EVALUATION OF IMMUNE RESPONDERS IN HUMAN SCHISTOSOMIASIS

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By

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List of abbreviations

aa	:	**a**mino **a**cid
Ab	:	**A**nti**b**ody
Ag	:	**A**nti**g**en
BFA	:	**B**re**f**eldin **A**
BSA	:	**B**ovine **S**erum **A**lbumin
CD_4^+	:	**C**luster of **D**ifferentiation **4**
CD_8^+	:	**C**luster of **D**ifferentiation **8**
cpm	:	**C**ounts **P**er **M**inute
DNA	:	**D**eoxyribo**n**ucleic **A**cid
ELISA	:	**E**nzyme **L**inked **I**mmuno**s**orbent **A**ssay
ERDC	:	**E**gyptian **R**eference **D**iagnostic **C**enter
Fc	:	**F**ragment **C**rystallizable
FITC	:	**F**luorescein **I**so**t**hio**c**ynate
FSC	:	**F**orward **Sc**atter
HCl	:	**H**y**d**rochloric **A**cid
hr	:	**H**ou**r**
IFN-γ	:	**I**nter**f**ero**n**- γ
Ig	:	**I**mmuno**g**lobulin
IL-10	:	**I**nter**l**eukin-10
IL-2	:	**I**nter**l**eukin-2
IL-4	:	**I**nter**l**eukin-4
IL-5	:	**I**nter**l**eukin-5
kDa	:	**K**ilo **Da**lton
min	:	**Min**ute
mL	:	**M**illi**l**iter
MoAb	:	**Mo**noclonal **A**nti**b**odies
mRNA	:	**m**essenger **R**ibo**n**ucleic **A**cid
NK	:	**N**atural **K**iller
nm	:	**N**ano**m**eter
NP	:	**N**ative **P**rotein
N-Para	:	**N**ative **Para**myosin
O/N	:	**O**ver**n**ight

List of Abbreviations

OD	:	**O**ptical **D**ensity
p.i.	:	**P**ost-**I**nfection
Para	:	**Para**myosin
PBMCs	:	**P**eripheral **B**lood **M**ononuclear **C**ell**s**
PBS	:	**P**hosphate **B**uffered **S**aline
PCR	:	**P**olymerase **C**hain **R**eaction
PE	:	**P**hyco**e**rythrin
PerCp	:	**Per**idin **c**hlorophyll **P**rotein
pg	:	**P**ico**g**ram
PHA	:	**P**hyto**h**em**a**gglutinin
PI	:	**P**ropidium **I**odide
PMTs	:	**P**hoto**m**ultiplier **T**ube**s**
R	:	**R**esistance
RP	:	**R**ecombinant **P**rotein
R-Para	:	**R**ecombinant **Para**myosin
rpm	:	**R**ound **P**er **M**inute
S	:	**S**usceptible
SAWA	:	**S**oluble **A**dult **W**orm **A**ntigen
SD	:	**S**tandard **D**eviation
SDS-PAGE	:	**S**odium **D**odecyl **S**ulphate **P**oly**a**crylamide **G**el **E**lectrophoresis
SWAP	:	**S**oluble **W**orm **A**ntigen **P**reparation
t	:	**T**ime
TcR	:	**T c**ell **r**eceptor
Th0	:	**T h**elper 0
Th1	:	**T h**elper 1
Th2	:	**T h**elper 2
UV	:	**U**ltra**v**iolet
WBC	:	**W**hite **B**lood **C**ells
WHO	:	**W**orld **H**ealth **O**rganization
wks	:	**W**eeks

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ABSTRACT

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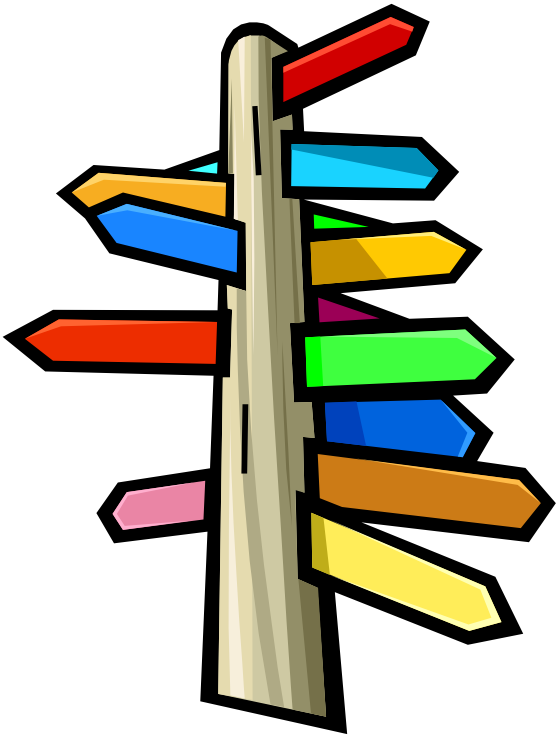
Cellular immune responses to *Schistosoma mansoni* recombinant and native antigens were investigated in a defined study population of 64 individuals in Egypt, where data collected from a 2-year observation of exposure, infection and reinfection pattern were used to categorically classify putative resistant (R) and susceptible (S) individuals. Using peripheral blood mononuclear cells (PBMCs) of individuals enrolled in the study, *in vitro* lymphocyte proliferation and cytokine (IL-5, IL-10 and IFN-γ) production in response to defined recombinant and native paramyosin antigens (R-Para and N-Para, respectively) and native soluble adult worm antigen preparation (SAWA) were measured. Cellular response to the R-Para and SAWA Ag suggest that the Th1 type of response appears to be important in predicting resistance in this population. Of the recombinant Ag tested, R-Para induced significant levels of IFN-γ. The production of IL-10, a Th2-type cytokine was strongly implicated in immune regulation. Of importance was the evidence found for SAWA and R-Para induced IFN-γ responses in predicting resistance. The association was significant even after the effect of infection which was accounted for in a multivariate analysis. This implies that other than acquired immunity to infection, some immunological state-dependent host factors may also play an important role in the overall changes of reinfection patterns seen in *Schistosomiasis mansoni* in this Egyptian population.

Keywords: *Schistosoma mansoni*; Schistosomiasis; "Resistant" and "Susceptible" individuals; Cellular immune responses; Peripheral blood mononuclear cells; Cell proliferation; Cytokines; IL-5; IL-10; IFN-γ; Recombinant and native paramyosin.

INTRODUCTION AND AIM OF THE WORK



Introduction and Aim of the Work

Schistosomiasis, also known as bilharzia, after Theodor Bilharz who first identified the parasite in Egypt in 1851, is caused by parasitic trematode worms (flukes) of the genus *Schistosoma* **(Hagan and Sharaf, 2003)**. Schistosomiasis is an important parasitic disease that affects more than 200 million people worldwide causing more than 250 000 deaths per year **(van der Werf *et al.*, 2003)**. The pathology characteristic of this disease is a granulomatous reaction around parasite eggs within the liver and other organ **(Boros, 1989)**. Currently, schistosomiasis control strategy is mainly based on the treatment of infected individuals by chemotherapy with safe and effective drugs **(Harder, 2002)**. In spite of decades of chemotherapy, the number of infected people remains almost the same **(Bergquist, 1995)**. Large extension of endemic areas and constant reinfection of individuals together with poor sanitary conditions in tropical countries requires other controlling strategies besides drug treatment **(Bergquist, 1998)**. Therefore, vaccination as a way to control schistosomiasis would contribute enormously to disease eradication. In the case of schistosomiasis, a sterilizing vaccine, although desirable, is not essential. Since schistosomes do not multiply within the final host, a vaccine that induces even a partial reduction in worm burdens could considerably reduce pathology, limit parasite transmission and be less expensive than repetitive drug treatment **(Chitsulo *et al.*, 2004)**. The best long-term strategy to control

schistosomiasis is thought to be the immunization with an antischistosomiasis vaccine **(Bergquist, 2002)**. Many world health agencies agree that the development of an antischistosomiasis vaccine should be sought. Several studies are in progress in this field, testing different antigens (Ag) of the parasite and different vaccination strategy **(Chitsulo *et al*., 2004)**.

Six schistosome Ags have been selected by the World Health Organization (WHO) as candidates to compose a subunit vaccine against schistosomiasis **(Bergquist, 1995)**. One of these Ags is paramyosin, a filamentous, α-helical, coiled-coil protein of approximately 100 kDa that is present in the muscle of invertebrates, including *Schistosoma mansoni* **(Bergquist, 1995; Pearce *et al*., 1988)**. Paramyosin was identified as the major Ag recognized by sera of mice vaccinated intradermally with an *S. mansoni* adult parasite extract **(Lanar *et al*., 1986)**. Purified paramyosin used in mice immunization conferred significant resistance against challenge infection **(Bergquist, 1995; Pearce *et al*., 1988)**. The resistance induced by immunization involved interferon (IFN)-γ production by stimulated T cells, suggesting that during challenge of vaccinated hosts paramyosin may elicit a protective T cell response as a result of its release from migrating parasite larvae **(Bergquist, 1995)**. It thus appears that both

cellular and humoral immune responses are important mechanisms in the protective immunity induced by paramyosin immunization.

Studies of human immune responses to paramyosin support the potential of this protein as a vaccine candidate. *S. mansoni* paramyosin is strongly recognized by sera of individuals naturally resistant to infection and it also induced high levels of IFN-γ production by peripheral blood mononuclear cells (PBMC) from these individuals **(Ribeiro de Jesus *et al.*, 2000; Correa-Oliveira *et al.*, 1989)**. Paramyosin also induced significant lymphocyte proliferation and interleukin (IL-2) and IL-5 production in individuals resistant to *S. mansoni* reinfection **(Al-Sherbiny *et al.*, 2003)**. These results suggest the involvement of paramyosin in natural resistance and in resistance to reinfection against *Schistosoma mansoni*.

The identification of immunodominant epitopes within vaccine candidate Ags is extremely important, not only because it makes immunization possible, excluding cross reactivity and the accompanying risk of autoimmunity or allergy, but also because it allows the construction of vaccines with relevant peptides from different candidate Ags, improving the chance of achieving high levels of protection.