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Is the development of falciparum malaria in the human host limited by the availability of uninfected erythrocytes?

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Abstract

Background: The development and propagation of malaria parasites in their vertebrate host is a complex process in which various host and parasite factors are involved. Sometimes the evolution of parasitaemia seems to be quelled by parasite load. In order to understand the typical dynamics of evolution of parasitaemia, various mathematical models have been developed. The basic premise ingrained in most models is that the availability of uninfected red blood cells (RBC) in which the parasite develops is a limiting factor in the propagation of the parasite population.

Presentation of the hypothesis: We would like to propose that except in extreme cases of severe malaria, there is no limitation in the supply of uninfected RBC for the increase of parasite population.

Testing the hypothesis: In this analysis we examine the biological attributes of the parasite-infected RBC such as cytoadherence and rosette formation, and the rheological properties of infected RBC, and evaluate their effects on blood flow and clogging of capillaries. We argue that there should be no restriction in the availability of uninfected RBC in patients.

Implication of the hypothesis: There is no justification for the insertion of RBC supply as a factor in mathematical models that describe the evolution of parasitaemia in the infected host. Indeed, more recent models, that have not inserted this factor, successfully describe the evolution of parasitaemia in the infected host.

Introduction

Parasites often have a mechanism which regulates parasite load according to parasite density. Although this auto-regulation is not fully understood, it maintains an equilibrium between activated specific and non-specific host defense processes, the sensitivity of red blood cells (RBC) to invasion and the virulence of the parasite [1]. Understanding of this auto-regulation would be facilitated by the development of a suitable mathematical model. Several attempts have been made in the past to generate mathematical models of the process of malaria infection in non-immune individuals [2–7]. The formulation of a model is essential since at some stages of their development in the host the malaria parasites cannot be seen, either because they are sequestered in the deep blood vasculature or else because detection limits are too high. The usefulness of such models is obvious as they could disclose the evolution of antimalarial immunity, anaemia

that may be life threatening, the significance of antigenic variation to in-host and population evolution of the disease. Eventually, such models could be used for the assessment of drug response and the effects of vaccine to the point that they could advise the selection of vaccine target and the timing of drug treatment for optimization of both ways of medical intervention.

All models are derived from a paper by Anderson *et al* (1989) [8] in which the following basic assumptions have been made: 1) Uninfected cells are released from the bone marrow at a constant rate and have a natural life expectancy; 2) Red blood cells (RBC) are infected by a rate that is proportional to the density of uninfected RBC. 3) The death of infected cells due to maturation of schizonts is rapid compared to the above-mentioned rates. 4) The released merozoites either die or successfully infect new RBC.

Assumption 1) has recently been shown to be inadequate as the RBC survival time is only 1/3 that of healthy controls [9] and this is a major contributor to anaemia [10], in addition to impaired erythropoiesis [11]. These effects, however, have no bearing on the present discussion. Assumptions 3) and 4) are correct, but the dependence of the formation of infected cells on the concentration of uninfected cells (assumption 2) seems to be questionable. Its introduction into the model implies that in extreme cases of anaemia, the availability of uninfected RBC may rate-limit (by self-limiting) the evolution of infection. We would like to test this consideration in the broader context of the rheological effects of cytoadherence and invasion of RBC by merozoites *in vivo*.

Cytoadherence and rosetting

Cytoadherence is defined as the ability of parasitized red blood cells (PRBC) to attach to specific receptors on the endothelial cells of the microcapillaries, and rosetting is defined as the ability of PRBC to bind to uninfected RBC. Both processes could influence the invasion of RBC by merozoites emerging from the mature rupturing schizont. Although there are numerous works on cytoadherence, rosetting and invasion in cultures, very little is known about the details of invasion *in vivo*.

Let us examine the case of *Plasmodium falciparum* infection. Here, most if not all infected RBC harbouring mature parasite stages are sequestered in the post-capillary venules of the host due to their ability to cytoadhere to the endothelial cells of the venules [12–16]. This sequestration, on one hand, prevents the passage of the rigidified PRBC through the spleen and their ensuing removal by resident macrophages, and on the other hand, these cell-cell interactions could increase the probability of invasion.

Reports on the pathology of malaria show post-capillary venules clogged with uninfected RBC and PRBC [17–21]. Most of these are based on post-mortem histology and are, hence, probably relevant for severe cases of malaria. Here, we shall address only non-severe infection since, the situation in severe disease may be much more complex [22]. The first generation of post capillary venules range in size from 10 μm to 30 μm and the second generation from 40 μm to 200 μm, whereas the diameter of the spherical PRBC is not larger than 4.4 μm. There is, therefore, plenty of room for circulation to continue, especially with vasodilatation, for which there is some evidence of occurrence in malaria infections [23–25]. PRBC are more rigid and less deformable than uninfected RBC (although these are also less deformable than normal RBC) [26,27] and thus increase blood viscosity [28–30]. However, we have to consider the phenomenon of rosetting as well, that is, the adherence of uninfected RBC to PRBC [31,32]. Studies show that the cell-cell attachments within rosettes are strong and suggest that rosettes might survive both the arterial circulation and passage through microvessels and thus could contribute to the ischaemic complications of falciparum malaria [33,34]. However, it has been recently suggested that rosettes cannot withstand arteriolar shear stress but do endure venular shear stress [35]. Hence, rosettes can be formed in the venules and reduce cytoadherence. This could be due to the masking of attachment ligands by uninfected RBCs from their receptors on the endothelial cells, or to competition for ligands between receptors on RBC and on endothelial cells. Yet, even if rosettes are only partially being formed during the passage through the venules, they would still slow down blood flow and thereby enhance cytoadherence, in as much as reduced flow is known to increase it [36]. However, if cytoadherence occurred before rosetting, then adherent cells should not efficiently form rosettes [37].

Effects of sequestration of PRBC on blood flow

Obviously then, cytoadherence with or without rosetting should increase resistance to blood flow through the venules. This was indeed shown to be the case under flow conditions in various experimental systems [36,38–42]. Histology, however, indicates that even in severe malaria not all venules are obstructed. Vasodilatation, which has been observed by some investigators in malaria patients [23,43], is known not to affect venules directly, but feeding arteries and arterioles. Flow in capillaries in the healthy host is typically intermittent and not all capillaries necessarily allow blood flow all the time. Vasodilatation of arterioles tends to reduce intermittency and recruit more capillaries into flow. Flow in the venules does not usually stop altogether, but in the smallest first generation venules there will be fluctuations depending on the capillaries supplying them. These fluctuations, along with other factors such as the tissue-specific distribution of

cytoadherence receptors, could explain the differential sequestration of PRBC in some venules but not in others. It is also possible to infer on these phenomena from the behavior of white cells during inflammatory response: these cells adhere to the wall of venules as they migrate through [44]. White cells are much larger and more resistant to deformation than parasitized red cells and yet they do not stop flow. Nonetheless, it has been shown that microvascular resistance does increase when white cells adhere to the wall, most probably because the lumen is effectively narrowed.

Impaired blood flow in malaria patients

Investigations have shown that in malaria patients' blood flow is restricted through the kidney [23,45,46], the liver [47,48], the brain [49,50], the placenta [51] and that in *Plasmodium berghei* infection there is a general failure of capillary flow and disruption of venous outflow tracts by aggregates of infected and uninfected cells [52]. Another report could not find evidence for hypoperfusion in cerebral malaria [53]. This could be due to the autoregulation of local blood flow by anoxic blood that can accelerate flow by up to 200-fold or to neural regulation that can displace blood from a vasoconstricted area to a region of vasodilatation. In fact, in children with cerebral malaria cerebral blood flow is increased considerably [50]. It must be underscored that all these perturbations in blood flow were observed only in severely ill patients, and in extreme cases that are often fatal, could even lead to hypoglycemia and lactic acidosis [54–57].

Complete clogging of venules is incompatible with the evolution of parasitaemia

It is very likely therefore that in non-severe and even in acute malaria patients, the post-capillary venules are never fully clogged. In all the aforementioned pathological studies, histological inspection of post-mortem tissue sections reveals venules filled with PRBC. This filling could occur only if cytoadhering PRBC do not obstruct flow completely [58], allowing for bypassing cells to submit the PRBC to attach to endothelial receptors thus filling all potential cytoadhering sites. Reduced flow velocity due to partial clogging could assist cytoadherence whose efficiency is inversely related to the speed of flow [36]. Complete clogging is probably inconsistent with the nutritional requirements of the growing parasite (supply of metabolic substrates and removal of waste products), and most importantly, with effective invasion of RBC by released merozoites. Let us consider what happens in a fully clogged vessel when segmenters burst to release merozoites. It is tempting to suggest that the rupture of PRBC reinstates blood flow in the venules, and merozoites are entrained by the renewed blood flow into larger capillaries where they meet uninfected RBC supplied from parallel, unclogged venules, and invade them. This process would require that all segmenters rupture simultaneously. Otherwise, the merozoites that emerge from one segmenter will encounter only PRBC that are refractory to invasion. However, even in synchronous infection, rupture of segmenters and reinvasion takes well over one hour, suggesting that not all segmenters rupture at the same time. These considerations indicate that complete clogging of the post capillary venules by cytoadhering PRBC is disadvantageous for the optimal propagation of the parasites. Thus, total clogging probably never occurs. In fact, the rate of increase in parasitaemia in naive individuals (as deduced from clinical studies of malariotherapy patients) suggests that all released merozoites successfully reinvade [2,9]. An analysis of patients data indicates that infection is not restricted by the availability of RBC [58] although disease contributes to increased destruction of both RBC and PRBC [1].

The case of rosetting is somewhat different: the emerging merozoites will encounter uninfected RBC of the rosette that are available for invasion. Rosettes may obstruct microvessels to the flow of uninfected RBC more efficiently than cytoadhering PRBC, but this is compensated for by the availablilty of rosetting RBC for reinvasion. If the entire microvessel is filled with rosettes, quantitative considerations imply that the rate of parasite propagation will be lower compared to non-rosetting parasites: PRBC is surrounded in the rosette by 4–7 RBC, while each schizont can release up to 20 merozoites. Once again, since the evolution of parasitaemia indicates that all merozoites successfully invade, rosetting cannot be a quantitatively important phenomenon in falciparum malaria.

The availability of uninfected RBC to merozoites is not restrained

As we have just argued, if total clogging of venules never occurs, does the availability of uninfected RBC limit the evolution of parasitaemia in non-immune patients as suggested by mathematical models of within-host parasite dynamics (see Introduction)? Such limitation is based on the assumption of the mass action law that is applicable to a closed system. The anatomy of post capillary venules, the regulation of blood flow and cytoadherence of PRBC to the endothelium of the venules in conjunction with the parasite cycle, implies that reinvasion occurs in an open system. In this system merozoites emerge from sequestered segmenters and are exposed to a continuous flow of uninfected RBC within the venule. In fact, the partial clogging of the capillaries and the decreased deformability of uninfected RBC could reduce the velocity of flow thereby increasing the chances of invasion. Merozoites could also be entrained by the blood flow to second generation venules where they would meet even larger numbers of RBC coming from merging first generation venules that may not contain sequestered PRBC at all. Therefore the

merozoites enjoy a pure source of RBC, with much higher concentration than that in the total bloodstream. Thus, it must be concluded that reinvasion is not limited by the availability of uninfected RBC. The reduction of parasite propagation observed in natural infections that parallels increasing anaemia [1] must result from other factors such as increased immune response, virulence of the parasite and its preference for sub-sets of RBC [59,60]. Merozoites released from schizonts lose their invasive capability within minutes [61,62], and from studies in cultures of *P. falciparum* it is known that agitation reduces the levels of reinvasion [63]. This would imply that reinvasion must occur before meorozoites reach large veins where the velocity of blood flow is considerably accelerated. Here again, the reduced rate of blood flow in venules lined with cytoadhering PRBC is an advantage for efficacious reinvasion that may be favored by decreased agitation.

The general observation that anaemia is associated with the severity of infection [64], implies that parasitaemia is not limited by anaemia, i.e, the reduced availability of RBC. The ability of virulent parasite strains to multiply so rapidly as to outpace the evolution of effective defense by the host [59] indicates that the supply of RBC is not restricted. The slower evolution of parasitaemia with avirulent strains that have a preference for a sub-set of RBC [59,60] may result from the loss of the ability to invade due to encounter with unsusceptible RBC, and not from a general limited supply of RBC.

Models of within host dynamics describing the dynamics of parasite evolution and auto-regulation that do not assume limitation of RBC availability were shown to successfully describe the clinical picture [65,66].

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