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VERSITA

Identifying phenotypes involved in susceptibility to *Schistosoma mansoni* infection in F1B6CBA mice

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Abstract

Schistosomiasis is a disease with a strong genetic component influenced by socioeconomic and ecological factors. Epidemiological studies have identified several genetic regions involved in the schistosomiasis susceptibility. However, it is not well known what physiological traits are predisposing to the disease. The study of experimental infections in *inbred* mouse strains with variable genetic susceptibility to the disease offers a good opportunity to tackle this question. F1B6CBA hybrid between the most divergent strains was infected in order to characterize the immunophenotypes that correlate with the susceptibility of schistosomiasis disease in mice. Complete blood counts and immunophenotype were determined at 0, 3, 6, and 9 weeks post infection. Nine weeks after cercariae exposure, animals were perfused and worm recovery was assessed. A large number of hepatic lesions, a reduction in the eosinophil and basophil count in the acute phase of infection and the decreased number of monocytes, neutrophils and B-lymphocytes are phenotypes associated with increased susceptibility to *S. mansoni* infection.

Keywords

Schistosomiasis susceptibility; *S. mansoni* infection; F1B6CBA hybrid; immunophenotypes; experimental crosses

Introduction

Schistosomiasis is an infectious disease with a strong genetic component influenced by socioeconomic, environmental and ecological factors (Campino *et al.* 2006). The immune response plays a central role on the development of severe forms of schistosomiasis, although others factors like infection intensity, nutritional status or genetic background of patients may influence the severity of schistosomiasis (Pearce and MacDonald 2002). *S. mansoni* may cause markedly varied patterns of immune response during the course of the infection (Gryseels *et al.* 2006; Pearce and MacDonald 2002; Stadecker *et al.* 2004). The immunopathology of *S. mansoni* infection consists of skin inflammation (cercariae dermatitis) and granulomatous reaction in response to the tissue-trapped parasite eggs (Ross *et al.* 2002). Therefore, *S. mansoni* infection causes most of the damage in the liver where maximum egg accumulation occurs (Gryseels *et al.* 2006).

Mononuclear cells, neutrophils and eosinophils play a main role in the early stages of granuloma, being an important source of proinflammatory chemokines (Burke *et al.* 2009). However, the development of long-lived organized granulomatous lesions around eggs is wholly dependent on CD4+ T lymphocytes (Gause *et al.* 2003). An effective T-cell response is known to be critical for the development of the granulomatous response and host survival. Although the role of CD8+ cells in the immune response against *S. mansoni* is still unclear, this subtype of lymphocytes around the granuloma has been identified suggesting that CD8+ cells act as suppressors of Th2 cell function thereby regulating granulomatous inflammation (Pedras-Vasconcelos and Pearce 1996).

The susceptibility to schistosomiasis presents the classic complex disease phenotype (Bethony and Quinnell 2008; Cooke and Hill 2001). Thus, mouse experimental crosses have been a suitable strategy for mapping genes involved in different complex diseases including the immune response against *S. mansoni* (Rutitzky *et al.* 2005; Smith *et al.* 2009). Several studies report differences in the hepatic fibrosis or granuloma size in different inbreed mouse lines

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(Cheever 1986; Cheever *et al.* 1983; Cheever *et al.* 2002). C57/BL6 mice show the highest degree of resistance to schistosomiasis, whereas CBA/2J mice develop the severe form of murine schistosomiasis (Fanning *et al.* 1981; Bosshardt *et al.* 1997). Hybrids between those strains (F1B6CBA) mice are heterozygous for those loci that differ between both parental strains but are genetically uniform. In spite of the participation of different white blood cells subpopulations in schistosomiasis pathogenesis, blood parameters have not been explored as putative biomarkers of schistosomiasis susceptibility. The analysis of these phenotypes could help to understand the differences in the susceptibility to *S. mansoni* infection. In the current study, we evaluated the degree of susceptibility to schistosomiasis of F1B6CBA2J hybrids and their parental counterparts (CBA/2J and C57BL/6); we also characterized the immuno- and haematological phenotypes that correlate with susceptibility of schistosomiasis in mice.

Materials and Methods

Ethics statement

Animal procedures in this study complied with the Spanish (Real Decreto RD1201/05) and the European Union (European Directive 2010/63/EU) guidelines on animal experimentation for the protection and humane use of laboratory animals, and were conducted at the accredited Animal Experimentation Facilities (Servicio de Experimentación Animal) of the University of Salamanca (Register number: PAE/SA/001). Procedures were approved by the Ethics Committee of the Salamanca University (protocol approval number 12/4508).

Parasite and mice

Mice were housed and handled under standard animal laboratory conditions according to European Union (EU) regulations. The animals were maintained in plastic boxes with food and water ad libitum with a regular 12 h light–dark periods and a temperature of 20°C. We used 11 male, 10 female from parental strains CBA/2J and C57BL/6J, respectively; and 19 male and 20 female F1 hybrid mice (F1B6CBA2J) hybrid. Cercariae of *S. mansoni* were obtained from infected *Biomphalaria glabrata* snails breeding in the Laboratory of Immunological and Molecular Parasitology, University of Salamanca. Each mouse was subcutaneously infected with 150 *S. mansoni* cercariae. Blood samples were collected at 0, 3, 6 and 9 weeks after infection. Three mice of each strain and sex were used as healthy controls animals. Infected animals were perfused and adult parasites were counted with a dissecting microscope at 10× magnification 9 weeks after infection. At the time of perfusion, the liver was also collected for measurement of egg counts. Then, liver tissue was digested in 4%KOH, as previously described (Siles-Lucas *et al.* 2007). Macroscopic lesions of liver were quantified as granuloma affected surface per 100 mm2 in each animal using imageJ software (Schneider *et al.* 2012).

Quantification of specific antibodies against S. mansoni

Serum was collected 0, 3, 6 and 9 weeks after infection and immunoglobulin levels were determined. The Specific Worm Antigen Product (SWAP) was obtained as previously described (Pardo *et al.* 2004). ELISAs were performed by coating polystyrene plates (Costar) with 0.1 ml of 10 mg/ml SWAP in Phosphate Buffer Saline (PBS) overnight at 4°C. Plates were washed in PBS-Tween 20 and blocked with 10% Fetal Bovine Serum (FBS) in PBS for 1 hr. at 37°C. Plates were again washed and sera added at 1:1000 dilution and incubated overnight at 4°C. Antibodies were detected by the addition of peroxidase-labelled anti-mouse IgG1 or IgG2a antibodies at 1:2000 dilution (Sigma). The reaction was developed with H2O2 and orthophenylenediamine (Sigma) and measured at 492nm on an Ear400FT ELISA reader (Lab Instruments). The results are expressed as means of the optical density from all the animals of each group plus the standard error.

Haematological analysis

Blood was collected in EDTA-coated tubes (Vacutainer®). Fifty microliters of blood were analysed using HEMAVET system®. The following haematological parameters were measured: White Blood Cell (WBC, 103cel/µL), neutrophil (NE, 103cel/µL), lymphocyte (LY, 103cel/µL), monocyte (MO, 103cel/µL), eosinophil (EO, 103cel/µL) and basophil count (BA, 103cel/µL). Red Blood Count (RBC, 106cel/µL), Haemoglobin (Hb, g/L), Haematocrit (HCT, %), Mean Corpuscular Volume (MCV, µ3), Mean Haemoglobin Corpuscular (MCH, pg), Mean Corpuscular Haemoglobin Concentration (MCHC, g/dL), Red Cell Distribution Width (RDW) and platelet (PLT, 103cel/µL).

Flow cytometry analysis

Red blood cells were lysed using red cell lysis buffer (Sigma-Aldrich, St Louis, MO). Following centrifugation the white blood cell pellet was washed in PBS with 2% FBS and then stained with appropriate combinations of the following antibodies purchased from BD Biosciences: CD45-PERCP-Cy5 (553092), CD8b-PE (550798), CD4 FITC (553047), B220-APC (553092). After 30 minutes of antibody incubation, samples were washed again and then stored on ice in the dark until analysed by flow cytometer. Acquisition was performed using a FACSCalibur flow cytometer; at least 10.000 cells per sample were acquired. Data analysis was performed using FlowJo software (Tree Star). Steps involved in the analyses of immunophenotypes were included in Fig. 1.

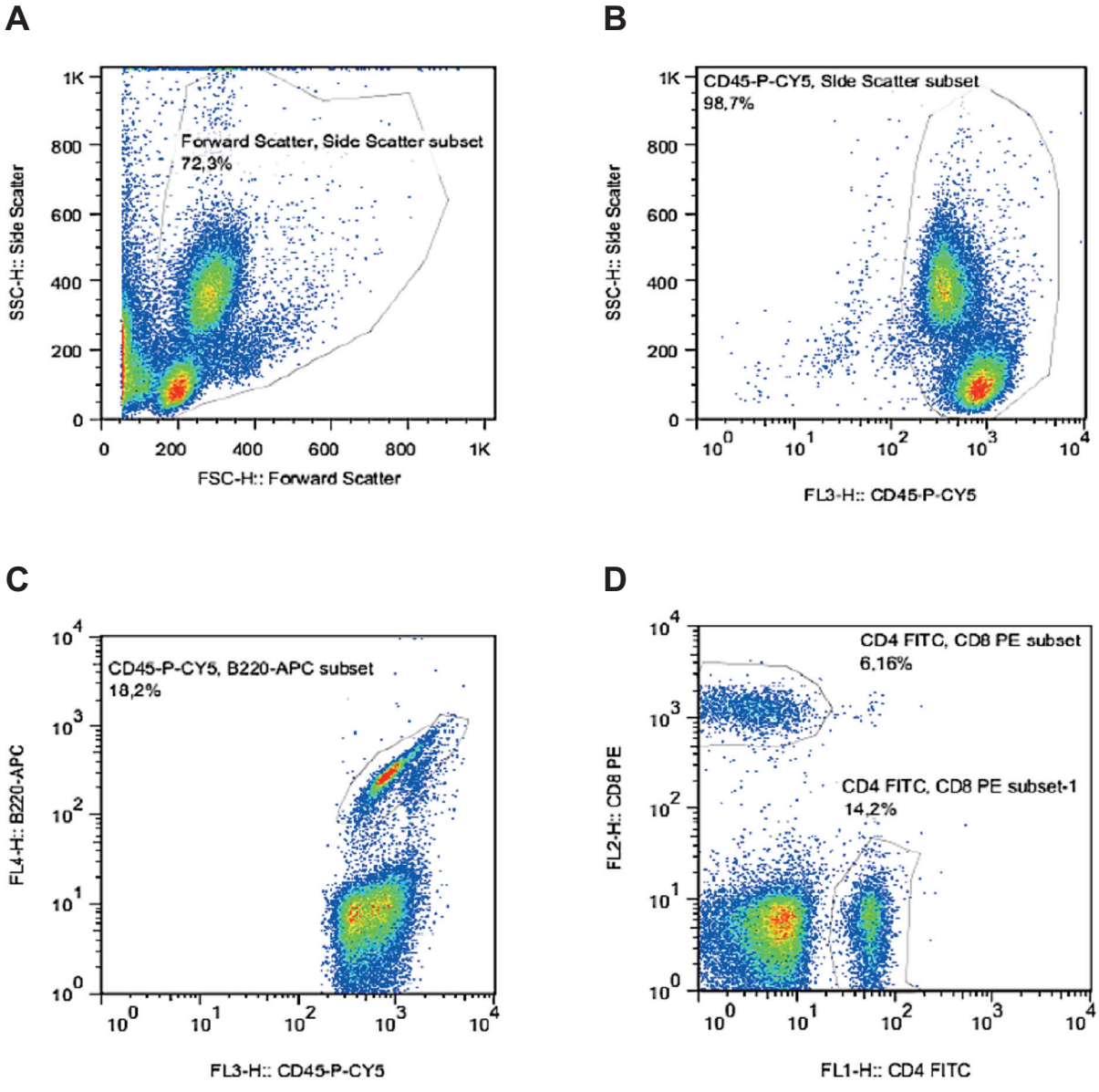

Fig. 1. A – Forward and side scattering selected profiles **B –** complete leukocytes populations **C –** B-Lymphocytes **D –** T-CD4+ and T-CD8+ Lymphocytes. Inside the gate displays the frequency of the gated population

Table I. Worm recovery, eggs counts and hepatic lesions in CBA/2J, C57/BL6 and F1CBAB6

Strain	Worm recovery			Egg density		Lesions/cm²
	Male	Female	Total	Liver	Intestine	
CBA/2J	20.0 (2.37)	19.40 (2.01)	39.40 (4.38)	21153.63 (2924.13)*	12307.46 (2547.65)	48.9 (8.7)*
C57/BL6	31.29 (5.08)	18.86 (3.35)	50.14 (8.34)*	10620.23 (2017.79)	10131.00 (1644.47)	24.8 (2.7)
F1CBAB6	18.27 (1.85)	15.00 (1.27)	33.23 (3.01)	11007.58 (926.12)	8034.48 (837.40)	25.4 (2.6)

Mean (SEM), *p<0.05

Statistical analysis

Results were expressed as median and standard deviations for continuous variables. Differences between groups were examined for statistical significance using the Kruskal-Wallis test. Spearman correlations were computed between each pair of phenotypes. Differences with a p-value <0.05 were considered as statistically significant and all test were two-sided. Statistical analyses were performed using R 3.1.0 (R Foundation for Statistical Computing; http://cran.r-project.org/).

Results

Mortality and parasite burden in F1CBAB6 compared with their parental progenitors

No deaths were observed on C57BL/6 compared with a 37.5% mortality rate in CBA/2J mice. Meanwhile, F1B6CBA hybrid mice presented a 15.2% of mortality. In the present study, we have observed the highest parasite recovery in C57BL/6 compared with CBA/2J and F1B6CBA hybrid mice under experimental *S. mansoni* infection. Meantime, CBA/2J was the strain with the highest egg counts ($p<0.001$) and hepatic lesions in liver ($p<0.001$). These data confirm that CBA/2J mice had a significantly higher hepatic damage than C57/BL6. Although F1CBAB6 hybrid mice tend to have similar values to C57/BL6 mice in egg density, no significant differences were observed with CBA/2J parental strain as shown in Table I.

Haematological response to *S. mansoni* infection in CBA/2J, C57/BL6 and F1CBAB6 hybrid mice

The haematological parameters were measured following the schedule of determinations at 0, 3, 6 and 9 weeks after infection. We compared infected F1CBAB6 hybrid with both infected parental counterparts.. We further included the white and red cells parameters of control mice in Table II and Table III. Regarding the red blood parameters, we observed that infected CBA/2J mice showed anaemia induced by the infection as judged by the significant decrease of the Hb content ($p = 0.003$) and the RBC count ($p = 0.007$) at nine week after infection compared with infected C57BL/6J strain. In addition, infected CBA/2J mice showed a significant decrease in PLT count ($p = 0.03$). Meanwhile, infected F1B6CBA hybrid showed intermediate levels of RBC, Hb and PLT (Fig. 2).

On the white cells parameters, we noted that the levels of lymphocytes and neutrophils in infected C57BL/6J were significantly higher ($p<0.05$) than in infected CBA/2J along the entire period of the infection ($p<0.05$); furthermore, at 9 week after infection C57BL/6J also presented higher levels of monocytes ($p<0.001$). The hybrid F1B6CBA showed sim-

Table II. Summary of haematological parameters measured in healthy mice

Time point	Strain	WBC 10^3cel./µL	MO 10^3cel./µL	LY 10^3cel./µL	NE 10^3cel./µL	EO 10^3cel./µL	BA 10^3cel./µL	Hb g/dL	RBC 10^6cel./µL	PLT 10^3cel./µL
Initial	CBA/2J	6.6 (1.0)	0.34 (0.1)	6.5 (0.7)	0.83 (0.1)	0.14 (0.02)	0.06 (0.01)	14.0 (1.3)	8.97 (0.3)	353 (179)
	C57/BL6	4.36 (1.5)	0.26 (0.1)	3.5 (1.2)	0.51 (0.2)	0.03 (0.01)	0.01 (0.0)	10.7 (1.1)	7.19 (0.5)	452 (258)
	F1CBAB6	2.88 (0.8)	0.29 (0.1)	2.15 (0.6)	0.40 (0.1)	0.04(0.02)	0.02 (0.01)	11.88 (0.6)	8.02 (0.60)	609.8(186.6)
3th week	CBA/2J	8.7 (1.2)	0.50 (0.2)	7.0 (0.9)	1.1 (0.12)	0.09 (0.03)	0.01 (0.0)	13.90 (2.0)	9.82 (0.4)	451 (190)
	C57/BL6	7.6 (1.9)	0.41 (0.1)	5.1 (1.3)	1.9 (0.6)	0.4 (0.1)	0.03 (0.0)	12.3 (0.5)	8.68 (0.2)	471 (208)
	F1CBAB6	4.9 (1.5)	0.42 (0.2)	3.31 (1.1)	1.13 (0.4)	0.42 (0.2)	0.01 (0.0)	13.88 (0.5)	9.34 (0.43)	664.8 (83.84)
6th week	CBA/2J	8.1 (1.2)	0.47 (0.1)	6.7 (0.2)	0.87 (0.1)	0.06 (0.02)	0.02 (0.01)	13.5 (0.3)	9.72 (0.3)	662 (104)
	C57/BL6	8.1 (0.7)	0.55 (0.2)	5.6 (0.4)	1.91 (0.2)	0.07 (0.0)	0.01 (0.0)	12.2 (0.7)	9.4 (0.1)	714 (65)
	F1CBAB6	3.05 (0.9)	0.37 (0.1)	2.15 (0.6)	0.50 (0.3)	0.02 (0.02)	0.01 (0.01)	12.3 (2.55)	8.1 (1.5)	602.6 (301.3)
9th week	CBA/2J	7.3 (1.1)	0.51 (0.1)	6.23 (1.5)	0.51 (0.2)	0.04 (0.01)	0.01 (0.0)	13.9 (1.0)	9.92 (1.2)	726 (183)
	C57/BL6	7.14 (1.7)	0.83 (0.2)	5.1 (1.0)	1.1 (0.6)	0.06 (0.0)	0.02 (0.0)	13.6 (0.1)	8.9 (0.1)	613 (137)
	F1CBAB6	3.96 (1.5)	0.40 (0.1)	3.20 (1.3)	0.34 (0.1)	0.02 (0.02)	0.01 (0.01)	13.88 (0.26)	8.91 (0.27)	708.8 (166.6)

WBC: White Blood Cells; Mo: Monocytes count, LY: Lymphocytes count; NE: Neutrophils counts; EO: Eosinophils count, BA: Basophils count.

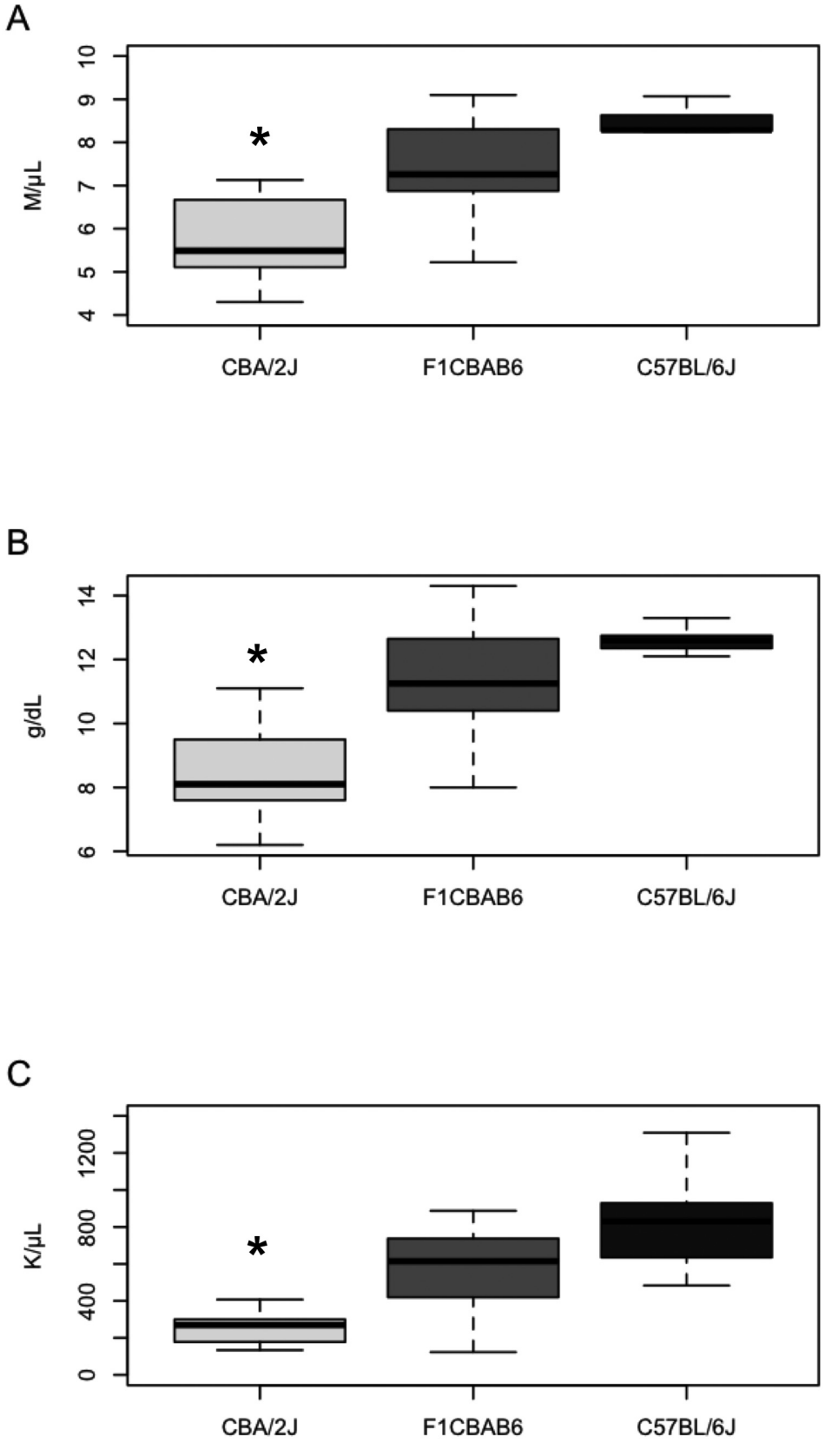

Fig. 2. Assessment of red blood parameters related with anaemia during *S. mansoni* infection in CBA/2J, C57/BL6 and F1CBAB6 hybrid mice. **A –** RBC: Red blood cells; **B –** Hb: Haemoglobin; **C –** PLT: Platelets. Results are plotted as mean ± SEM

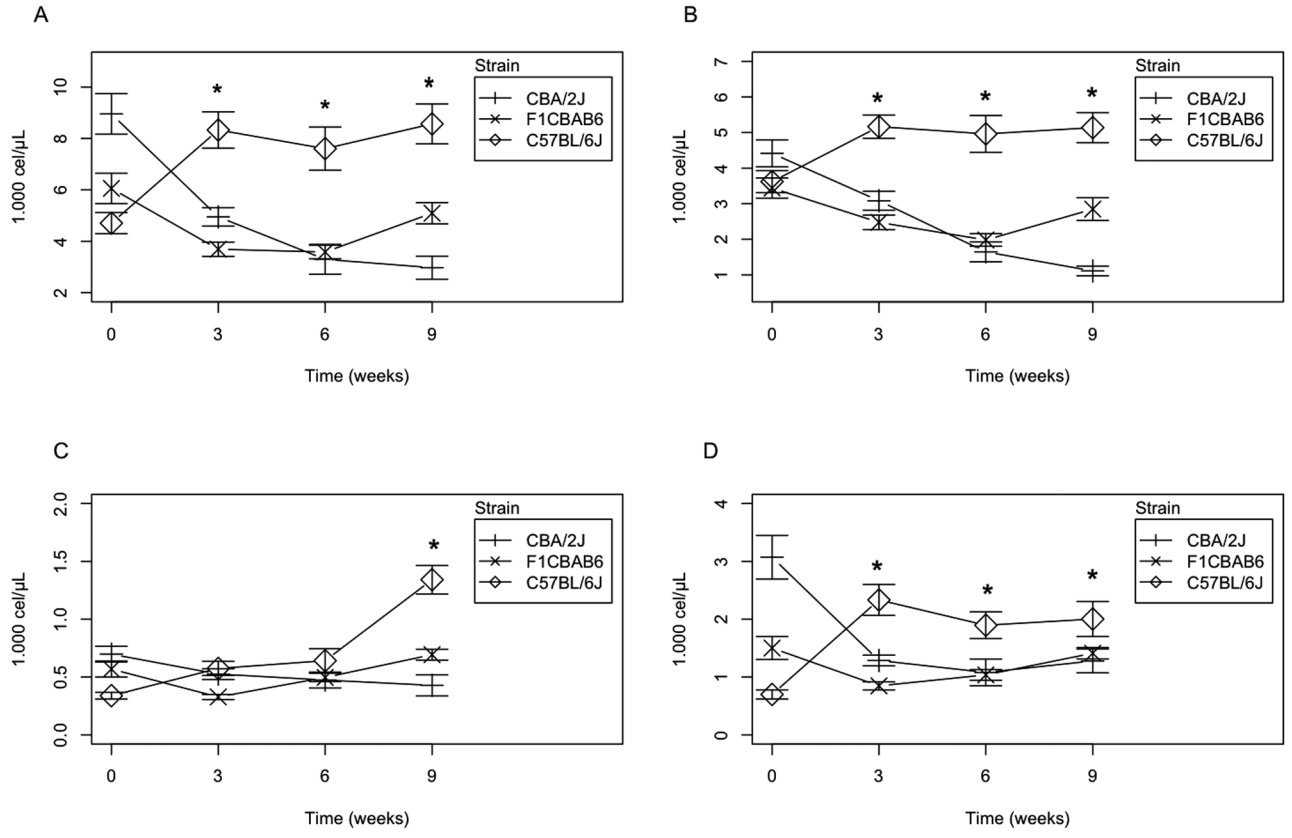

Fig. 3. Variation of white cells counts in response to *S. mansoni* infection in CBA/2J, C57/BL6 and F1B6CBA mice. **A –** White Blood Cells, **B –** Lymphocytes count, **C –** Monocytes count, **D –** Neutrophils counts. Results are plotted as mean ± SEM

ilar levels of lymphocytes, monocytes and neutrophils than CBA/2J, as shown in Fig. 3. Interestingly, C57BL/6J strain showed a maximum peak of eosinophil and basophil levels at the third week after infection, something that was not present in F1B6CBA hybrids and CBA/2J strain (Fig. 4).

Specific antibodies response and Lymphocytes subpopulation analysis to *S. mansoni* infection in F1CBAB6 compared with their parental progenitors

With respect to humoral response, Fig. 5 illustrates the level of SWAP-specific IgG, IgG1 and IgG2a antibodies in F1CBAB6

Table III. Summary of Cytometry values and red blood parameters measured in healthy mice

Time point	Strain	B220%	T-CD4%	T-CD8%
Initial	CBA/2J	39.5 (3.1)	28.1 (0.5)	17.6 (0.9)
	C57/BL6	57.53 (1.04)	11.23 (0.47)	12.67 (0.12)
	F1CBAB6	34.06 (6.67)	15.96 (2.97)	11.04 (1.36)
3th week	CBA/2J	42.1 (2.0)	27 (1.0)	14.2 (1.2)
	C57/BL6	49.87 (1.5)	20.93 (2.3)	12.67 (0.1)
	F1CBAB6	34.92 (2.8)	30.2 (2.3)	15.10 (1.5)
6th week	CBA/2J	49.8 (5.2)	23.8 (1.7)	12.6 (0.9)
	C57/BL6	56.33 (1.72)	14.67 (1.18)	15.80 (0.92)
	F1CBAB6	34.92 (2.83)	30.22 (2.33)	15.10 (1.52)
9th week	CBA/2J	51.8 (3.34)	19.0 (0.9)	11.7 (0.2)
	C57/BL6	58.70 (2.33)	9.87 (0.55)	15.03 (0.55)
	F1CBAB6	43.26 (6.54)	19.14 (5.36)	12.24 (2.06)

B220%: Limphocytes B; T-CD4%: Limphocytes T-CD4%; T-CD8%: Limphocytes T-CD8%

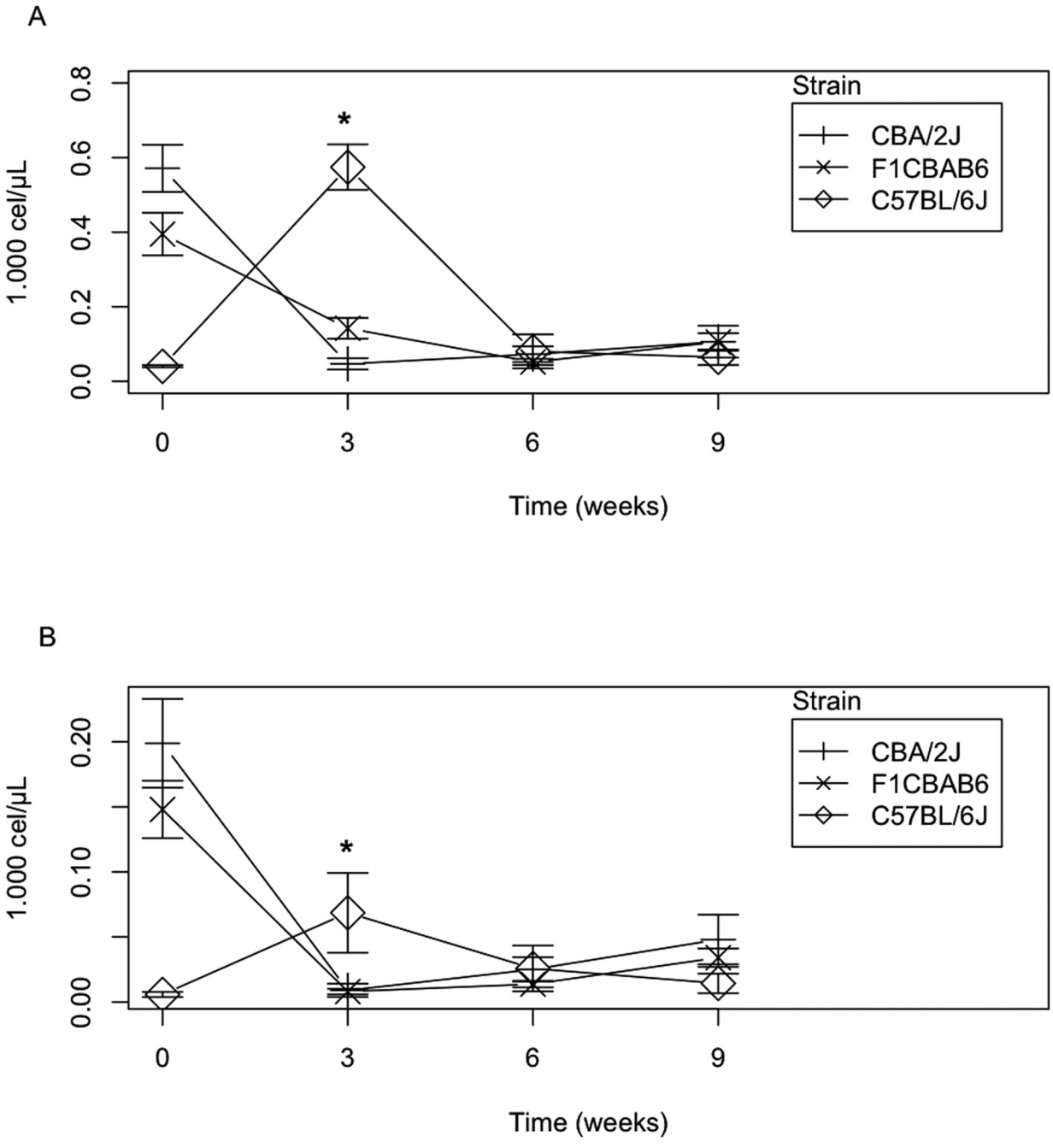

Fig. 4. Variation of eosinophils and Basophils counts in response to *S. mansoni* infection in CBA/2J, C57/BL6 and F1B6CBA mice. **A** – Eosinophils count, **B** – Basophils count. Results are plotted as mean ± SEM

hybrid and both parental strains. We describe the highest differences between the parental strains at 9th week after infection. At this time the levels of IgG, IgG1 and IgG2a were significantly reduced ($p<0.05$) in CBA/2J mice compared to C57BL/6 and F1CBAB6, suggesting that C57/BL6 presents a more effective humoral immune response against *S. mansoni.*

We analysed lymphocyte subpopulations on F1CBAB6 mice and their parental counterparts at different times of infection (Fig. 6). Furthermore, we included the evolution of lymphocyte population in control mice in table III. Globally, it was observed that CD4 T-lymphocyte count increased at the 3th week post-infection, however at 6th and 9th weeks after infection in C57BL6, CBA/2J and hybrid F1CBAB6 mice it was observed a decrease on CD4 T-lymphocytes. Interestingly, CBA/2J susceptible strain showed a higher percentage of CD4 T-lymphocytes compared with C57BL/6 at 3 weeks post-infection ($p<0.05$), while the hybrid F1CBAB6 presented an intermediate count. No significant differences between parental strains were observed at week 6 and 9 post-infection. Regarding CD8+ lymphocytes we noted that

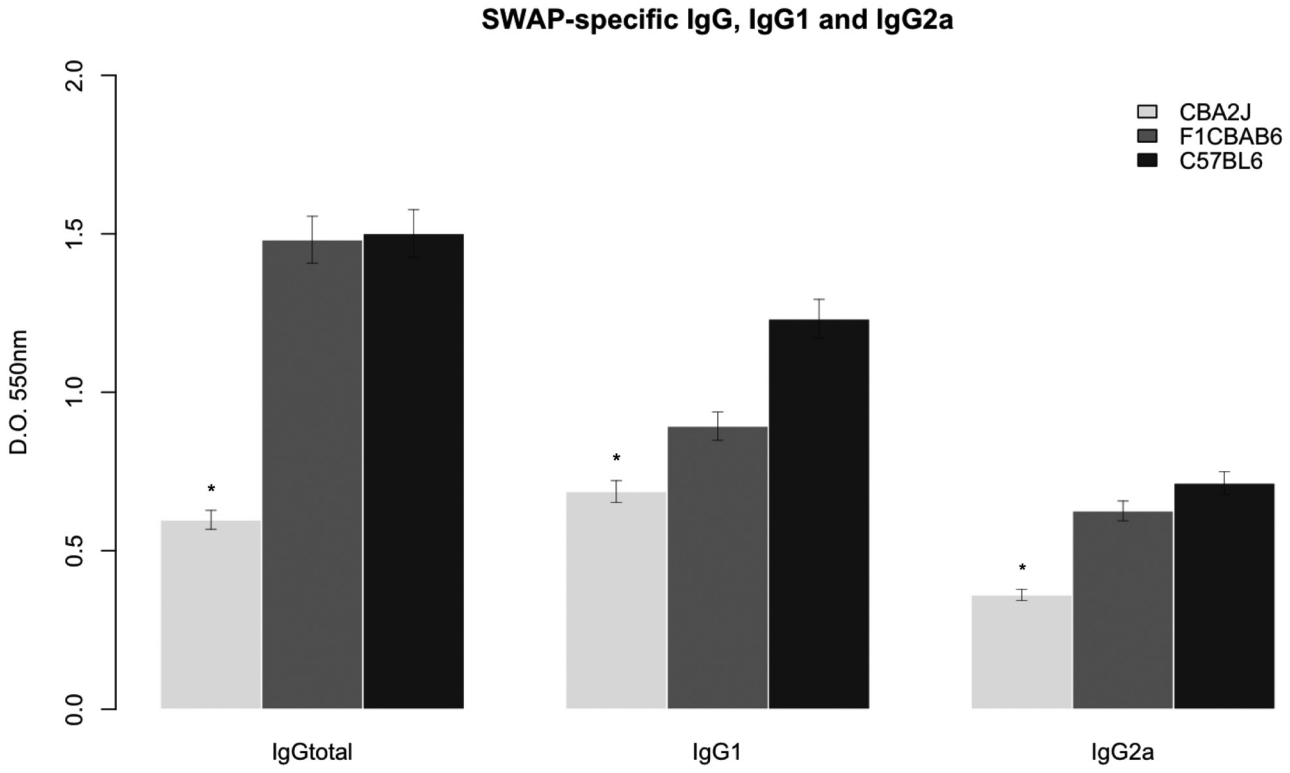

Fig. 5. Total serum profile of anti-*Schistosoma mansoni* antibodies (IgG, IgG1 and IgG2a) expressed as optical density in CBA/2J C57/BL6 and F1CBAB6 against soluble worm antigens preparation (SWAP) of *S. mansoni*

CBA/2J showed significant diminished CD8+ levels at 3 and 6 weeks post-infection compared with C57/BL6 strain ($p<0.05$). Meanwhile F1CBAB6 presented intermediate levels of CD8+ lymphocytes. With respect to circulating lymphocytes B, it was noticed that C57BL/6J mice showed a significantly higher level of circulating B cells compared with CBA/2J and F1CBAB6 hybrid ($p<0.05$) along *S. mansoni* infection. The high levels of circulating B cells in C57/BL6 are highly correlated with the production of total IgG levels ($p = 0.003$). Moreover, CBA/2J strain presented a decrease of circulating B cells after the third week after infection, whereas F1CBAB6 hybrid increased this lymphocyte population at six and nine weeks after infection.

Discussion

In this study we confirmed the differences of *S. mansoni* infection between C57BL/6 and CBA/2J inbreed mouse strains and we described the degree of schistosomiasis susceptibility in F1B6CBA hybrid. The results obtained clearly showed that C57BL/6 tolerate better *S. mansoni* infection than CBA/2J.

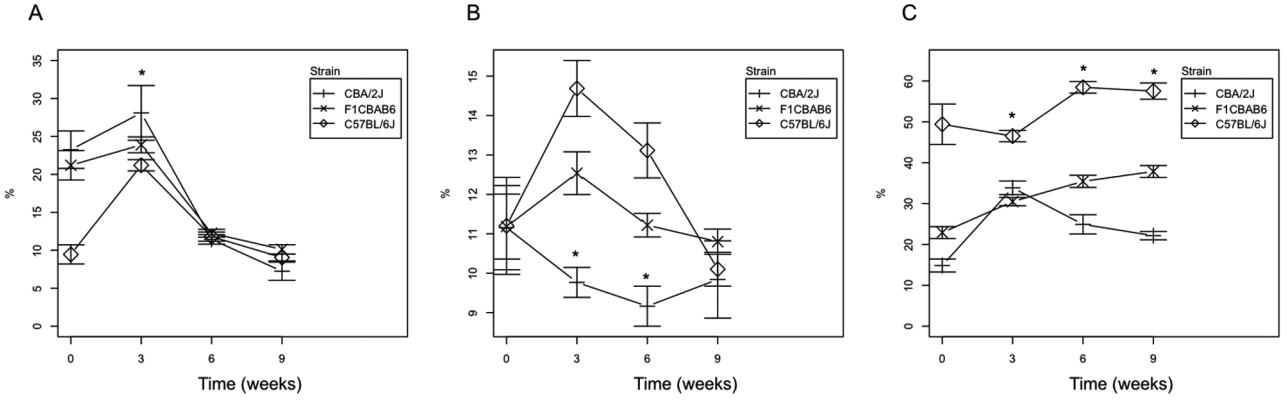

Figure 6. Lymphocyte subpopulation during *S. mansoni* infection in CBA/2J, C57/BL6 and F1CBAB6. **A** – %CD4 T lymphocytes, **B** –%CD8 T lymphocytes, **C** – %B lymphocytes. Results are plotted as mean ± SEM

Among the different parameters studied, the first finding related with the resistance/susceptibility to experimental infection was the difference on mortality rates; indeed, C57BL/6 survived longer than CBA/2J strain. The mortality of hybrid F1B6CBA showed intermediate values between both parental strains, and therefore indicates a degree of susceptibility to infection also intermediate between the parental strains.

The second important finding associated with the resistance/susceptibility to *S. mansoni* infection is the schistosomiasis-related iron deficiency. Our results showed that CBA/2J presented high level of anaemia compared with C57/BL6 mice. Different aetiologies could explain the relationship between schistosomiasis and anaemia: (i) iron deficiency due to extra-corporeal loss; (ii) splenic sequestration; (iii) autoimmune haemolysis and (iv) anaemia and inflammation playing a simultaneous role (Friedman *et al.* 2005). Thus, experimental cross could be an appropriate methodology for mapping genes involved in the development anaemia in *S. mansoni* infection.

Thirdly, regarding the intensity of infection, C57BL/6 mice were able to harbour a higher number of adult worms than CBA/2J strain. According to other authors, the behaviour of the CBA/2J strain could be explained because most of the mice with high numbers of adult worms have died during the experiment, and only those mice containing fewer would have survived (Cheever *et al.* 1987). On the other hand, we observed that CBA/2J presented higher number trapped eggs in liver. In addition, we also observed that the CBA/2J strain presented a significantly higher granuloma affected surface compared with C57BL/6. The evaluation macroscopic granulomatous lesions are positively correlated with the intensity of infection and it is useful for evaluating *S. mansoni* experimental pathology (Shariati *et al.* 2011). These results confirm that CBA/2J presented high grade of susceptibility against *S. mansoni* infection showing a high grade of hepatic damage (Rutitzky and Stadecker 2011).

In the present study, we also characterized haematological features and immunophenotypes correlating with susceptibility of experimental Schistosoma infection. The variables studied were the magnitudes associated with the immune response: cell blood counts, lymphocyte subpopulations (B-cells, CD4 and CD8 T lymphocytes), and detection of IgG, IgG1, IgG2a isotype-specific antibodies and subclasses. B-cells are required for Th2 T-cell responses but not for granuloma formation late in infection (Ji *et al.* 2008). In addition B-cells are responsible for producing specific antibodies against multicellular pathogens. For this reason, high levels of B-cells correlate with high levels of immunoglobulins in C57BL/6 resistant strain. Studies in mice have shown that the Th2 response is associated with the production of IL-4 and high levels of IgG1 while the Th1 response is associated with the production of high levels of IFNγ and IgG2a (Lawrence *et al.* 1994). We should remark the low level of IgG1 and IgG2a antibodies in CBA/2J mice. Recently, it has been reported that CBA/2J have a Th17-type dominant response against *S. mansoni*, which could be the cause of their increased vulnerability to schistosomiasis (Anthony *et al.* 2007).

Multiple studies conducted in mice showed that CD4 T-lymphocytes are essential for granuloma formation and to orchestrate the immune response to *S. mansoni* infection (Davies *et al.* 2001). In this sense, we found an increase in the T-CD4 levels at initial stages of *S. mansoni* infection followed by a deep suppression of T-CD4+ expression in F1CBAB6 and their parental strains. Several studies have indicated that schistosomes are able to induce the death of CD4+ T cells through the binding of Fas-ligand (Rutitzky *et al.* 2003). Interestingly, we found that C57/BL6 presented higher levels in monocytes population at nine weeks after infection than CBA/2J. Monocytes population to mobilize and traffic to where they are needed is central for their functions in promoting immune response during infection and in driving inflammatory diseases, and play an important role mediating host antimicrobial response. In our study, we described a higher number of neutrophils in C57/BL6 resistant strain compared with CBA/2J and F1CBAB6 along the *S. mansoni* experimental infection. This type of granulocyte population has also a key role in the pathogenesis of schistosomiasis. Published studies have shown that mast cells and eosinophils are able to produce IL-4 and modulate granuloma formation (Gessner *et al.* 2005; Mohrs *et al.* 2005). Our results showed high levels of peripheral blood eosinophils and basophils at the early stage of infection (third week after infection) in C57/BL6 strain. Both cells types are present in the initiation and maintenance of chronic allergic inflammation, and they could play a protective role in the immune response against Schistosoma mansoni (Rumbley *et al.* 1999).

CD8 T-Lymphocytes are a cell population traditionally associated with Th1 immune response against intracellular pathogens. However, it has been shown that this cell population can process an immune response against extracellular pathogens before antigen presentation by Major Histocompatibility Complex I (MHCI) (Kovacsovics-Bankowski and Rock 1995; Bevan 1995). In addition, the murine model of schistosomiasis reported the presence of infiltrating CD8 cytotoxic T-lymphocytes in the granuloma reaction around eggs (Pedras-Vasconcelos and Pearce 1996). In our study C57BL/6 strain presented high levels of CD8 cytotoxic T-lymphocytes at 3 and 6 weeks after infection. Therefore, this subtype of lymphocytes could be involved in the modulation of Th-2 immune response characteristic of the chronic phase of this disease, probably minimizing the damage associated with the immune granuloma formation.

Taken together, comparative analysis on the immunophenotype showed the following data associated with resistance in C57BL/6 inbreed mice: (1) high level of lymphocytes, neutrophils and monocytes during *S. mansoni* infection, (2) high level of eosinophils and basophils 3 weeks after infection, and (3) an efficient Th1/Th2 humoral immune response with increased IgG1 and IgG2a. The results in F1CBAB6 hybrid mice demonstrated an intermediate survival and middle Th1/Th2 hu-

moral immune response. Futures studies will be performed in a backcross population in order to identify Quantitative Trait Loci involved in the susceptibility to schistosomiasis.

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