Anti-disease therapy for malaria – 'resistance proof'?

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Abstract:

Antimalarial drugs have in the past fallen prey to resistance and this problem is likely to continue in the future. One approach to developing drugs that might be less prone to resistance might be to target the disease rather than the parasite itself. The rationale for this idea, which has been somewhat developed in antibacterial chemotherapy, is that drugs that can alleviate disease pathogenesis while not compromising the survival, growth or transmission of the pathogen should not exert selective pressure that would encourage the emergence and spread of resistance. This review considers (concentrating on possible interventions at the parasite level) whether such 'anti-disease' therapy could be developed for severe *Plasmodium falciparum* malaria, and if so whether it might be less prone to resistance. Several anti-adhesive treatments, aiming to reduce the tissue sequestration of *P. falciparum*-parasitised erythrocytes that is associated with cerebral malaria and other complications, have been investigated as 'adjunctive' therapies. These therapies are however unlikely to be 'resistance proof' because sequestration appears to enhance parasite survival in the host. Severe malarial anaemia is another potentially fatal complication of malaria that results not only from lysis of host erythrocytes by intracellular parasites but to a greater extent from lysis of unparasitised erythrocytes and impaired erythropoiesis. The possibility of therapy interfering with the last of these processes, which may be more 'resistance proof', is discussed in detail.

INTRODUCTION

Malaria therapy has been compromised repeatedly through the years by the emergence of resistant parasites [1-3]. This has necessitated the development of new drugs to replace those lost to resistance. If those new drugs in turn lose their effectiveness within a few years, their development will only have allowed a short 'breathing space' rather than a long-term solution. Furthermore, the investment made in developing the drugs may not be recouped by a short period of therapeutic use. Hence the need for drugs less prone to resistance [4].

One approach that might offer new drugs that are relatively or even completely 'resistance-proof' is the targeting of pathogenic mechanisms in malaria rather than the malarial parasites themselves. Such approaches have been discussed for many years and in some cases shown to have merit in experimental systems and even in clinical trials. The issue of likely resistance emergence has not however been much addressed. In this short essay, we shall briefly review the major features and mechanisms of malaria pathogenesis before asking whether modulators of any of these mechanisms might be expected to be less prone to resistance. Following this general discussion we shall focus on one pathophysiological feature contributing to severe malaria, namely inefficient erythropoiesis, and the prospect that modulators of the mechanisms causing this effect might be less prone to resistance than conventional antimalarial drugs.

It is clear that the pathogenesis of malaria is dependent on both parasite and host factors as well as geographic and social ones [5]. Here we shall concentrate mainly on possible interventions at the parasite level but host targets are also of considerable interest, especially as they are outside the genetic control of the parasite [6].

LESSONS FROM BACTERIA

Before proceeding to review the pathogenesis of malaria, which is caused by apicomplexan protozoa of the genus *Plasmodium*, it may be useful to refer to another group of pathogens for which the concept of anti-disease therapy and its possible 'resistance to resistance' is better developed. Bacterial pathogens have a variety of virulence mechanisms, some of which could potentially be the subject of therapeutic intervention. These include delivery and function of toxins, expression of virulence-related genes, quorum sensing, adhesion to host tissues and biofilm formation [7, 8]. One perceived advantage of targeting such virulence mechanisms is reduced killing of the normal bacterial flora, which is increasingly recognized as crucial to health [9]; this is not as far as we know relevant to malaria. Another potential advantage is reduced selection pressure leading to slower emergence of resistance. This advantage is as yet unproven and would only be expected where the intervention did not lead to reduced survival, fitness, or spread to new hosts of the pathogen. In some instances this appears to be a realistic prospect. For example some bacterial pathogens – *Clostridium botulinum, C. tetani, Bacillus anthracis, Corynebacterium diphtheriae, Vibrio cholerae* and *Shigella* spp. – produce toxins that can be partly or wholly responsible for pathogenesis [7]. Some of these appear not to offer any advantage to the organism. Interference with the action of these toxins, their production by the bacterium or delivery to the host (e.g. secretion) might therefore exert little or no selective pressure. An example of an experimental

toxin-targeted therapy is the small molecule virstatin, which inhibits expression of the ToxT transcription factor required for production of the toxin and toxin-coregulated pilus in *V. cholerae* [10]. Tetanus and diphtheria toxoids are the basis of current vaccination for these diseases. In other cases there may be a selective advantage for organisms with the targeted phenotype, in which case interventions targeting it would be expected to exert selective pressure. Some types of adhesion to host surfaces might come into this category. There has been considerable interest in bacterial adhesion blockers, for example pyridone-based compounds that inhibit assembly of type-1 and P pili of *Escherichia coli* [11]. An important issue therefore, which also applies to malaria, is to have knowledge of the significance of the virulence factor or other disease-causing feature not only to the host but also to the pathogen expressing it.

Possible disadvantages of anti-disease therapies for bacterial infections have also been discussed. One that might apply to malaria as well is the difficulty in determining 'susceptibility' to an anti-disease therapy [8]. How would one predict the likely effectiveness of the therapy in a given population or geographic area without an accessible susceptibility test (in which the pathogen is incubated in different concentrations of drug and then scored for growth and/or survival) to apply to the relevant clinical isolates? Another disadvantage that may be of relevance to malaria is the possible need for long-term treatment to keep the infecting organism in a non-pathogenic state if it is not quickly eliminated [9]. Would clinicians be satisfied with a drug that did not clear parasites from the body of the patient? None of the proposed virulence-targeting antibacterial therapies has yet reached clinical use so there is no proven precedent that can be exploited for malaria.

PATHOGENESIS OF MALARIA

Human malaria is caused by any one of five species of *Plasmodium: P. falciparum, P. vivax, P. ovale, P. malariae* or *P. knowlesi.* Many other *Plasmodium* species cause malaria in various vertebrates and some of these, especially rodent parasites such as *P. berghei*, are used as models for the study of pathogenesis and treatment *in vivo.* The classical clinical picture of uncomplicated malaria involves high fever with malaise, chills, sweats, headache, fatigue, vomiting and/or other 'flu-like' symptoms [12]. The symptoms usually appear in waves (paroxysms) whose periodicity reflects the duration of the asexual, intra-erythrocytic cycle of the particular species: namely 24 hours for *P. knowlesi*, 72 hours for *P. malariae* and 48 hours for the other species. This periodicity comes from the largely synchronous growth of the parasite population in the host, resulting in synchronized release of merozoites (the form that invades erythrocytes [RBC]) and parasite waste products from the broken RBC at the end of the asexual cycle.

While most malaria cases are uncomplicated, interest has focussed on the pathogenesis of severe malaria, which can have a mortality rate of 15–20% and leave even the patients who recover with serious long-term sequelae [13]. The vast majority of severe malaria, and malarial mortality, is caused by *P. falciparum.* The clinical profile can include severe anaemia, shock, impaired consciousness, convulsions, long-term neurological deficits, respiratory distress, metabolic acidosis, pulmonary oedema, hypoglycaemia, renal failure, and disseminated intravascular coagulation [14, 15]. In addition pregnant women can experience placental

infection, low birth weight and foetal loss. Severe malaria is a complex multi-system disorder with a variety of contributing mechanisms [14]. In general three overlapping syndromes are however recognized: severe anaemia, cerebral malaria and metabolic acidosis.

Severe anaemia (<5 g/dl haemoglobin or <0.15 haematocrit with $>10^4$ parasites/μl of blood according to World Health Organisation [WHO] criteria) occurs most often in areas of high malaria transmission and is especially common in young children and pregnant women [16]. It can be associated with long-term infection of low parasitaemia as well as with the higher parasitaemias that are often a feature of acute *P. falciparum* malaria [15]. Severe malarial anaemia is complex and multifactorial (**Fig. (1)**). It results not only from loss of RBC due to parasite release or immune-mediated destruction of parasitised RBC (pRBC), but also to destruction of unparasitised RBC due to clearance in the spleen, by macrophages and by immune lysis [16, 17]. Destruction of unparasitised RBC appears to be caused by oxidative damage, inflammation and parasite products and may exceed destruction of pRBC by up to 12-fold [18]. Aggravating this condition is ineffective erythropoiesis [16, 19]. Bone marrow abnormalities and reticulocytopaenia are consistently seen, indicating that the response to haemolysis in malaria is inadequate [16]. The causes of this problem are not fully known but probably include cytokine imbalances and the effects of parasite products such as glycosylphosphatidylinositol (GPI)-linked molecules and haemozoin, a waste product of the parasite's extensive digestion of RBC haemoglobin. Other factors contributing to the severity of malarial anaemia include pre-existing low haemoglobin, diet, drug therapy, other infections and certain genetic traits [16].

Mature intraerythrocytic *P. falciparum* parasites, in contrast to those of other species, are rarely seen in the peripheral circulation. This is due to sequestration of these pRBC in the microvasculature of the brain, heart, lung, muscle, adipose tissue and (in pregnant women) the placenta [13]. The direst consequences seem to occur in the brain. Cerebral malaria is marked by convulsions, coma, and (often) death. It is usually a consequence of accumulation of large concentrations of pRBC in cerebral capillaries leading to microvascular obstruction, metabolic disturbances such as local acidosis, and release of damaging pro-inflammatory mediators such as tumour necrosis factor (TNF)-α [13]. Some combination of these factors causes endothelial stress, cell apoptosis, and loss of the integrity of the blood-brain barrier [15]. Sequestration is the result of several adhesive phenomena. The most important are believed to be (i) cytoadherence of pRBC to endothelial cells, (ii) adherence of pRBC to multiple unparasitised RBC, forming 'rosettes' and (iii) platelet-mediated clumping of pRBC [13]. pRBC can also adhere to each other (autoagglutination), to placental syncytiotrophoblasts and to a variety of immune cells. Adhesion is mediated by specific, tissue- and parasite-strain-dependent molecular interactions. Host cells have a wide array of receptors: these include CD36, intercellular adhesion molecule (ICAM)-1, P-selectin, thrombospondin, platelet endothelial cell adhesion molecule (PECAM)-1, E-selectin, vascular cell adhesion molecule (VCAM-1), heparan sulphate and others on endothelial cells; complement receptor 1 (CR1), A and B blood group antigens and heparan sulphate-like molecules on RBC; CD36, the complement receptor gC1qR and P-selectin on platelets; and chrondroitin sulphate A in the placenta [13]. Endothelial receptors may be upregulated or relocated in response to parasite factors that stimulate the production of TNF-α and interferon (IFN)-γ [15]. Corresponding parasite adhesins

are chiefly members of the variant erythrocyte membrane protein-1 (PfEMP-1) family located on the pRBC surface [20].

The presence of metabolic acidosis and associated respiratory distress is a key predictor of death in severe malaria [21]. It is believed to be caused mainly by increased anaerobic metabolism by the host, with parasite metabolism playing a lesser role. Various factors contribute to increased oxygen consumption by and decreased oxygen supply to tissues. These include anaemia, sequestration, parasite products, pulmonary pathology and pro-inflammatory cytokines [14, 21].

WOULD MODULATORS OF MALARIA PATHOGENESIS BE LESS PRONE TO RESISTANCE?

If modulators of the pathogenesis of severe malaria were to make a significant therapeutic impact they would presumably have to interfere with the processes that lead to severe anaemia and/or cerebral malaria (and other manifestations of sequestration). Such interventions would be expected also to be of benefit for the metabolic complications of the disease, which appear to derive in large part from anaemia and sequestration [21]. Interventions targeting severe anaemia are discussed in the next section.

In terms of 'resistance-proof' therapy, any drug that (like conventional drugs) interfered with malarial parasite invasion, growth and division within erythrocytes, merozoite release or formation of gametocytes (the pre-sexual forms responsible for transmission via mosquito vectors) would presumably exert a strong selective pressure for resistance and would therefore be irrelevant. Two key parasite factors believed to contribute to the severity of *P. falciparum* malaria in comparison with other malarias are fast growth rate [15] and ability to invade RBCs of all ages [22]. These factors lead to high parasite burdens, which together with capacity to sequester (see above) make *P. falciparum* exceptionally virulent. Unless the patient dies, high parasite burdens are likely to be associated with prolonged high blood gametocyte levels and more efficient transmission; so again it is unlikely that moderating either growth rate or ability to target all RBC ages, if it could be done, would be less prone to resistance.

Reduction of sequestration would presumably ease the severity of cerebral malaria and other sequestration-dependent syndromes. The idea of adjunctive therapy with adhesion blocking agents has been extensively investigated and in a few cases has reached clinical trials. The agents used include specific peptide- or protein-based blockers such as PfEMP-1 fragments or soluble complement receptor 1, small chemical blockers such as N-acetyl cysteine, and anticoagulants such as heparin derivatives [13, 23-44]. None has yet been licensed as an antimalarial therapy. Problems include the multifarious nature of the molecular interactions causing sequestration and the lack of understanding of the role of each interaction in malaria pathology [13, 22, 45]. Worse, there may be a danger in manipulating adhesive interactions: for example, binding of pRBC to macrophages via CD36 was found to aid phagocytic clearance [13].

Whether adhesion blockers, or other approaches such as interference with switching of expression of PfEMP-1 types [46], might be less prone to parasite resistance would be expected to depend on the benefit (if any) derived by the parasite from sequestration. The process is generally supposed to offer protection from splenic clearance [13] but this has been disputed [47]. Alternative explanations for what drove the evolution of this trait include the superior environment of the microvasculature for parasite survival – e.g. in terms of partial pressure of oxygen, protection from or modulation of the immune response, or promotion of erythrocyte re-invasion or gametocyte development – in comparison with the peripheral circulation [48]. Whatever the explanation, there is likely to be a benefit to the parasite accruing from removal of mature pRBC from the general circulation. This contention is supported by a recent study by Fonager et al [49] in which mutants of *P. berghei* ANKA deficient in sequestration via CD36 had substantially reduced growth in mice. The disadvantage of the mutant relative to the wild-type strain was only partially mitigated by splenectomy. An alternative possibility is that severe malaria results from dysregulation of *var* gene expression in response to host stress [50], something that might be corrected without necessarily reducing the fitness of the parasites in their hosts. In conclusion, there is evidence that removing the benefit of sequestration might confer some selective pressure so there must be serious doubts as to whether drugs blocking adhesion or antigenic variation would be 'resistance proof'. Nonetheless it would be important to identify the precise nature of the advantage(s) conferred by sequestration, considering that some *Plasmodium* species have not been shown to display this behaviour, before coming to a conclusion on this point.

Schofield and co-workers have argued that most if not all of the symptoms of severe malaria can be attributed to an inflammatory cascade set in motion by a single parasite 'toxin', which they have identified as the GPI moiety that anchors a number of parasite surface proteins [51]. Experimental GPI-related vaccines have shown promise in mouse models of severe malaria [51]. GPI is believed to be an essential parasite component so (unlike some bacterial toxins) interfering with its synthesis would presumably be simply parasiticidal and therefore not 'resistance proof'. In any case, the 'single toxin' theory of malaria pathogenesis has yet to enjoy wide acceptance [5].

MALARIA-INDUCED INTERFERENCE WITH ERYTHROPOIESIS AS A POSSIBLE THERAPEUTIC TARGET

The effect of *Plasmodium* infection on erythropoiesis can occur both on a quantitative level, i.e. ineffective erythropoiesis where insufficient numbers of erythroid precursors proliferate or differentiate, or qualitatively (termed dyserythropoiesis) where erythroid cells present with morphological and frequently nuclear abnormalities [18, 52, 53]. Anaemia can be severe and persistent despite low or undetectable levels of parasitaemia and may persist for weeks after parasite clearance from the system [52, 54], suggesting dysregulated erythropoiesis rather than the destruction of infected or uninfected red blood cells as a principle cause in persisting anaemia. In predominantly 'benign' infections with *P. vivax* with characteristically low parasitaemias, suppressed erythropoiesis is emerging as a significant contributor to malarial anaemia that may

have previously been underestimated [55]. Tackling the problem of malarial anaemia thus extends beyond successful parasite treatment and could therefore present a promising target for 'resistance-proof' therapy.

In children, who show a higher prevalence of malarial anaemia than adults, erythropoietin (EPO) concentrations seem to be adequately increased in response to the level of anaemia whereas adults most frequently have reduced EPO levels [56-58]. These observations suggest fundamental differences in the mechanisms causing anaemia in children or adults, most likely due to differences in immunity levels between naïve and semi-immune/immune states, and may require different treatment strategies. The use of recombinant EPO for treatment of malarial anaemia in adults, where EPO production is insufficient, has been suggested [58]. Yet, others proposed a protective effect of an anti-EPO antibody in malaria due to the abrogation of erythropoiesis in the liver, which has been suggested as essential for the continuation of malaria infection [59].

Iron deficiency is a common condition in malaria-endemic areas due to nutrient or micro-nutrient insufficiencies or concomitant infections. Anaemia can develop as a result of decreased availability of serum iron but is also frequent in relation to chronic infectious diseases [17]. Increased levels of inflammatory cytokines such as interleukin (IL)-6 can stimulate the secretion of hepcidin, which inhibits the uptake of iron via enterocytes and prevents iron release by macrophages, leading to hypoferraemia and increased iron storage in the reticuloendothelial system [60, 61]. A recent study suggests that blood-stage parasites actually upregulate host hepcidin expression to divert available iron from competitor liver stage parasites and prevent a superinfection that would threaten their own niche [62]. Anaemia also commonly occurs post-malarial infection as a result of compromised iron recycling by the reticulo-endothelial system [63] and decreased iron absorption [64] and can render iron supplementation directly post-infection inefficient. Iron supplementation in malaria endemic regions was found to prevent severe anaemia episodes in about 30% of cases, corresponding to the average prevalence of iron deficiency [65], and such supplementation improved anaemia synergistically with anti-malarial treatment in several studies, with the synergistic effect being dependent on the efficacy of the antimalarial drug [66, 67]. Universal iron supplementation in resource poor settings had been recommended for infants and children by the WHO [68, 69] until it was implicated in increased morbidity and mortality in relation to malaria infections. A large randomized clinical trial on the supplementation of iron and folate was prematurely terminated due to safety concerns after increased occurrence of hospitalization and death due to malaria in supplemented individuals [70]. This led the WHO to revise its recommendations for iron supplementation in malaria endemic regions, suggesting pre-screening for iron deficiency might be essential [71]. A systematic database review of different available studies has however since come to the conclusion that iron supplementation does not increase the risk of clinical malaria and death as long as sufficient malaria surveillance is present, and does not pose an increased risk to individuals with sufficient iron levels [72]. While iron and micro-nutrient supplementation appear beneficial in alleviating malarial anaemia, when deficiencies are present, a concomitant successful antiparasite treatment (which would be susceptible to the emergence of resistance) seems essential to render it efficacious.

Suppression of the erythropoietic response appears to be predominantly caused by the release of pro- and anti-inflammatory cytokines, growth factors and effector molecules by the host immune system, an effect that is

293 also observed in chronic inflammatory diseases and cancer [73, 74]. Cells of the innate immune system
294 including monocytes/macrophages, neutrophils, natural killer or dendritic cells respond to the exposure to
295 parasitic antigens, GPIs or haemozoin through the secretion of pro-inflammatory cytokines such as TNF-α,
296 interferon (IFN)-γ, IL-1β, IL-12, IL-6, and tumour-necrosis-factor-related apoptosis-inducing ligand (TRAIL),
297 many of which interfere with proliferation or differentiation of erythroid precursor cells or promote their
298 apoptosis [19, 74, 75]. The factors produced furthermore act synergistically and/or form paracrine loops, and
299 can induce the production of nitric oxide via inducible NO synthase, which in turn is involved in parasite
300 killing but also directly inhibits erythropoiesis [74, 76].
301
302 TNF-α is one of the major inflammatory mediators that inhibit erythropoiesis and it induces cleavage of the
303 main erythroid transcription factor GATA-1 [19]. Early studies suggest that its anti-erythropoietic effect can in
304 part be reversed using anti-TNF sera [77]. However, TNF-α and many of the other pro-inflammatory factors
305 are at the same time essential for killing or preventing replication of parasites [74]. While conflicting evidence
306 exists for the effects of certain factors on the erythropoietic response (macrophage inhibitory factor [MIF], IL-
307 12) [78, 79], a cytokine imbalance and the failure to attenuate the initial type 1 immune response by type 2
308 cytokines such as IL-10, IL-4 or tumour growth factor (TGF)-β have been suggested as principle problems
309 [19, 74]. Interference with the pro-inflammatory response poses the risk of promoting parasite survival but
310 supporting a timely onset of the anti-inflammatory response could provide an approach for alleviating anaemia,
311 particularly in cases of undetectable parasitaemia. This would, nonetheless, depend on successful parasite
312 treatment, and is furthermore limited by the fact that some of these anti-inflammatory cytokines also possess
313 anti-erythropoietic activity [80].
314
315 The malarial pigment haemozoin, which is produced by *Plasmodium* as an insoluble crystalline degradation
316 product of erythrocyte haemoglobin and serves in detoxifying haem [81], had long been believed to be a rather
317 inert substance. Yet recent studies have suggested that haemozoin may play a very active role in the
318 development of malarial anaemia either through direct action on erythroid progenitor cells or via cells of the
319 host immune system [82-84]. Haemozoin has been found directly to inhibit erythroid precursors or promote
320 apoptosis at concentrations between 1 and 10 mg/ml, concentrations similar to those found in individuals with
321 severe malaria [16, 85]. Additionally, haemozoin non-enzymatically catalyzes the conversion of fatty acids
322 (lipid peroxidation) to long-chain aldehydes such as 4-hydroxynonenal (4-HNE), which also hinder the
323 proliferation of erythroid progenitors or induce their apoptosis [83, 84, 86]. In addition, recent investigations
324 suggest a transfer of 4-HNE from parasitised to unparasitised RBC within rosettes, which may mark the
325 unparasitised cells for destruction [87]. The ingestion of haemozoin by monocytes/macrophages and its
326 deposition in the bone marrow show important erythropoiesis-inhibiting effects via the production of pro-
327 inflammatory factors [88]. However it still remains a matter of debate whether pure haemozoin is
328 immunologically active [89] or these effects are mediated by adherent parasite DNA [90], proteins [91] or
329 lipids [92]. Haemozoin has been suggested as an adjuvant for potential malarial vaccines to boost the immune
330 response [93] but its potential erythropoiesis-inhibiting properties should limit such an advance. At the same
331 time, the production of haemozoin cannot be targeted without exerting selective pressure on the parasite and

trying to modulate the resulting inflammatory response may be a more promising approach, though it faces the above-mentioned limitations of interfering in the host immune response.

The causes for the reduced erythropoietic response seen in malaria appear to be multi-factorial, resulting from altered balances of cytokines and inflammatory factors and changes to the erythropoietic microenvironment, and are often aggravated by iron deficiency. However, these aspects currently need further investigation as the underlying mechanisms that inhibit erythropoiesis remain poorly understood and may present the down-side of important mechanisms designed to reduce parasite growth. The distinct differences in erythropoietic response between children and adults require elucidation and the question has been raised whether inadequate erythropoiesis may actually be an adaptive host immune response aimed at limiting parasitaemia.

Severe malarial anaemia has been blamed for between 190,000 and 974,000 deaths per year in children under 5 and the successful use of blood transfusion in severe malarial anaemia [94] suggests that therapeutic interventions to limit the loss of RBC would be likely to save lives. For the reasons mentioned in the preceding section, interfering with parasite destruction of pRBC would not be 'resistance proof'. Interfering with destruction of unparasitised RBC or with the effects of infection on erythropoiesis would by contrast not be expected to select parasites that would bypass the intervention, as there would be no (obvious) benefit in their doing so. Despite representing a treatment of end-product rather than cause, tackling inefficient erythropoiesis thus seems an attractive approach to anti-disease therapy. Balanced against its presumed benefit would be the consideration that increased supply of RBC or elevated reticulocyte regeneration rates may lead to an increase in parasite burden, particularly if the parasite preferentially infects reticulocytes (as in *P. vivax*) [95]. Such a view finds support in a study conducted by Chang et al, who observed that untimely administration of EPO and resulting reticulocytosis could actually worsen parasitaemia and increase mortality in *P. chabaudi*-infected mice [96]. Ginsburg & Hoshen [97] have however argued that supply of RBC is only in exceptional circumstances a limiting factor for parasite multiplication. Overall, approaches to treating severe malaria differ fundamentally in their implications for erythropoiesis. While the use of erythropoietic stimulants such as EPO, iron supplementation or folate has been suggested for reducing the accompanying anaemia, other studies postulate the exact opposite in an effort to reduce parasitic burden. Given the important implications for the involvement of the host immune system in limiting the erythropoietic response, important lessons may be learned from studies of the anaemias of chronic inflammation and offer novel treatment approaches via immunomodulatory therapies. As a key element in the anaemia of chronic disease the hepcidin pathway is a promising target for therapeutic intervention either directly through hepcidin antagonists or neutralizing antibodies (of which some are in clinical trials for cancer-related anaemia) or through the modulation of its regulators such as bone morphogenic proteins (BMP), haemojuvelin and IL-6 [98-100]. Such an approach could however prove ambiguous in the case of malaria where a role for hepcidin in limiting parasitaemia is suspected [62]. Results on hepcidin-independent pathways involved in the inhibition of erythroid proliferation/differentiation could nevertheless open new potential therapeutic routes in the future [101]. In any case, therapeutic agents restoring RBC would presumably be used as adjunctive therapies [102], i.e. in conjunction with conventional drugs that kill or inhibit parasites.

CONCLUSIONS

'Anti-disease' therapies offer one approach to the development of new antimicrobial drugs that might be less prone to resistance. These agents would however only be 'resistance-proof' if they did not exert selection pressure favouring the emergence of less susceptible forms of the pathogen. In severe malaria, treatments abrogating the effects of parasite sequestration (e.g. cerebral malaria) and loss of erythrocytes (severe malarial anaemia) have been explored, chiefly as 'adjunctive' therapies, i.e. as a complement to antiparasitic drug therapy. Inhibition of sequestration by blocking adhesion of mature *P. falciparum*-infected erythrocytes to host cells is not likely to be 'resistance-proof' because sequestration appears to offer survival advantages to the parasite. Reversal of the processes that lead to interference with erythropoiesis in malaria may be more promising from the point of view of lack of selective pressure for resistant parasites. Whether drugs can be developed that can achieve this without detrimental effects remains to be seen. Any anti-disease therapy is likely to have a rather long response time and, as mentioned above in the context of bacterial infections, would not actually clear parasites from the body. It would therefore most likely be deployed in combination with an antiparasitic agent.

What are the directions for future research and the issues that have to be addressed in order to show that anti-disease therapies really could be less prone to resistance? In addition to the trials taking place at the moment, we need to find out more about the biology of sequestration and inhibition of erythropoiesis by parasites in order to establish the evolutionary drivers for these traits and whether they could be eliminated without affecting parasite fitness in the host or transmissibility through the vector. As with other putative 'resistance proof' therapies, the speed of emergence of resistance to the experimental therapies would need to be measured in comparison with other agents [103] in suitable animal models. Finally, new insights into malaria pathogenesis such as the roles of 'toxins' may open new avenues for the development of anti-disease therapies.

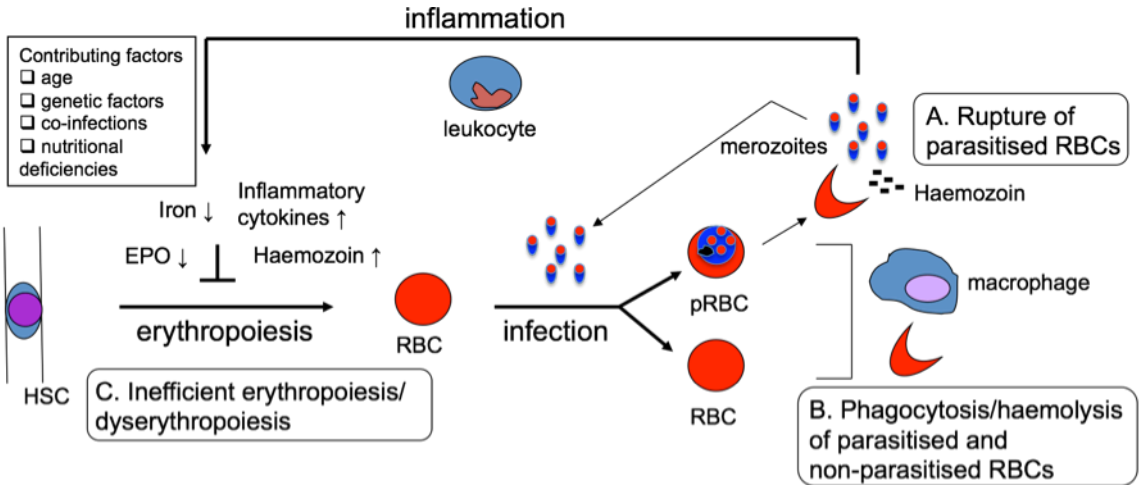

Fig. (1). Causes of malarial anaemia. Erythrocytes (RBC) are depleted as parasitised RBCs (pRBC) rupture releasing merozoites and haemozoin (A). Both parasitised and non-parasitised RBC undergo phagocytosis by macrophages or haemolysis (B). Haematopoietic stem cells (HSC) of the bone marrow give rise to insufficient numbers of functional RBCs due to the inhibitory effects of haemozoin and inflammatory cytokines, reduced availability of iron or decreased erythropoietin (EPO) levels.

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