Dendritic cells subsets mediated immune response during *Plasmodium berghei* ANKA and *Plasmodium yoelii* infection

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**A B S T R A C T**

The roles of dendritic cells (DCs) in mediating immunity against *Plasmodium* infection have been extensively investigated, but immune response during pathogenesis of malaria is still poorly understood. In the present study, we compared the splenic DCs phenotype and function during *P. berghei* ANKA (PbA) or *P. yoelii* (*P. yoelii*) infection in Swiss mice. We observed that PbA-infected mice developed more myeloid and mature DCs capable of secreting IL-12, while *P. yoelii*-infected mice had more plasmacytoid and immature DCs secreting higher levels of IL-10. Expression of FoxP3, IL-17, TGF-β and IL-6 were also different between these two infections. Thus, these results suggest that the phenotypic and functional subsets of splenic DCs are key factors for regulating immune responses to PbA and *P. yoelii* infections.

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1. Introduction

The spleen is a convoluted organ with numerous functions [1], including immuno-pathologic involvement of this organ in human [2], rodent models [3] and in experimental malaria models [4]. *Plasmodium falciparum* infection is a major cause of malaria deaths accompanied by severe clinical symptoms, comprising cerebral malaria (CM), circulatory collapse, severe anemia, metabolic acidosis and multiorgan failure [5]. Animal models and particularly rodents offer us the only opportunity to examine the initiation of immune responses in the T-cell areas and germinal centers of the spleen, a major site for the control of blood-borne infections during malaria [6]. In response to Plasmodium parasites, DC plays a significant role at the host-pathogen interface and delivers a crucial link between innate and adaptive immune responses. DCs are diverse and distinct specialized population of antigen presenting cells (APC), divided into different subpopulations myeloid DCs (CD11c+CD11b+) and plasmacytoid DCs (CD11c−CD45R/B220+) [7–11]. Previously it was indicated that among the two subsets of DC, depletion of myeloid/conventional DCs resulted in reduced activation of malaria-specific T cells and inhibition of experimental CM development [12]. However the pathogenesis of CM in the context of DC immune regulation is yet to be revealed.

On the uptake of antigen, DCs process and present peptides to naive T cells and secrete pro-inflammatory or anti-inflammatory cytokines that influences the nature of the response [13]. Interleukin-10 (IL-10) have been reported to be a potent regulator of DC maturation and effector function. Further, up-regulation of co-stimulatory molecule, secretion of proinflammatory cytokines such as IL-12 and tumor necrosis factor (TNF-α) and DC capacity to stimulate T cells are effectively suppressed by IL-10 [14–20]. In addition, APC function can also be influenced by IL-10 secreted by DCs in an autocrine manner [21,22]. Importantly, the production of cytokines by DCs and the presence of co-stimulatory molecules on the DC surface play a precarious role in promoting the differentiation of Th0 cells into phenotypes such as Th1, Th2, Th17, or regulatory T (Treg) cells [23–25].

The abilities of cytokines either pro or anti-inflammatory to promote or protect from severe malaria are likely to depend on many factors, including the hosts’ ability to DC maturation and the immune status of an infected individual. Moreover, exposure of DCs to IRBC may lead to expansion of DC subsets that differ in their ability to induce effector CD4+ T cells [26,27]. In addition, there are reports that the increase in mDC/pDC ratio is associated with the activation of the Th1 pathway, whereas activation of the Th2 pathway is associated with decreased ratio [28,29]. Therefore, it is very tempting to invoke a role for DC subsets to possibly accomplish a delicate balance between control of overwhelming infection and prevention of immune-pathology during malaria [30].

Consistent with findings that the ratio of mDC to pDC was found much lower, along with high level of Tregs in acute *Plasmodium vivax* patients than those of naïve and immune controls [31], in our study we observed difference in mDC/pDC ratio between PbA and *Plasmodium yoelii* infection. However, the functions of APC...
subsets are dependent on a number of limits, comprising source of the mouse strain, regional localization along with parasitic infections and T cell/APC ratio [32,33]. We therefore investigated the effect of PbA and P. yoelii infection on DC subsets, DC maturation and CD11c+IL-12, CD11c+IL-10, FoxP3, IL-17, TGF-β and IL-6 expression in Swiss mice. In our previous work we have shown that TGF-β and IL-6 might play an important role in modulating the balance between the differentiation of Treg and Th17 cells in malaria [34]. In this study, it is generally assumed that PbA infection might promote DC phenotypic maturation and expresses IL-12, IL-6 and IL-17, while suppressing IL-10, TGF-β and FoxP3 whereas, the reverse phenomenon during P. yoelii infection was observed. Besides, we investigated that variations in DC subsets possibly play an important role in immune response, and the subsequent disparity in infection outcome of different strains.

2. Materials and methods

2.1. Chemicals and reagents

RPMI 1640 medium with 2.05 mM L-glutamine, HEPES buffer, Antimycotic solutions, Gentamycin solution and fetal calf serum (FCS) were purchased from Hyclone, USA. The following antibodies FITC-anti-CD11c (#117305), PE-anti-B220 (#103207), PE-anti-CD86 (#105007), PE-Cy5.5-anti-CD40 (124624), FITC-anti-CD69 (#104505), FITC-anti-CD62L (#104405), FITC anti-MHC class II (#107605), PE-IL-10 (#505007), purified anti mouse-IL-17 (#506905), purified Foxp3 (#320001), purified IL-6 (#504505) and IgG Abs were obtained from Bio legend (New Delhi, India). PE-IL12 (#554479) was procured from BD Biosciences. Primary antibodies against TGF-β and alkaline phosphatase (AP)-conjugated anti mouse and anti-rabbit secondary antibodies were obtained from Cell Signaling technology, Inc. (Danvers, MA). HRP (Horseradish peroxidase) conjugated secondary anti mouse, anti-rabbit antibodies, pre-stained protein molecular weight marker and 3,3’-diaminobenzidine tetrahydrochloride (DAB) system were bought from Bangalore GeNei (Bangalore, India). All the remaining chemicals were purchased in an analytical grade of the highest purity (India).

2.2. In vivo malaria infection

Male Swiss albino mice obtained from the National Institute of Nutrition (Hyderabad, India). Mice 6–8 weeks old were maintained in sterilized cages and absorbent media; food and water were provided ad libitum. Animal experiments were carried out at the Animal Housing Unit in the Department of Zoology, University of Calcutta as per the guidelines as approved by the Institutional Animal Ethics Committee (IAEC) University of Calcutta, and conforms with the “Principles of Laboratory Animal Care” (NIH publication 144 No. 85-23, revised in 1985).

Mice were infected with Plasmodium berghei ANKA and P. yoelii 17XNL pRBC obtained from National Institute Malaria Research Center, New Delhi, and blood parasitemia, survivability and weight change were studied as described previously [34].

2.3. Isolation of splenocytes & cell viability assay

Spleens from PbA and P. yoelii infected mice were aseptically removed in 4, 6 and 8 dpi along with spleen from uninfected age matched as controls and single cell suspensions were made in RPMI 1640. Then the splenic cell suspension was allowed pass through nylon mesh with 50 μm pore size (Becton Dickinson, Franklin Lakes, NJ). The cell suspension was centrifuged at 1500 rpm at 4°C, the respective cell supernatants were discarded and the pellet was resuspended in 2 ml RBC lysis buffer (1×) for

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**Fig. 1.** (A) Parasitemia, (B) survival rate and (C) weight change of Swiss albino mice after infection with P. berghei ANKA and Plasmodium yoelii. Mice were separately infected with $1 \times 10^6$ infected RBCs intraaperitoneally obtained from a homologous passage mouse that had been infected with PbA and P. yoelii. Matched controls received an equal amount of uninfected erythrocytes (suspended in PBS). For monitoring of parasitemia, a Giemsa-stained thin smear was made daily as shown in (A). Values represent the mean with SEM ($n = 5$ mice per group). Results are representative of five independent experiments. (D) Mice of respective experimental groups along with matched controls were sacrificed in respective dpi and splenocytes were counted. Data is representative of five ($n$) comparable experiments and represented as mean and ±SD values. The results were evaluated by ANOVA. Significant differences between infected and control mice are marked "p < 0.05;" and differences between infected groups on respective days are marked (by letters). The same letter indicates no significant differences and different letters indicate significant differences.
5 min at room temperature. Further, an equal volume of PBS (1×) was added to the splenic cell suspension to stop the reaction and centrifuged at 1500 rpm at 4 °C. The supernatants were discarded and pellets were resuspended in PBS and the viable splenocytes for each animal were counted in a hemocytometer by trypan blue exclusion test.

2.4. Flow cytometry analysis of dendritic cell maturation markers

Spleens were dissected out from the respective mice group, and single cell suspension was prepared as described earlier. After accurate cell count, cells were divided into 10⁶ cells/100 μl in cell staining buffer (CSB) or in flow buffer, containing 3% FCS. These
cells were then incubated with anti-CD16/32 (Fc-block) for 5 min at 4 °C to block nonspecific binding. Intracellular staining for PE-IL12 and PE-IL-10 was performed as described previously [34]. Further the cells were incubated with the titrated amount of APC-CD11c, FITC-CD11c, FITC-CD11b, FITC-B220, FITC-CD80, PE-CD86, FITC-CD40 and FITC-MHC-II fluorochrome conjugated primary antibody in the dark, and incubated for 30 min to 1 h. Finally the cells were washed and the pellets were resuspended in 100 µl CSB and were analyzed using FACS aria III in the FACS diva software (BD bioscience).

2.5. Immunohistochemical analysis of TGF-β and IL-6

Immunohistochemistry was performed according to Keswani and Bhattacharyya [34]; briefly, spleen tissue from the paraffin embedded blocks were cut into 5 µm sections and mounted on positively charged super frost slides (Export Mengel CF, Menzel, Braunschweig, Germany). First the tissues were deparaffinised and rehydrated through graded alcohols, then endogenous peroxidase was quenched in 3% hydrogen peroxide solution. Tissues were then pre blocked in Tris-buffered saline (TBS) containing 0.3% Triton, and 0.5% BSA (#832095, SRL, India,) to inhibit the background staining. Further the sections were incubated with TGF-β and IL-6 primary antibody (1:30) in a humid chamber overnight at 4 °C. After PBS-Tween20 was sections were incubated with HRP conjugated anti-sera specific for TGF-β and IL-6 and were diluted 1:30 in Tris-buffered saline containing 0.3% Triton, and 0.5% blocking agent for 2 h at room temperature. Further, immunoreactive complexes were detected using DAB system (Bangalore GeNei Pvt Ltd, Cat #SFE5). Sections were counterstained briefly in haematoxylin (MERCK) and cover slipped with DPX mounting medium (MERCK).

2.6. Preparation of cell lysates

Spleen tissues were recovered from control, 4, 6 and 8 dpi respectively during PbA and P. yoelii infection. Tissues were homogenized in ice cold RIPA Lysis buffer (150 mM sodium chloride, 1.0% Triton-X–100, 50 mM Tris (pH 8.0), 0.01% SDS, and 0.5% sodium deoxycholate) containing 1 mM PMSF (phenyl methanesulfonyl fluoride), 1 µg aprotonin/ml, and 1 µg leupeptin/ml (all Sigma, St. Louis, MO) followed by sonication and then incubated for 30 min. The samples were then centrifuged at 14,000 rpm for 15 min at 4 °C and the supernatants were collected and stored at −20 °C. Protein estimations were performed using the Bradford reagent (Sigma) and subsequent measures of absorbance at 595 nm in a UV-1700 PharmaSpec spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD).

2.7. Western blot analysis

Western blot analysis of Foxp3 and IL-17 expression was done according as described previously [34]. Briefly, cell lysates (40 µg) of each spleen were separated on 10–12% polyacrylamide gels. Electro blotted onto nitrocellulose membrane, and the membranes were blocked with 5% non-fat dry milk in TBS (0.1% Tween-20) at 4 °C for 30 min. Respective primary antibody was diluted in 1:1000 to 5% BSA and applied to the membrane, overnight at 4 °C.

Fig. 3. Proportions of splenocyte expressing CD80 and CD40 CD11c+ DCs during PbA and P. yoelii infected Swiss. Mice were infected with 1 × 10^7 infected RBCs and Lymphocytes from spleen were obtained from uninfected control, 4, 6 and 8 dpi. Cells were stained with APC-labeled anti-CD11c+, Fitc labeled anti-CD80 and Fitc labeled anti CD40. (A) Expression of CD11c+CD80+ on splenocytes was analyzed and flow plot was determined (B) along with the percentage change of CD11c+CD80+ cells in splenocytes. (C) Expression of CD11c+CD40+ on splenocytes was analyzed and flow plot was determined (B) along with the percentage change of CD11c+CD40+ cells in splenocytes. Quadrant markers were set based on staining with respective isotype controls. Data is representative of five (n) comparable experiments and represented as mean and ±SD values. The results were evaluated by ANOVA. Significant differences between infected and control mice are marked ("p < 0.05; "p < 0.01), whilst differences between infected groups on respective days are marked (by letters). The same letter indicates no significant differences and different letters indicate significant differences.
After overnight incubation at 4°C, the membrane was washed in non-fat dry milk in TBS/Tween-20 and then incubated with AP-conjugated secondary antibodies at a dilution of 1:1000 ratios in 5% BSA for 2 h at room temp. The membranes were then developed with NBT/BCIP (nitroblue tetrazolium chloride/5-Bromo-4-chloro-3-indolyl-phosphate; Hi-Media, Mumbai, India) and the band intensity was measured by densitometry following β-Actin as loading control.

2.8. Statistical analysis

Values between groups of same or different dpi were analyzed using one or two way ANOVA. All values are shown as mean ± SEM, except where otherwise specified. Data were analyzed and, when applicable, the significance of the differences between mean values was determined using Student’s test. P values < 0.05 were considered significant for all statistical analysis, otherwise stated.

3. Results

3.1. Characteristics of PbA and P. yoelii infection during Swiss albino mice

To study the DCs phenotype and function in spleen, we used the PbA and P. yoelii model. Infection was induced by i.p. injection of 1 × 10^6 PbA and P. yoelii infected erythrocytes respectively in 6–8 week old Swiss mice. In accordance with our previous studies, infections were monitored daily and parasitemia raised gradually till 3 dpi in both PbA infected and P. yoelii infected mice. Parasitemia level during P. yoelii reaches a maximum of approximately 35% in peripheral blood approximately 7–8 days after the start of infection, but all mice survived until natural clearances of parasites. In contrary, PbA infected mice developed lower parasitemia and the point of mean median survival was around 4–9 dpi where most of the mice succumbed to CM during this period (Fig. 1A and B). Significant differences between weight changes were also observed during PbA infection compared to P. yoelii infection with respect to control mice (Fig. 1C). Statistically significant differences in the development of parasitemia, the percentage of survival and weight change were observed (Fig. 1A–C).

3.2. Comparison of number of splenic DCs expressing IL-10 and IL-12 during PbA and P. yoelii infection

CD11c is an integrin receptor commonly used as a marker to define DCs, flow cytometric analysis was performed by staining with anti-CD11c-FITC monoclonal antibody to detect CD11c+DCs expressing from splenocytes of Swiss mice infected with PbA and P. yoelii. Because of the sharp increase in the total number of cells in the spleen resulting from the splenomegaly induced by PbA with respect to non-lethal P. yoelii infection within 6 dpi (Fig. 1D), we observed the percentage change of CD11c+DCs in the spleen. There was decrease in percentage of total splenic DCs on 8 dpi during PbA infection, whereas no significant difference at each detected time point was noted during P. yoelii infection. These results indicate that although P. yoelii infection induces increased numbers of splenic DCs on 8 dpi, this may be associated with different

Fig. 4. The proportions of splenocytes expressing CD86, and MHCII CD11c+ DCs during PbA and P. yoelii infected Swiss. Mice were infected with 1 × 10^6 infected RBCs and Lymphocytes from spleen were obtained from uninfected control, 4, 6 and 8 dpi. Cells were stained with APC-labeled anti-CD11c and PE labeled anti-CD86 and Fitc labeled anti MHCII. (A) Expression of CD11c+CD86+ on splenocytes was analyzed and flow plot was determined (B) along with the percentage change of CD11c+CD86+ cells in splenocytes. (C) Expression of CD11c+ MHCII+ on splenocytes was analyzed and flow plot was determined (B) along with the percentage change of CD11c+CD486+ cells in splenocytes. Quadrant markers were set based on staining with respective Isotype controls. Data is representative of five (n) comparable experiments and represented as mean and ±SD values. The results were evaluated by ANOVA. Significant differences between infected and control mice are marked (*p < 0.05; **p < 0.01), whilst differences between infected groups on respective days are marked (by letters). The same letter indicates no significant differences and different letters indicate significant differences.
outcomes of PbA and P. yoelii infection in Swiss mice (Fig. 2A and B). Maturation and the antigen-presenting cell (APC) function of DCs are dependent on immune-regulatory IL-10. During the course of PbA infection, a notable decrease in CD11c*IL-10* production was detected, in contrast, increases in the production of CD11c*IL-10* was observed in Swiss mice following P. yoelii infection. It is likely that splenic DCs from PbA and P. yoelii infected mice are functionally different in terms of the induction of cytokines during the course of infection (Fig. 2C). In contrary, when we studied the expression of CD11c*IL12*, we observed a significant increase during PbA infection whereas no significant change during P. yoelii infection (Fig. 2D).

3.3. Comparison of DC maturation profile in Swiss mice during PbA and P. yoelii infection

To investigate the DC maturation profile, we studied MHC class II, CD86, CD40 and CD80 expression on splenic CD11c* DCs during PbA and P. yoelii infection. Interestingly, during PbA infection we observed increased expression of CD11c*CD80* and CD11c*CD40* on 4, 6 and 8 dpi, whereas only CD11c*CD80* expression was increased during P. yoelii infection. On the other hand, no significant change in CD11c*CD40* expression was observed during P. yoelii infection (Fig. 3A–D). Interestingly CD11c*CD86* expression was also high at 8 dpi during PbA infection, whereas delayed rise during P. yoelii infection (Fig. 4A and B). Further, we observed increased expression of CD11c*MHCII* on splenic DCs during PbA infection, whereas no significant change was observed during P. yoelii infection (Fig. 4C and D).

3.4. Comparison of DC subpopulations in Swiss mice during PbA and P. yoelii infection

Spleen from naive, PbA and P. yoelii infected mice were isolated from control, 4, 6 and 8 dpi and were submitted to CD11c*B220* and CD11c*CD11b* cell phenotyping by flow cytometry. We compared the change in numbers of subpopulations of splenic DCs from PbA and P. yoelii infected Swiss mice during the course of infection. As shown in Fig. 5, the population of splenic CD11c*B220* DCs decreased on 6 dpi during PbA infection, whereas, there was increase in CD11c*B220* DC population during P. yoelii infection compared with naive mice. Interestingly, on 8 dpi there was a further significant decrease in percentage of CD11c*B220* DC population during PbA infection and significant increase during P. yoelii infection was observed (Fig. 5A and B). The percentage change of CD11c*CD11b* double positive DCs in spleen was analyzed by flow cytometry. The blood stage of a PbA infection induces a significant increase in the percentages of CD11c*CD11b* cells in 4, 6 and 8 dpi spleen with respect to control. On the other hand, P. yoelii infection only showed significant increase on 4 and 6 dpi in percentage of CD11c*CD11b* DCs cells with respect to control (Fig. 5C and D). Interestingly a decrease in percentage of CD11c*CD11b* DCs cells on 8 dpi was observed with respect to 4 and 6 dpi during P. yoelii infection. We next demonstrated the ratio of mDC to pDC in the spleen of Swiss mice infected with PbA and P. yoelii.
Yoelii. As shown in Fig. 5E, the ratio of mDC to pDC was significantly increased in the spleen of PbA infected group, while there was no significant increase in P. yoelii infected group except a steep rise on 4 dpi. Taken together, these results suggest that the mDC/pDC may be involved in the immunopathogenesis during PbA and P. yoelii infection.

3.5. Comparison of TGF-β, IL-6, FoxP3 and IL-17 expression in spleen during PbA and P. yoelii infection

To observe the role of pro and anti-inflammatory cytokines in the context of DCs response on spleen during PbA and P. yoelii infected mice, we evaluated the expression of key immune-regulators. The results illustrated that the IL-6 expression levels increased during PbA infection with respect to control, on the other hand, decreased expression on 8 dpi spleen was observed during P. yoelii infection (Fig. 6A). In contrary, we observed decreased expression of TGF-β during PbA infection, whereas the opposite was observed during P. yoelii infection (Fig. 6B). Next we observed the expression pattern of FoxP3 for Treg cells and IL-17 for Th17 cells in both PbA and P. yoelii infected mice [34]. During PbA infection in 8 dpi spleen we observed down regulation of FoxP3 expression with respect to control, whereas, upregulation of FoxP3 in P. yoelii infection (Fig. 6C and D). We further observe IL-17 expression during the course of malaria infection. Interestingly, supporting our previous results we observed upregulation of IL-17 by western blot on 8 dpi in lethal PbA infection whereas, downregulation during resolving P. yoelii infection (Fig. 6E and F). However, the involvement of Treg and Th17 cell types, the possible reasons and consequences need to be investigated.

4. Discussion

In this study, we investigated the splenic DCs phenotype and function in Swiss mice during PbA and P. yoelii infection. Along with differences in parasitemia evolution and survivability during these infections, distinct values for the parameters measured in this study also showed markedly differently. DCs play an elementary role in the initiation of immune responses against pathogens, they reside in almost all tissues significantly inspecting and sensing foreign antigens. During malaria infection, antigen presentation is
known to be compromised [35] and subsequently immature DC undergoes maturation possibly stimulated by pathogens or cytokines. In the present study; we investigated the role of DC subpopulation, mDC and pDC in spleen, vital organ for protective immunity against Plasmodium [36–38]. We observed that during the peak parasitemia, P. berghei-infected mice developed more myeloid DCs, while P. yoelii-infected mice had more plasmacytoid DCs in the spleen. Although the contributions of myeloid/plasmacytoid DCs to PBA and P. yoelii infection need to be clarified in involving depletion studies.

Stimulation of T-cell responses, and more prominently, induction of Th1 cell development is associated with production of Th1 cytokines as well as DCs maturation [39–41]. DC maturation, characterized by up-regulation of MHC II and co-stimulatory molecules is critical for antigen presentation to CD4+ Th cells [39–42]. Further, imbalance in Th1/Th2 immune response by blocking the CD80/CD86 signaling pathway was shown in P. chabaudi AS malaria model [43]. CD40 is also necessary to elicit effector functions, such as IFN-γ secretion from CD4+ T cells [44] moreover, CD80/CD86 signaling pathway was shown in stage PbA CD11c+ infection. In the present study, we found that blood-stage PBA infection induced increased numbers of splenic CD11c+DCs positive for MHC class II, CD40 and CD80 from Swiss mice on 8 dpi, on the other hand P. yoelii infected mice exhibited these alterations in the magnitude and timing DC maturation in spleen may account for the subsequent immune response patterns and different outcomes of the two rodent parasite strains when infected with the same strain of mice.

A major attribute of mature DCs is the cytokine secretions, which have important modulator functions in T cell differentiation. Mature DCs are capable of producing a variety of cytokines, such as IL-6, IL-12, IL-23, IL-10, IL-1β, and TNF-α [45]. In our study, we showed PBA-infected mice developed more myeloid and mature DCs capable of secreting IL-12. In contrast, during P. yoelii-infection we observed more plasmacytoid and immature DCs secreting higher levels of IL-10. Therefore, suggesting that the cytokine environment might play an important role in spleen immune response. Furthermore, the effect of IL-10 in reducing antigen presentation by trapping peptide loaded MHCII molecules and expression of co-stimulatory molecule CD80/CD86 have already been described [46], but impertuous response in pathogenesis of malaria is still poorly unstated. Meanwhile, as described previously, levels of TGF-β and Foxp3, key regulators in the induction of Treg cells were high during resolving P. yoelii infection, whereas decreased expression were observed during non-resolving PBa infection. Interestingly, we observed increased expression of IL-17 and IL-6, modulators of Th17 induction in spleen during PBA infection at 8 dpi whereas, decreased expression in resolving P. yoelii infected mice at 8dpi [34]. Finally, we propose that during malaria, mode of parasite infection, DC subsets, DC maturation and contrasting effects of IL-10 and IL-12 possibly regulated the immune responses.

In conclusion, our finding suggests the distinct response in profiles of splenic DCs during PBA and P. yoelii infection and also highlighting important questions concerning that how DCs might act at apparently crossed purposes to do this. Mechanistically, the activation status of mDC and pDC following IL-10, IL-12 separately or together in vitro study and using specific inhibitors will be assessed in more details. Once these mechanisms will be better assessed and understood, attempts to try and link an observed dendritic cell phenotype with functional outcome will be made during infection. Significantly, our findings will possibly throw some light on how splenic DCs phenotypical and functional subsets are key factors for regulating immune responses to PBA and P. yoelii infections.

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References


