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Wave expansion of CD34$^+$ progenitor cells in the spleen in rodent malaria

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ABSTRACT

Defense against malaria depends upon amplification of the spleen structure and function for the clearance of parasitized red blood cells (pRBC). We studied the distribution and amount of CD34$^+$ cells in the spleens of mice infected with rodent malaria. We sought to identify these cells in the spleen and determine their relationship to infection. C57BL/6J mice were infected with self-resolving, *Plasmodium chabaudi* CR, or one of the lethal rodent malaria strains, *P. chabaudi* AJ and *P. berghei* ANKA. We then recorded parasitemia, mortality, and the presence of CD34$^+$ cells in spleen, as determined by immunohistochemistry and flow cytometry. In the non-lethal strain, the spleen structure was maintained during amplification, but disrupted in lethal models. The abundance of CD34$^+$ cells increased in the red pulp on the 4th and 6th days p.i. in all models, and subsided on the 8th day p.i. Faint CD34$^+$ staining on the 8th day p.i., was probably due to differentiation of committed cell lineages. In this work, increase of spleen CD34$^+$ cells did not correlate with infection control.

1. Introduction

Malaria is an endemic disease transmitted by *Anopheles* sp. and caused by the parasitism of red blood cells by *Plasmodium* sp. Malaria is associated with a high morbidity of two million deaths per year. Malaria especially affects children and pregnant women in developing nations (WHO, 2007). Immunity against malaria infection is neither protective, nor is it long-lasting. Exposure is associated with the development of symptoms followed by subsequent benign disease (Smith et al., 1997). Splenomegaly is one of the effects that individuals with immunity may experience after the first malaria attack (Soe-Soe et al., 2001). When infected individuals leave the endemic area, the absence of malaria infection is followed by return of the spleen to normal size, and a risk of severe malaria infection, similar to the first episode (Bacaner et al., 2004).

The spleen is a complex organ with several functions, including the removal of senescent or aberrant red blood cells (RBC) from circulation, as well as the removal of circulating pathogenic organisms (Krücken et al., 2005). Its importance in controlling malaria infection has been demonstrated in both human (Faucher et al., 2006) and rodent models (Oster et al., 1980), with reports also demonstrating an immunopathologic involvement of this organ in experimental malaria models (Eling, 1980).

In malaria infection, the spleen is responsible for parasite clearing, which is achieved through the filtration meshwork localized mainly in the marginal zone located between white and red pulp (Engwerda et al., 2005). As parasitemia increases, a large number of infected RBCs (iRBC) are retained in the red pulp. These iRBCs lack outer membrane flexibility and do not squeeze through the inter-endothelial slit in the venous sinus. Therefore, they are retained in the marginal zone and remain at the red pulp perimeter, causing acute, intense and significant congestion (Groom et al., 1991).

To control the overt infection, the splenic structures rapidly enlarge, increasing filtration capacity and iRBC removal. This enlargement involves the participation of several cell types, as well as endothelial progenitor cells (Hristov and Weber, 2004) and hematopoietic stem progenitor cells (HSPCs) (Nakayama et al., 2007). The CD34 antigen appears on the surface of these progenitor cells during early stages of cell differentiation and, afterward, decreases in intensity in relation to endothelial and hematopoietic cell differentiation (Krause et al., 1996). The HSPC migration and proliferation promotes hematopoiesis in organs with extramedullar hematopoiesis, such as the spleen, in stress situations (Li and Li, 2006).

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Due to ethical issues and the lack of safe, non-invasive methods to study human spleen amplification, experimental animal malaria models are used to study the role this process plays in malaria control. Rodent malaria models are especially useful for this goal because they allow for sequential and controlled study of parasitemia (Nitcheu et al., 2003) and splenic structure (Weiss et al., 1986) (Fávila-Castillo et al., 1996). There are several models of rodent malaria available that either allow for spontaneous control of infection, such as the CR strain of *P. chabaudi chabaudi* (Taylor et al., 1997) or lethal models that continuously evolve, such as the ANKA strain of *P. berghei* (Pamplona et al., 2007) or the AJ strain of *P. chabaudi chabaudi* (Long et al., 2006).

In these models, most studies are performed in splenectomized animals in order to study malaria in the absence of spleen function. Conflicting results have been reported, but these studies consistently describe an immunopathology associated with spleen function leading to longer survival of splenectomized mice infected with *P. yoelli* 17XL (Weiss, 1989) and *P. berghei* (Eling, 1980), and an absence of infection in controls in other models with non-lethal infection of *P. yoelli* and *P. chabaudi adami* (Oster et al., 1980).

The mechanisms controlling the amplification and organization of the splenic structure remain unclear. To understand this process, all spleen compartments must be studied by detecting migration and homing of the progenitor cells. These cells play an important role in the early stages of the enlargement process in other diseases, such as myeloproliferative disease (Baldwin et al., 2007). Our group reported the homing of immune progenitor cells, and described an organized array of chemokine expression in non-lethal malaria models. This array was disorganized in fatal models, but could be partially restored by chemokine supplementation (Garnica et al., 2002). We also studied dendritic cell populations related to the immune response in the amplification process, and in particular we focused on dendritic cells expressing surface $CD11c^+$ antigens (Garnica et al., 2005). These cells are involved in an innate immune response and an adequate adaptive immune response that is mediated by antigen presentation and immune cell selection, and occurs principally in the white pulp (Sponaas et al., 2006). The amplification of the filtration and phagocytic meshwork depends on growth and differentiation of cords of Billroth, localized in the red pulp. This could produce cells capable of generating new vascular structures for the filtration process. The study of cells that produce phagocytes and endothelial structures is essential for understanding the early stages of the rapid amplification of the spleen filtration meshwork. Furthermore, these cells also participate in homing of hematopoietic cells for blood production, which is essential for recovery from loss of blood cells during malaria infection. This blood recovery is dependent on undifferentiated cells with surface CD34 antigens. These cells have been reported to arise from extramedullar hematopoiesis occurring in the spleen and liver (Kiel et al., 2005). Using immunohistochemistry and flow cytometry, we studied the distribution and proliferation of $CD34^+$ cells in the spleen of mice infected with lethal and non-lethal malaria to better define the role of these cells in spleen amplification and infection control.

2. Materials and methods

All solutions were prepared with commercial reagents of at least pro-analysis quality and with sterilized 18 MΩ milliQ® water. When necessary, the specific origins of reagents are listed in the text.

2.1. Experimental group and parasites

Groups of 5–6 week old female Wild Type C57BL/6J mice were provided by the Biotério Central da Faculdade de Medicina da Universidade de São Paulo (FMUSP). The animals were maintained in sterilized cages and absorbent media; food and water was provided *ad libitum*. All animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals (Clark, 1996). Parasite strains, *P. chabaudi chabaudi* AJ, lethal, *P. chabaudi chabaudi* CR, self resolving, and *Plasmodium berghei* ANKA, lethal, (generous gifts of Dr. Walliker, University of Edinburgh, UK) were stored as stabilates in liquid nitrogen between experiments in order to avoid any selection of virulent strains.

2.2. Experimental malaria models, parasitemia determination and spleen removal

Parasitized mouse red blood cells (pRBC) from a liquid N_2 preserved stabilate were injected into mice of the same background. After amplification, 1×10^6 pRBC of each strain were injected i.p. into individual mice to induce a regular experimental infection. Groups at least 40 mice per parasite strain were infected for each experiment. Parasitemia, expressed as a percentage of infected erythrocytes, was monitored by Giemsa-stained tail-blood films, and determined in at least 1000 red blood cells by microscopic examination. Each day, groups of three mice were killed by carbon dioxide inhalation and their spleens were aseptically removed, weighed and immediately processed.

2.3. HE and CD34 immunochemistry

The spleens were fixed by immersion in at least 20 vol. of a 4% formaldehyde aqueous solution with 0.05 M $NaPO_4$, pH 7.2, overnight for subsequent paraffin embedding. Sections of these preserved spleen fragments were stained by routine hematoxylin–eosin (HE) staining and also fixed in silane treated slides for immunohistochemistry. The slides were incubated for 30 min in NH_4OH for endogen peroxidase destruction, and for 1 h in oxalic acid for malaria pigment destruction. For antigen recuperation, the slides were incubated in Tris–EDTA solution, at 96 °C for 30 min. After blocking endogenous biotin by incubation with streptavidin (DAKO Denmark A/S) for 15 min, followed by incubation with biotin (DAKO) for 15 min, $CD34^+$ detection was performed using a three step antibody amplified reaction. Sections were sequentially incubated overnight with rabbit polyclonal anti-CD34 (Santa Cruz Biotechnology, Inc., sc9095) at a 1:10 dilution, followed by incubation for 30 min with goat anti-rabbit IgG secondary polyclonal antibody (Sigma–Aldrich Co.) at a 1:200 dilution, and finally incubated for 30 min with 1:200 anti-goat IgG peroxidase conjugate (Sigma). The reaction was detected by DAB oxidation. Slides were counterstained with hematoxylin and representative fields were micrographed.

2.4. Spleen pulp morphometry

Red and white pulp portions of spleen were determined by modification of a previously described method for morphometry (Cardoso et al., 1996). Briefly, digital images with two mega pixels of resolution of randomized 10× fields of HE stained spleen sections were obtained with a plain achromatic objective, using a Canon Power Shot G5 digital camera (Canon Inc.). Images were analyzed with a superimposed 70-point grid using Adobe Photoshop CS2 software (Adobe Systems Inc.). Images were analyzed by a trained pathologist (Andrade Jr.). Marginal sinuses were used as the frontier of white pulp. Counts of the points observed above each pulp were determined, discarding those on fibrous stroma or without spleen tissue, in at least 10 fields of each animal. This resulted in at least 600 points for each animal and we used those data to determine the volume density (Vv) of white and red pulp. The estimated experimental error was 0.01 for the total computed *n*. The estimated weight for each pulp was determined using this Vv proportion of the total wet spleen weight determined at sacrifice.

2.5. CD34 flow cytometry

Spleens were weighed and mechanically dissociated in 10 vol. (w/v) of PBS containing 5% BSA and 2 mM of sodium azide. Spleens were then centrifuged through a gradient (*Ficoll-paque Plus*) at 700*g* at room temperature for erythrocyte exclusion. After two more washes with PBS buffer, performed by centrifugation at 400*g*, at 4 °C, cell suspensions (10^6 cells/ml, 500 μL) were incubated for 30 min with anti-CD34$^+$ mAB conjugated with phycoerythrin (Santa Cruz) at a concentration of 1 μg/10^6 cells and fixed with 70% ethanol at −20 °C. The acquisition of relevant events was performed using FacScalibur (Becton Dickinson immunocytometry Systems) with a minimum of 20,000 events. The analysis was performed using the Summit software (DAKO) by applying a variation of the Milan–Mulhouse protocol for HSPCs isolation (Gratama et al., 1998). This protocol modified to suit our experimental conditions. The FL2 and FL3 parameters were adequately compensated before any analysis.

2.6. Statistical analysis

Data describing the medians of parasitemia, spleen weight and CD34$^+$ cells were compared using the non-parametric Kruskal Wallis test, due to variances of significant differences, using Prisma 3.0 (Statsoft Inc.) software.

3. Results

3.1. Parasitemia and spleen growth

Here we present data from one of three similarly performed experiments. Parasitemia evolution and spleen growth in the studied models are shown in Fig. 1, and were statistically different. Parasitemia associated with infection by *P. chabaudi* AJ or CR strain underwent a rapid initial ascension, peaking on the 7th day and resolving at the end of experiment, but with distinct peak values. The AJ strain infection presented elevated levels (>60%) of parasitemia, with erratic values after the 8th day of infection, when most of the infected mice died (>75%). The CR strain infection showed self-resolution and was associated with lower levels of parasitemia than the AJ strain (>40%). Infection with *P. berghei* ANKA resulted in lower parasitemia than the *P. chabaudi* strains (>20%), and presented a slow parasitemia evolution, with continuous growth and without control until the end of experiment. No early death due to cerebral involvement or any other cause, was detected during the ANKA strain infection.

The spleen presented a similar enlargement during all infections, but achieved distinct values, as shown in Fig. 1C and D. In the acute phase, the AJ strain infected mice presented larger spleens (>0.4 g) until the 7th day of infection. At the end of the experiment, ANKA strain infection presented larger spleens (>0.7 g) than the other strains, despite lower parasitemias. Morphometric data showed that the red pulp increase was more accentuated in ANKA strain infection in the initial days of the experiment, but at the end of experiment, red pulp growth was more accentuated in AJ strain infection (>0.5 g). The white pulp in ANKA strain infection presented continuous growth until the end of the experiment, showing values higher than 0.2 g. Infection by other strains did not present the same evolution.

3.2. CD34$^+$ cell immunochemistry

Spleens of all models were submitted to HE and CD34 immunohistochemistry, as described in Section 2. HE of normal spleen is

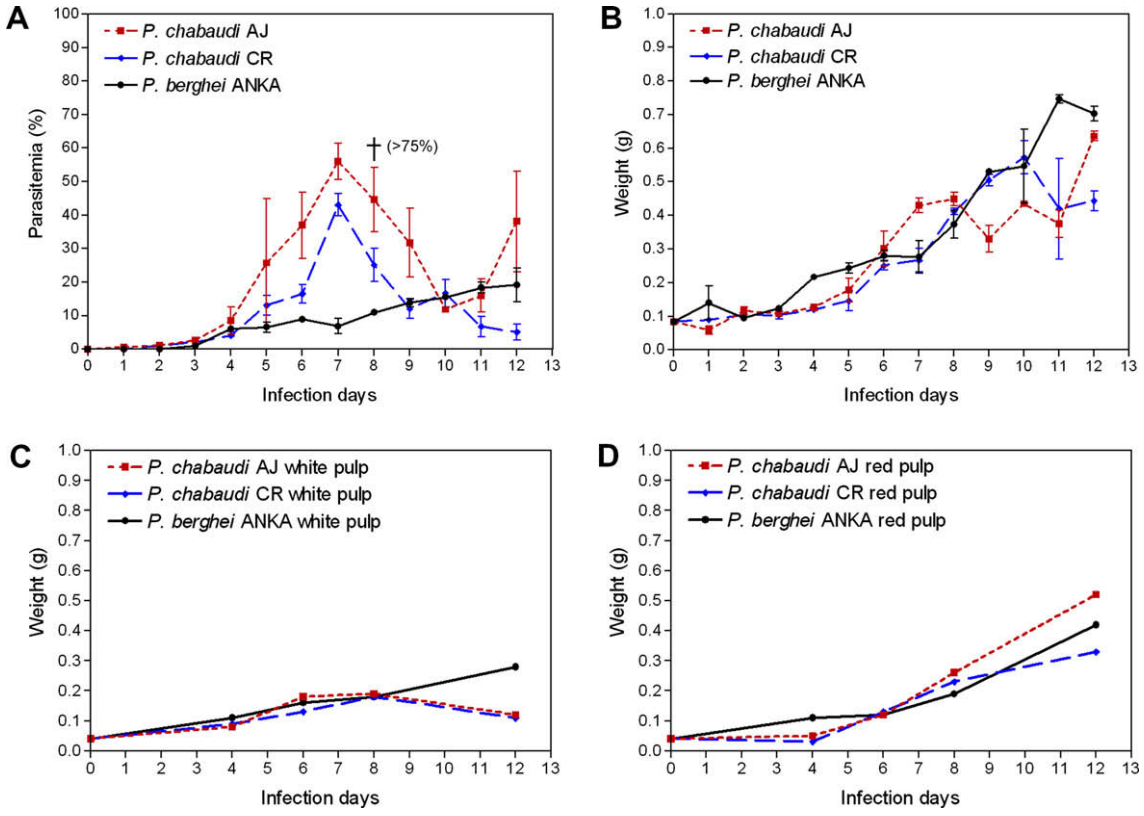

Fig. 1. Parasitemia (A) spleen growth (B) white pulp growth (C) and red pulp growth (D) of 5–6 week old C57BL/6J mice infected with 10^6 *P. chabaudi* AJ (dotted line), *P. chabaudi* CR (traced line) or *P. berghei* ANKA (continuous line) parasitized erythrocytes by i.p. route (n = 40 mice/group; 3 mice/time point). Parasitemia errors bars represent the 95% confidence interval of the median. The symbol (+) represents the day of infection when the first death by *P. chabaudi* AJ infection was registered.

shown in Fig. 2A. The efficiency of immunohistochemistry was tested in the absence of primary CD34 mabs, and Fig. 2B shows an absence of non-specific staining. Normal spleen submitted to the complete reaction is shown in Fig. 2C. These samples show clearly stained cells in two main areas. We observed several faintly stained cells in the white pulp and scattered large cells with gross and intense staining in the red pulp. Sequential evolution of the spleens of mice infected with malaria strains are shown in Fig. 3. In the mice infected with the CR self-controlling strain, the spleen structure was maintained during spleen amplification, as shown in Fig. 3 lane B, and this was not seen in lethal models. The immunohistochemistry of $CD34^+$ cells in spleens of mice infected with the malaria strains is shown in Fig. 4. We focused our analysis on gross stained $CD34^+$ cells in the red pulp. The spreading of $CD34^+$ cells was increased in the red pulp on days 4 and 6 p.i. in all models (Lanes A–C), as compared to non-infected spleens. On the 8th day p.i., $CD34^+$ staining was faint in most cells of the red pulp in all strains, suggesting a differentiation of red pulp infiltrating cells in committed cell lineages. On the 12th day p.i., grossly stained $CD34^+$ cells reappeared in red pulp, always associated with the extramedullary erythropoiesis foci. Foci of extramedullary erythropoiesis and germinal centers were observed in all models, but were only highly abundant in *P. chabaudi* strain infections (Lanes A and B), and not seen in the spleens of *P. berghei* infected mice (Lane C). Our data indicate an increase in $CD34^+$ cells in the red pulp and probably a differentiation of these cells during malaria infection.

3.3. $CD34^+$ flow cytometry

We used flow cytometry to determine the total amount of free $CD34^+$ cells and free HSPCs $CD34^+$ in spleen tissues. The first cell population was selected by a protocol in which only events with fluorescence above the first log of axis were counted and unviable events were excluded by FSC and SSC parameters. To determine the HSPCs $CD34^+$, we used a modified Milan–Mulhouse protocol. The results of flow cytometry are shown in Fig. 5. The total number of free $CD34^+$ cells and free $CD34^+$ HSPCs in spleens of mice infected with the studied strains are reported according to post infection evolution. Total number of free $CD34^+$ cells in spleen tissues increased on the 4th, 8th, and 12th days p.i. in all infection models used in this experiment. Free progenitor round and smooth $CD34^+$ cells (HSPCs) appeared as a wave on the 4th day p.i. in all models. On the 8th day, the number of these cells decreased in *P. chabaudi* CR and *P. berghei* ANKA infections, but not in *P. chabaudi* AJ infection. On the 12th day p.i., these cells reappeared in all models, but at lower levels in mice infected with *P. chabaudi* CR and *P. berghei* ANKA. *P. chabaudi* models presented equivalent numbers of these cells at the 4th day p.i., which was larger in the *P. berghei* mice despite absence of malaria control. These data are consistent with the immunohistochemistry findings in the red pulp, which suggested a wave influx of free $CD34^+$ HSPCs in spleen during rodent malaria infection.

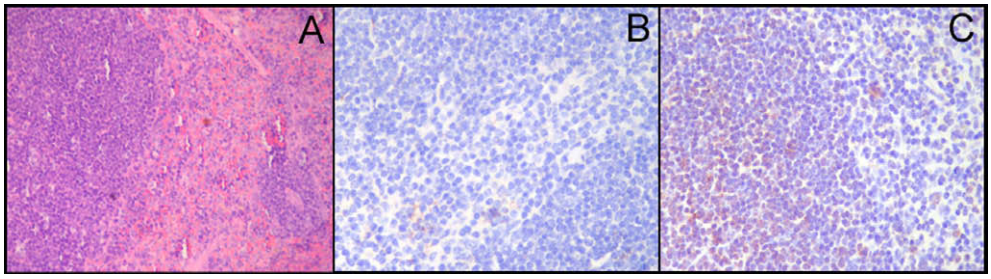

Fig. 2. HE (A) and immunochemistry of $CD34^+$ cells in pre-infection spleen mice stained with primary antibody (C) and without (B) to verify the efficiency of the reaction. Note the clear marginal sinus in HE stained sections (arrows).

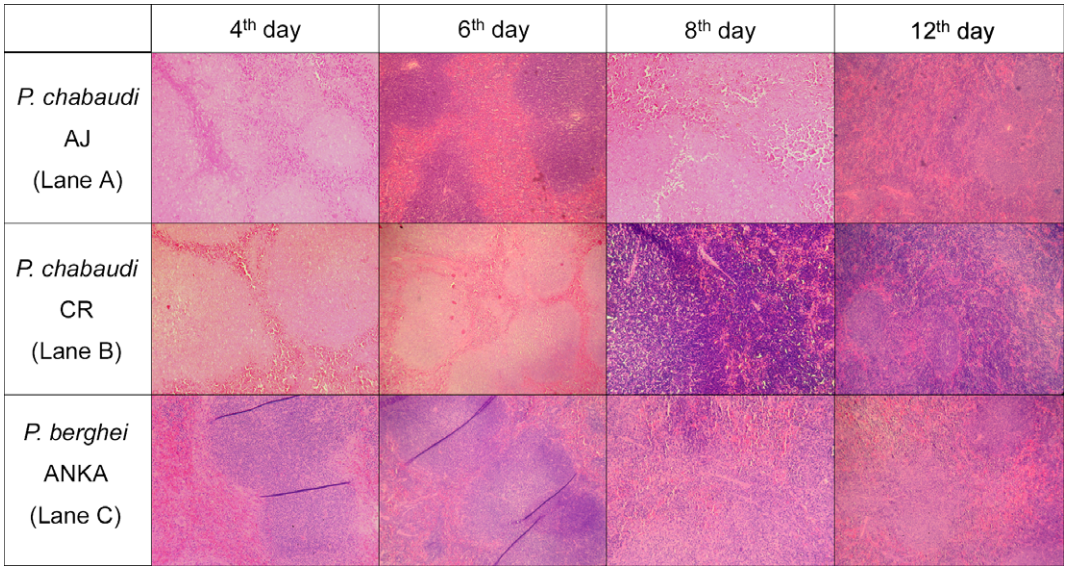

Fig. 3. HE in spleen of C57BL/6J mice infected with 10^6 *P. chabaudi* AJ (Lane A), *P. chabaudi* CR (Lane B) and *P. berghei* ANKA (Lane C) parasitized erythrocytes on days 4, 6, 8 and 12 p.i.

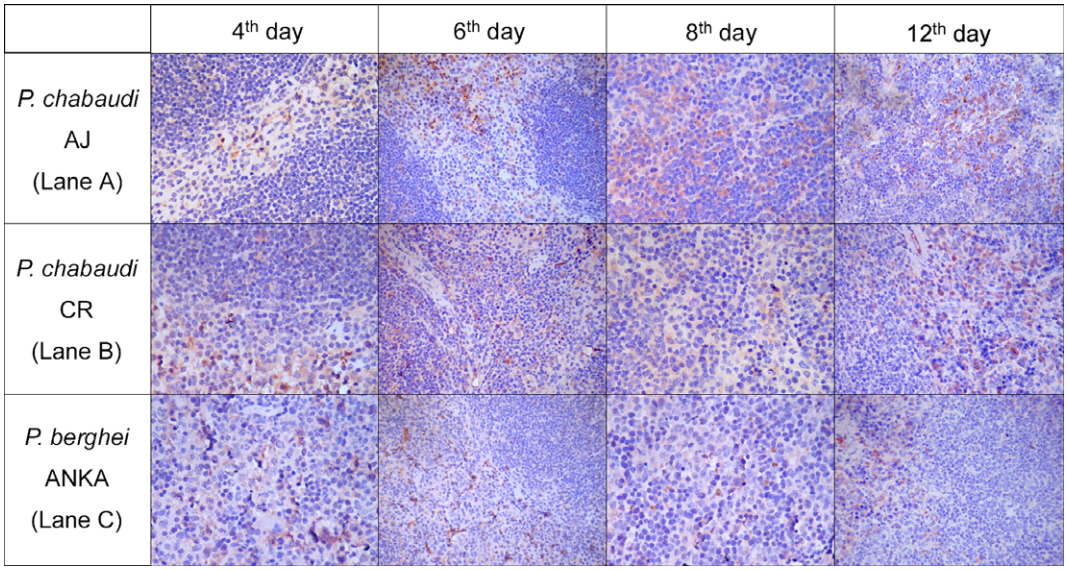

Fig. 4. Immunochemistry of CD34$^+$ cells in spleen of C57BL/6J mice infected with 10^6 *P. chabaudi* AJ (Lane A), *P. chabaudi* CR (Lane B) and *P. berghei* ANKA (Lane C) parasitized erythrocytes on days 4, 6, 8 and 12 p.i.

4. Discussion

In this study, we investigated the distribution and number of CD34$^+$ cells in C57BL/6J mice spleen in different rodent malaria models, as well as parasitemia evolution and spleen growth during these infections. *P. chabaudi* AJ and CR strains presented similar parasitemia evolution, but had distinct values for the parameters measured in this study. This fact may be explained by the high synchronicity and virulence that the AJ strain presents in its asexual erythrocyte cycle (Cavinato et al., 2001). This AJ strain regularly causes high activation of the immune system, an increased clearance of normal and parasitized cells (McDevitt et al., 2006) and, consequently, intense anemia (Chang and Stevenson, 2004). In the latter stages, when levels of parasitemia were more than 50%, non-infected erythrocytes were present only at hematopoietic sites, such as bone marrow and spleen. This condition can lead to intense anemia and death of the infected mouse. The mice surviving this stage of AJ infection presented minor parasitemia levels and may represent a specific group with a different biological response to distinct inoculums or host comportment.

Plasmodium berghei ANKA infection presented a slower parasitemia than that observed for *P. chabaudi* infection, showing lower values and the absence of resolution until the end of the experiment (Garnica et al., 2002). However, despite minor levels of parasitemia, this parasite offers a large antigenic load, due to large schizonts, polyparasitism and enlargement of the infected cell (Killick-Kendrick, 1974). These effects were not seen in *P. chabaudi* pRBCs (Landau and Killick-Kendrick, 1966).

The spleen amplification was similar in infections by the strains used in this study, though it was slightly more intense in *P. berghei* ANKA infection than in *P. chabaudi* infection at the end of experiment. This could be related to the intense congestion that these species cause in the spleen. *P. berghei* causes erythrocyte hypertrophy (Killick-Kendrick, 1974), which affects cell transit through the inter-endothelial slit (IES) located in the venous sinuses (Groom et al., 1991), resulting in early congestion in this region. Comparatively, the AJ strain infection caused more splenic amplification in the acute phase, evident on the 7th day p.i., before death.

The splenic pulp evolution also showed different patterns in the three models used in this experiment. In *P. berghei* ANKA infection, the red pulp increased more than it did in *P. chabaudi* infected mice at the 4th day p.i. This may be due to the deformity that *P. berghei* causes to erythrocytes, as well as the high efficiency of the filtration meshwork that is localized in red pulp, for capturing aberrant erythrocytes (Groom et al., 1991). At the end of the experiment, the red pulp in AJ infected mice showed a greater increase than red pulp in CR and ANKA infected mice. This may correlate with the high levels of parasitemia that this infection causes. This fact may be related to the intense involvement of the spleen in erythrocyte replacement, as was described in human cases of myelodysplasia (Barosi et al., 2004).

In *P. berghei* infection, the white pulp grew throughout the experiment whereas the *P. chabaudi* strains did not exhibit this constant growth. This fact may be related to the high antigenic load that this species may offer per parasite. This could consequently lead to a higher stimulation and ampliation of spleen lymphoid tissues. In *P. chabaudi* infection, white pulp amplification might be efficient or interrupted at any specific moment, resulting in death of the AJ infected host, or survival of the CR infected host.

By using immunohistochemistry to detect CD34$^+$ cells, we observed two distinct behaviors in spleen pulps. In the white pulp, the CD34$^+$ cells were occasionally dispersed in the lymphoid follicles, with some intense labeling in the spleens of control mice as previously described (Pusztaszeri et al., 2006). Using these data, we could not determine that an influx had truly occurred according to the requirements of marked-cell infusion experiments (Miyata et al., 2008). However, we could hypothesize that staining would be obliterated by an increased efflux rate and/or death of this population.

In the red pulp, the phenomenon was more intense, with no CD34$^+$ staining detected in control mice. On the 4th day p.i., intense staining appeared on both isolated cells and vascular stroma-cell regions. This could be related to the HSPCs and/or the erythroid progenitor cell populations that express the CD34 antigen, and might increase red pulp during malaria infection (Lamikanra et al., 2007).

At the time of these experiments, we initially observed intense marking of multiple types of CD34$^+$ cells in the red pulp and marginal zone in all models. This was probably the result of the bone marrow mobilization that occurs in response to an increase of CXCL12 expression in spleen tissues during *P. chabaudi* CR and *P. berghei* ANKA infection (Garnica et al.,2002, 2003). This chemokine

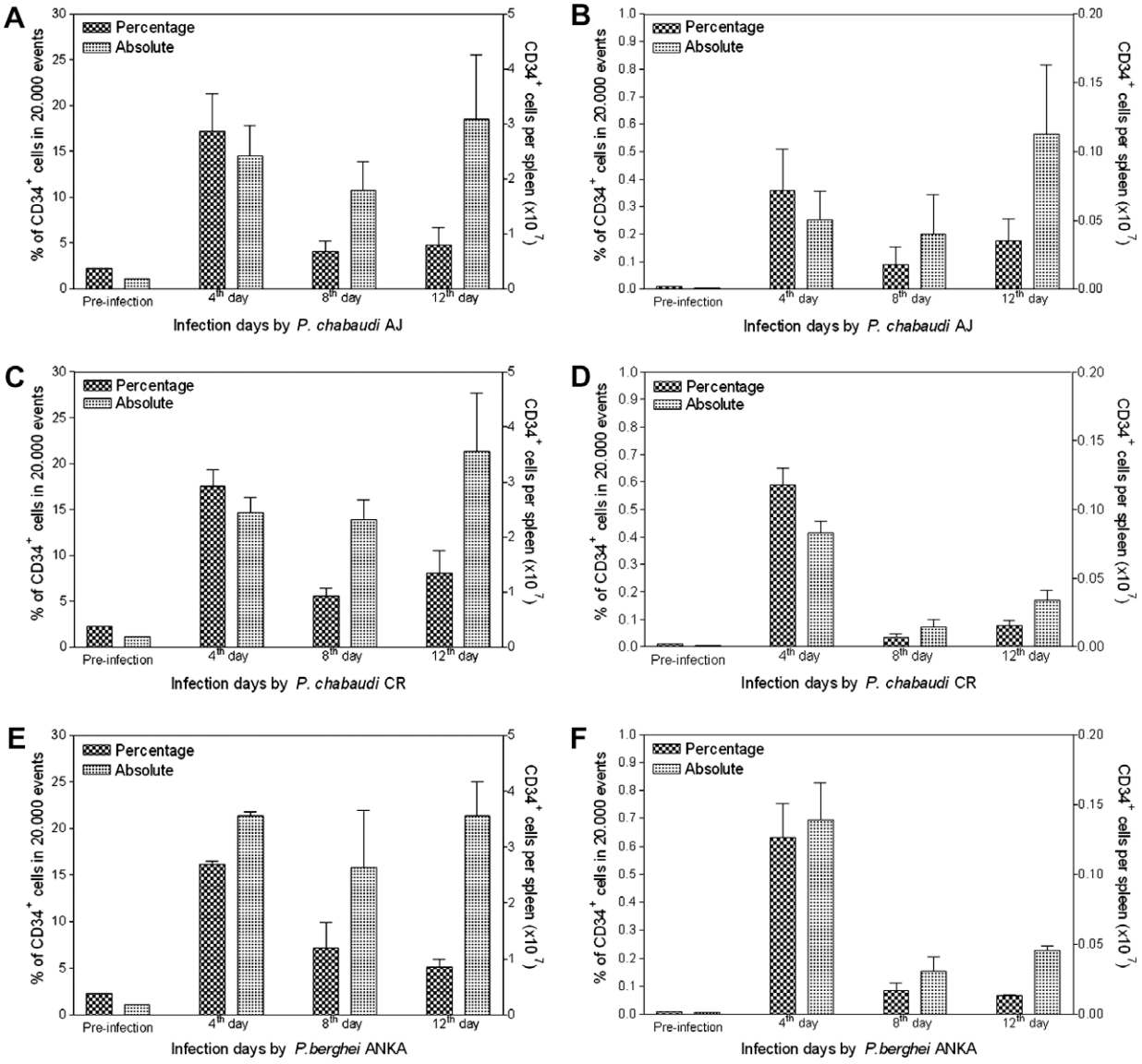

Fig. 5. Flow cytometry of the amount of free CD34$^+$ cells (A, C and E) and HSPCs CD34$^+$ (B, D and F) in spleen-dissociated cell suspensions of C57BL/6J mice infected with 10^6 *P. chabaudi* AJ (A and B), *P. chabaudi* CR (C and D) and *P. berghei* ANKA (E and F) parasitized erythrocytes at day 4, 8 and 12 p.i. Dark gray bars, percentage of CD34$^+$ cells in 20,000 events; Light gray bars, absolute number of CD34$^+$ cells per spleen.

promotes migration and adhesion of HSPCs in hematopoietic niches (Kopp et al., 2005). Concurrent with the earlier appearance of extramedullary hematopoietic foci, the CD34$^+$ cell staining diminished over time, suggesting differentiation of progenitor cells in other cell lineages that might play a role in spleen ampliation and cell clearance.

These immunochemistry findings were corroborated by flow cytometry, since we observed an increase of free CD34$^+$ on the 4th, 8th and 12th days p.i., and an increase of free CD34$^+$ HSPCs on the 4th and 12th days p.i. In *P. chabaudi* infection, the amount of free CD34$^+$ HSPCs and free CD34$^+$ cells, and their distribution in spleen tissues on 4th day p.i., were similar in both strains tested, with a wave distribution during early spleen amplification. On the 8th and 12th days p.i., the survivor fraction of the AJ infected mice presented a larger amount of free CD34$^+$ HSPCs than the CR infected mice. This was similar to that found in *P. berghei* ANKA infections. Despite the wave presented in *P. berghei* infection, this parasite caused disorganized spleen amplification, probably due to CD34$^+$ destruction during bone marrow parasite schyzogony and a consequent innate immune response. However, this infection presented a larger amount of free CD34$^+$ cells and CD34$^+$ HSPCs than *P. chabaudi* infection on the 4th day p.i. Moreover, lethal infections presented greater amounts of CD34$^+$ cells, unrelated to infection resolution and malaria control.

Based on colony forming assays, and without identification by specific markers, a wave of production of progenitor cells appeared on the 4th of *P. berghei* infection (Asami et al., 1992). For mice that are deficient in progenitor cell function, rodent malaria causes high mortality, without spleen amplification, demonstrating the importance of spleen hematopoiesis (Asami et al., 1991). Other reports have suggested that defective spleen extramedullary erythropoiesis implantation was directly related to mortality, since levels of erythropoietin and other hematopoietic factors were normal (Villeval et al., 1990; Chang and Stevenson, 2004; Lamikanra et al., 2007). This fact explains some of the bone marrow alterations in patients with malaria and anemia, such as interruption of erythropoiesis despite adequate stimulation by erythropoietin (Abdalla and Wickramasinghe, 1988). These alterations were attributed to

soluble factors in serum from malarial patients from Thailand. This complex defect of erythropoiesis was attributed to interaction between pro-inflammatory cytokines, like IL-12, TNF and MIF, and erythropoietic progenitor cells (Jootar et al., 1993). IL-12 was studied in experimental models and exhibited an inverse relationship with dyserythropoiesis (Mohan and Stevenson, 1998). TNF was indicated as inducing anemia (Odeh, 2001), but this fact has been questioned in recent works, ascribing only a supplementary function to TNF in the process, both in human malaria (Casals-Pascual et al., 2006) and experimental models (Hernandez-Valladares et al., 2006). Our work studied the participation of $CD34^+$ cells in splenic amplification. This is a crucial process in parasitemia control. However, no evidence indicates a cell infiltration block in lethal models that present this pattern of non-lethal model. This data indicate that other mechanisms, not related to $CD34^+$ cells, are evolved in inducing death or cure of infection. Some works have described an infiltration of $CD11c^+$ cells (Ing et al., 2006), a typical lymphoid progenitor in spleen. This may be related to the enhancement of CXC12 expression in spleen tissues during malaria infection (Garnica et al., 2002). CXC12 also promotes migration of $CD11c^+$ dendritic cells to spleen tissues when exogenously administered in malaria experimental models (Garnica et al., 2005). Another study has also reported this migration during *P. chabaudi* AS infection (Leisewitz et al., 2004), but we did not exclude the reciprocal effect between these precursor cells. An enhancement of $CD34^+$ cells in spleen tissues may indicate an enhancement in hematopoiesis that may contribute to malaria control, especially when this enhancement represents activation of immunologic cells. Hematopoietic precursor cells may boost the immunologic cells by diverse mechanisms since these cells are also precursors of immunologic lineages and hosts in integration and differentiation processes (Kobari et al., 2006).

In our lethal models, the isolated increase in $CD34^+$ cells did not relate to the infection control. Therefore, we could not attribute an essential function to these cells in disease resolution. One possibility is that these cells only replaced the erythroid cells that were destroyed during infection, but we could not determine whether this was activated by differentiation and/or division, entry into quiescence, or entry into apoptosis. We observed no indication of apoptosis in histology. Our immunochemistry and flow cytometry data demonstrated an influx of $CD34^+$ cells into the spleen on the 4th and 12th days p.i. during *P. chabaudi* and *P. berghei* infection, and interruption of differentiation on the 8th day, independent of the lethality of the strain. In the present work, an increase of spleen $CD34^+$ cells did not correlate with infection control in rodent malaria.

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