*Original Article*

# *Escherichia coli* shares T- and B-lymphocyte epitopes with *Schistosoma mansoni*

Mahmoud Mohamed Bahgat, Hanaa Mohamed Gaber, Amany Sayed Maghraby

*Therapeutical Chemistry Department, Immunology and Infectious Diseases Laboratory, the Center of Excellence for Advanced Sciences, the National Research Center Dokki, Cairo 12311, Egypt*

## Abstract

Background: In this study, we tested the cross-reaction between crude *Escherichia coli* antigen (ECA) and 3 crude *Schistosoma mansoni* antigens.

Methodology: The schistosomal antigens used were cercarial antigen preparation (CAP), soluble worm antigen preparation (SWAP), and soluble egg antigen (SEA). Four groups each of 3 mice received 2 intraperotineal immunizations with the above-mentioned antigens at a two-week interval. The dose of the ECA was 20 µg/100 µl PBS/mouse and that of any of the used schistosomal antigens was 50 µg/100 µl PBS/mouse. IgM and IgG reactivities and cross-reactivities were tested in individual immunized mice sera (IMS) against the above-mentioned antigens by ELISA and Western blotting. The changes in the B, $CD4^+$ and $CD8^+$-T cells' counts post immunization were recorded.

Results: Priming with ECA caused significant increases in IgM ($P < 0.05$) against CAP and SWAP, while both priming and boosting with ECA caused a significant elevation in the IgG only against SWAP. Priming and boosting with ECA or schistosomal antigens caused significant increases in IgM against ECA. Priming with ECA or SWAP caused significant elevation in IgG against ECA. In Western blotting, ECA-IMS recognized 16, 33, 38 and 94 kDa ECA peptides that cross-reacted with CAP-IMS. ECA peptides at 30 and 38 kDa cross-reacted among ECA, SWAP and SEA-IMS. CAP peptides at 40, 71, 85 and 97 kDa cross-reacted with ECA-IMS. A 59 kDa SWAP peptide cross-reacted with ECA. SEA peptides at ~55, 96 and 101 kDa cross-reacted with ECA-IMS. Immunization with ECA, CAP, SWAP or SEA caused significant increases in mesentric lymph nodes (MLN)-$CD4^+$, $CD8^+$-T cells and MLN-B cells. For thymocytes, $CD4^+$-T cells significantly increased upon immunization with ECA and SWAP while $CD8^+$-T cells significantly increased upon immunization with SWAP.

Conclusion: It is necessary to include *E. coli* antigens as controls while establishing schistosomal antigens-based diagnostic tests to ensure the specificity of the detected immune responses. Characterization of the cross-reactive ECA antigens with protective potential against *S. mansoni* infection remains a future research objective.

**Keywords:** *Escherichia coli*, *Schistosoma mansoni*, T cells, B cells, epitope

*J Infect Developing Countries* 2009; 3(3):206-217.

Received 29 September 2008 - Accepted 10 February 20



## Introduction

The established immune reactions to antigens from previous infections influence immune responses to helminthes infection [1-3]. Cross-reaction and/or cross-protective immune responses between *Escherichia coli* and the parasite *Schistosoma* were poorly studied except for a preliminary report by our group [4].

Infection with high loads of non-pathogenic *E. coli* inhibits degranulation of mast cells [5] that are known to play an important role in killing *Schistosoma mansoni* cercariae while penetrating the host's skin and exerting cytotoxic response on schistosomula [6]. In addition, peritoneal mast cells are involved in early acute hepatic and intestinal granuloma formation resulting from schistosomiasis [7].

We previously reported that immunization with crude *Saccharomyces* antigens induces cross-reactive IgM and IgG responses against several crude *S. mansoni* antigens and elevation in $CD4^+$, $CD8^+$T and B-cells [8].

Another clear example for the interrelationship between microbial and parasitic infections is the associated morbidity and mortality among *Mycobacterium tuberculosis* infected patients in areas with high prevalence of helminth infections [9-10].

*In vitro* studies showed that immune cells derived from humans suffering from intestinal helminth or chronic *S. mansoni* infections show impaired responses to antigens from *M. tuberculosis* [11-12]. Since mycobacterial infections are controlled by a Th1 type response [1-3,13], the elevated bacterial load in chronically *S. mansoni*

infected mice might be due to the established Th2 response during chronic schistosomal infection that inhibits mycobacterial antigen specific-Th1 response and increase susceptibility to mycobacterial infections [13-14].

The rationale of the present study is based on our previous observation of an apparent cross-reaction between IgG in sera from *S. mansoni* infected mice with the *E. coli* antigen (ECA) used in the present work [4] which was attributed to either the presence of common antigens among the parasite and the bacteria or super-infection of the tested mice by *E. coli* that elicited such IgG response. To validate the hypothesis of the presence of common antigens between both pathogens, we compared the immune responses resulting from immunizing pathogen free mice with antigens derived from *E. coli* or the *S. mansoni* independently.

## Materials and methods

*Antigens and experimental animals*

ECA from non-pathogenic *E. coli* (ECA; Bio-Rad Laboratories, Richmond, CA) was reconstituted to the desired concentration in water as recommended by the manufacturer and stored at -80$^{o}$C till use. This *E. coli* lysate is usually used to reduce background during the immune screening of a phage cDNA library where *E. coli* is used as the host cell. The used cercarial antigen preparation (CAP), soluble worm antigen preparation (SWAP) and soluble egg antigen (SEA) from the Egyptian strain of *S. mansoni* were all purchased from the parasite life cycle facility at the Theodore Bilharz Research Institute (Giza, Egypt). These antigens were prepared as described by Jassim *et al*. [15].

Female pathogen-free Swiss albino mice, 18-20 g, were obtained from the animal facility at the National Research Center of Egypt and used throughout this study. Animals were fed on standard rodents chew, supplied with water, and maintained at 23$^{o}$C ambient temperature. Four groups each of 3 mice received two intraperotineal (IP) immunizations with the following antigens at a two-week interval. The first received ECA (20 µg/100 µl PBS/mouse); the second received CAP (50 µg/100 µl PBS/mouse); the third received SWAP (50 µg/100 µl PBS/mouse); and the fourth received SEA (50 µg/100 µl PBS/mouse). A fifth group of control mice injected with PBS (100 µl/mouse) was included. Blood samples were collected from both immunized and control animals by penetrating the retro-orbital plexus/sinus with a heparin-treated glass capillary tube one week post priming and booster and centrifuged at 14,000 *g* at 4$^{o}$C for 20 minutes. Sera were separated, aliquoted, and frozen at -80$^{o}$ C until used.

*Enzyme-linked immunosorbent assay*

The assay was performed as previously described [16] to determine levels of IgG and IgM in individual sera of different mouse groups. Individual u-shaped wells of polyvinyl 96-well-plates (Alto, Italy) were coated each with 100 µl of either (1) ECA (10 µg/ml), (2) CAP (25 µg/ml), (3) SWAP (50 µg/ml) or (4) SEA (25 µg/ml) diluted in coating buffer (20 mM sodium carbonate; 50 mM sodium bicarbonate, pH9.6) followed by overnight incubation at room temperature. Plates were washed three times using 0.01M PBS, pH 7.4, containing 0.05% Tween-20 (PBST). Antigen-free sites were blocked against nonspecific binding using 200 µl/ well of PBST containing 1% bovine serum albumin (PBST-BSA), and then left for one hour at 37$^{o}$C. After three washes with PBST, 100 µl/ well of diluted sera 1:100 in PBST-BSA were incubated in the plate wells at 37$^{o}$C for two hours. Following incubation, plate wells were washed three times with PBST then peroxidase-labeled anti-mouse IgM (whole molecule) or IgG (g chain) conjugates (Sigma, St. Louis, Mo., USA) were added 100 µl/well at a dilution of 1:5000 and 1:500 respectively in PBST-BSA and incubated with the plate wells for one hour at 37$^{o}$C. For visualization of the antigen antibody reaction, the substrate *O*-phenylenediamine (Sigma, St. Louis, Mo., USA) containing $H_2O_2$ was applied 100 µl/well and plates were left at room temperature for 10 minutes to allow colour development. To avoid an increase in the background in the control wells, the reaction was stopped by adding 25 µl/ well of 2 N HCl and the change in optical densities was measured at λ max 490 nm with a micro-well plate ELISA reader (TECAN-SUNRISE, Austria). Reactivities of both IgM and IgG were tested in individual sera against the four antigens in duplicate wells.

*Electrophoresis Western blotting*

Sodiumdodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the ECA, CAP, SWAP, and SEA was conducted [17] through 4% stacking and 10% resolving mini slab-gels in an electrophoresis chamber (Mini-protean II; Bio-Rad Laboratories, Munich, Germany). Resolved proteins were visualized by silver staining [18].

**Table 1.** Detection of IgM response in sera from mice immunized with ECA or different *S. mansoni* antigens using ECA-coated multi-well plates.

| IgM response | | | |
|---|---|---|---|
| The first immunization with ECA | | | |
| | Control | ECA | P-value |
| Mean ±S.D. | 0.15 ±0.039 | 0.71 ±0.39 | 0.0726 |
| The second immunization with ECA | | | |
| | Control | ECA | P-value |
| Mean ±S.D. | 0.15 ±0.03 | 0.42 ±0.19 | 0.09 |
| The first immunization with CAP | | | |
| | Control | CAP | P-value |
| Mean ±S.D. | 0.13 ±0.005 | 0.38 ±0.05 | 0.001 |
| The second immunization with CAP | | | |
| | Control | CAP | P-value |
| Mean ±S.D. | 0.11 ±0.06 | 0.34 ±0.09 | 0.05 |
| The first immunization with SWAP | | | |
| | Control | SWAP | P-value |
| Mean ±S.D. | 0.11 ±0.01 | 0.39 ±0.08 | 0.006 |
| The second immunization with SWAP | | | |
| | Control | SWAP | P-value |
| Mean ±S.D. | 0.11 ±0.01 | 0.29 ±0.04 | 0.006 |
| The first immunization with SEA | | | |
| | Control | SEA | P-value |
| Mean ±S.D. | 0.21 ±0.066 | 0.56 ±0.23 | 0.06 |
| The second immunization with SEA | | | |
| | Control | SEA | P-value |
| Mean ±S.D. | 0.21 ±0.06 | 0.35 ±0.05 | 0.08 |
| | | | |

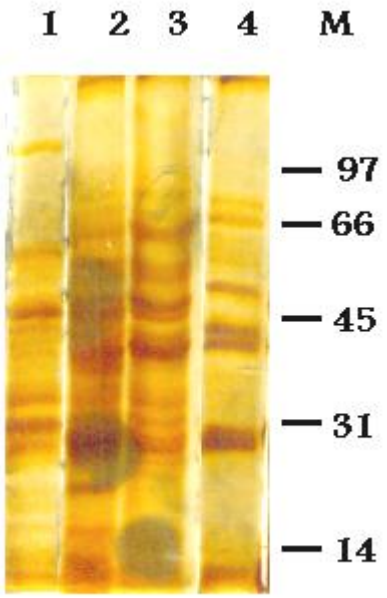
S.D. "Standard Deviation"

**Table 2.** Detection of IgG response in sera from mice immunized with ECA or different *S. mansoni* antigens using ECA-coated multi-well plates.

| IgG response | | | |
|---|---|---|---|
| The first immunization with ECA | | | |
| | Control | ECA | P-value |
| Mean ±S.D. | 0.44 ±0.27 | 1.31 ±0.6 | 0.09 |
| The second immunization with ECA | | | |
| | Control | ECA | P-value |
| Mean ±S.D. | 0.44 ±0.27 | 1.3 ±0.05 | 0.02 |
| The first immunization with CAP | | | |
| | Control | CAP | P-value |
| Mean ±S.D. | 0.17 ±0.01 | 0.22 ±0.02 | 0.04 |
| The second immunization with CAP | | | |
| | Control | CAP | P-value |
| Mean ±S.D. | 0.17 ±0.01 | 0.27 ±0.05 | 0.03 |
| The first immunization with SWAP | | | |
| | Control | SWAP | P-value |
| Mean ±S.D. | 0.09 ±0.01 | 0.17 ±0.02 | 0.007 |
| The second immunization with SWAP | | | |
| | Control | SWAP | P-value |
| Mean ±S.D. | 0.11 ±0.04 | 0.15 ±0.01 | 0.34 |
| The first immunization with SEA | | | |
| | Control | SEA | P-value |
| Mean ±S.D. | 0.3 0.02 | 0.3 0.05 | 0.8 |
| The second immunization with SEA | | | |
| | Control | SEA | P-value |
| Mean ±S.D. | 0.3 0.02 | 0.34 0.09 | 0.5 |

S.D. "Standard Deviation"

**Figure 1**. Protein profiles via SDS-PAGE fractionation.




Protein profiles of the ECA (lane 1), SEA (lane 2), SWAP (lane 3) and CAP (lane 4) fractionated by SDS-PAGE followed by silver staining. The visualized ECA fractions had molecular weights of 16, 26, 30, 38, 47, 62, 71 and 101 kDa. The molecular weights of the resolved SEA fractions were 16, 30, 35, 38, 71 and 76 kDa. The SWAP fractions were seen at 20, 24, 27, 30, 38, 40, 46, 48, 59, 71 and 83 kDa. The CAP fractions were at 24, 26, 38, 43, 51, 71 and 77 kDa. Noteworthy, peptides at 38 and 71 kDa were common among the four antigens. Peptides at 30 kDa were common between ECA, SEA and SWAP. Peptides at 26 kDa were common between the ECA and CAP. Peptides at 16 kDa were common between the ECA and SEA. Peptides at 24 kDa were common between the CAP and SEA.

Immunoblotting was conducted [19] to detect cross-reactivity between the ECA and crude *S. mansoni* antigens. Resolved proteins were electro-transferred from gel to nitrocellulose sheets (BA85, pore size 0.45 µm; Schleicher and Schull, Dassel, FRG) at 60 volts for three hours in transfer buffer. Membranes were cut into individual strips that were washed three times each for five minutes with PBS-0.3% T followed by blocking against nonspecific binding for one hour in PBS-0.3% T-1% BSA at room temperature. Strips were washed three times as described above and incubated for two hours with sera from immunized mice with different antigens as well as control sera (1/100 in PBS-0.3%T). After three successive washes each for five minutes, strips were rinsed for two hours in peroxidase-conjugated anti-mouse IgG (1/500 in PBS-0.3%T) at room temperature. Visualization of immune reaction on the nitrocellulose strips was done by incubation with peroxidase specific substrate (22 mg diaminobenzedine and 2.2µl $H_2O_2$ 30% in 22 ml PBS).

*Immunofluorescence staining of mesenteric lymph nodes cells and thymocytes*

Mesenteric lymph nodes (MLN) and thymus were excised from each mouse and cleaned from adipose tissue. The MLN capsules and thymus were gently teased apart using two glass slides on PBS. The number of cells in the MLN and in the thymus was counted using a heamocytometer. The cell suspension was diluted 1:1 in 4% trypan blue dye to test cell viability.

Percentage viability was calculated using the following formula:

- (Number of viable cells/total number lymphocytes) x 100

Total number of lymphocytes was calculated using following formula:

- N x 2 x $10^4$/ml

  N: Number of lymphocytes counted in 16 large squares of the haemacytometer

  2: Dilution factor since the cell suspension was diluted 1:1 in 4% trypan blue

T-cell subsets were identified using fluorescein isothiocyanate (FITC)-conjugated monoclonal anti-mouse $CD4^+$, $CD8^+$ -cells. B-cells were detected using FITC labeled anti-mouse IgM µ-chain. The percentage of positive cells was determined by counting a minimum of 100-200 viable cells using a fluorescence microscope at a magnification of 40X.

*Statistical analysis*

Means and standard deviations were deduced by the student's t-test using GraphPad InStat software. $P < 0.05$ was considered statistically significant.

## Results

*Immune responses in immunized mice with ECA and cross-reactivity with various* S. mansoni *antigens*

One week following the first immunization with ECA, mice sera showed a significant ($P < 0.05$) increase in IgM reactivity against both CAP and SWAP over the control sera, but the increase in the reactivity was not significant against SEA (Table 1). One week after the second immunization with ECA, mice sera showed a significant elevation in the IgM level ($P < 0.05$) only against SWAP when compared with un-immunized sera (Table 1). One week following both the first and second immunizations with ECA, mice sera showed a significant ($P < 0.05$) elevation in IgG levels only against SWAP when compared to the control sera (Table 2).

*Immune responses in mice immunized with various* S. mansoni *antigens and cross-reactivity with ECA*

One week following both first and second immunizations with either ECA or each of the schistosome antigens (CAP, SWAP or SEA), mice sera showed significant (Table 1; $P < 0.05$) increase in the IgM levels against ECA in comparison to the control sera. After priming, the IgM response to ECA (Table 1) was highest in immunized mice with CAP ($P < 0.001$) followed by SWAP ($P < 0.006$), SEA ($P < 0.06$) and ECA ($P < 0.07$). After the second immunization, IgM response to ECA (Table 1) was highest in immunized mice with SWAP ($P < 0.006$) followed by CAP ($P < 0. 005$), SEA ($P < 0.08$) and ECA ($P < 0.09$).

One week post priming with either of the used antigens, only sera from immunized mice with CAP or SWAP showed significant increase ($P < 0.05$) in the IgG levels (Table 2) against ECA when compared with control mice sera, and the significance was higher upon immunization with SWAP ($P < 0.007$) than with CAP. One week after boosting with either of the four antigens, only sera from mice immunized with ECA or CAP showed a significant increase ($P < 0.05$) of IgG level against ECA (Table 2) when compared with control mice sera, and the

significance was higher upon immunization with ECA ($P < 0.02$) than CAP ($P < 0.03$).

*Sodiumdodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the 4 antigens*

Figure 1 shows the protein profile results of ECA (lane 1), SEA (lane 2), SWAP (lane 3) and CAP lane (4) fractionation by SDS-PAGE and silver staining. The visualized ECA fractions had molecular weights of 16, 26, 30, 38, 47, 62, 71 and 101 kDa. The molecular weights of the resolved SEA fractions were 16, 30, 35, 38, 71 and 76 kDa. The SWAP fractions were seen at 20, 24, 27, 30, 38, 40, 46, 48, 59, 71 and 83 kDa. The CAP fractions were at 24, 26, 38, 43, 51, 71 and 77 kDa.

*IgG reactivity in immunized mice sera against SDS-PAGE fractionated ECA*

When ECA-immunized mice sera (IMS) were tested for the IgG reactivity against SDS-PAGE fractionated ECA on Western blots (Figure 2A), results clearly indicated that individual sera from mice that received single (strips 1 & 2) or booster immunizations (strips 3 and 4) as well as un-immunized mice sera (UIMS; strip 5) showed more or less identical profiles as they recognized ECA peptides at ~16, 30, 33, 34, ~38, 56 and ~94 kDa, except for the individual serum tested on the strip 1 that uniquely reacted to a 26 kDa protein that was not recognized by other sera. Cross-reactivity of CAP, SWAP, and SEA-IMS that were IgG reactive in ELISA was further tested against SDS-PAGE fractionated ECA (Figures 2B, C and D respectively) where strips 1-3 were treated with sera from individual mice that received single immunizations, while strips 4-6 were treated with individual booster immunized mice sera, and strip 7 with control mouse serum.

Primed or booster CAP-IMS as well as UIMS generally recognized immunogenic ECA-peptides at 16, 38 and 94 kDa. Two sera from booster immunized mice recognized antigenic bands at 33 and 46 kDa (strips 5 and 6), while only one sample from the same group recognized two extra bands at 25 and 28 kDa (strip 5) that were not recognized by the rest of the used sera (Figure 2B). It is noteworthy that the 16, 33, 38 and 94 kDa were also previously recognized by the ECA-IMS. Priming or booster SWAP-IMS, as well as UIMS, generally recognized immunogenic ECA-peptides at 26, 28, 30, 33 and 38 kDa. The intensities of the recorded bands were higher with the booster immunization sera (Figure 2C) and the last two peptides were cross-reactive with ECA-IMS.

Primed or booster SEA-IMS, as well as UIMS, generally recognized immunogenic ECA-peptides at 16, 26, 29, 38 and 91 kDa. The bands' intensities were higher with the booster immunization sera (Figure 2D). Only three sera samples recognized peptide at 33 kDa (strips 1, 2 and 5) that was not observed with other sera. Both the 33 & 38 kDa peptides were previously recognized by both the ECA and SWAP-IMS.

*IgG reactivity in sera from ECA-immunized mice towards SDS-PAGE fractionated* S. mansoni *antigens*

Cross-reactivity of IgG in ECA-IMS towards SDS-PAGE fractionated CAP; SWAP and SEA (Figures 3A, B and C respectively) was investigated. Individual sera from mice that received single (A; strips 1 and 2) or booster (A; strips 3 and 4) immunizations with the ECA cross-reacted with CAP peptides at 65, 71, 80, 85, 91 and 97 kDa, while only one single immunization serum strongly reacted to an additional peptide at ~ 40 kDa (strip 1). UIMS (A; strip 7) recognized immunogenic CAP-peptides at 71 and 85 kDa.

Individual sera from mice that received single (B; strips 1 and 2) or booster (B; strips 3 and 4) immunizations with the ECA recognized immunogenic SWAP peptides at 59 and 68 kDa that were not detected by un-immunized mouse serum (B; strip 6).

When crossed with SEA fractionated antigen, individual sera from mice that received single (C; strips 1 and 2) or booster (C; strips 3 and 4) immunizations with the ECA recognized common peptides at 40, ~55, 75, 98, 101 and 104 kDa. The UMIS (C; strip 5) did not react to any of the above mentioned bands.

*Cellular response*

Immunization with the ECA caused significant ($P < 0.05$) increases in the mean percentage of MLN-$CD4^+$, $CD8^+$-T and B-cells in comparison to levels in the control animals (Table 3). Immunization with CAP caused significant ($P < 0.05$) increases in the mean percentage of MLN-$CD4^+$, $CD8^+$-T and B-cells compared to levels in the control mice (Table 3).

**Table 3.** Mean percentage of mesenteric lymph node lymphocytes from mice immunized with ECA, CAP, SWAP, and SEA antigens and un-immunized mice.

| Crude antigens | $CD4^+$-T lymphocytes Mean ± S.D. | $CD8^+$-T lymphocytes Mean ± S.D. | $CD4^+/CD8^+$-T lymphocytes Mean ± S.D. | B-lymphocytes Mean ± S.D. |
|---|---|---|---|---|
| ECA | 40.3± 4.1* | 22.8± 3.4* | 1.76 | 31.4 ±6.8* |
| CAP | 40.8 ±8.2* | 40.2 ±6.5* | 1.4 | 30.7± 1.5* |
| SWAP | 34.1 ±3.8* | 28.5± 3.7* | 1.19 | 30.9± 2.8* |
| SEA | 28.6 ±2.2* | 25.2± 2.2* | 1.1 | 29.5± 0.8* |
| Unimmunized cells | 17.3 ±2.7 | 15.8± 2.5 | 1.1 | 18.4 3.6 |

*P<0.05, S.D. "Standard deviation

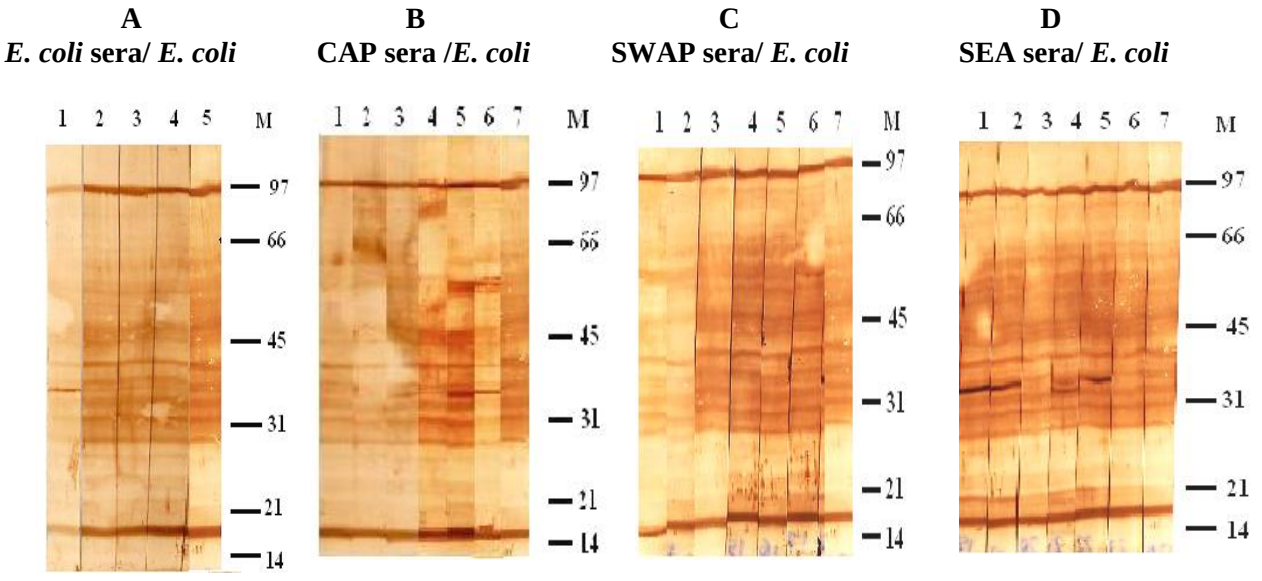



**Figure 2.** IgG reactivity in IMS against SDS-PAGE fractionated ECA. ECA-IMS were tested for total IgG reactivity against SDS-PAGE fractionated ECA antigens (Figure 2A) compared to cross-reactivity of IgG in CAP, SWAP and SEA-IMS (Figures 2B, C and D respectively) against the same SDS-PAGE fractionated ECA as detected by Western blotting. In Figure 2A**,** strips 1 and 2 were incubated with sera from mice that received single ECA-immunization, while strips 3 and 4 were incubated with sera from mice that received booster ECA-immunization, and strip 5 with UIMS. In Figure 2B, C and D, strips 1-3 were incubated with individual mice sera that received single immunization, while strips 4-6 were incubated with booster IMS, and Strip 7 with control mouse serum. It is noteworthy that the 16, 33, 38 and 94 kDa were recognized by both the ECA and CAP IMS (Figures 2 A and B respectively). Moreover, both the 30 and 38 kDa were recognized by the ECA, SWAP and SEA-IMS (Figures 2 A, C ,and D respectively).

**Table 4.** Mean percentage of thymocytes from mice immunized with ECA, CAP, SWAP, and SEA antigens and un-immunized mice.

| Crude antigens | $CD4^+$-T lymphocytes Mean ± S.D. | $CD8^+$-T lymphocytes Mean ± S.D. | $CD4^+/CD8^+$-T lymphocytes Mean ± S.D. |
|---|---|---|---|
| ECA | 46.3± 4.04* | 21.6± 2.6 | 2.14 |
| CAP | 32.9 ±5.5 | 25.3 ±2.4 | 1.3 |
| SWAP | 35.9 ±2.6* | 34.2± 5.8* | 1.04 |
| SEA | 31.2 ±1.1 | 27.9± 2.8 | 1.1 |
| Unimmunized cells | 23.3 ±6.11 | 22.8± 2.6 | 1.02 |

*P<0.05, S.D. "Standard deviation"

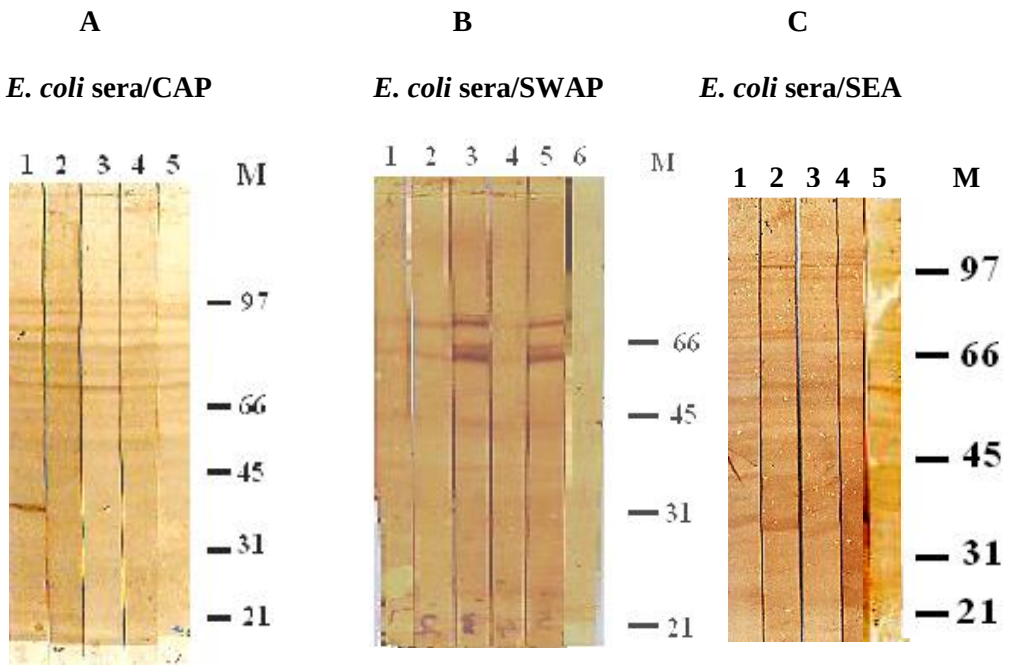



**Figure 3.** Cross-reactivity of IgG in ECA-IMS towards SDS-PAGE fractionated CAP, SWAP, and SEA (Figures 3A, B, and C respectively). Sera from mice that received single (A; strips 1 and 2) or booster (A; strips 3 and 4) immunization with ECA cross-reacted with CAP peptides at 65, 71, 80, 85, 91 and 97 kDa, while only one single immunization serum strongly reacted to an additional peptide at ~ 40 kDa (strip 1). UIMS (A; strip 7) recognized immunogenic CAP-peptides at 71 and 85 kDa. Sera from mice that received a single (B; strips 1&2) or booster (B; strips 3 and 4) immunization with ECA cross-reacted with immunogenic SWAP peptides at 59 and 68 kDa that were not detected by un-immunized mouse serum (B; strip 6). When crossed with SEA fractionated antigen, sera from mice that received a single (C; strips 1&2) or booster (C; strips 3&4) immunization with the ECA cross-reacted with SEA-peptides at 40, ~55, 75, 98, 101 and 104 kDa. The UMIS (C; strip 5) did not react to any of the above-mentioned bands.

Immunization with SWAP caused a significant ($P < 0.05$) elevation in the mean percentage of MLN-$CD4^+$, $CD8^+$-T cells and B-cells when compared to levels in control mice (Table 3). Immunization with SEA caused a significant ($P < 0.05$) increase in the mean percentage of MLN-$CD4^+$, $CD8^+$T and B-cells compared to levels in the control mice (Table 3). The resulting $CD4^+$/$CD8^+$T lymphocytes balance upon immunization with either of the used antigens was shifted towards the $CD4^+$T cells subsets and the highest ratio was recorded in case of ECA (Table 3). Also, immunization with ECA caused a significant ($P < 0.05$) increase in the mean percentage of $CD4^+$-thymocytes and non-significant (1.1 fold) increase in $CD8^+$-thymocytes when compared to control animals (Table 4). Immunization with CAP caused non-significant (1.47, 1.1 fold) increases in the mean percentage of both $CD4^+$ and $CD8^+$-thymocytes respectively compared to levels in control mice (Table 4). Immunization with SWAP caused significant ($P < 0.05$) increases in both $CD4^+$ and $CD8^+$-thymocytes compared to levels in control mice (Table 4). Immunization with SEA caused a non-significant (1.4, 1.2 fold) elevation in the mean percentage in both $CD4^+$and $CD8^+$-thymocytes respectively compared to levels in control mice (Table 4). The $CD4^+$/ $CD8^+$T lymphocytes balance was generally shifted towards the $CD4^+$T cells subsets and the highest ratio was recorded in the case of ECA (2.14) followed by CAP (1.3), SWAP, (1.04) and SEA (1.1).

## Discussion

ELISA results clearly demonstrated presence of common antigens among *S. mansoni* and *E. coli* that supports our previous observation of an apparent cross-reaction between IgG in sera from *S. mansoni* infected mice with the ECA [4]. Immunization with ECA resulted in significant increase in both IgM and IgG reactivity mainly against SWAP but not other crude schistosomal antigens or ECA itself, allowing the conclusion that the majority of the cross-reactive antigens are present in the adult worm stage.

Interestingly, the highest IgM and IgG measurements against ECA were not recorded in sera from immunized animals with the ECA but in CAP or SWAP-IMS, reflecting the stronger capacity of the cross-reactive antigens present in the cercariae and adult worms to stimulate humoral immune responses than the ECA itself.

Silver staining of the resolved four antigens by SDS-PAGE reflected presence of peptides of identical migration properties. Common peptides among the four antigens were visualized at 38 and 71 kDa. Common peptides between ECA, SEA and SWAP were recorded at 30 kDa. Common peptides between the ECA and CAP were seen at 26 kDa. Common peptides between the ECA and SEA were resolved at 16 kDa. Although similarity in the molecular weights of the resolved peptides by SDS-PAGE is not adequate evidence that such peptides are of the same identity, this finding led us to further compare the cross-reactivity of the SDS-PAGE antigens against either the ECA-IMS or schistosomal antigens-IMS.

ECA-IMS, as well as UIMS, showed a more or less identical profile as they recognized ECA peptides at ~16, 30, 33, 34, ~38, 56 and ~94 kDa, except for individual ECA-IMS that uniquely reacted to a 26 kDa protein. CAP-IMS, as well as UIMS, generally recognized immunogenic ECA-peptides at 16, 38 and 94 kDa. Only two CAP- booster IMS recognized antigenic bands at 33 and 46 kDa. Of those two samples, one recognized two extra bands at 25 and 28 kDa. It is noteworthy that the 16, 33, 38 and 94 kDa were also previously recognized by the ECA-IMS. SWAP-IMS, as well as UIMS, recognized immunogenic ECA-peptides at 26, 28, 30, 33 and 38 kDa. The last two peptides were cross-reactive with ECA-IMS. SEA-IMS, as well as UIMS, recognized immunogenic ECA-peptides at 16, 26, 29, 38 and 91 kDa. Only three SEA-IMS recognized ECA-peptide at 33 kDa. Both the 33 and 38 kDa peptides were previously recognized by ECA and SWAP-IMS.

Vaccinating mice with an *S. mansoni* hydrophobic polypeptide 30 kDa antigen elicited high levels of IgG antibodies (predominantly IgG2a and IgG2b) and resulted in significant protection against infection [20]. Also immunizing mice with *S. mansoni* 31 and 32 kDa antigens resulted in significant protection against infection [21-23].

Immunized rats with *S. mansoni* irradiated cercariae strongly reacted to schistosomula surface proteins of 38 kDa [24]. Also, passive transfer of sera, containing mainly IgG2, from immunized mice with this *S. mansoni* peptide resulted in a high degree of protection against cercarial challenge [25-26]. The importance of the 38 kDa *S. mansoni* peptide as a potent immunogen in man came from the data that 97% of Brazilian *S. mansoni* infected patients recognized this peptide and exhibited strong *in vitro* lymphokine production in response [27].

These results identified the molecular weights of the cross-reactive *E. coli* peptides with the induced IgG responses upon immunization with different schistosome antigens. The increase in the intensities of the ECA cross-reactive bands upon using sera from animals that received booster immunization with different schistosomal antigens confirms the cross-reactivity. The observation that many of the above-mentioned bands were also recognized by the UIMS suggests that control mice are frequently exposed to *E. coli* antigens and as a result they showed IgG reactivity against fractionated ECA antigen.

ECA-IMS recognized common CAP peptides at ~ 40, 65, 71, 80, 85, 91 and 97 kDa. Immunogenic adult *S. mansoni* glycoproteins of approximately similar molecular weights were previously recognized by Western blotting using sera from mice vaccinated with radiation-attenuated cercariae [28]. A 41 kDa cercarial antigen was previously described as an early diagnostic antigen for infection [29]. *S. mansoni* antigens at 42 [30] and 70 kDa [31] were previously characterized by Western blotting using sera from multiple vaccinated mice with irradiated cercariae with the 70 kDa antigen further identified as heat shock protein. Also, the 80 kDa large subunit of calpain was previously identified by Western blotting in *S. japonicum* ceracariae [32]. In addition, both 73 and 81 kDa cercarial antigens were previously recognized using naturally infected rat sera [33]. A 45 kDa *S. mansoni* cercarial specific Ca-binding protein was characterized by Western blotting [34]. Moreover, 60, 85, and 94 kDa were previously recognized in *S. mansoni* cercarial proteins using infected mice sera [35].

ECA-IMS recognized immunogenic SWAP peptides at 59 and 68 kDa. Immunogenic adult *S. mansoni* glycoproteins of approximately similar molecular weights were previously recognized by Western blotting using sera from mice vaccinated with radiation-attenuated cercariae [28]. A 57 kDa antigenic protein was detected in *S. mansoni* SWAP using sera from mice immunized by either virulent or radiation-attenuated cercariae [36]. A 62 kDa, calreticulin, antigen was recognized in *S. mansoni*-SWAP using an infected patient's sera [37] which was defined as a good T- and B-cell antigen and represents a potential vaccine candidate. A 70 kDa heat shock protein was characterized in *S. mansoni* as a major immunogen that invariably elicited an antibody response in infected humans [38].

ECA-IMS cross-reacted with 40, ~55, 75, 98, 101, and 104 kDa SEA peptides. An immunogenic peptide of a molecular weight ~97 kDa was recognized by sera of mice vaccinated with an *S. mansoni* adult parasite extract [39]. This was characterized as paramyosin and included as one of the six candidate vaccines against schistosomiasis [40]. In addition, 45, 47, 49, 64, and 92 kDa *S. mansoni* egg immunogenic peptides were previously recognized using naturally infected rat sera [33]. An immunogenic peptide of ~75 kDa molecular weight was previously recognized in *S. mansoni* egg secretory excretory antigens using polyclonal antisera raised in rabbits against processed urine of *S. mansoni*-infected patients [41] or in feces of *S. mansoni*-infected patients [42]. These results identified the molecular weights of the cross-reactive schistosome antigens with the induced IgG responses upon immunization with the crude *E. coli* peptides.

The $CD4^+/CD8^+$T lymphocytes ratio was used as an indicator for the possible immunomodulatory effects of the four antigens used as it is known that if the ratio is greater than one, then the antigen has an immunostimulatory effect; and if it is less than one, then the antigen has an immunosuppressive effect. Since the $CD4^+/CD8^+$T lymphocytes ratio was higher in MLN and thymocytes from ECA immunized mice than in those immunized with schistosomal antigens, one might concluded that the cellular immunostimulatory effect of ECA is stronger than that of the schistosome antigen.

The capacity of an antigen to induce protective immunity depends on its T-cell activation potential as such cells are required for both cell-mediated immunity and for eliciting antibody formation. Our results agree with those of van Schaik and Abbas [43], who found that infection with *E. coli* induced large proportions of both CD4+ and CD8+-T cells. Early schistosome infection is associated with type 1 response with expansion in both cytotoxic (Tc1) and helper (Th1) T-cells. At week seven after infection, a type 2 response is established, indicated by an increase in Th2 cells and a reduction in Tc1 cells [44]. Pemberton and Wilson [45] demonstrated that a Th1 response dominated the lymph node environment upon immunization with irradiated *S. mansoni* cercariae.

Immunomodulatory mechanism induced by *S. mansoni* worms was shown to be dependent on an IL-10-producing B-cell population [46]. Several SWAP-purified peptides stimulated proliferation of lymph node T-cells from immunized mice with irradiated *S.*

*mansoni* cercariae [47]. *S. mansoni* SWAP duplet peptides of 62/60 kDa were found to contain immunodominant T-cell immunogen(s) in irradiated cercariae-immunized mice. Further characterization indicated that the 60 kDa molecules are poorly immunogenic in mice and rabbits, whereas the 62 kDa, *S. mansoni* calreticulin is a good T- and B-cell antigen and represents a potential vaccine candidate [48]. Our Western blotting results showed that ECA-IMS recognized SWAP peptide at 59 kDa.

A 26 kDa egg antigen induced significant $CD_4^+$ T-cell response [49]. Interestingly an ECA peptide of the same molecular weight cross-reacted to sera from mice immunized with SEA. Mesenteric lymph node cells from B-cell depleted mice (JHD mice) displayed lower proliferative responses to SEA [50]. SEA was reported to stimulate cytotoxic $CD_8^+$T-cell response as well as T-cell-mediated granulomatous that is principally responsible for the pathology of the disease in mice [51]. When electroeluted SEA fractions (less than 21, 25 to 30, 32 to 38, 40 to 46, 50 to 56, 60 to 66, 70 to 90, 93 to 125, and greater than 200 kDa) were used in an *in vitro* lymphoproliferation assay, T-cell-enriched spleen cells from acutely infected mice responded to all nine fractions, while those from chronically infected mice responded to only the 50 to 56 and the 60 to 66 kDa fractions [52].

In addition, depleting $CD4^+$ T-cells from acute and chronic-infection spleen cells abrogated the response while depleting $CD8^+$ T-cells enhanced proliferation of acute-infection T cells in response to all the above-mentioned fractions and facilitated responsiveness to inactive SEA fractions in chronic-infection T cells [52]. Moreover, acute-infection $CD4^+$ granuloma T cells responded to the 40 to 46, 50 to 56, 70 to 90, 93 to 125, and greater than 200 kDa fractions, while the chronic-infection granuloma T cells responded only to the greater than 200 kDa fraction of SEA [52]. Depletion of the $CD4^+$ T-cell subset abrogated proliferation of acute-infection granuloma lymphocytes, whereas subset depletions of either $CD4^+$ or $CD8^+$ T cells from chronic-infection granuloma cells revealed that both subsets respond to > 200kDa fraction [52]. Interestingly, the 40, ~55, 75, 98, 101 and 104 kDa SEA immunogenic fractions were recorded in our hands by sera from ECA immunized mice, supporting the stimulation of both $CD4^+$ or $CD8^+$ T cells upon immunization with either the SEA or the ECA obtained by us.

In conclusion, we quantified the cross-reactive IgM and IgG in sera from mice immunized with *S. mansoni* antigens by ELISA against crude *E. coli* antigen and vice versa. We also characterized the molecular weights of the cross-reactive *E. coli* peptides with sera from mice immunized with antigens from different developmental stages of the parasite *S. mansoni*. We also characterized the molecular weights of the cross-reactive *S. mansoni* antigens with sera from mice immunized with crude *E. coli* antigen. Approximately similar molecular weights were previously reported by others either as immunogenic molecules or diagnostic markers and some were described to contain T-or B-cells proliferation epitobes and evaluated as vaccine candidates.

Both the ECA and the 3 *S. mansoni* antigens induced MLN and thymus $CD4^+$, $CD8^+$-T and B-cells, and the $CD4^+/CD8^+$ ratio was always higher in ECA immunized mice than in those immunized with the schistosomal antigens, suggesting that the immuno-stimulatory effect of ECA is stronger than the schistosome antigen. These results indicate that it is very important to include *E. coli* antigens as necessary controls in ELISA and Western blot analyses of sera from vaccination experiments with particular antigens to ensure that the measured immune response is specific. Furthermore, the detectable immune response in sera from the control mice against the ECA and the presence of a high degree of cross-reaction between the ECA and *S. mansoni* antigens add new criteria for selecting control mice which have to be both IgM and IgG negative in all serological tests.

Characterization of cross-reactive ECA antigens of protective potential against *S. mansoni* infection remains a future research objective. Future studies may help solve the problem of purifying antigens with protective potential from different parasite stages. While one needs enormous numbers of the particular parasite stage to end up with a couple of micrograms of pure antigen, non-pathogenic *E. coli*, can be easily grown *in vitro* and used as factories to produce large amounts of common protective antigens.

## Acknowledgment

This work was supported by a grant (3/5/6) awarded from the National Research Center of Egypt.

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**Corresponding Author**:
Mahmoud Mohamed Bahgat, Therapeutical Chemistry Department, Immunology and Infectious Diseases Laboratory, the Center of Excellence for Advanced Sciences, the National Research Center Dokki, Cairo 12311, Egypt
Email: mbahgatriad@yahoo.com
Fax: 00202-33370931
Tel: 0020183979699 or 00202-25766126

**Conflict of interest:** No conflict of interest is declared.