Microreview

The role of the spleen in malaria

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Summary

The spleen is a complex organ that is perfectly adapted to selectively filtering and destroying senescent red blood cells (RBCs), infectious microorganisms and Plasmodium-parasitized RBCs. Infection by malaria is the most common cause of spleen rupture and splenomegaly, albeit variably, a landmark of malaria infection. Here, the role of the spleen in malaria is reviewed with special emphasis in lessons learned from human infections and mouse models.

The spleen consists of distinct microanatomical zones and microcirculations exquisitely adapted to performing different functions ranging from induction of adaptive immune responses, recycling of iron and phagocytosis of erythrocytes as well as selective destruction of senescent or damage red blood cells (RBCs) and pathogens including Plasmodium (Bowdler, 2002; Engwerda et al., 2005; Mebius and Kraal, 2005; Buffet et al., 2009). Such filtering capacity is related to its trabecular structure formed by: (i) white pulp, lymphoid tissue containing the majority of immune effector cells; (ii) red pulp, a reticular meshwork where destruction of senescent, aberrant RBCs and Plasmodium-parasitized RBCs (pRBCs) occurs; and (iii) a marginal zone lying between the white pulp and red pulp, where inert particles, bacteria and viruses are eliminated (Fig. 1). In addition, blood enters the spleen through a central artery that branches into capillaries, some of which directly bypass the filtration beds of the cords as closed fast microcirculation whereas others empty into the filtration beds of the red pulp as open slow microcirculation before reaching the venous system.

Infections by malaria parasites induce a dramatic, albeit variable splenic response mostly characterized by splenomegaly. In fact, spleen size has been used as a tool to determine the intensity of malaria transmission in endemic regions (Neva et al., 1970; Snow et al., 1989; Chaves et al., 2011). Of note, during the erythrocytic stages of malaria infection, the spleen is the main organ involved in the development of the immune response and in elimination of pRBCs (Engwerda et al., 2005). However, parasites counteract by establishing chronic infections through evasion and modulation of the immune response and through remodelling the spleen, sometimes provoking imbalanced immune responses that may cause severe disease (Buffet et al., 2009). This dual role on protection and pathology highlights the need for further research on the mechanisms involved to understand fully the role of the spleen in pathology associated with malaria infections.

Structure and function of the spleen

White pulp

The white pulp, lymphoid tissue containing the majority of immune effector cells, contains two major lymphocyte populations segregating into different zones forming sheaths around branching arterial vessels. The inner zone, T-cell zone or periarteriolar lymphoid sheath, is where T cells reside and can interact with interdigitating dendritic cells (DC) and transiting B cells to develop adaptive immune responses. It is surrounded by the B-cell zone, organized in follicles that undergo antigen-specific B-cell clonal expansion and immunoglobulin isotype switching upon interaction with follicular DC, DC and helper T cells (Bowdler, 2002).
Marginal zone

The marginal zone forms a rim around the white pulp and supports transit of cells from the blood to this region. It consists of a marginal sinus lined by reticular cells and banded by specific subsets of macrophages that play important functions in the uptake of particulate matter and bacteria from the blood: the marginal metallophilic macrophages (MMM), situated at the white pulp border, and the marginal zone macrophages (MZM), adjacent to the red pulp (Dijkstra et al., 1985). Other cells localize in between, such as B cells involved in T-cell-independent responses and a variety of trafficking cells including DC and T cells (Engwerda et al., 2005; Mebius and Kraal, 2005). Recirculating lymphocytes enter the spleen within this region and are directed to the white pulp via fibroblast channels without crossing endothelium (Steiniger et al., 2001; Bajenoff et al., 2010). Moreover, similar to lymph nodes, rapid transport of small molecules such as cytokines, chemokines and antigens directly to the white pulp (Lokmic et al., 2008) can take place via a tubular network, termed conduit system (Gretz et al., 2000; Nolte et al., 2003).

Red pulp

The red pulp comprises 70–80% of the volume of the spleen (van Krieken et al., 1985). It consists of sinuses and cords that display unique architectural and microcirculatory features related to its function. The splenic cords, or cords of Billroth, are open spaces, devoid of endothelial lining, populated by highly active macrophages along with reticular fibres and reticular cells (Chadburn, 2000). Blood particles and cells, including erythrocytes, haematopoietic cells, granulocytes, circulating monocytes and activated lymphocytes, flow from arterioles within these spaces, thus facilitating immune surveillance of blood-borne material (Saito et al., 1988). Normal red blood cells are rapidly collected from the cords by passing through the internen-
Microcirculation and clearing function

Blood flow in the spleen is complex with distinct circulation patterns adapted to enable filtration and immune clearance. Blood enters the spleen through the splenic artery, at the hilus. It then divides into trabecular arteries and enters the parenchyma where small arterioles branch and enter the red pulp (Groom et al., 1991). Early studies identified two types of circulation, open and closed (Schmidt et al., 1993). Ninety percent of the total splenic blood flow enters the marginal zone and travels through the adjacent venous sinuses, bypassing the reticular meshwork of the red pulp in a fast pathway, called closed circulation. The other 10% percolates through the marginal zone or directly enters the red pulp meshwork, filling the cordal open system, and will follow a slow circulation through collection by venous sinuses. Blood collected in sinuses merges in the trabecular veins and is drained through the efferent splenic vein at the hilus (Bowdler, 2002; Cesta, 2006). The splenic flow closely affects clearing functions. The open circulation provides two checkpoints at the red pulp and marginal zone where macrophages and other immune cells can survey red blood cells and blood-borne particles. Hence, mechanisms exist to regulate mass and velocity of cell passage through these compartments. First, innervation of the splenic capsule and trabeculae makes it responsive to sympathetic stimuli to regulate entry of red blood cells. Second, the myoelastic tissue of efferent splenic veins confers elasticity to change its diameter and modulate efflux. Third, blood flow through the filtration beds of the red pulp in human spleens is controlled by modulating the size of interendothelial slits in venous sinuses, through contractility of fibres. In addition, the presence of contractile fibroblasts cells located in filtration beds has been described to grant normal basal filtration in human and murine spleens (Weiss, 1991).

Role of the spleen in malaria infections

'So powerful is the splenic response, so nicely does it interweave host and parasite, that one is moved to speculate that the very structure of the spleen, as that of haemoglobins, may have been evolutionarily driven by malaria' (Weiss, 1991).

Mechanical trapping

Deformability of erythrocytes was found to be critical for their removal in sinusoidal spleens, i.e. human and rat spleens. Early studies in Plasmodium berghei-infected rats showed impaired splenic trapping of infected and heat-induced abnormal erythrocytes during precrisis (Wyler et al., 1981), a period characterized by splenomegaly, decreased cordal blood flow and extramedullary erythropoiesis, which was restored before the onset of crisis, a period of massive destruction of pRBCs. The authors hypothesized that two defence mechanisms might act in concert during precrisis: (i) obstruction of the cords by erythroid precursors might cause closing of the circulation and (ii) secretion of soluble factors by cordal macrophages might retard intracellular development of the parasite (Wyler et al., 1981). In the crisis period that follows, the altered rheologic properties of erythrocytes are a major determinant of their trapping and removal by the spleen (Wyler et al., 1981). Of interest, studies on microcirculation and splenic function during acute falciparum malaria in patients revealed that patients with splenomegaly accelerated clearance of radioactively labelled erythrocytes as opposed to patients with normal spleen sizes (Wyler et al., 1981; Looareesuwan et al., 1987). Yet, after antimalarial chemotherapy, patients with normal spleen sizes accelerated clearance of radio-labelled erythrocytes. Together, these results clearly showed that splenomegaly in sinusoidal spleens affects blood microcirculation and consequently the filtering capacity of this organ. Whether enhanced splenic function has any clinical benefit for patients with Plasmodium falciparum or other species remains to be determined.

Insights from splenectomy

Humans

Examination of a Plasmodium infection in splenectomized hosts has revealed that the spleen plays an important role both in parasite destruction and in modulating expression
of parasite antigens on the surface of the infected RBC. Plasmodium surface proteins may have important functions for parasite survival. They are implicated in antigenic variation, i.e. the ability to escape immune response by changing antigen expression over time (Scherf et al., 2008) and are involved in pRBC adherence to host cells and parasite sequestration in inner organs (Rowe et al., 2009). During a P. falciparum infection this phenomenon results in removal of mature stages (trophozoites and schizonts) from the peripheral blood, and is thought to be one of the major causes of complications of the disease.

Splenectomized patients invariably show an increase in parasitaemia during an infection with P. falciparum regardless of the antimalarial agent used (Demar et al., 2004; Bach et al., 2005; Bachmann et al., 2009) demonstrating the crucial importance of the spleen in parasite clearance. Furthermore, P. falciparum schizonts and trophozoites are found, albeit not always (Ho et al., 1992), in peripheral blood in the absence of a spleen, suggesting lack of sequestration. This phenomenon is illustrated by a study of a Cameroonian patient who revealed P. falciparum-infection with large parasitaemia (24%) and the presence of circulating mature stages 3 weeks after being splenectomized (Bachmann et al., 2009). RT-PCR analysis of blood samples could not demonstrate expression of multigene families, var, rif-A and stevor, and the pRBCs were unable to bind to host receptors (CD36, ICAM-1, VCAM-1 and P-selectin) in vitro. Interestingly, both of these functions were restored after 29 days of in vitro culture. In this study, MSP-1 genotyping indicated that changes observed in vitro were unlikely to be the result of outgrow of an initially under-represented parasite population.

As mentioned (Bachmann et al., 2009), two hypotheses for the changes of parasite phenotype observed in the absence of spleen can be made. (i) The asplenic environment could alter or reduce the expression of the parasite surface antigens responsible for sequestration. Although no mechanism has yet been identified, this could explain the appearance of mature parasites in peripheral blood of splenectomized patient. (ii) Antibodies against parasite antigens in immune patients could inhibit sequestration and increase parasite clearance. This may also select for parasites, which do not express the proteins responsible for sequestration at the surface of pRBC. In an intact host, as those parasites would have a reduced or no ability to sequester, only few of them would be able to survive or bypass the spleen. However, after splenectomy these non-sequestering parasites would be able to expand.

In naive splenectomized patients, expression of parasite surface proteins and the ability of pRBC to bind endothelial cells in vitro is not altered and cases of cerebral malaria have been observed (Ho et al., 1992; Buffet et al., 2011). These results would seem to indicate that splenectomy does not affect antigen expression or sequestration. In that case, observations made above in immune patients are more likely to be due to a selection of parasites not expressing antigens before splenectomy. However, it still does not explain why the expression of surface adhesins is then reactivated in vitro (Bachmann et al., 2009). Clearly, further studies are needed to confirm or invalidate those hypotheses on the impact of splenectomy on parasite phenotype.

Splenectomy has also revealed the importance of the spleen in infections with Plasmodium vivax. In fact, pathological examinations of post-surgery spleens due to P. vivax infections revealed extensive remodelling, intense phagocytosis and local infarction (Lubitz, 1949). In addition, the P. vivax genome, like that of P. falciparum, contains subtelomeric multigene families putatively involved in antigenic variation (del Portillo et al., 2001; Carlton et al., 2008). Noticeably, contrary to current views, recent reports have described cytoadherence of P. vivax-infected reticulocytes to endothelial receptors partly mediated by VIR proteins (Carvalho et al., 2010). Whether expression of these families is also influenced by the presence of the spleen remains to be determined.

Monkeys

Pioneering experiments in Macaca mulatta and Macaca sinica, infected with Plasmodium knowlesi and Plasmodium fragile respectively, showed lower or no recognition of pRBC by immune sera, and loss of pRBC cytoadherence to host cells after several infections of splenectomized animals (Barnwell et al., 1982; 1983; David et al., 1983; Handunnetti et al., 1987). As changes in pRBC phenotype was seen only after few passages in splenectomized monkeys, observations made could be the result of a gradual selection in this context. However, detection of surface proteins by immune serum was restored after only two erythrocytic cycles in intact monkeys (Barnwell et al., 1982; 1983; David et al., 1983; Handunnetti et al., 1987). The authors hypothesized that the spleen had influenced expression of surface antigens. Although the interpretation of these results is still not clear, they confirm the observations made in humans, showing that expression of surface antigens in a population of parasite can be modulated by the presence or the absence of the spleen.

Plasmodium falciparum in the experimental host, the squirrel monkey, expresses different serotypes on the surface of the red cell in splenectomized animals compared with those expressed in intact monkeys (Hommel et al., 1983). Furthermore, splenectomy affects the cytoadherence properties of the parasite such that the tissue sequestration is reduced in splenectomized animals. In vitro, pRBCs from intact monkeys bind to
CD36, in contrast to pRBCs from splenectomized monkeys that do not (David et al., 1983).

**Rodents**

Splenectomy of mice and rats at the time of crisis (a period of rapidly decreasing parasitaemia described in rodent malaria models) abrogates immunity and increases parasitaemia (Quinn and Wyler, 1980; Yadava et al., 1996), highlighting the role of the spleen in parasite killing and/or in generating and expanding the immune response. Splenectomy aggravates 17X non-lethal and ameliorates 17XL lethal malaria (Weiss, 1991) and the genetic background of the mouse affects the outcome of a Plasmodium yoelli 17X after splenectomy: there is no effect in DBA/2 mice by removal of the spleen, whereas C57BL/6 or BALB/c mice fail to resolve infection. Thus, host genotype may influence the contribution of the splenic response to control the infection.

The influence of the spleen on expression of parasite antigens on the surface of RBCs has also been assessed in rodents. Indeed, as for *P. knowlesi* and *P. fragile* in monkeys and *P. falciparum* in humans, several passages of a cloned line of Plasmodium chabaudi chabaudi AS in splenectomized CBA/Ca mice results in the absence of surface antigens as detected by immune serum, and the resultant parasites no longer sequestered in organs (Gilks et al., 1990).

Altogether, data from humans, monkeys and rodents unambiguously demonstrate the pivotal role of the spleen in malaria infections. Whether loss of surface antigen expression and pRBC cytoadherence is a phenotype appearing de novo as a direct consequence of the lack of the spleen (Barnwell et al., 1983; David et al., 1983; Handunnetti et al., 1987; Gilks et al., 1990) or whether there is a parasite sub-population lacking expression of surface antigens and cytoadherence, which upon splenectomy expands (Bachmann et al., 2009), is still a matter of debate. A better understanding of the host/parasite interactions in the spleen and studies of parasite phenotype in immuno-deficient hosts with an intact spleen could help to answer those questions. Combined use of modern molecular biology techniques with experimental systems, such as rodents and monkeys, in which the spleen has a similar influence on parasite biology as in human infection, offers an ideal approach to dissect these mechanisms.

**Immune responses**

One of the first lines of defence against a blood-stage malaria infection are the red pulp macrophages, which are highly phagocytic and are able to remove pRBC (Yadava et al., 1996). Splenic marginal zones in mice also contain cells of the innate immune system such as MZM, MMM and migrating DC that are uniquely placed to intercept and destroy pRBC. However, although MZM and MMM are crucial for the clearance of blood-borne particles and some pathogens (Seiler et al., 1997; Aichele et al., 2003), they have not yet been shown to remove pRBC. The location of the marginal zones also makes them a likely environment for the capture of malaria antigens by antigen-presenting DC, which then migrate into the T-cell areas. DC are the critical antigen-presenting cell (APC) in the spleen for activating T-cell responses. They require activation via a PRR interaction with parasite molecules to promote phagocytosis and upregulation of the antigen-processing machinery and molecules such as MHC class II, CD80, CD86 and CD40. These molecules are upregulated on splenic DC early in infections in rodent models of blood-stage malaria (Perry et al., 2004; Sponaas et al., 2006; Wilson et al., 2006) indicating that engagement of PRR(s) has taken place. The exact PRR requirements for activation of DC are not known. Toll-like receptors such as TLR2, which recognize the GPI (Krishnegowda et al., 2005; Zhu et al., 2005) on several parasite molecules, may be one possibility. Other PRR such as TLR9 (Wu et al., 2010) and the recently described AT-rich sensor (Sharma et al., 2011), which recognize Plasmodium DNA are intracellular and cytoplasmic respectively, and therefore would require prior phagocytosis of the pRBC for their activation.

**DC and T-cell activation in the spleen**

There are several populations of DC in the spleen: classical CD11c DC, which co-express or not CD8 and CD4, and plasmacytoid DC (pDC). Plasmacytoid DC do not appear to take up pRBC or present malarial peptides in vitro to T cells (Voisine et al., 2010). Similarly pDC isolated from *P. chabaudi*-infected spleens are not presenting peptides from the malaria antigen, merozoite surface protein 1 (Voisine et al., 2010). However, viable *P. berghei* parasites have been observed in pDC in vivo (Wykes et al., 2011), although it is yet to be determined whether this could result in parasite destruction and antigen presentation. Both populations of classical DC can take up parasite material, process and present Plasmodium peptides to CD4 T cells (Sponaas et al., 2006; Lundie et al., 2010) but with different kinetics. CD8+ DCs are effective APC early in infection. They produce IL-12 and induce proliferation and IFN-γ production in CD4 T cells and NK cells (Ing and Stevenson, 2009). In *P. chabaudi* infections, these DC apoptose, and the major APCs for CD4 T cells at the peak of infection are the CD8− DC (Sponaas et al., 2006). At this stage in a *P. chabaudi* infection CD4 T cells are activated to produce additional cytokines such as IL-10 and IL-4 and it has been suggested that this change...
in the Ag-presenting splenic DC population is responsible for the switch away from predominantly IFN-γ-producing T cells present in acute infection (Sponaas et al., 2006).

IFN-γ produced as a result of T cell or NK activation induces a small population of atypical progenitor cells in the bone marrow, giving rise, among other cells, to inflammatory monocytes, which migrate into the spleen during an acute P. chabaudi infection phagocyte pRBC in the spleen and contribute to the control of acute parasitaemia (Sponaas et al., 2009; Belyaev et al., 2010). There are several reports showing that splenic DCs during infection are unable to activate T cells effectively in vitro (Luyendyk et al., 2002; Ocana-Morgner et al., 2003; 2008; Millington et al., 2006; Wilson et al., 2006). Loss of DC function could have several causes. DC may have lost phagocytic ability and thus their processing and presentation capacity as a result of maturation (Lundie et al., 2010), the necessary costimulatory molecules are not upregulated (Carapau et al., 2007), and/or they secrete inhibitory cytokines such as IL-10 (Carapau et al., 2007). This downregulation could be viewed as part of a normal process of immune regulation within the spleen (Wilson et al., 2006). However, it is possible the acute inflammatory response induced by the infection may accelerate DC maturation and/or downregulation of DC functions such that activation of T cells may be prematurely curtailed (Lundie et al., 2010).

**Splenic architecture during malaria**

Splenic architecture is altered by malaria, which may also adversely affect developing immune responses (Fig. 2). The red pulp expands, and in mice is the site of significant haematopoiesis (Freeman and Parish, 1978; Alves et al., 1996; Achtman et al., 2003). Marginal zones are transiently lost (Beattie et al., 2006), and T- and B-cell zones

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**Fig. 2.** Structural remodelling of the mouse spleen during malaria infection. A and B. Haematoxylin-Eosine staining of the naive (A) and infected (B) spleen of C57BL/6 mice infected with P. chabaudi chabaudi AS showing hypercellularity induced by infection. C and D. Cryostat sections of the spleen from naïve (C) and day 10 (D) infection of C57BL/6 mice infected with P. chabaudi stained with IgD (red) and CD138 (green) evidencing the large increase of plasma cells (Cadman et al., 2008a). Cryostat sections of the spleen from day 1 (E) and day 5 (F) of infection of BALB/c mice infected with P. yoelli 17X non-lethal strain stained with DAPI (blue) and fibroblast growth factor 8 (red) showing the formation of a spleen barrier cells of fibroblastic origin (Martin-Jaular et al., 2011). Different cell markers in spleen studies are described in Table S1.
can be become indistinct with extrafollicular development of plasma cells (Achtman et al., 2003) (Fig. 2D). It is not clear which parasite moieties effect these changes but they are independent of signalling through Myd88, the adaptor molecule for many TLR (Cadman et al., 2008b). All of these changes can impact on the magnitude and nature of the malaria-specific immune response that is being generated while this is taking place. T cells cannot migrate into the B-cell areas thus impeding B-cell help (Millington et al., 2006), many short-lived plasma cells, which remain in the spleen, may contribute to a short-lived Ab response (Ndungu et al., 2009), and affinity maturation of Ab can be transiently impeded (Cadman et al., 2008b). Despite these dramatic but transient changes, a robust splenic immune response to the parasite is generated, including prominent germinal centre formation (Achtman et al., 2003) and generation of a long-lived memory B and CD4 T-cell response as well as long-lived plasma cells and protective antibodies (Ndungu et al., 2009; Stephens and Langhorne, 2010).

Comparison of splenic immune responses in mouse and human

To what extent data obtained on splenic immune response to malaria infection can be extrapolated to humans is still a difficult question to answer. Because of ethical issues, biopsies of spleen during acute infection cannot be performed, and lymphocytes and myeloid cells of peripheral blood cannot be directly compared with those in the spleen. Animal models and particularly rodents offer us the only opportunity to examine the initiation of immune responses in the T-cell areas and germinal centres of the spleen. DC and macrophages are normally tissue-resident cells, and although they can be derived from blood monocytes in vitro using cytokines, they represent only subsets of the splenic populations. Nevertheless, monocyte-derived DC or blood DC subsets can be both activated (Ndungu et al., 2009), or inhibited by parasite products or P. falciparum-infected RBC (Urban et al., 1999) similar to the responses of some splenic DC in mice.

In mice there are clear differences in the distribution and composition of the B-cell populations between spleen and blood. Immature and transitional B cells emigrating from the bone marrow to lymphoid organs as well as circulating naïve and memory B cells are found in peripheral blood, whereas naïve and differentiating activated B cells and plasmablasts, and germinal-centre B cells are present in large numbers in spleens (Ndutai et al., 2011). Thus, B cells in blood and spleen are not easily compared. However, the memory B-cell pool increases in the blood after repeated infections of children with P. falciparum (Weiss et al., 2009), similar to the increases in splenic memory B cells in mice reinfected with P. chabaudi (Ndungu et al., 2009); the major difference being that it appears to take several infections in children for the expansion of memory B cells, whereas this is apparent with two infections in mice.

There is only very limited information on cells of the innate and adaptive immune response in spleens of malaria patients. Immunohistochemical analysis of a spleens from post-mortem organs of fatal P. falciparum malaria in children show marked disorganization of the architecture, with loss of marginal zones, relative loss of B cells, and ill-defined T-cell zones (Urban et al., 2005) with some similarities to that observed in rodents. An analysis of a spleen from an active non-treated P. vivax infection has revealed extensive plasmablastic proliferation in vascular and perivascular spaces as well as significant increases in B cells and plasma cells, confirming that like the rodents models, a robust humoral immune response is taking place (M.V.G. Lacerda and H.A. del Portillo, unpublished), thus resembles polyclonal B-cell activation and extracellular plasma cell foci observed in mice infected with P. berghei, and P. chabaudi.

Structural remodelling

Seminal studies from Leon Weiss and collaborators elegantly documented the structure and function of the mouse spleen under steady state and during a blood-stage malaria infection (Weiss et al., 1986; Weiss, 1989; 1991). In a non-lethal malaria infection of BALB/c mice with the reticulocyte-prefering P. yoelii 17X, the ‘open’ circulation of the spleen is suddenly and temporarily changed to a ‘closed’ circulation. One of the consequence of this remodelling is the appearance of a syncitial layer of fibroblasts forming a physical barrier; barrier cells (Weiss et al., 1986). Such cells putatively contain receptors for the specific cytoadherence of P. yoelii 17X infected reticulocytes allowing macrophage-clearance escape and establishment of chronic infections (Martin-Jaular et al., 2011) (Fig. 2E and F). The existence of barrier cells in the human spleen remains controversial in malaria even though their presence in the human spleen in other pathologies has been verified (Bowdler, 2002). Moreover, extensive remodelling of the human spleen in malaria infections, characteristically consisting of white pulp expansion and a diffuse hypercellularity in the splenic red pulp, has also been reported from morphological studies of post-mortem or spleen rupture cases (Urban et al., 2005). Prospective studies using immunohistochemical and electron microscopy analyses from patients with malaria who have their spleen removed because of a pathological splenic rupture should clarify whether barrier cells are present and give us a more
detailed morphological view of the remodelling of the human spleen due to malaria infections.

Clinical aspects

Anaemia

Anaemia is probably the most frequent complication of malaria, even in areas where *P. vivax* predominates (Quintero *et al*., 2011). Classical work already has suggested that after complete eradication of malarial parasites RBC lifespan is reduced for 4–5 weeks and that reduction is associated with the presence of complement-containing complexes on the RBC surface, leading to increased eryrophagocytosis rate in the spleen (Woodruff *et al*., 1979). More recent data on histologic analysis in an *ex vivo* human spleen model show that more than 90% of artesunate pretreated pRBCs are retained and processed in the red pulp, providing the first direct evidence of a zone-dependent parasite clearance by the human spleen (Buffet *et al*., 2006). Because of their altered mechanical properties, less deformable ring forms are retained in the spleen, thereby reducing the parasite biomass available to sequester in vital organs, thus influencing the risk of severe complications, such as cerebral malaria or severe anaemia (Safeukui *et al*., 2008). However, prospective studies with small number of patients have shown that despite presenting a higher parasitaemia, splenectomized patients living in *P. falciparum*-endemic areas do not necessarily present more severe disease, likely because in previously immune patients the spleen is almost non-essential for protection against severe malaria (Bach *et al*., 2005).

Epidemiological data confirm that children from *P. falciparum* endemic areas develop splenomegaly over time in parallel to parasite density in peripheral blood and anaemia, both phenomena being reversed after 5 years of constant exposure, assuming therefore that premunition in malaria has also a reflection upon the spleen size and its role on anaemia pathogenesis (Bjorkman, 2002). It is hypothesized that co-evolution resulting in increased splenic clearance of *P. falciparum*-altered RBCs in children favours the survival of the host and, ultimately, sustained parasite transmission (Buffet *et al*., 2011). However, this information is largely unknown for *P. vivax*.

Anaemia is one of the major presentations of hyper-reactive malarial splenomegaly (formerly ‘tropical splenomegaly’) (Bryceson *et al*., 1983), which is the major cause of massive splenomegaly in Africa, followed by B-lymphoproliferative disorders, posing a not straightforward differential diagnosis. This clinical entity is characterized by a polyclonal activation of B-lymphocytes (increase of IgM) triggered by cumulative malarial infections, leading to immense spleen enlargement with signs of hypersplenism, positive antimalarial antibody, negative or weakly positive parasitaemia by conventional tests, and dilutional anaemia (Alecrim *et al*., 1982; Buffet *et al*., 2009).

Thrombocytopenia

Thrombocytopenia is a common complication in malaria (Lacerda *et al*., 2011). Some data suggested that platelets were sequestered in the spleen during the acute infection (Skudowitz *et al*., 1973); however, a more elegant methodology using scintigraphy with 111In-labelled platelets suggested that the sequestration or pooling of platelets from patients with uncomplicated malaria was non-splenic (Karanikas *et al*., 2004). In *P. chabaudi* infection in mice, thrombocytopenia was absent in splenectomized mice, reinforcing the essential role of the spleen in thrombocytopenia (Watier *et al*., 1992). The term hypersplenism was proposed to describe the clinical picture of an enlarged spleen followed by decrease of one or more peripheral blood cell populations, probably due to sequestration or destruction of cells inside the spleen. This is reverted by splenectomy. Spleen enlargement does not explain *per se* destruction of cells. Patients with both *falciparum* and *vivax* acute malaria followed daily for 7 days after the beginning of standard treatment demonstrate fast recovery of platelet count, which does not parallel the reverse in spleen enlargement measured by ultrasound, as illustrated in Fig. S1. In patients with malaria, the increase in the macrophage-colony stimulating factor is associated to thrombocytopenia, suggesting that macrophages play a role in the destruction of these particles (Lee *et al*., 1997).

Spleen rupture

If a patient with malaria is complaining of left upper quadrant abdominal pain, pleuritic left lower chest pain and/or enlarging tender splenomegaly (before or even during antimalarial treatment), splenic haematoma or splenic infarct should be suspected and managed accordingly to avoid the further life-threatening spleen rupture complication (de Lacerda *et al*., 2007; Imbert *et al*., 2009). Lack of prior immunity to malaria appears to be a major predisposing factor (Zingman and Viner, 1993). Because spleen biopsies are not ethically acceptable in patients with acute malaria, spleen samples taken at post-mortem after fatal complications such as sepsis are probably the only way to study this organ in malaria. However, only in a few studies was the histopathology of the spleen partially described. The mechanisms relating to the formation of splenic haematomas mostly associated to *P. vivax* infection and the interface with coagulation disorders and/or thrombocytopenia are noted to be imprecise.

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Future perspectives

As reviewed above, investigation of the mechanisms underlying spleen infections in humans is mostly restricted to clinical observations, exploration of feasible biopsies and post-mortem analysis of fixed tissue sections. Moreover, even studies using animal models, although being highly valuable, represent unlinked pieces that need to be puzzled out. With the advent of new technologies in the biomedical field, the understanding of the spatio-temporal events that malaria parasites undergo in the spleen is beginning to be understood and challenged.

Ex vivo model of human spleen

Buffet and collaborators developed a system for ex vivo perfusion of intact human spleens that preserved parenchymal structure, vascular flow and metabolic activity for up to 6 h (Buffet et al., 2006). These studies showed for the first time the ‘ex vivo’ physiology of this blood filtratory organ and added important insights into the mechanisms of pRBC clearance in an intact spleen, independent from parasite-priming and serum factors. Continuous usage of this model for P. falciparum, as well as for P. vivax (once a continuous in vitro culture system for blood stages have been developed), further guarantees a deeper insight into spleen physiology in malaria infections.

Imaging

Recent advances in imaging are accelerating the possibility of quantifying clinical aspects of the spleen in human and experimental infections. Thus, arterial phase CT scans revealed an abnormal enhancement of malarial splenic parenchyma that progressively regressed to a normal pattern, where differences in blood flow between red and white pulp become apparent (Karakas et al., 2005) after antimalarial treatment. Both P. falciparum and P. vivax malaria cases have been reported to induce splenic infarction based on CT images showing hypodense heterogeneous multifocal areas (Bonnard et al., 2005; Kim et al., 2007).

Implementation of confocal microscopy to image the spleen was first accomplished by Grayson et al. to assess T-cell recruitment in the white pulp (Grayson et al., 2001), which migration was later suggested to be directed by fibroblast channels originating in the marginal zone (Grayson et al., 2003; Bajenoff et al., 2008). Other studies using two-photon microscopy have reported T-DC synapses in the marginal zone and red pulp of the spleen (Morelli et al., 2003; Mittelbrunn et al., 2009). Noticeably, the use of intravital and magnetic resonance imaging to study the dynamic passage of P. yoelii 17X through the spleen and its remodelling has been recently reported (Martin-Jaular et al., 2011). In contrast to the dogma that malaria parasites sequester in the deep capillaries to avoid spleen clearance, these studies demonstrated active cytoadherence of infected reticulocytes to a spleen blood barrier of fibroblastic origin (Fig. 2E and F). The use of intravital and magnetic resonance imaging of the human spleen are yet to be reported but advances in optics and enhanced sensitivity of single-molecule probes for imaging will soon allow non-invasive in vivo human studies of the dynamic passage of malaria through the human spleen.

Concluding remarks

The spleen is a vital organ for the development of immune responses to the malaria parasite and for protection against severe malarial disease upon re-exposure. On the other hand, uncontrolled inflammatory responses and reticuloendothelial hyperplasia in the spleen during Plasmodium infection may cause vascular dysfunction and severe organ failure (Choudhury et al., 2008; Imbert et al., 2009; Buffet et al., 2011). The complex microcirculation comprising both closed and open circulation and the compartmentalization with different cells and functions limit our present 2D view of this organ. However, recent advances in bioengineering and microfluidics, are paving the way to construct 3D organs-on-a-chip, including the spleen (Baker, 2011; Deplaine et al., 2011; Herricks, et al., 2011). Although many challenges remain ahead, it is envisaged that a combination of all these new innovative technologies along with clinical observations will soon give us the full dynamic view of the role of the spleen in normal and pathological conditions caused by Malaria. This information in turn should help in the design of novel approaches in malaria vaccine development.

Conflict of interest

The authors declare no conflict of interest.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Seven-day follow up of four patients with vivax and falciparum malaria, after the beginning of antimalarial treatment, evidencing the fast recovery of platelet counts and the slower modification of the bigger axis of the spleen measured through single-observer ultrasound exam. Dashed lines represent the cut-off of normality for platelet count (150 000/mm3) and splenomegaly (12 cm) (Adapted from [Lacerda et al., 2007].

Table S1. Cell markers in spleen studies.

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