Study Of Il_1b, Il_4, Il_5 And Ige Before And After Mirazid Therapy In Children With Intestinal Schistosomiasis And Fascioliasis

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Abstract: This study was planned to evaluate the effectiveness of Myrrh in children with hepatic fascioliasis and intestinal schistosomiasis through study of clinical and parasitologic cures and assessment of serum total IgE and production of IL-1, IL-4 and IL-5 by peripheral blood mononuclear cells (PBMCs) before and after Mirazid therapy. The study included 21 children with fascioliasis, 8 children with Schistosoma mansoni infection in addition to 10 healthy children with matched age and sex as control. Diagnosis was based on the detection of Fasciola hepatica or Schistosoma Mansoni eggs in stool analysis. Patients were given the recommended dose of Mirazid. Clinical evaluation and stool analysis were done initially and at 2, 4, 12 weeks post treatment to evaluate cure. Rectal snip was done for responding schistosomiasis cases to confirm recovery. Total IgE was measured in serum by enzyme immunoassay. PBMNCs were separated and cultured for 48 hours and cytokines production in response to PHA stimulation was assessed in culture supernatants by solid phase sandwich enzyme-linked immunosorbent assay. Parasitologic cure was 90.9% in the fasciola group and 100% in the schistosoma group at 4weeks post treatment. After a second dose the fasciola patients who remained positive were also cured. Total IgE was significantly higher in both fasciola and schistosoma groups before treatment compared with control and decreased significantly with. IL-1β was higher in both patient groups than control and it did decrease significantly 12 weeks after therapy. Similarly, IL-5 was high before treatment in both groups and decreased significantly after 12 weeks of treatment. IL-4 on the other hand did not differ from control before therapy but it increased significantly after treatment in both fasciola and schistosoma group. Therefore, Mirazid is an effective fasciolicidal and schistosomicidal drug. IL-1 and IL-5 are high in fasciola and schistosoma infection and decrease with therapy that may denote a role in immunopathogenesis. Cytokines level but not total IgE may be taken as criteria of cure.
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Introduction

The parasitic trematode Fasciola hepatica is a liver fluke that infects a wide variety of mammal usually through ingestion of vegetations on which infective metacercariae have encysted. It is estimated to infect over 17 million people worldwide causing significant morbidity and mortality ***(Haseeb et al, 2003)***. Schsitosomiasis, a disease caused by another trematode parasite of the genus schistosoma affects over 200 million people worldwide and can lead to several chronic syndromes among which is intestinal schistosomiasis ***(Jiz et al., 2009)***. Most helminth infections of human and animals induce similar immune response which is characterized by production of type 2 cytokines, IgG1 and IgG2, IgE antibodies and eosinophil and mast cell activation ***(Hoffman et al., 2002)***. However, there is limited information on the immune responses to F.hepatica and schsitomiasis especially on the type of T cell induced with these parasites ***(O'Neill et al., 2000)***. In fascioliasis, despite infection with this parasite was accompanied with elevated IgE, eosinophils and Th2 immune responses***(Berquist et al., 2005)***, other studies detected depressed Th2 cytokines as IL-4***(Allam et al., 2000 and Mutapi et al., 2007)***. In schistosomiasis, granulomogenesis and fibrogenesis are found to be mediated by immunologic events involving Il-1, Il-4 and IL-3***(Jacobs and Marck 1998)***. IL-1 production in schistosomiasis patients was similar to normal control and blood samples taken from schistosome endemic areas showed inconsistent IL-4 production ***(Scott et al., 2000)***. Treatment of F.hepatica and schistosoma infections remains highly problematic. F.hepatica treatment requires high or multiple doses of drugs with frequent side effects. In schistosomiasis, praziquantil which has been in use for more than 20 years is faced with increasing resistance and serious side effects ***(Ismail et al., 1994)***. Recently, Myrrh (Mirazid), which is an oleo gum resin obtained from the stem of commiphor molmol (family Burseraceae), has been licensed for medical use in Egypt and several countries as a trematodical drug with high efficacy

and safety ***(Barakat et al., 2005)***. The aim of this work was to evaluate the effectiveness of Mirazid therapy in children with fascioliasis and schistosomiasis mansoni through assessment of clinical and parasitologic cure. Also, immunologic parameters reported to have a role in these invasive parasitic infections such as total IgE, IL-1β, IL-4 and IL-5 were assessed in relation to Mirazid therapy.

Subjects and Methods:

Subjects:

This work is an experimental study that was carried out in the Unit of Infectious Diseases and Malnutrition, Mansoura University Children's Hospital, Egypt. The study included 21 children with F.hepatica infection (8males and 13 females) with mean age±SD of 10.14±2.05 years, 8 children with intestinal schistosomiasis (6males and 2 females) with mean age of 11.37±1.99 years in addition to 10 healthy children with matched age and sex as control. Patients were recruited from rural health care units, Talkha Center, Dakahlia Governorate, Egypt and from patients attending the outpatient clinic of Mansoura University Children's Hospital. Diagnosis of patients was based on detection of Fasciola hepatica or Schistosoma mansoni egg in stool. Patients with fascioliasis were maintained on liver free diet for 1 week before the diagnosis was confirmed. Patients with immune deficiency or on immunosuppressive therapy or those receiving recent trematocidal drugs were excluded from the study.

Methods:

Patients were given the recommended doses of Mirazid therapy which are 10 mg/kg/day half an hour before breakfast for 3 consecutive days in schistosomiasis and 6 days for patients with fascioliasis. This dose was repeated for non responders after 4 weeks. All patients and controls were subjected to thorough history and clinical examination. Stool analysis was done by Kato Katz technique for quantitative assessment of egg excretion per gram stool ***(Katz et al., 1972)***. It was done initially and 2, 4 and 12 weeks after treatment to follow the effect of therapy. Rectal snip was done to children with schistosomiasis to confirm parasitologic cure. Routine investigations which included complete blood count with manual assessment of eosinophil count, erythrocyte sedimentation rate and abdominal ultrasound were done for all subjects and repeated for patients after 12 weeks of therapy. In addition, serum and 10 ml heparinized blood samples were withdrawn from all patients and controls under sterile conditions initially and from patients after 12 weeks of treatment for assessment of serum total IgE and vitro production of IL-1β, IL-4 and IL-5 by peripheral blood mononuclear cells (PBMCs). Total IgE was measured in serum by enzyme immunoassay (EIAgen Total IgE, Biochem Immunosystems, Italy). Heparinized blood samples were used for PBMCs separation and culture.

Peripheral Blood Mononuclear Cells (PBMCs) Culture:

PBMCs were separated by density gradient centrifugation on ficol hypaque (Biokit-Germany). Cells ($2x10^6$/ml) were cultured in PRMI 1460 (Hyclone-Utah) with 10% fetal calf serum (High Veld Biological-Kelvin 2054 RSA), 1% L-glutamine 200nM (Bioscience) and 2% penicillin/streptomycin 10,000 IU/ml (Life Technology-Scotland) and incubated in 5% CO2 at 37°C humidified atmosphere. After 48 hours of incubation with phytohaemaglutinin (PHA) (Biocheme-Berlin) at a dose of 25 µg/ml, culture supernatants were collected, centrifuged to remove cells and store in aliquots at -70°C till cytokine assay***(Boyum 1976)***.

Cytokine assay:

IL-1β, IL-4 and IL-5 were assessed in culture supernatants by solid phase sandwich enzyme-linked immunosorbent assay (ELISA) supplied by DIACLONE, France.

Principle: A monoclonal antibody specific for the assayed cytokine has been coated on to wells of the microtiter strips provided. Samples including standards are pipetted into these wells. During the first incubation, the cytokine antigen and mAb specific for this cytokine are simultaneously incubated. After washing, the enzyme is added. After incubation and washing to remove the unbound enzyme, a substrate is added to induce a coloured reaction product. The intensity of this colour is directly proportional to the concentration of tested cytokine.

Results:

Table (1) shows clinical data of studied patients. Abdominal pain and pallor were the most frequent manifestations. Therapy results in significant clinical improvement (table 2). Parasitologic cure was 100% in schistosomiasis patients by Kato after 4 weeks of therapy which was also confirmed by rectal snip at the end of the study. Parasitologic cure in children with fascioliasis was 90.9% at 4 weeks post-treatment where 2 children were still passing ova and they did receive a second dose so that by 12 weeks all cases were cured. Hemoglobin increased significantly after therapy in fascioliasis and schistosomiasis groups ($p=0.002$, 0.009 respectively). Absolute eosinophilia was detected in fasciola group but it decreased significantly after therapy ($p=0.023$) (table 3). Serum total IgE levels were significantly higher in both fascioliasis and schistosomiasis before treatment compared with control ($p<0.001$, 0.005 respectively). After treatment, these levels declined significantly in

both patient groups (p=0.001, 0.036). However, although total IgE level did not differ in schistosomiasis after treatment from control (p=0.08), it remained significantly higher in fasciola group than control (p<0.001) (table 4). In vitro IL-1β and IL-5 production by PBMCs were significantly higher in fasciola and schistosoma group than control before treatment and they decreased significantly after therapy to reach the control level after 3 months of therapy. IL-4, on the other hand did not differ significantly in both groups of patients before treatment from control (p=0.58, 0.79) but it showed significant rise after treatment (p=0.04, 0.012). In fascioliasis post treatment IL-4 was significantly higher than control (p=0.028) whereas in schistosomiasis it did not differ from the control (p=0.083) (table 5).

Table (1) clinical data of studied groups

Variable	Schistosomiasis (N=8)	Fascioliasis (N=21)
Age±SD (years)	11.37±1.99	10.14±2.05
Male/Female	6/2	8/13
Asymptomatic	3 (37.5%)	10 (47.6%)
Symptomatic	5 (62.5%)	11 (52.3%)
Abdominal pain	4 (50%)	7 (33.3%)
Diarrhea	2 (25%)	3 (14.28%)
Anorexia	0	1 (4.76%)
Fatigue	0	3 (14.28%)
Pallor	1 (12.5%)	10 (47.6%)
Hepatomegaly	0	1 (4.76%)
Egg count/gm stool Median (range)	72 (48 – 168)	60 (24 – 96)

Table (2) comparison of the frequency of clinical manifestations before and after treatment in studied groups (chi-square)

group	Before treatment	after treatment	significance
schistosomiasis (n=8)			
Asymptomatic	3	7	x2=4.5
Symptomatic	5	1	p=0.034*
Fascioliasis (n=21)			
Asymptomatic	10	18	x2=10.71
Symptomatic	11	3	p=0.001*

*p significant if <0.05

Table (3) comparison of CBC parameters in studied groups before and after 3 months of Mirazid therapy

Variable	before treatment	after treatment	significance
Hb gm/dl (mean±SD)			
Fasciola grp (n=21)	11.61±0.74	12.23±0.79	P=0.002*
Schistosoma grp (n=8)	12.12±0.94	13.05±0.91	P=0.009*
***WBCx10^3/mm^3 (median range)**			
Fasciola grp (n=21)	8.6 (5.2 – 11.7)	10 (4.8 – 10)	P=0.63
Schistosoma grp (n=8)	10.45 (6.3 – 11.2)	9.05 (6.3 – 10.1)	P=0.036*
Eosinophil count/mm^3 (median range)			
Fasciola grp (n=21)	520 (189 – 2013)	285 (80 – 1848)	P=0.023*
Schistosoma grp (n=8)	303 (102 – 1890)	242 (63 – 455)	P=0.16

*P is significant if <0.05 ** non parametric tests (median, range, Mann-Whitney)

Table (4) comparison of total IgE levels in fascioliasis and schistosomiasis groups (before and 3 months after Mirazid therapy) and control

variable	IgE before treatment	IgE after treatment	control IgE (n=10)	significance	
Fasciola grp (n=21) mean±SD	525.04±438.02	407.66±362.82	50.37±18.05	t_1=3.03 t_2=2.75 t_3=3.68	p_1<0.001* p_2<0.001* p_3=0.001*
**Schistosoma grp (n=8) Median (Rorge)	400 (56 – 1238)	153 (49 – 1109)	57.5 (40 – 80)	Z_1=2.73 Z_2=1.78 Z_3=2.1	P_1=0.005* P_2=0.083 P_3=0.036*

*P is significant if <0.05 **non parametric tests (median, rande, Mann-Whitney)

P_1: comparison of patients before treatment versus control P_2: comparison of patients after treatment versus control

P_3: comparison of patients before treatment versus patients after treatment

Table (5) comparison of in vitro IL-1, IL-4 and IL-5 production in children with fascioliasis, schistosomiasis(before and 3 months after Mirazid) and control (Mann-Whitney)

Variable group	before treatment	after treatment	control (n=10)	
IL-1β pg/ml (median range)				Z_1=3.32 P_1<0.001* Z_2=1.02 P_2=0.32 Z_3=3.82 P_3<0.001*
Fasciola grp (n=21)	110 (10.4 – 975.9)	13.09 (9.8 – 608)	10.6 (9.4 – 18.7)	
Schistosoma grp (n=8)	60.07 (11.92 – 1146.2)	11.25 (10.3 – 17.21)		Z_1=2.84 P_1=0.003* Z_2=0.59 P_2=0.064 Z_3=2.38 P_3=0.017*
IL-4 pg/ml (median range)				Z_1=0.55 P_1=0.58 Z_2=2.19 P_2=0.028* Z_3=2.7 P_3=0.04*
Fasciola grp (n=21)	1.05 (0.83 – 11.5)	4.12 (0.87 – 99.0)	4.12 (0.76 – 7.49)	
Schistosoma grp (n=8)	1.03 (0.75 – 2.28)	4.78 (1.05 – 43.7)		Z_1=0.75 P_1=0.79 Z_2=1.78 P_2=0.083 Z_3=2.24 P_3=0.02*
IL-5 pg/ml (median range)				Z_1=2.05 P_1=0.041* Z_2=0.68 P_2=0.51 Z_3=2.79 P_3=0.005*
Fasciola grp (n=21)	16.3 (4.75 – 80.18)	6.03 (4.59 – 33.6)	8.52 (2.55-18.07)	
Schistosoma grp (n=8)	16.32 (6.71 – 273.8)	7.26 (5.62 – 15.66)		Z_1=2.21 P_1=0.027* Z_2=0.52 P_2=0.57 Z_3=2.52 P_3=0.012*

P_1: comparison of patients before treatment versus control P_3: comparison of patients before treatment versus patients after treatment

P_2: comparison of patients after treatment versus control *P is significant if <0.05

Discussion:

Myrrh Arabian or Somalian is one of the oldest known medicines and was widely used by ancient Egyptians for purposes as well as for mummification. Myrrh contains a resin (23-40%), a volatile oil (2-8%), gum (40-60%) and a bitter principle. This plant extract was approved by FDA for food use ***(Ford et al., 1992)***. In this study, the parasitologic cure rate in fascioliasis was 90.9% at 4 weeks after Mirazid treatment and reached 100% after a second dose given for 2 cases with still positive stool. Patients with S.mansoni were completely cured after a single dose of the drug. Clinically, both patient groups showed a significant clinical improvement after therapy. The residual symptoms were just mild non specific abdominal pain that could be attributed to other causes. No side effects were encountered during the 12 weeks of study. Our results agree with previous studies showing high efficacy and safety of Mirazid therapy in fascioliasis with parasitologic cure rate of 92-100%. Similar results

were also obtained in schistosomiasis. ***Botros et al., 2005***, obtained a parasitologic cure rate of 96.4% in a schistosomiasis field study that compromise 364 cases. Other studies achieved a cure rate of 91.7% that increased to 98.9% after a second dose. Complete blood count was done to our patients before treatment to evaluate the impact of infection and after therapy to assess its effect. Hemoglobin, though not markedly decreased in studied patients, it showed a significant increase after treatment. The decline in hemoglobin in these parasitic infections may be explained by consumption of blood cells by the parasite, toxic suppression of bone marrow or pituitary or adrenal trophic hormones by the parasite products. Anorexia, malnutrition and blood losses may be other factors especially in schistosomiasis***(Ohnmacht et al., 2007)***. Eosinophilia is a well known haematologic clue to the presence of helminth infection although its absence does not exclude such infection. These cells were found to be potent effectors in parasite killing in vitro ***(Weller 2001)***. Absolute eosinophilia was found in children with fascioliasis in our study unlike schistosomiasis and it did decreases significantly after therapy. The improvement of haematologic derangement after Mirazid was also reported by other studies. Total IgE elevation has been documented in parasitic infections especially in invasive helminthes ***(Jiz et al., 2009)***. This finding was also documented in our study where serum total IgE levels were significantly higher in both patient groups than control. This high IgE level decreased significantly after therapy in both groups although it remained significantly higher than control value in fasciola group. High level of total IgE was also found in fascioliasis patients that decreased after triclabenzadole but did not reach the control level ***(Allam et al., 2000)***. In addition, IgE was higher in patients with schistosomiasis than normal individuals especially children and was more mixed S.mansoni and haematobium and this high level fell back to normal 4 month after therapy***(Khalil et al., 1995)***. Increased IgE level in parasitic infection appears to be protective. It was detected that increased IgE, eosinophil count were associated with resistance to re-infection with schistosomiasis ***(Wilson et al., 2007)***. Also, animal studies suggested a protective role for IgE in baboons infected with filarial parasites or S.mansoni. IL-1 and TNF were considered the primary mediators of cellular aggregations in schistosoma granuloma ***(Cham et al., 2008)***. IL-1β production by PBMCs assessed in our patients was higher than control and it decreases significantly after therapy reaching the control value. These results may be in contrast to ***Allam et al., 2000***, who detected depressed IL-1 production in fascioliasis. However, increased IL-1 and TNF alpha by murine peritoneal macrophages was documented in experimental schistosomiasis***(Seger et al., 1993)***. In addition, children and adolescents with S.haematobium cystitis had 56 fold greater TNF production which is another pro-inflammatory cytokine with overlapping activity with IL-1***(King et al., 2001)***. Experimentally in schistosoma-infected mice, IL-1 production increased 8 weeks after infection and was normalized after 12 weeks ***(Moreels et al., 2001)***. These experimental studies may explain the heterogeneity in IL-1 results in this parasitic infection. The time of infection, despite it can be easily controlled in experimental studies, it is very difficult in natural human infections due to immunity of exposure. IL-4 mediates important pro-inflammatory functions including induction of IgE isotype switch, promotion of eosinophil transmigration and differentiation of Th2 lymphocytes ***(Jiz et al., 2008)***. Although IL-4 production in our study was insignificantly lower than the control level, it did increase significantly after therapy in both patient groups and even become more than control value in fascioliasis denoting a state of depression during infection. These finding are similar to those found in fascioliasis before and after triclabendazole ***(Allam et al., 2000)***. Also, T cell proliferation and cytokine production as IL-4 were elevated with treatment after initial depression in schistosomiasis. In contrast, experimental fascioliasis in rats showed increased frequencies of cells producing IL-4 and IL-10 two weeks after infection ***(Tliba et al., 2002)***. Similar findings were also detected in cattle's infected with F.hepatica. Whether IL-4 is elevated or depressed it may depend on the acuteness or chronicity of infection. This was clarified by ***Waldvogel et al., 2004***, who detected increased IL-4 levels in claves with acute fascioliasis that decreased later on when the infection became chronic. The role of IL-4 in parasitic infection has been studied previously. It appears to be protective as it regulates granuloma formation as well as lymph node maturation events in experimental schistosomiasis mansoni ***(Zhang & Mutapi 2006)***. The protective role of IL-4 was also documented in IL-4-/-mice infected with S.mansoni which developed a Th1 response and an acute lethal syndrome ***(Patton et al., 2002)***. On the other hand, IL-4 and TGF-β were risk factors for periportal fibrosis in S.mansoni-infected baboons. Thus, while IL-4 can permit survival in the phase of continuing helminth infection, it is implicated in the immunopathology during such infections. So, the depressed IL-4 in our patients may be an immune evasion mechanism by the parasites which have different immune evasion strategies**,** or it may be a host immune regulatory mechanism to minimize the squeals of the immune reaction. IL-5 is a cytokine primarily involved in the pathogenesis of atopic and parasitic disease. It specifically controls production activation and localization of eosinophils. In our study, IL-5 was high in patients with fascioliasis and schistosomiasis

and decreased significantly to normal values after Mirazid therapy. High levels of IL-5 were also detected in other studies on chronic schistosomiasis***(Reiman et al., 2006)***. Also, IL-5 was produced by spleen cells from fasciola-infected mice ***(Magalhaes et al., 2009)***. The increased level of IL-5 in parasitized patients is protective. This was evident in schistosomiasis where resistant subjects produced higher IL-5***(Medhat et al., 1998)***. In this study, there was dissociation between IL-4 and IL-5 production which are both Th2 cytokines so that IL-5 was high unlike IL-4 which was relatively depressed. The same finding was also detected in children infected with S.haematobium where in vitro PBMC culture produced either IL-4 or IL-5 but rarely both***(Scott et al., 2000)***. In addition, the fasciola-infected IL-4-/-mice produced higher IL-5 than wild type mice ***(Mutapi et al., 2007)***. These results denote that both IL-4 and IL-5 are not necessarily coproduced. It can be concluded that Mirazid is an effective and safe fasciolocidal and schistosomicidal drug at clinical, parasitologic and immunologic levels. IL-1β and IL-5 are elevated in Fasciola hepatica and Schistosoma mansoni infection and decline with therapy suggesting a role in the immunopathogenesis of these disease. IL-4 production is depressed that may be an immune evasion mechanism by the parasite or a host regulatory mechanism. Normal levels of IL-1, IL-4 and IL-5 production rather than total IgE may be taken as adjuvant criteria of cure. We recommend the wide use of Mirazid in treatment of F.hepatica infection and S.mansoni infection. Prospective immune trials to study the effect of Th2 and IL-4 enhancement on the course of fasciola and schistosoma infection.

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References:

1. **Allam AF, Osman MM, El Sayed MH, et al:** IL-1, IL-4 production and IgE levels in acute and chronic fascioliasis before and after trilcabendazole treatment. *J Egypt Soc Parasitol,2000.30(3): 781-90*
2. **Barakat R, El-Morshedy H and Fenwick A:** Efficacy of myrrh in the treatment of human schistosomiasis mansoni. *Am J Trop Med Hyg,2005.73(2): 365-367*
3. **Berquist NR, Leonardo LR, Mitchell GF:** Vaccine-linked chemotherapy: can schistosomiasis control benefit from integrated approach? *Trends Parasitol,2005.21: 112-117*
4. **Botros S, Sayed H, El-Dusoki H, Sabry H, Rabie I, et al:** Efficacy of mirazid in comparison with pranziquantel in Egyptian Schistosoma mansoni-infected school children and households. *Am J Trop Med Hyg,2005.72(2): 119-123*
5. **Boyum A:** Isolation of lymphocytes granulocytes and macrophages. *Scand J Immunol,1976.5 suppl, 5*
6. **Cham GK, Kurtis J, Lusingu J, Theander TG, Jensen AT and Turner L:** A semi-sutomated multiplex high-throughput assay for measuring IgG antibodies against Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) domains in small volumes of plasma. *Malar J,2008.7: 108*
7. **Ford RA, Api AM, Letizia CS:** Monographs on fragrance raw material. *Food Chem Toxicol,1992.30 (suppl):1s-138s*
8. **Haseeb AN, El Shazly AE, Arafa MA et al:** Evaluation of secretory/excretory fasciola antigen in diagnosis of human fascioliasis. *J Egypt Soc Parasitol,2003.33(1): 123-138*
9. **Hoffman KF, Wynn TA, Dunne DW:** Cytokine-mediated responses during schistosome infections; walking the fine line between immunological control and immunopathology. *Adv Parasitology,2002.52:265-307*
10. **Ismail MM, Taha SA, Farghaly AM et al:** Laboratory-induced resistance to pranziquantil in experimental schistosomiasis. *J Egypt Soc Parasitol,1994.24(3): 685-95*
11. **Jacobs W and van Marck E:** Adhesion and costimulatory molecules in the pathogenesis of hepatic and intestinal schistosoma mansoni. Mem Inst Oswaldp Crus **,***1998.93(4): 523-9*
12. **Jiz M, Wu HW, Meng R, Pond-Tor S, Reynolds M, Friedman JF, Olveda R, Acosta L and Kurtis JD:** Pilot-scale production and characterization of paramyosin, a vaccine candidate for schistosomiasis japonica. *Infect Immun.2008.76: 3164-3169*
13. **Jiz M, Friedman J, Leenstra T, Jarilla B, et al:** Immunoglobulin E (IgE) responses to paramyosin predict resistance to reinfection with Schistosoma japonicum and are attenuated by IgG4. *Infection and Immunity,2009. 2051-2058*
14. **Katz N, Chaves A, Pellegrino J:** A simple device for quantitative stool thick-smear technique in S.mansoni. *Rev Inst Med Trop Sao Paulo,1972.14-397*
15. **Khalil HM, Sadek MS, El-Baki MH et al:** Detection of cellular and humoral markers (IL-2-IFN gamma and IgE) in sera of patients with urinary schistosomiasis. *J Egypt Soc Parasitol,1995.25(2):513-24*

16. **King CL, Malhotra I, Mungai P et al (2001):** Schistosoma haematobium-induced urinary tract morbidity correlates with increased TNF alpha and diminished IL-10 production. *J Infect Dis, 184(9): 1176-82*
17. **Magalhaẽs ES, Paiva CN, Souza HSP, Pyrrho AS, Mourao-Sa D et al:** Macrophages migration inhibitory factor is critical to interleukin-5-driven eosinophilopoiesis and tissue eosinophilia triggered by schistosoma mansoni infection. *FASEB J, 2009.23: 1262-1271*
18. **Medhat A, Shehata M, Bucci K et al:** Increased IL-4 and IL-5 production in response to S.haematobium adult worm antigens correlates with lack of reinfection after treatment. *J Infect Dis,1998.178(2):512-9*
19. **Moreels TG, De Man JG, Bogers JJ et al:** Effect of S.mansoni-induced granulomatous inflammation of GI motitlity. *Am J Physiol Gastrointest Liver Physiol,2001.280(5):G1030-42*
20. **Mutapi F, Winborn G, Midzi N, Taylor M, Mduluza T and Maizels RM:** Cytokine responses to Schistosoma haematobium in Zimbabwean population: contrasting profiles for IFN-γ, IL-4, IL-5 and IL-10 with age. *BMC infectious Disease,2007.7: 139*
21. **Ohnmacht C, Pullner A, van Rooijen N and Voihringer D:** Analysis of eosinophil turnover in vivo reveals their active recruitment to and prolonged survival in peritoneal cavity. *J Immunol.2007.179: 4766-4774*
22. **Patton EA, La Flamme AC, Pedras-Vasoncelos JA et al:** Central role for IL-4 in regulating NO-mediated inhibition of T cell proliferation and IFN gamma production in schistosomiasis. *Infect Immun,2002.70(1): 177-84*
23. **Reiman RM, Thompson RW, Feng CG, Hari D, Knight R Cheever AW, Rosenberg HF and Wynn TA:** Interleukin-5 (IL-5) augments the progression of liver fibrosis by regulating IL-13 activity. *Infection and Immunity,2006.74(3): 1471-1479*
24. **Scott M, Turner CM, Mutapi F et al:** Dissociation of IL-4 and IL-5 production following treatment for S.haematobium infection in humans. *Parasite Immunol,2000.22(7):341-8*
25. **Seger M, Gold D, Lengy J et al:** The schistosomulicidal activity and the production of IL-1 and TNF alpha by peritoneal macrophages from infected mice. *Parasite Immunol,1993.15(6): 339-47*
26. **Tliba O, Sibille P, Boulard C:** Early hepatic cytokine mRNA expression in experimental rat fascioliasis. *Vet Parasitol, 2002.103(3):237-49*
27. **Waldvogel AS, Lepage MF, Zakher A et al:** Expression of IL-4, IL-4 splices variants and IFN gamma mRNA in calves experimentally infected with F.hepatica. *Vet Immunol Immunopathol,2004.97(1-2): 53-63*
28. **Weller PE:** Eosinophilia, In: Rich RRm Fleisher TA, Shearer WT, Kotzin BL, Schroeder HW (eds), *Clinical Immunology Principles and Practice,2001.2nd ed, Vol 2, England, 56*
29. **Wilson MS, Mentink-Kane MM, Pesce JT, Ramalingam TR, Thompson R and Wynn TA:** Immunopathology of Schistosomiasis. *Immunol. Cell Biolo.,2007.85: 148-154*
30. **Zhang P and Mutapi F:** IgE: a key antibody in schistosoma infection. *Electronic Journal of Biology,2006.vol. 2(1): 11-14*

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