrocytes infected with *P. falciparum* rosette efficiently with uninfected erythrocytes that have normal copy number of CR1, but not with cells in which the number <60.[7] As the number of CR1 molecules on RBCs increases, so does their propensity to form rosettes.[7] Rosette formation is inhibited by sCR1.[7] Differences in the expression of CR1 on erythrocytes might determine susceptibility of an individual towards development of cerebral malaria and severe malaria-associated anemia. In one of the studies it was suggested that young children may be more susceptible to SMA because of their lower levels of RBC complement regulatory proteins, which make them less equipped to handle IC formation and complement activation.[40] Previously same group of researchers had proved that a decline in levels of CR1 and increase in immune complex levels significantly associates with SMA.[41] The mechanism for the loss of CR1 from the surface of erythrocytes is being investigated. A series of experiments indicated that CR1 present in the form of clusters on RBC surface, undergoes unclustering due to the binding of IgM -C3b complexes to glycophorin A.[42] Unclustering might promote rapid loss of CR1 from the surface of erythrocytes infected with the malaria parasite.

CR1 polymorphisms: Are they determinants of malarial susceptibility?

CR1 is a highly polymorphic glycoprotein. Three different polymorphic forms of CR1 have been identified, namely structural (size variation 160-250 kDa), density (high and low expression on RBCs controlled by alleles H and L) and knops blood group (McC (a^+)/McC (b^+); Sl (a^+)/Sl (a^-); Kna/ Knb).

a) Structural polymorphism: Four different structural polymorphic forms of CR1 are known, namely A, B, C and D (CR1*1, CR1*2, CR1*3, CR1*4) with respective

molecular weights of 190, 220, 160 and 250 kDa (under non-reducing conditions).[15] This polymorphism is regulated by four autosomal co-dominant alleles.[15] A polymorphism in the CR1 transcripts with incremental differences of 1.4 kb in mRNA was present in donors expressing the various polymorphic forms. This difference corresponds to the size of one LHR and 40 kDa difference, seen among allotypic forms of CR1. Therefore on the basis of this observation it was suggested that the insertion or deletion forms the basis of structural polymorphism.[16] Analysis of restriction fragment length polymorphism (RFLP) suggested that intragenic duplication rather than alternate mRNA splicing is responsible for the allotypic differences.[13] Various studies have proved that structural polymorphism of CR1 occurs at different frequencies in distinct populations. Van Dyne *et al*. had reported the gene frequencies for A and B alleles to be 0.87 and 0.12 for whites, 0.74 and 0.22 for blacks and 0.98 and 0.02 for Orientals.[43] Moulds *et al*. reported the gene frequencies for A and B alleles to be 0.82 and 0.11 for African-Americans based in Houston and 0.87 and 0.11 for Caucasians based in Houston.[44] Dykman *et al*. in 1984 had reported the gene frequencies to be 0.83 and 0.16 for A and B alleles respectively.[45] In Indian subjects the percentage distribution of AA, AB and BB structural phenotypes had been previously found to be 94.9%, 5.1% and 0% respectively with relative gene frequencies of 0.975 for A allele and 0.02 for B allele, homozygous BB phenotype had not been identified in this study.[46] In one of our previous studies, we found that out of 117 normal subjects, 82.9% had AA pattern, 16.24% were AB and 0.85% had the BB genotype. The gene frequencies for A and B alleles were found to be 0.911 and 0.088 respectively.[47] Although the gene frequencies for different structural alleles of CR1 differ for distinct populations, but on the whole allele A is the most prevalent in all populations.

b) Density polymorphism: Second type of polymorphism is a Hind III RFLP, which in Caucasians but not in Africans, correlates with CR1 copy number on erythrocytes.[13] Homozygotes for the L (low expression) allele usually express fewer than 200 copies of CR1, homozygotes for the H (high expression) allele express several times this number and heterozygotes are intermediate.[13] This polymorphism arises due to a single base change in the intron of d1d2 segment within the LHR-D (Long homologous repeat) region resulting in the generation of a polymorphic Hind III site within this region.[14] The gene frequencies for H and L alleles of CR1 have been found to be different in different populations. Mitchell *et al* had shown the gene frequencies to be 0.75 and 0.25 for healthy controls.[48] Wilson *et al.* found the percentage distribution for HH, HL and LL density polymorphic genotypes to be 65%%, 25% and 10% respectively in case of normal individuals.[49] Moldenhauer *et al.* had also reported gene frequencies of 0.27 and 0.73 for the L and H allele respectively.[50] Study on the percentage distribution of HH, HL and LL phenotypes in Indian subjects had shown the distribution to be 63%, 29% and 8% respectively. Gene frequencies for H and L alleles in this study were 0.77 and 0.23 respectively. HH had been found to be the predominant form in this study.[51] Genotypic frequencies of HH, HL and LL forms have also been studied in the malaria endemic and non-endemic groups in different populations. In non-endemic Caucasian and Choctaw population groups in USA, the gene frequencies for H and L alleles were found to be 0.82, 0.18 and 0.84, 0.16 respectively. In endemic Black Africans the gene frequencies for H and L alleles were 0.85 and 0.15; in S. Chinese-Taiwanese 0.71 and 0.29; in Pacific Asians 0.42 and 0.58 and in Cambodians 0.53 and 0.47 respectively.[52] Previously we also conducted a study to ascertain the distribution of H and L alleles in 117 normal healthy Indian individuals. In our study, gene frequencies for H and L alleles were 0.53, 0.47 respectively.[47] This indicates that the genotypic frequencies observed for Indian population is similar to that of Pacific Asians and Cambodian population.

c) Knops polymorphism: The third type of polymorphism represented by Knops blood group system is of particular interest.[53] In this system, Mc^a and Mc^b is one allelic antigen pair and Sl^a and Vil is another pair. The corresponding phenotypes for the first pair are McC (a+) and McC (b+) and for the second pair are Sl (a+) and Sl (a-).[53] Studies have now established the molecular basis for Knops polymorphism. These antigens have been localized on the LHR-D segment of CR1. Single nucleotide polymorphisms occurring in SCR 25, which lead to amino acid substitutions, result in generation of these polymorphic forms [Figure 3].[17] Population based studies have been carried out to determine the distribution of different types of Knops polymorphic

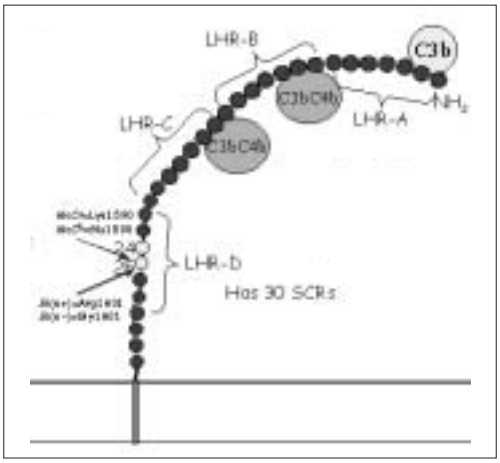

Figure 3: Location of Knops antigen system on complement receptor 1 molecule

Sl - Swain Langley blood group, McC - McCoy blood group, LHR - Long homologous repeat, SCR - Short consensus repeat

forms in different populations. The gene frequencies for Sl (a+) and Sl (a-) in African American persons are almost equal (0.48 vs. 0.52) wheras Sl (a-) is greatly increased in Africa.[17] On the contrary, Sl (a-) is almost completely absent in Caucasian and Asian persons.[54] Data from India however, is still not available on this aspect of CR1 polymorphism and we are investigating the distribution of Knops polymorphic forms of CR1 in the Indian population.

Out of the three polymorphic forms, size polymorphism has not been found to play a role in determining susceptibility to severe malaria. With regard to density polymorphism, some studies suggest that low-density allele confers protection against malaria[18] whereas another[19] suggested that low- density allele might be a risk factor for severe forms of malaria. Erythrocytes with low CR1 expression (because of the homozygous LL genotype of CR1) have been shown to form reduced number of rosettes with *Plasmodium falciparum* infected cells.[55] In Africa, rosetting has been shown to correlate with disease severity.[3] Since erythrocytes having low CR1 form fewer rosettes, it has been postulated that low E-CR1 might protect from severe malaria.

Recently, Cockburn *et al.* suggested that homozygous form of L allele confers protection against severe malaria possibly due to reduced rosetting mediated micro vascular obstruction.[18] On the contrary Nagayasu *et al.* had observed a significantly higher frequency of the LL genotype in the severe malaria group compared to the uncomplicated malaria group. They reasoned that the presence of circulating immune complexes in the *P. falciparum* infected individuals contributes to pathogenesis. The authors had suggested that the rate of clearance of immune complexes is thought to be related to the number of CR1 molecules on erythrocytes. Therefore, decreased expression of CR1 (due to L allele) may contribute to the pathogenesis of severe malaria through the deposition of immune complexes and resulting tissue damage.[19] Therefore the role of L allele in the pathogenesis of severe malaria still remains controversial.

Knops polymorphic forms Sl (a^-) and McC (b^+) have been found to be present at increased frequency in malaria endemic regions[17] and hence suggested to be protective against rosetting and severe malaria. The role of these polymorphic forms in affecting the rosette formation has also been investigated.[18.53] Knops phenotype Sl (a^-) has been found to rosette less efficiently than Sl(a+) and hence suggested to be more protective.[53] Because of less rosetting of uninfected Sl(a^-) with infected erythrocytes, there might be less parasite sequestration in the microvasculature in such individuals and consequently protection against obstruction of blood flow. Recently it was found that children with genotype Sl (a^-) are less likely to have cerebral malaria, than those children with Sl (a+). In particular, individuals with Sl (a^-) / McC(a/b) genotype were less likely to have cerebral malaria than individuals with Sl (a^+)/ McC(a/a). This observation led the researchers to hypothesize that the Sl (a^-) allele and, possibly, the McCb allele evolved in the context of malaria transmission and that in certain combinations probably confer a survival advantage on these populations[20] [Table 2].

DNA sequence comparison of CR1 revealed that single amino acid variations in SCR 25 of LHR D are responsible for different knops phenotypes.[56] It has also been found that it is the SCR 8, 9, 10 and SCR 15, 16 and 17 of LHR B and LHR C, which interact directly with PfEMP1.[2] SCR 25 has not been experimentally been shown to be involved in direct interaction with PfEMP1.

The molecular mechanism for the exact role of Sl(a-) and McCb alleles in conferring protection against malaria has still not been worked out. Various possible explanations have however been given, first being the bringing together of SCR 25 in the vicinity of SCR 8, 9, 10 and/or SCR 15, 16, 17 because of secondary and tertiary folding

Table 2: Complement receptor 1 polymorphisms associated with protection or susceptibility to malaria

Polymorphic forms of complement receptor 1 (Chromosomal map position- 1q32)	Plausible affect	Reference
LL	Risk factor for development of severe malaria	19
	Reduced rosetting, protective against malarial infection	18
Sl (a^-)	Reduced rosetting capability of erythrocytes, increased protection against severe malaria	53, 17
Sl (a+)	Increased rosetting and clumping of erythrocytes, development of severe malaria	53
McC (b^+)	Protective against malaria	17
Sl (a^-) / McC(a/b)	Protective against cerebral malaria	74

of CR1 protein. Second explanation that has been given is that changes in SCR 25 might affect binding of C1q or mannan binding lectin to LHR-D and possibly these proteins might be interacting with PfEMP1. Another hypothesis that has been given is the role of SCR 25 in the CR1 clustering which is the process required for clearance of immune complexes by erythrocytes.[53]

Discussion

Many unsolved mysteries still remain. One of these is the potential role of Mc (b+) and Sl (a-) in conferring protection against malaria. PfEMP1 binds to the active site of CR1, it might therefore inhibit CR1 functions i.e. ligand binding, cofactor activity or that of the decay-accelerating activity. This in-turn could lead to excessive complement activation and hence more of inflammation. On the other hand, the CR1-PfEMP1 interaction could be a potential therapeutic target. Development of a vaccine against PfEMP1 may prevent some cases of severe malaria. Monoclonal antibodies can be developed, that can reverse rosette formation in patients of SMA. However, in order to achieve any of these desired targets, it is absolutely essential to get a more thorough understanding of the interaction of CR1 with PfEMP1 and the role of CR1 polymorphisms in malarial pathogenesis.

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