

Original Research Article

<https://doi.org/10.20546/ijcmas.2020.903.005>

Dendritic Cells as an Adjuvant to some *Schistosoma mansoni* Antigens for Vaccination in Experimental Schistosomiasis

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ABSTRACT

Keywords

Dendritic cells,
Vaccination,
Adjuvant,
*Schistosoma
mansoni* antigens,
Experimental
Schistosomiasis

Article Info

Accepted:
05 February 2020
Available Online:
10 March 2020

Schistosomiasis is a major disease of the developing world. Despite integrated control measures, schistosomiasis continues to spread to new regions. So, there is a pressing need to identify new antigens and to explore new adjuvants to improve vaccine efficacy. The aim of the present study was to evaluate the efficacy of an adjuvant dendritic cell (DC) combined with *S. mansoni* SEA and SWAP antigens as vaccination in mice experimentally infected with *S. mansoni*. The study was carried out on 70 laboratory bred Swiss albino male mice. The mice were divided into seven groups each contained 10 mice. The vaccine was given in three doses injected subcutaneously at two weeks' interval. Porto-mesenteric worm burden, hepatic and intestinal egg counts, hepatic granuloma number and diameter, and oogram pattern were assessed to evaluate the antischistosomal effect of vaccines. Ig G, IL-10 & IL-12p40 levels were tested to assess immunological effect. Results revealed that the use of SEA + DCs were associated with a significant decrease in worm burden and tissue egg load together with an increased percentage of dead eggs. In addition, there was a significant reduction in granuloma formation. Also percent of CD4 +ve cells in liver and IL-10 & IL 12p40 was the highest in SEA+DCs group. Importantly, our data suggest a need to re-evaluate host immune responses to many schistosome antigens. There is also a need for a standardized & effective adjuvant formulation and schistosomiasis vaccine development.

Introduction

Schistosomiasis is the second-most widespread human parasitic disease after malaria (Duval *et al.*, 2015). It is estimated that nearly 258 million people are infected worldwide with up to 700 million at risk of being infected, leading to an estimated 280000 deaths annually (WHO, 2017; Toor *et al.*, 2018). The antischistosomal praziquantel

(PZQ) is cheap, effective and widely available, but drug fails to treat all cases and does not prevent reinfection. Thus, in spite of the wide-scale use of PZQ in the past 40 years, the numbers of infected individuals, especially in sub-Saharan Africa, remain at an unsatisfactorily high level (Hagan *et al.*, 2004). Many consider that the best long-term strategy to control schistosomiasis is through immunization combined with drug treatment

(Bergquist, 2002; Morais *et al.*, 2018). The development of a protective vaccine against schistosomiasis still remains potentially the most effective mean for controlling the disease (Merrifield *et al.*, 2016). The current *Schistosoma* vaccine candidates prove not to be the most effective, so it is important to identify new antigens and to explore alternative vaccination strategies, including new adjuvants to improve vaccine efficacy (McManus and Loukas, 2008). Adjuvant selection has a large impact on the effectiveness of the vaccine, and the use of adjuvants to aid in the stimulation of the immune system is a critical step and a major variable affecting vaccine development (Stephenson *et al.*, 2014).

Soluble egg antigen (SEA) coming from seeded eggs in liver tissue activates Th1-polarized response (Pearce, 2005), while *S. mansoni* adult worm antigen (SWAP) modulates the effects of Th1 and Th2 responses through immunosuppressive cytokines as interleukin-10 (IL-10) and transforming growth factor beta (TGF- β). The balance between Th1, Th2 determines the outcome of *Schistosoma* infections (Milner *et al.*, 2010). Dendritic cells (DCs) are specialized antigen-presenting cells that play a central role in cellular immunity and immunotolerance (Boeckh and Ljungman, 2009). DCs are critical in the activation of T cells, providing antigen presentation, costimulatory signals, and inflammatory cytokine stimulation to direct an effector T cell response (Constantino *et al.*, 2017). DCs are activated and acquire an inflammatory profile and have the ability to direct the profile of helper T (Th) cells towards Th1, Th2, Th17, Th9 and regulatory (Treg) cells (Perona, 2018). DCs facilitate cross talk between the innate and adaptive immune system. Targeting vaccines to DCs thus provides a great deal of opportunities for influencing the humoral immune responses, by fine-tuning the T cell

response as well as regulating antigen availability for B cells (Tesfaye, 2019). DCs vaccines are widely utilized in cancer therapy (Palucka and Banchereau, 2013) and infection control (García *et al.*, 2011) as well as in basic research to analyze T-cell response (Roy and Klein, 2012). DCs primed with whole parasites: mimic the natural infection and may increase the success of vaccine approaches in the sense that they are activated directly by pathogens; and stimulate a broader repertoire of T cells which increases the possibility of a suitable immune response against parasites (Colino and Snapper, 2003).

The current study aimed to evaluate the efficacy of an adjuvant dendritic cell vaccination combined with *S. mansoni* SEA and SWAP antigens in experimentally infected mice with *S. mansoni*.

Materials and Methods

Experimental animals

Mice were obtained from Schistosome Biological Supply Program (SBSP), Theodor Bilharz Research Institute (TBRI), Giza, Egypt and kept under standard housing conditions in the animal house of TBRI. All procedures related to animal experimentation met the International Guiding Principles for Biomedical Research Involving Animals as issued by the International Organizations of Medical Sciences (www.cioms.ch/).

Experimental design

The study was carried out on 70 laboratory bred CD-1 Swiss albino male mice weighing about 20 ± 2 gm at the beginning of the experiment. The mice were divided into seven groups each contained 10 mice as follows: Group I control none infected injected by PBS, group II control infected by ± 80 *S. mansoni* cercariae, group III injected by SEA

and then infected by ± 80 *S. mansoni* cercariae, group IV injected by SEA + DCs and then infected by ± 80 *S. mansoni* cercariae, group V injected by SWAP and then infected by ± 80 *S. mansoni* cercariae, group VI injected by SWAP + DCs and then infected by ± 80 *S. mansoni* cercariae and group VII mice were injected with dendritic cells only and then infected by ± 80 *S. mansoni* cercariae.

Antigen preparation (Bradford, 1976 and El-Ahwany *et al.*, 2012)

The crude antigens preparations were purified, sterilized by filtration through 0.45 μm filters (Nalgene Brand Product, Sybran Corp., Rochester, New York, USA) and the protein content was estimated spectrophotometrically using the Bio-Rad kit (Bio-Rad Laboratories, Hercules, California, USA). Then, it was dissolved in Phosphate Buffered Saline (PBS) for immunization.

Dendritic cells preparation

Generation of immature DCs (Ma *et al.*, 2017)

Isolation of bone marrow cells from the femurs of three mice. Every femur's bone marrow was cultured in a separate flask (5ml), six femurs in six flasks. They were cultured in complete media containing 80% complete-RPMI 1640 medium, 20% fetal bovine serum, 100 U/ml penicillin, 100 mg/ml streptomycin supplemented with 100 nano gram (ng) /ml granulocyte macrophage colony stimulating factor (GM-CSF) and 100 ng/ml interleukin 4 (IL-4) at 37 °C and 5% carbon dioxide (CO₂) for 3 days. On the 3rd day, 2 ml of the culture supernatant was carefully removed, and 3 ml of fresh complete culture medium was added to each well and continued to culture for another 2 days at 37 °C and 5% CO₂. One week later, another dose of GM-CSF and IL-4 after that antigens were added as the

follow: 300 μl SEA or SWAP containing 30 μg protein was added to mature DCs as the first dose of vaccine, 200 μl SEA or SWAP containing 20 μg protein was added to mature DCs as the second dose of vaccine. 100 μl SEA or SWAP containing 10 μg protein was added to mature DCs as the last dose of vaccine. After one week, the cells were collected, and immature DCs were isolated by using CD11c + MicroBeads (BD system, New Jersey, USA) according to the manufacturer's protocol. These bone marrow DCs were found to be > 95% CD11c + as detected by flow cytometry (FACS) (Beckman FC-500MPL, California, USA). After that count of the cells was done. All procedures were performed in the Egyptian Society for Progenitor Cell Research (ESPCR) at 34 el-khshid street, Minal el-roda, Cairo, Egypt (Fig. 1).

Vaccine regimen

The vaccine was given in three doses injected subcutaneously at two weeks' interval according to Etewa *et al.*, (2017): In groups III and V, the first dose is about 300 μl of the antigen containing 30 μg protein. The second dose is about 200 μl of the antigen containing 20 μg protein.

The third booster dose is about 100 μl of the antigen containing 10 μg protein. In groups IV, VI and VII, the dose is 100 μl cell suspension at density 10⁶/ml that were subjected to 30 μg protein in the first dose, 20 μg protein in the second dose and 10 μg protein in the last dose of the vaccine (Li *et al.*, 2010).

Experimental Infection of mice with *S. mansoni* according to Lopes Ida *et al.*, (2006)

After two weeks of final immunization, mice were infected with ± 80 *S. mansoni* cercariae (Egyptian strain) by subcutaneous injection.

Evaluation of the effect of vaccine on murine schistosomiasis

After 45 days' post exposure to the cercariae, all mice were sacrificed and subjected to the following parameters:

Parasitological studies

S. mansoni worm burden, Oogram pattern and Ova count per gram tissue (liver and intestine).

Porto-mesenteric worm burden and reduction % (Duvall and DeWitt, 1967)

Saline Perfusion of *S. mansoni* adult worms from the hepatic vascular compartment was performed. The number of male and female *schistosomes* as well as the number of couples were counted and recorded for each mouse. The percentage reduction of adult worms after treatment was calculated according to Tendler *et al.*, (1986) using the formula $R = C - V / C \times 100$, where R = reduction %, C = mean number of adult worms from infected non-treated mice, and V = mean number of adult parasites from treated mice.

Oogram pattern

This method was performed to detect the percentage of different developmental stages of *Schistosoma* eggs according to Pellegrino *et al.*, (1962).

Ova count per gram tissue (liver and intestine) (Cheever, 1970; Cheever and Anderson, 1971).

After scarification and perfusion of mice, representative portions of liver and intestine were dried on a filter paper and placed in a glass test tube containing 5 ml of 5% KOH solution incubated at 37°C for 24 hours until the tissue was completely digested. The

samples were then examined and eggs were counted under a light microscope at $\times 40$ magnification

Histopathological study

Representative specimens of the liver were taken from each sacrificed mouse and sections were prepared and stained with hematoxylin and eosin (H and E) (Harris, 1900).

Evaluation of histopathological study

The number of granulomas in five successive low power fields (10 \times 10) was counted and recorded. Reduction % of number of granulomas = $(C - V / C) \times 100$, where C is the mean number of granulomas in control infected mice and V is the mean number of granulomas in vaccinated infected mice. Measurement of granuloma diameter (μm): Granulomas were measured by a graduated lens and scale. The mean diameter of each granuloma was calculated by measuring two diameters of the lesion at right angles to each other and the arithmetic mean of the two measurements was then calculated (Mahmoud and Warren, 1974).

For each mouse 40-50 granulomas were measured, the percent reduction in granuloma diameter relative to the *S. mansoni* infected control group was calculated as follows:

Reduction % of granuloma diameter (El-Ahwany *et al.*, 2012)=

Mean granuloma diameter (MGD) of infected control group – MGD of infected vaccinated group $\times 100$

MGD of control infected group

Immunohistochemical study: through detection of CD4 expression in murine liver tissues.

Immunohistochemical study for CD4+ cells

All steps were carried out in Pathology Department Menoufiya University according to Hald *et al.*, (2013).

Immune-staining interpretation

Scoring of the entire section was carried out by using a binocular Olympus light microscope with wide angle (field size of 0.274 mm², and field diameter of 0.59 mm) at x400 magnification.

The presence and intensity of the staining of CD4 immune marker in the cells of liver tissues was evaluated by:

CD4 marker expression

Positive CD4 staining was identified when the cell membrane alone or together with the cytoplasm showed brown staining, whereas, negativity was considered when no membrane staining was noticed (Hald *et al.*, 2013). The mean percent of CD4 positive cells: Cells were counted per ten high power fields (h.p.f) and percent was evaluated.

H-Score of CD4 marker as reported by Nie *et al.*, (2013)

H-score Formula= strong intensity (3) x percentage + moderate intensity (2) x percentage + mild intensity (1) x percentage + negative staining (0) x percentage. The final score ranges from 0 to 300.

Detection of Ig G Level

It was determined according to the instruction of the ELISA kits (eBioscience). Detection of IgG level was in serum samples of the groups vaccinated before infection, groups (I, III, IV, V, VI & VII).

Detection of IL-10 and IL-12p40 levels

They were determined according to the instruction of the ELISA kits (eBioscience). In brief, mouse IL-10 and IL-12p40 were detected by biotinylated monoclonal antibodies, which were evidenced by avidin-conjugated horseradish peroxidase followed by incubation with TMB. Absorbance of the samples and controls was read at 450nm (ELx808 Absorbance Microplate Reader; BioTek).

Statistical analysis

Data are presented using SPSS version 22.0 (SPSS Inc., Chicago, Illinois, USA) on an IBM compatible computer. Variable parameters were presented as the mean \pm SD. Group comparisons were assessed by ANOVA test for statistically significant differences, defined as a p value \leq 0.05 using GraphPad Prism software (Version 7.02) (p value \leq 0.05; p value \leq 0.001; p value \leq 0.0001). The Kruskal–Wallis test was applied to study the difference between the groups having nonparametric variables (worm burden).

Results and Discussion

The development of a protective vaccine against schistosomiasis still remains potentially the most effective mean for controlling the disease (Merrifield *et al.*, 2016). The current *Schistosoma* vaccine candidates prove not to be the most effective, so it is important to identify new antigens and to explore alternative vaccination strategies, including new adjuvants to improve vaccine efficacy (McManus and Loukas, 2008).

Parasitological parameters

The highest reduction of total *S. mansoni* worm burden was 77.8%, 70.2%, in group IV and group VI respectively and all tested

groups showed significant reduction in comparison to control group (P6: 0.001) (table 1). Regarding schistosomal oogram pattern, the least percentage of immature eggs in oogram pattern were 21.1 ± 3.41 in group IV (SEA+DCs) followed by 25.9 ± 3.72 in group VI (SWAP+DCs) compared to 52.8 ± 1.98 for control infected group II with the presence of statistically significant difference. There was a significant reduction in the percentage of mature ova where the least percentage was 14.1 ± 2.42 in group IV (SEA+ DCs) followed by 17 ± 1.63 in group VI (SWAP+ DCs) versus 42.1 ± 1.66 for control infected group II with a highly statistically significant difference ($p < 0.001$). There was highly significant difference to all groups in comparison to control group where the highest percentage of dead eggs were achieved in group IV (SEA+DCs) 64.8 ± 4.31 followed by group VI (SWAP+DCs) 57.1 ± 3.37 versus 5.1 ± 1.19 for control infected one and there was highly significant difference between group III (SEA) and group IV (SEA + DCs), group V (SWAP) and group VI (SWAP + DCs) as P values were, P6: 0.001 and P13:0.001, respectively (table 2). Egg viability is an important parameter for the evaluation of efficacy of vaccines. Accordingly, in the present study, there were changes in the oogram pattern, including a significant reduction in the percentage of immature and mature ova and the highest percentage of dead eggs was in DCs combined groups. These results are in agreement with results obtained by Ismail, (2005) and those by Etewa *et al.*, (2014). The reasonable explanation for these results may be the presence of predictable antibodies IgM, IgG, and IgA classes in response to worm antigens as reported by Dunne *et al.*, (1993). Furthermore, a decrease in the intestinal egg burden might reflect a consequent reduction in disease transmission by reducing the passage of *Schistosoma* eggs into the intestinal lumen from the mesenteric veins (Ranasinghe *et al.*, 2018).

Also, combined groups showed the least number of ova/gram tissue of the liver; 1345 ± 738.4 with reduction percent 87% in group IV (SEA+ DCs) followed by 1560 ± 337.3 with reduction percent 84.9% in group VI (SWAP+ DCs) versus 10355 ± 3020.7 for control infected group II. The least number of ova/gram tissue of the intestine was also in the same combined groups, 2103 ± 617.8 with reduction percent 82.7% in group IV (SEA+ DCs) followed by 2410 ± 483.6 with reduction percent 80.3% in group VI (SWAP+ DCs) versus 12215 ± 3539.6 for control infected group II (Table 3). According to WHO benchmark for progression of a *Schistosoma* vaccine antigen into clinical assessment, a molecule that can consistently induce 40% protection or better is considered as an optimal anti-schistosome vaccine candidate, and a significant reduction in adult worm numbers represents a high standard when considering an effective anti-schistosome vaccine target (McManus and Loukas, 2008).

In the present study there were significant reductions in all assessed parasitological parameters, the adults' worm burden, hepatic and intestinal egg load with increased percentage of dead eggs in combined vaccinated groups with DCs. This could be due to enhancement of immune response. As, DCs are critical in the activation of T cells, providing antigen presentation, costimulatory signals and inflammatory cytokine stimulation to direct an effector T cell response (Constantino *et al.*, 2017). These results are supported by a number of previous studies on other antischistosomal vaccines (Abdameleket *et al.*, 2014, Etewa *et al.*, 2014 and Selim *et al.*, 2016). In addition, Li *et al.*, (2010) reported that dendritic cells pulsed with Glutathion S-transferase in combination with CpG oligodeoxynucleotide induce significant immunoprotection against the infection of *S. japonicum*.

Histopathological results

Haematoxylin and eosin sections of liver tissue showed that the mean number of hepatic granulomas in combined groups was significantly lower than other groups. Hence, the reduction percentages of hepatic granuloma number reached 80.7% in group IV (SEA+ DCs) and 68.1% in group VI (SWAP+ DCs) with significant difference between combined groups (P11: 0.011) (Table 4&Fig. 2). Also, size of granulomas decreased in combined groups with reduction percentages of hepatic granuloma diameter reached 54.2% for group IV (SEA+ DCs) and

45.6% for group VI (SWAP+ DCs)(Table 4&Fig. 3). These results indicated the increase in Th1 response by its specific cytokines which was reflected positively on the number and size of hepatic *Schistosoma* granulomas leading to decrease in number and size of them (Etewa *et al.*, 2017). These are in harmony with those of Ismail, (2005), Oliveira *et al.*, (2012) and Etewa *et al.*, (2017). These observations can be explained by the statement of Chitsulo *et al.*, (2004), who pointed out that a vaccine that induces even a partial reduction in worm burdens could reduce pathology and limit parasite transmission.

Table.1 Comparison between the studied groups regarding the mean total worm load (N=60)

Studied groups		Total worm load		Reduction percent (%)	Kruskal Wallis test	P value	Post hoc p value
		Mean ±SD	Range				
Group II (control infected)	N=10	35.6±9.13	23 – 47	0%	44.5	0.001**	P1:0.001**
Group III (SEA)	N=10	13.4±4.11	8 – 18	62.3%			P2:0.001**
Group IV (SEA+DCs)	N=10	7.90±1.85	5 – 11	77.8%			P3:0.001**
Group V (SWAP)	N=10	16.1±2.92	11 – 21	54.6%			P4:0.001**
Group VI (SWAP+DCs)	N=10	10.6±3.02	7 – 17	70.2%			P5:0.002*
Group VII (DCs)	N=10	20.8±6.61	11 – 30	41.6%			P6:0.004*
							P7:0.147
					P8:0.126		
					P9:0.011*		
					P10:0.001**		
					P11:0.033*		
					P12:0.007*		
					P13:0.002*		
					P14:0.101		
					P15:0.001**		

**Highly significant

P1: Comparison between group II and group III
 P3: Comparison between group II and group V
 P5: Comparison between group II and group VII
 P7: Comparison between group III and group V
 P9: Comparison between group III and group VII
 P11: Comparison between group IV and group VI
 P13: Comparison between group V and group VI
 P15: Comparison between group VI and group VII

* significant

P2: Comparison between group II and group IV
 P4: Comparison between group II and group VI
 P6: Comparison between group III and group IV
 P8: Comparison between group III and group VI
 P10: Comparison between group IV and group V
 P12: Comparison between group IV and group VII
 P14: Comparison between group V and group VII

Table.2 Comparison between the studied groups regarding the mean number of *S. mansoni* eggs in oogram pattern (N=60)

Studied groups		Number of immature egg		ANOVA	P value	Post hoc p value
		Mean ±SD	Range			
Group II (control infected)	N=10	52.8±1.98	50 - 55	52.3	0.001**	P1:0.001** P2:0.001** P3:0.001** P4:0.001** P5:0.001** P6:0.001** P7:0.001** P8:0.149 P9:0.001** P10:0.001** P11:0.011* P12:0.001** P13:0.001** P14:0.003* P15:0.001**
Group III (SEA)	N=10	28.3±3.94	22 - 34			
Group IV (SEA+DCs)	N=10	21.1±3.41	16 - 27			
Group V (SWAP)	N=10	34.0±1.56	32 - 37			
Group VI (SWAP+DCs)	N=10	25.9±3.72	20 - 33			
Group VII (DCs)	N=10	38.6±2.98	34 - 43			
Number of mature egg						
Group II (control infected)	N=10	42.1±1.66	40 - 45	54.2	0.001**	P1:0.001** P2:0.001** P3:0.001** P4:0.001** P5:0.001** P6:0.001** P7:0.001** P8:0.023* P9:0.001** P10:0.001** P11:0.004* P12:0.001** P13:0.001** P14:0.001** P15:0.001**
Group III (SEA)	N=10	19.2±2.34	15 - 22			
Group IV (SEA+DCs)	N=10	14.1±2.42	12 - 20			
Group V (SWAP)	N=10	24.8±1.87	21 - 28			
Group VI (SWAP+DCs)	N=10	17.0±1.63	15 - 20			
Group VII (DCs)	N=10	29.2±1.47	26 - 31			
Number of dead egg						
Group II (control infected)	N=10	5.10±1.19	3 - 7	55.4	0.001**	P1:0.001** P2:0.001** P3:0.001** P4:0.001** P5:0.001** P6:0.001** P7:0.001** P8:0.017* P9:0.001** P10:0.001** P11:0.002* P12:0.001** P13:0.001** P14:0.001** P15:0.001**
Group III (SEA)	N=10	52.5±3.95	48 - 60			
Group IV (SEA+DCs)	N=10	64.8±4.31	55 - 69			
Group V (SWAP)	N=10	41.2±1.54	39 - 44			
Group VI (SWAP+DCs)	N=10	57.1±3.37	52 - 64			
Group VII (DCs)	N=10	32.2±3.35	27 - 36			

**Highly significant

P1: Comparison between group II and group III
P3: Comparison between group II and group V
P5: Comparison between group II and group VII
P7: Comparison between group III and group V
P9: Comparison between group III and group VII
P11: Comparison between group IV and group VI
P13: Comparison between group V and group VI
P15: Comparison between group VI and group VII

* significant

P2: Comparison between group II and group IV
P4: Comparison between group II and group VI
P6: Comparison between group III and group IV
P8: Comparison between group III and group VI
P10: Comparison between group IV and group V
P12: Comparison between group IV and group VII
P14: Comparison between group V and group VII

Table.3 Comparison between the studied groups regarding the mean number of ova / gram tissue of liver and intestine (N=60)

Studied groups		Number of ova / gram tissue of liver		Reduction percent (%)	K	P value	Post hoc p value
		Mean ±SD	Range				
Group II (control infected)	N=10	10355±3020.7	7750 -15500	0%	44.4	0.001**	P1:0.001** P2:0.001** P3:0.001** P4:0.001** P5:0.001** P6:0.121 P7:0.427 P8:0.173 P9:0.001** P10:0.041* P11:0.273 P12:0.001** P13:0.019* P14:0.001** P15:0.001**
Group III (SEA)	N=10	1840.0±450.1	1200 – 2500	82.2%			
Group IV (SEA+ DCs)	N=10	1345.0±738.4	550 – 2450	87.0%			
Group V (SWAP)	N=10	2050.0±392.2	1600 – 2800	80.2%			
Group VI (SWAP+DCs)	N=10	1560.0±337.3	1100 – 2050	84.9%			
Group VII (DCs)	N=10	4030.0±1119.5	2950 – 6800	61.0%			
Number of ova / gram tissue of intestine							
Group II (control infected)	N=10	12215.0±3539.6	8200 - 17700	0%	71.2	0.001**	P1:0.001** P2:0.001** P3:0.001** P4:0.001** P5:0.001** P6:0.198 P7:0.660 P8:0.558 P9:0.001** P10:0.141 P11:0.648 P12:0.001** P13:0.306 P14:0.001** P15:0.001**
Group III (SEA)	N=10	2804.5±658.5	1900 - 3850	77.0%			
Group IV (SEA+ DCs)	N=10	2103.0±617.8	1100 - 2900	82.7%			
Group V (SWAP)	N=10	3100.0±483.6	2250 - 3950	74.6%			
Group VI (SWAP+DCs)	N=10	2410.0±483.6	1750 – 2950	80.3%			
Group VII (DCs)	N=10	5985.0±1377.6	4300 – 8050	51.0%			

**Highly significant

P1: Comparison between group II and group III
P3: Comparison between group II and group V
P5: Comparison between group II and group VII
P7: Comparison between group III and group V
P9: Comparison between group III and group VII
P11: Comparison between group IV and group VI
P13: Comparison between group V and group VI
P15: Comparison between group VI and group VII

P2: Comparison between group II and group IV
P4: Comparison between group II and group VI
P6: Comparison between group III and group IV
P8: Comparison between group III and group VI
P10: Comparison between group IV and group V
P12: Comparison between group IV and group VII
P14: Comparison between group V and group VII

Table.4 Comparison between the studied groups regarding the mean number and diameter of hepatic granuloma (N=60)

Studied groups		Number of hepatic granuloma		Percent of reduction (%)	Kruskal Wallis test	P value	Post hoc p value
		Mean ±SD	Range				
Group II (control infected)	N=10	9.85±2.70	7 - 15	0%	44.6	0.001**	P1:0.001** P2:0.001** P3:0.004* P4:0.001** P5:0.007* P6:0.001** P7:0.173 P8:0.049 P9:0.001** P10:0.001** P11:0.011* P12:0.001** P13:0.015* P14:0.096 P15:0.001**
Group III (SEA)	N=10	4.25±1.17	2.5 – 6.2	56.8%			
Group IV (SEA+DCs)	N=10	1.90±0.61	1.4 – 3	80.7%			
Group V (SWAP)	N=10	5.58±2.53	2.50 - 11	43.3%			
Group VI (SWAP+DCs)	N=10	3.14±1.20	1.20 -5.20	68.1%			
Group VII (DCs)	N=10	6.91±2.04	4.60 – 11.8	29.8%			
diameter of hepatic granuloma(µm)							
Group II (control infected)	N=10	5801.3±891.3	4447.5 – 7360.5	0%	Anova	0.001**	P1:0.001** P2:0.001** P3:0.001** P4:0.001** P5:0.019* P6:0.002* P7:0.251 P8:0.070 P9:0.001** P10:0.001** P11:0.148 P12:0.001** P13:0.004* P14:0.016* P15:0.001**
Group III (SEA)	N=10	3770.4±1005.7	2321.0 – 5381.7	35.0%	62.2		
Group IV (SEA+DCs)	N=10	2658.8±560.3	1855 – 3509.3	54.2%			
Group V (SWAP)	N=10	4160.0±691.9	3028.3 – 5286.5	28.3%			
Group VI (SWAP+DCs)	N=10	3151.7±533.3	2045.0 – 3701.7	45.6%			
Group VII (DCs)	N=10	4990.6±1034.3	3588.5 – 7421.7	13.9%			

**Highly significant

P1: Comparison between group II and group III
P3: Comparison between group II and group V
P5: Comparison between group II and group VII
P7: Comparison between group III and group V
P9: Comparison between group III and group VII
P11: Comparison between group IV and group VI
P13: Comparison between group V and group VI
P15: Comparison between group VI and group VII

* significant

P2: Comparison between group II and group IV
P4: Comparison between group II and group VI
P6: Comparison between group III and group IV
P8: Comparison between group III and group VI
P10: Comparison between group IV and group V
P12: Comparison between group IV and group VII
P14: Comparison between group V and group VII

Table.5 Comparison between the studied groups regarding the mean percent of CD4 positive cells in liver (N=70)

Studied groups		Percent of CD4 positive cells in liver		ANOVA	P value	Post hoc p value
		Mean ±SD	Range			
Group II (control infected)	N=10	25.0±4.71	20 – 35	50.2	0.001**	P1:0.001**
Group III (SEA)	N=10	40.0±4.71	35 – 50			P2:0.001**
Group IV (SEA+DCs)	N=10	60.0±9.12	45 – 75			P3:0.001**
Group V (SWAP)	N=10	35.0±4.71	30 – 45			P4:0.001**
Group VI (SWAP+DCs)	N=10	50.0±4.71	45 – 60			P5:0.001**
Group VII (DCs)	N=10	30.0±4.71	25 – 40			P6:0.021*
						P7:0.256
				P8:0.112		
				P9:0.001**		
				P10:0.003*		
				P11:0.344		
				P12:0.001**		
				P13:0.006*		
				P14:0.001**		
				P15:0.001**		

**Highly significant

P1: Comparison between group II and group III
 P3: Comparison between group II and group V
 P5: Comparison between group II and group VII
 P7: Comparison between group III and group V
 P9: Comparison between group III and group VII
 P11: Comparison between group IV and group VI
 P13: Comparison between group V and group VI
 P15: Comparison between group VI and group VII

* significant

P2: Comparison between group II and group IV
 P4: Comparison between group II and group VI
 P6: Comparison between group III and group IV
 P8: Comparison between group III and group VI
 P10: Comparison between group IV and group V
 P12: Comparison between group IV and group VII
 P14: Comparison between group V and group VII

Table.6 Comparison between the studied groups regarding the mean H score of CD4 in liver (N=60)

Studied groups		H score of CD4 in liver		ANOVA	P value	Post hoc p value
		Mean ±SD	Range			
Group II (control infected)	N=10	55.0±8.81	45 - 70	57.4	0.001**	P1:0.001**
Group III (SEA)	N=10	110.0±10.0	100 - 125			P2:0.001**
Group IV (SEA+DCs)	N=10	170.0±9.12	155 - 185			P3:0.001**
Group V (SWAP)	N=10	95.0±4.71	90 - 105			P4:0.001**
Group VI (SWAP+DCs)	N=10	140.0±8.81	130 - 155			P5:0.001**
						P6:0.001**
Group VII (DCs)	N=10	80.0±4.71	75 - 90			P7:0.001**
				P8:0.001**		
				P9:0.001**		
				P10:0.001**		
				P11:0.001**		
				P12:0.001**		
				P13:0.001**		
				P14:0.001**		
				P15:0.001**		

**Highly significant

P1: Comparison between group II and group III
 P3: Comparison between group II and group V
 P5: Comparison between group II and group VII
 P7: Comparison between group III and group V
 P9: Comparison between group III and group VII
 P11: Comparison between group IV and group VI
 P13: Comparison between group V and group VI
 P15: Comparison between group VI and group VII

P2: Comparison between group II and group IV
 P4: Comparison between group II and group VI
 P6: Comparison between group III and group IV
 P8: Comparison between group III and group VI
 P10: Comparison between group IV and group V
 P12: Comparison between group IV and group VII
 P14: Comparison between group V and group VII

Fig.1(a) Dendritic cells in culture during maturation process (red arrows). (b): Mature unstained dendritic cells in culture (red arrows)

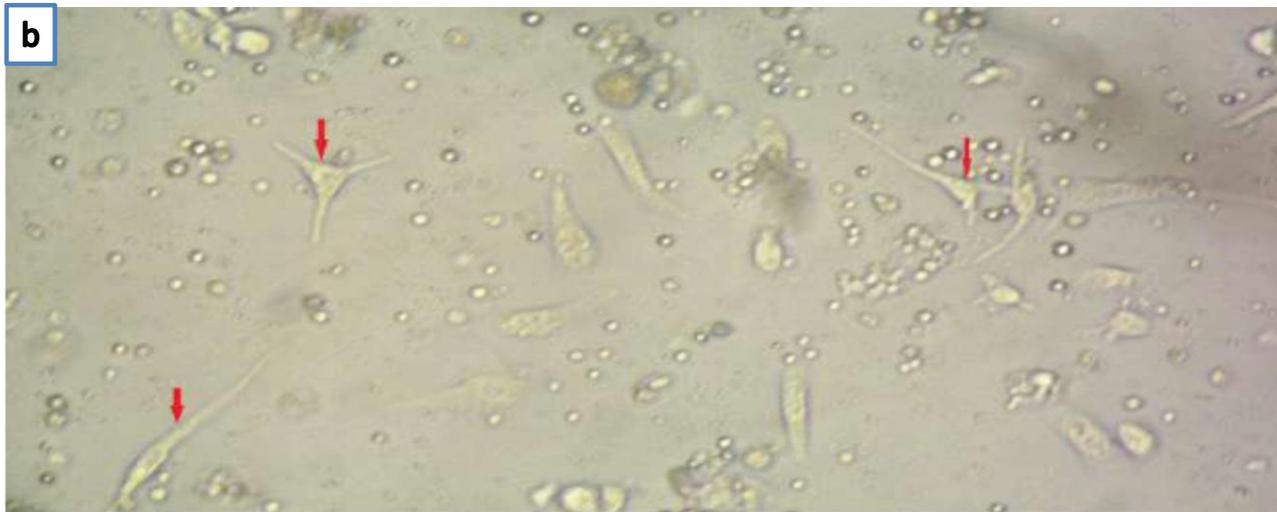
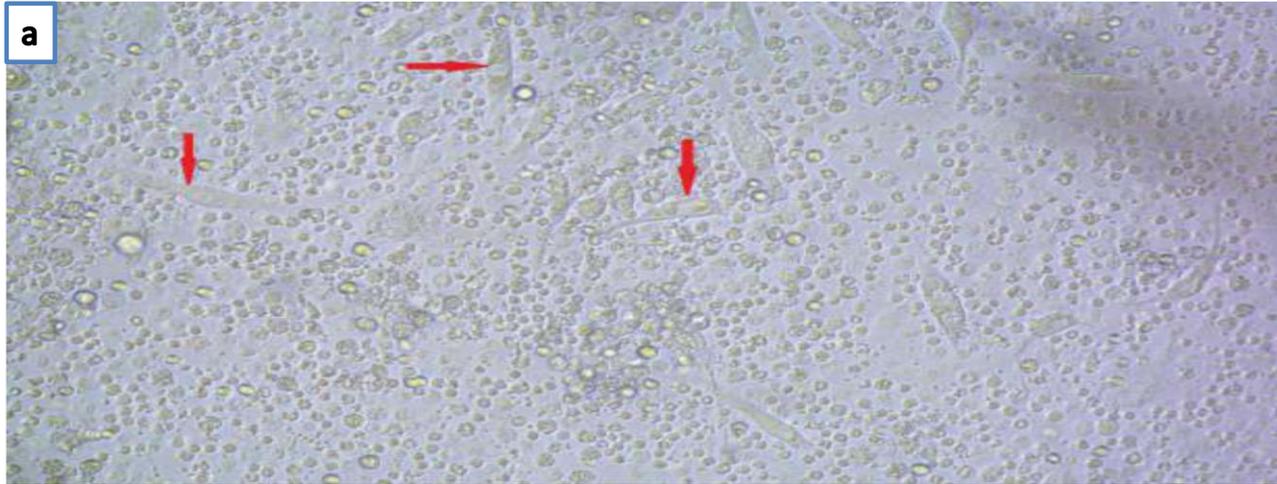


Fig.2(a) A control infected mouse liver tissue revealed nine granulomas as a mean number (x 40). (b): Liver section of a mouse vaccinated with SEA (group III) revealed five granulomas as a mean number (x 40). (c): Liver section of a mouse vaccinated with SEA and dendritic cells (group IV) revealed two granulomas as a mean number (x 40). (d): Liver section of a mouse vaccinated with SWAP (group V) revealed six granulomas as a mean number (x 40). Liver section of a mouse vaccinated with SWAP and dendritic cells (group VI) revealed four granulomas as a mean number (x 40). Liver section of a mouse vaccinated with dendritic cells (group VII) revealed seven granulomas as a mean number (x 40).

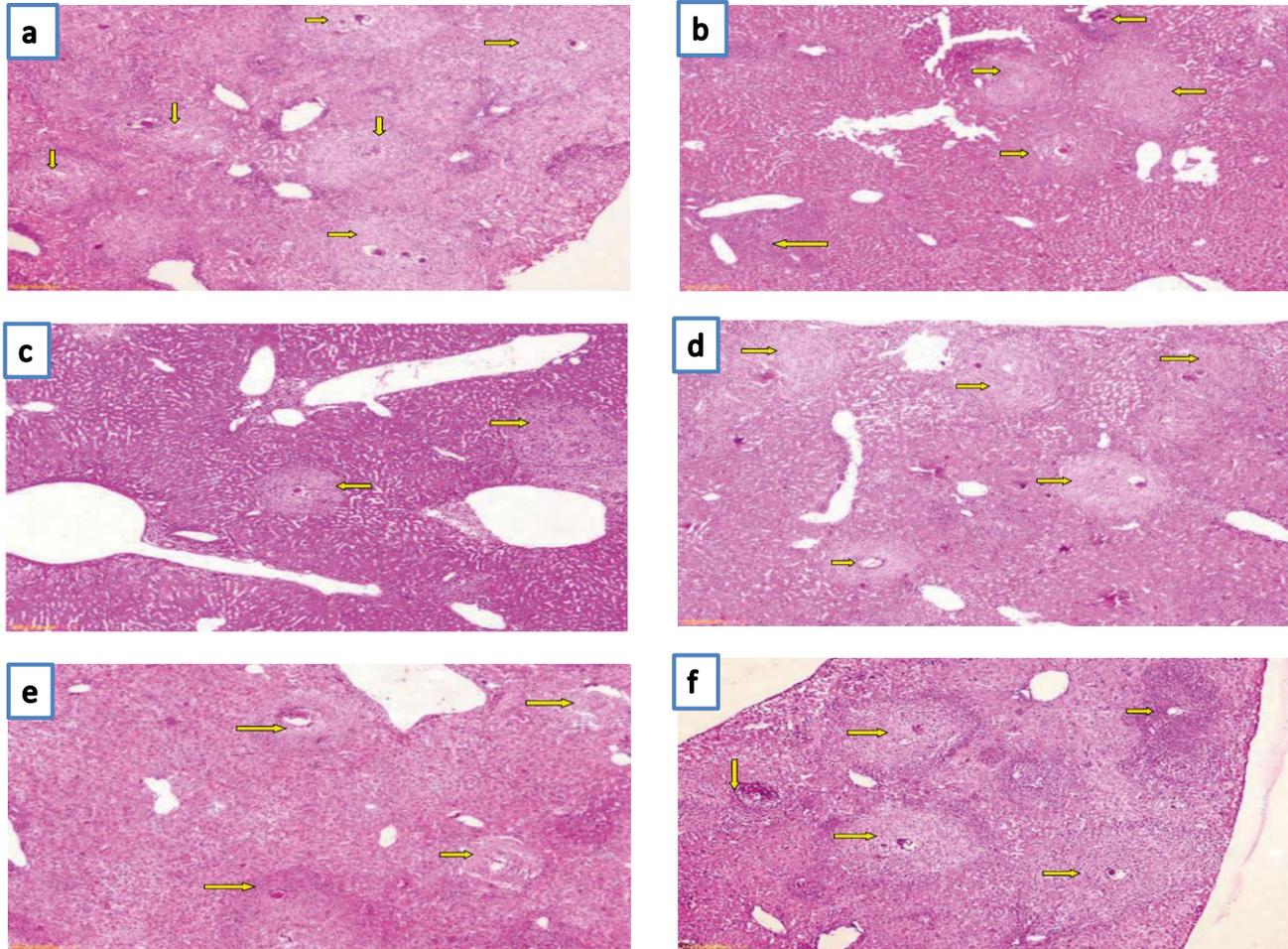


Fig.3(a) Liver section of an infected control mouse showing large size granuloma with massive cellular infiltrate surrounding multiple bilharzial ova (x100). (b): Liver section of an infected mouse vaccinated with SEA revealed the median size of granulomas (X 100). (c): Liver section of an infected mouse vaccinated with SEA and dendritic cells showing small size granuloma with massive cellular infiltrate surrounding multiple bilharzial ova (x100). (d): Liver section of an infected mouse vaccinated with SWAP revealed the median size of granulomas (X 100). (e): Liver section of an infected mouse vaccinated with SWAP and dendritic cells revealed the median size of granulomas (X 100). (f): Liver section of an infected mouse vaccinated with dendritic cells revealed the median size of granulomas (X 100)

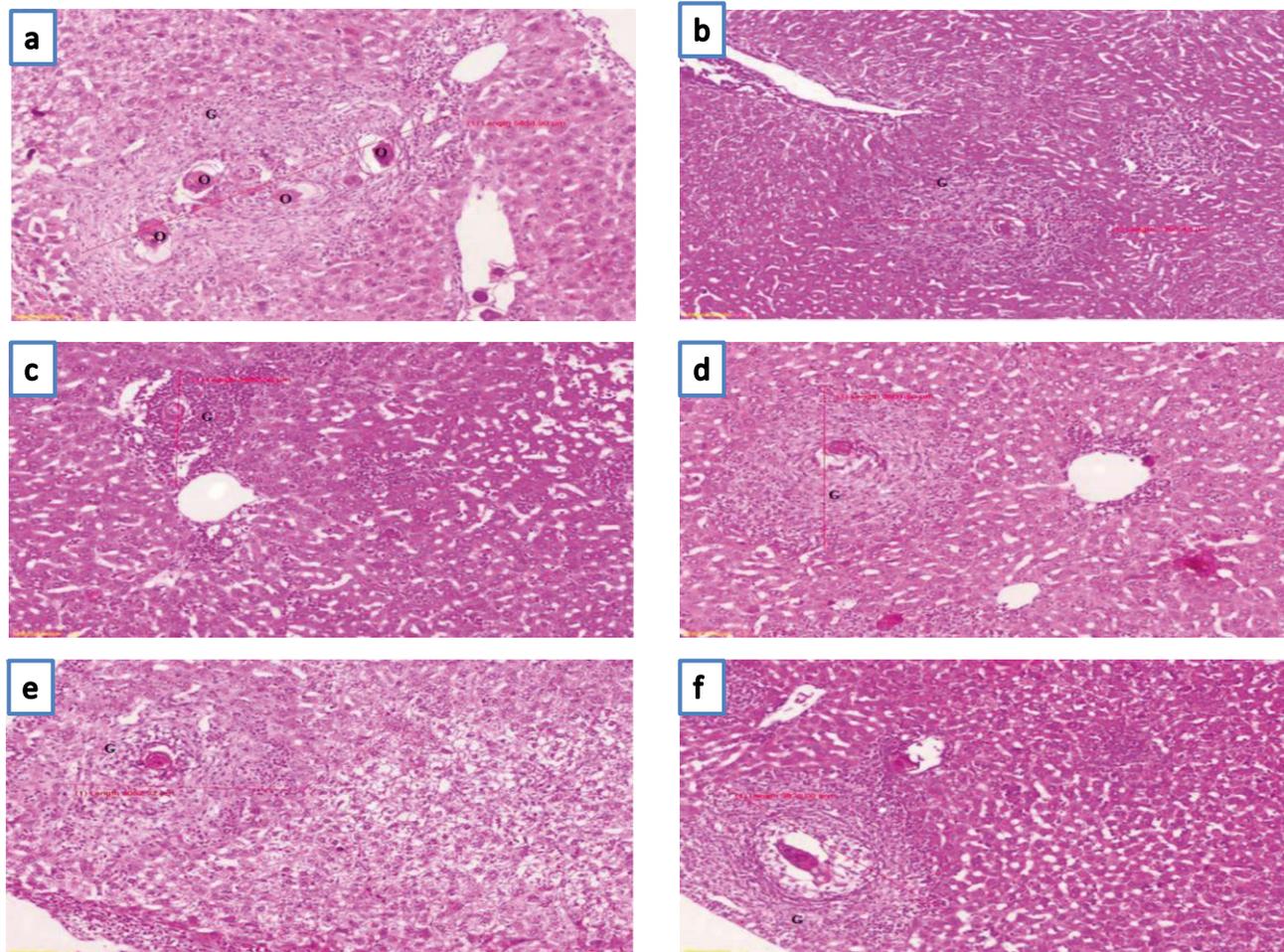
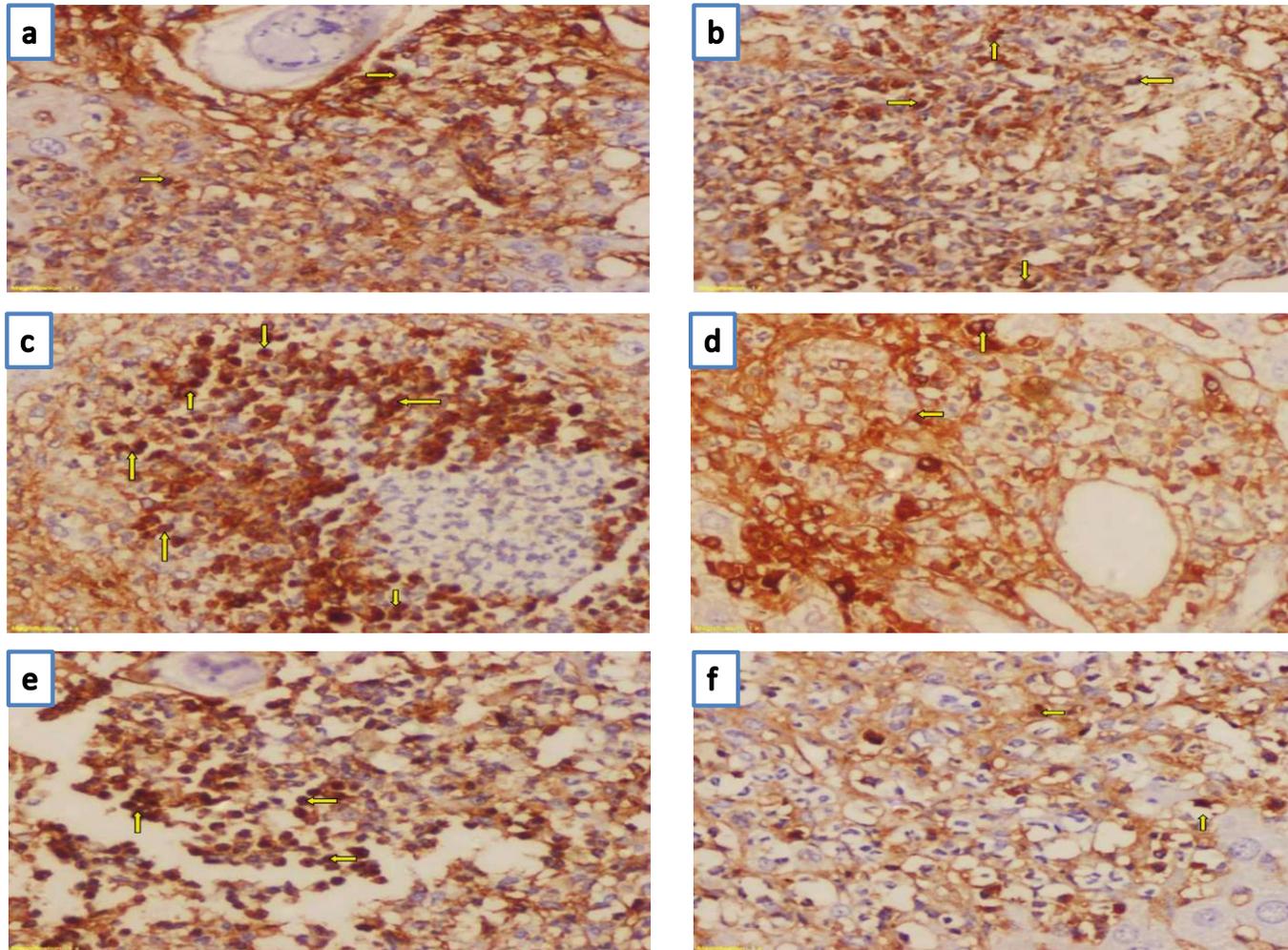
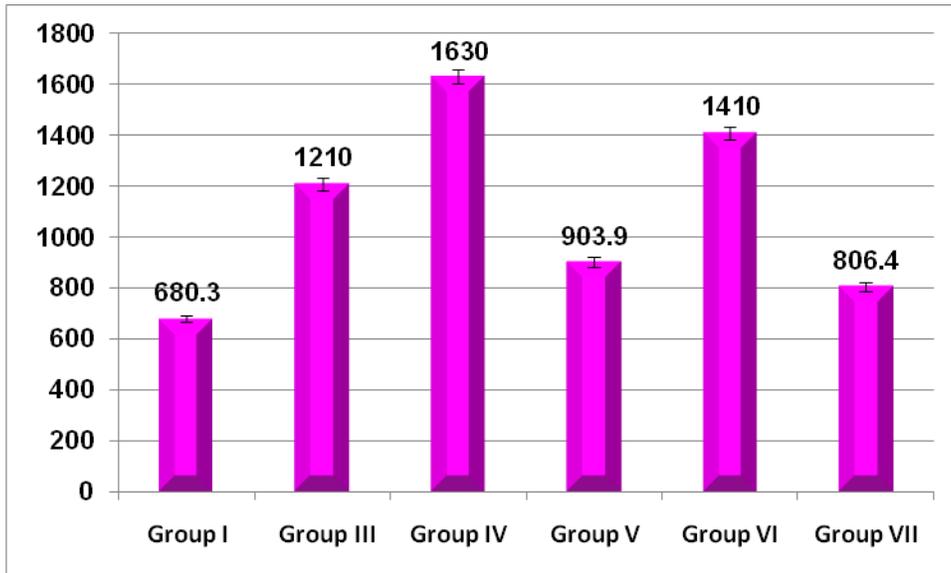


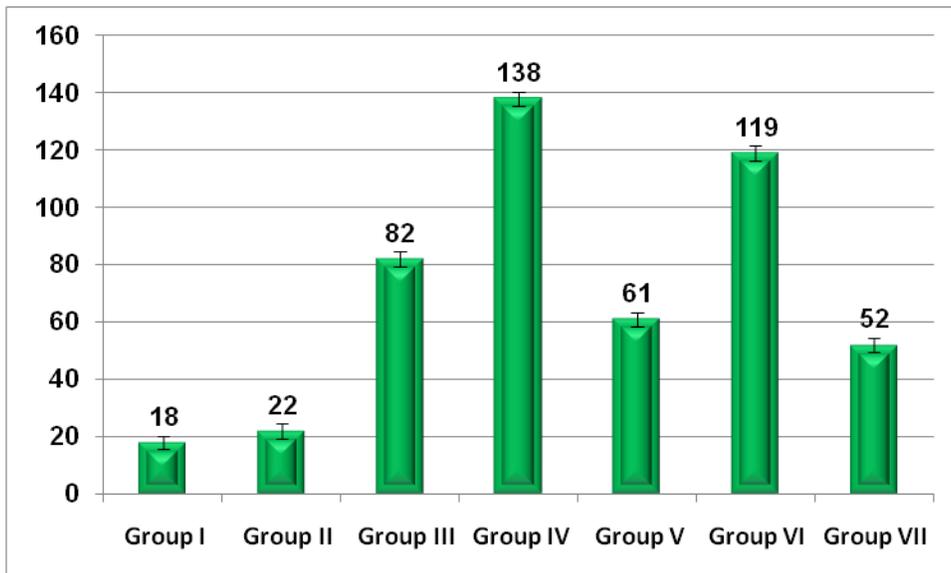
Fig.4(a) Liver section of control infected mouse showing positive CD4 membranous expression in few lymphocytes (x 400). (b): Liver section of infected mouse vaccinated with SEA showing positive CD4 membranous expression in 35 % of lymphocytes (x 400). (c): Liver section of infected mouse vaccinated with SEA and dendritic cells showing diffuse strong positive CD4 membranous expression in majority of lymphocytes (x 400). (d): Liver section of infected mouse vaccinated with SWAP showing positive CD4 membranous expression in 30 % of lymphocytes (x 400). (e): Liver section of infected mouse vaccinated with SWAP and dendritic cells showing positive CD4 membranous expression in 40 % of lymphocytes (x 400). Liver section of infected mouse vaccinated with dendritic cells showing positive CD4 membranous expression in 20 % of lymphocytes (x 400)



Graph.1 Mean serum IgG level (mg /dl) among the studied groups



Graph.2 Mean serum IL 10 level (pg /dl) among the studied groups



Graph.3 Mean serum IL 12P40 level (pg /dl) among the studied groups

Immunohistochemical outcomes:

The highest percent of CD4 +ve cells in liver was 60% in group IV (SEA+ DCs) followed by group VI (SWAP+ DCs) 50% in comparison with group II (control infected)

the percent was 25% with the presence of highly statistically significant difference ($p < 0.001$) (Table 5&Fig. 4). The mean H score of CD4 in liver was the highest (170 ± 9.12) in group IV (SEA+ DCs) which indicates strong expression of the stain followed by group VI

(SWAP+DCs) (140 ± 8.81) versus 55 ± 8.81 in group II control infected with the presence of highly significant statistical difference ($p < 0.001$) (Table 6). DCs play a key role in the generation of CD4 T cell responses to pathogens (Bizzell *et al.*, 2018). DC-mediated tailoring of the appropriate T cell programme ensures a proper cascade of immune responses that adequately targets the insult (Eisenbarth, 2019).

Antibodies profile

The mean IgG level was the highest in group IV (SEA+ DCs) 1630 ± 27.4 mg/dl followed by group VI (SWAP+DCs) 1410 ± 24.0 mg/dl versus group I (control normal) 680.3 ± 14.7 mg/dl with the presence of highly statistically significant difference ($p < 0.001$) (Graph 1). DCs facilitate cross talk between the innate and adaptive immune system. Targeting vaccines to DCs thus provides a great deal of opportunities for influencing the humoral immune responses, by fine-tuning the T cell response as well as regulating antigen availability for B cells (Tesfaye, 2019). IgG is the dominant immunoglobulin evoked as the result of an immune response (Tete *et al.*, 2013). It is known that Th2-type responses during helminthic infection can promote B-cell class switching to IgG1 (Harris and Gause, 2011). The increased IgG level recorded in the study suggests that there is an association between a reduction in worm burden and parasite-specific antibodies. These results were in agreement with that of Jin-ping *et al.*, (2004) who stated that there was increase in serum antibody level in mice immunized with SEA loading dendritic cell. And this indicated that SEA loading dendritic cell could induce protective immunity against infection of *S. japonicum*. The variation of IgG level results may be due to different *Schistosoma* species or different dose of infection. Moreover, Alves *et al.*, (2018) and Ranasinghe *et al.*, (2018) demonstrated that

the protective vaccine formulation induced a specific IgG response in the vaccinated mice compared with their controls.

The present study supports the idea that both cellular and humoral immune mechanisms may be important to control *S. mansoni* infection. In the initial stages of a *Schistosoma* infection, parasites induce mainly a Th1-type immune response, and at around 6 weeks, at the commencement of female worm egg deposition, the immune response shifts dramatically to Th2 (Wilson *et al.*, 2007).

Cytokines profile, the mean IL-10 and IL-12p40 levels

The highest mean level of IL-10 was in group IV (SEA+DCs) (138 ± 2.58 pg/dl) followed by group VI (SWAP+DCs) (119 ± 4.08 pg/dl) versus group I (control normal) (18 ± 2.31 pg/dl) and group II (control infected) (22 ± 2.58 pg/dl) with the presence of highly statistically significant difference ($p < 0.001$). Moreover, there was a highly statistically significant difference between both combined groups; group IV (SEA + DCs) and group VI (SWAP + DCs) (Graph 2). Also, the mean IL-12p40 level was the highest in group IV (SEA+DCs) (122 ± 2.58 pg/dl) followed by group VI (SWAP+ DCs) (99 ± 4.08 pg/dl) versus group I (control normal) (21 ± 2.10 pg/dl) and group II (control infected) (26 ± 2.58 pg/dl) with the presence of a highly statistically significant difference ($p < 0.001$) (Graph 3). DCs have the ability to direct the profile of helper T (Th) cells towards Th1, Th2, Th17, Th9 and regulatory (Treg) cells (Perona *et al.*, 2018). DCs are thought to play an important role in peripheral tolerance induction by various mechanisms, including production of soluble factors like IL-10, TGF- β or indoleamine 2,3-dioxygenase (Travis *et al.*, 2007). IL-10 has also been shown to play an important role in schistosomiasis by

preventing the development of Th1 and Th2-mediated pathologies (Pearce and MacDonald, 2002). These results were supported by previous other studies (Ricciardi *et al.*, 2016, Alves *et al.*, 2018, Egesaet *al.*, 2018 and Ranasinghe *et al.*, 2018). The vaccine that induces both Th1- and Th2-like responses would be necessary to achieve higher levels of protection. An additional concern is that a vaccine should not lead to a potentiated immunopathologic response, since the granulomatous reaction and resulting hepatic fibrosis in schistosomiasis are also immunologically mediated (Ribeiro De Jesus *et al.*, 2000). Overall, the protection induced in the present study was probably the result of a balanced Th1/Th2 response.

Ethical standards:

The authors assert that all procedures contributing to this work complies with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

Acknowledgment

The authors sincerely thank drHala Gaber Metwaly, clinical pathology department, Faculty of medicine CairoUniversity. For her help in dendritic cells preparation. We are also grateful to dr Dina Mohamed Sweed, pathology department, Liver Institute-MenoufiaUniversity.For her effort during examination of pathological specimens.

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How to cite this article:

Amany F. Atia, Amera F. Afifi, Mohammed M. Abdel Gaffer, Nadia S. ELNahas, Mona F. Abdel-Samee Faheem and Salwa F. A. Oshiba. 2020. Dendritic Cells as an Adjuvant to some *Schistosoma mansoni* Antigens for Vaccination in Experimental Schistosomiasis. *Int.J.Curr.Microbiol.App.Sci*. 9(03): 32-53. doi: <https://doi.org/10.20546/ijcmas.2020.903.005>