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Is malarial anaemia homologous to neocytolysis after altitude acclimatisation?

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ABSTRACT

Malaria patients frequently develop severe anaemia that can persist after *Plasmodium* infection has been cleared from the circulation. This puzzling phenomenon involves massive death of young uninfected erythrocytes at a time when parasitic infection is very low. We have observed striking similarities in erythrocyte homoeostasis during altitude acclimatisation and *Plasmodium* infection, both of which initially induce an increase in circulating erythropoietin (Epo). Decreasing levels of Epo after return to low altitudes induce the death of young erythrocytes, a phenomenon called neocytolysis. In a similar way, we propose that Epo, which peaks during acute malaria and decreases after parasite clearance, could be contributing to anaemia causing neocytolysis during recovery from *Plasmodium* infection.

Severe malarial anaemia (SMA) is one of the leading causes of mortality in malarial infections, being responsible for approximately half of the deaths associated with malaria (WHO, 2013; http://www.who.int/malaria/high_risk_groups/infants/en/). However, SMA has received relatively little attention compared with other areas of malaria research. SMA is recognised as a multifactorial pathology, involving impaired erythropoiesis and loss of circulating red blood cells (RBCs) (Chang and Stevenson, 2004; Schofield and Grau, 2005; Haldar and Mohandas, 2009). Erythropoiesis is considered to be impaired, even if the percentage of reticulocytes in peripheral blood is augmented during the acute phase of malaria, because it does not suffice to maintain adequate levels of RBCs (Chang et al., 2004). The mechanisms underlying impaired erythropoiesis in malaria remain unclear, although inflammatory cytokines (Ghosh, 2007) and other inflammatory mediators such as parasite-derived hemozoin have been postulated as major contributors (Awandare et al., 2011).

Loss of circulating RBCs is also considered a key factor in malarial anaemia. The anaemia characteristic of human malaria is frequently much greater than would be expected from the degree of RBC parasitism. Levels of parasitemia in malaria patients are normally lower than 1% and while some RBCs are ruptured as a result of parasite infection, this represents only a small proportion of the total loss of RBCs. Instead, the main cause of malarial anaemia is the loss of non-parasitised RBCs. It has been estimated that for each *Plasmodium*-infected RBC, eight uninfected RBCs are cleared during *Plasmodium falciparum* (Jakeman et al., 1999; Price et al., 2001) and 34 during *Plasmodium vivax* (Collins et al., 2003; Douglas et al., 2012) infections. It has also been observed that anaemia often persists after parasitemia subsides in experimental monkey infections (Egan et al., 2002; Jones et al., 2002) and in humans, where anaemia continues in some patients 1 month after parasite clearance (Woodruff et al., 1979; Biemba et al., 1998). These observations confirm that the death of parasitised RBCs plays a minor role in SMA but also indicate that the loss of RBCs can continue long after infection has been resolved.

The classical immune mechanisms for the clearance of infected RBCs, mediated by antibody recognition of parasite antigens expressed on the RBC surface and their subsequent phagocytosis by macrophages, play an important role in the outcome of infection (Stevenson and Riley, 2004). Phagocytosis of uninfected RBCs is also observed during malaria (Knüttgen, 1961; Wickramasinghe et al., 1989). Several complementary mechanisms have been proposed to contribute to the augmented phagocytosis of uninfected RBCs. Binding of soluble *Plasmodium* antigens to uninfected RBCs, followed by recognition by specific antibodies in the blood, might promote their phagocytosis by activated macrophages (McGregor et al., 1968; Waitumbi et al., 2000). Increased RBC phagocytosis might also result as a consequence of malaria-induced oxidative stress in the blood (Percario et al., 2012). Oxidative stress leads to changes in the structure of RBC membranes, such as Band 3 modifications (Arese et al., 2005) and exposure of phosphatidylserine in the outer membrane (Zwaal et al., 2005), which mark RBCs

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as targets for macrophage clearance. Additionally, loss of complement regulatory proteins on the surface of uninfected RBCs during malaria has also been suggested as a likely source of abnormalities in the RBC membrane that might explain the removal of these cells by monocytes and macrophages (Stoute et al., 2003). Recently, the transfer of 4-hydroxynonenal, generated through hemozoin-catalysed lipid peroxidation, from infected to uninfected RBCs has also been related to phagocytosis and clearance of uninfected RBCs during malaria (Uyoga et al., 2012).

In addition to changes in RBC membranes, as described above, increased macrophage phagocytic activity probably plays an important role in SMA since it may result in increased clearance of RBCs contributing to anaemia (Chua et al., 2013). Indeed, SMA is associated with high serum levels of neopterin, a marker of macrophage activation (Biemba et al., 1998). Interestingly, both macrophage activation and anaemia persist in some patients 1 month after clearance of parasitemia (Biemba et al., 1998).

We would like to propose a different point of view for the interpretation of the existing observations on SMA. We suggest that in order to understand the origin of SMA it is necessary to focus on how malaria interferes with well-known physiological mechanisms that regulate RBC populations. Under normal conditions up to 10^{11} RBCs are cleared each day by macrophages (Mosser and Edwards, 2008) as part of a homoeostatic process that governs the concentration of oxygen in the body tissues which, in turn, is under the control of the hormone erythropoietin (Epo) (Jelkmann, 2011).

In situations where oxygen levels change, RBC populations are rapidly adjusted to recover adequate oxygen supply to the tissues. A paramount example of this homoeostatic regulation is acclimatisation to changes in altitude. We have noted that there are conspicuous similarities between Epo dynamics in RBC homoeostasis during the climbing and descent of a mountain and a *Plasmodium* infection. During high altitude acclimatisation, the drop in oxygen availability raises the production of Epo (Jelkmann, 2011), which in turn stimulates erythropoiesis (Fang et al., 2007), increasing the population of circulating RBCs (Berglund, 1992) and, eventually, the amount of oxygen delivered to tissues (Bauer and Kurtz, 1989).

Similarly, an increase in the levels of Epo (Burchard et al., 1995; Burgmann et al., 1996; el Hassan et al., 1997; Vedovato et al., 1999; Diez-Padrisa et al., 2011) and, subsequently, in the proportion of reticulocytes (Leowattana et al., 2008) have also been described during acute malaria infection. The increase in Epo during malaria infection has been observed consistently in field studies as correlating directly with disease severity (Diez-Padrisa et al., 2011) and with anaemia (Burchard et al., 1995; el Hassan et al., 1997; Kurtzhals et al., 1997; Vedovato et al., 1999; Nussenblatt et al., 2001), suggesting that augmented Epo levels are not sufficiently high to counterbalance the massive loss of RBCs. Although the causes for this increase in Epo remain unclear, hypoxia might contribute to Epo increases during malaria infections. In *P. falciparum* infections, hypoxia may be caused by obstructed blood flow due to microvascular sequestration of infected RBCs, aggregation of infected and uninfected RBCs, and increased rigidity of RBCs (Dondorp et al., 2004). RBC rosetting and increased rigidity have also been observed in *P. vivax* infections (Udomsanpetch et al., 1995; Jayavanth et al., 2004). Insufficient oxygen supply to the tissues might be further complicated by increased oxygen consumption in the muscles during malaria infection (Yeo et al., 2013). It is also possible that acidosis, which is observed in malaria, impairs Epo production and may contribute to the insufficient levels of observed Epo (Eckardt et al., 1990).

Despite differences between the causes of hypoxia in high altitude acclimatisation and malaria, clear analogies can be drawn between Epo dynamics during the late stages of malaria infection and the return of a high altitude acclimatised individual to sea level. The return to lower altitudes entails comparatively higher oxygen availability and, consequently, increased RBC numbers are no longer needed to accomplish the supply of oxygen to body tissues. Accordingly, this situation leads to a decrease in Epo levels and erythropoiesis (Bauer and Kurtz, 1989; Eckardt et al., 1989). The ensuing lower production of RBCs is also accompanied by the selective death of young RBCs (Rice et al., 2001). This phenomenon, known as neocytolysis, continues until RBC homoeostasis is regained under the new oxygen conditions. Given its contribution to the decline of RBC populations, neocytolysis can be interpreted as an adaptive mechanism allowing for a much faster transition to a scenario of higher oxygen availability compared with reduced erythropoiesis alone (Handelman and Levin, 2010).

Since neocytolysis is triggered by a fall in Epo levels, we postulate that malarial anaemia can be caused, at least in part, by neocytolysis following a decrease in Epo during the recovery phase of a malaria infection. The key empirical evidence to support this assumption is that, as described above for the return to sea level after high altitude acclimatisation, the levels of Epo have been observed to decrease progressively for several weeks after *Plasmodium* clearance (Burchard et al., 1995; Burgmann et al., 1996; Leowattana et al., 2008). Inadequate levels of circulating Epo subsequently persist and coincide with persistent anaemia (Woodruff et al., 1979; Biemba et al., 1998). The reasons for the decline in Epo during malaria have not yet been elucidated; however, during a prolonged stay at high altitude or during chronic anaemia, Epo levels decline, probably via a feedback regulation mechanism (Jelkmann, 2011). This mechanism could also explain the inadequate levels of circulating Epo that are found during persistent malarial anaemia (Burgmann et al., 1996; Leowattana et al., 2008). It is also possible that Epo levels decrease progressively after parasite clearance as a consequence of recovered oxygen levels in tissues or a decrease in inflammation.

In agreement with our suggested role for neocytolysis in malarial anaemia, uninfected RBCs have a shortened life span compared with that of healthy individuals (Woodruff et al., 1979; Looareesuwan et al., 1991; Biemba et al., 1998). Morphologically, neocytolytic RBCs show increased levels of phosphatidylserine exposure in the outer membrane (a characteristic sign of senescence) both after

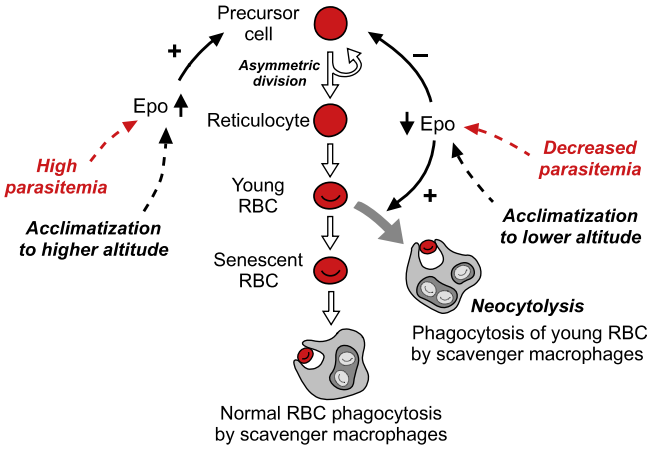

Fig. 1. Proposed model for homoeostasis of red blood cells (RBCs) populations during malaria. The relatively constant number of circulating RBCs is the consequence of a dynamic equilibrium resulting from a precise balance between cell production and removal (white arrows). The rate of production of new RBCs is continuously adjusted to maintain an adequate oxygen supply to the tissues by means of a feedback mechanism controlled by erythropoietin (Epo; solid black arrows). The reported similarities between malaria and altitude acclimatisation can be explained as analogous responses of this homoeostatic mechanism causing changes in Epo in both situations (dashed red and black arrows). In particular, the observed decrease in Epo in late stages of malaria infection may lead to severe malarial anaemia (SMA) by triggering neocytolysis.