STUDIES ON THE IMMUNOLOGICAL ROLE OF HAEMOCYTES IN HOST PARASITE RELATIONSHIPS DURING *SCHISTOSOMA MANSONI* INFECTION, IN EGYPT

Thesis

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LIST OF ABBREVIATIONS

A	Amebocytes
AB	Apoptotic bodies
ADC	Arginine decarboxylase
AG	Aminoguanidine
B. alexandrina	*Biomphalaria alexandrina*
B. glabrata	*Biomphalaria glabrata*
B. tenagophila	*Biomphalaria tenagophila*
B. straminea	*Biomphalaria straminea*
BH_4	Tetrahydrobiopterin
C	Cytoplasm
cNOS	Constitutive Nitric oxide synthases
EDRF	Endothelium-derived relaxing factor
eNOS	Endothelial Nitric oxide synthases
ERK	Extracellular signal-regulated kinase
ESPs	Excretory-secretory products
FAD	Flavin adenine dinucleotide
FREPS	Fibrinogen-Related Proteins
FSC	Forward scatter
G	Granulocytes
H	Hyalinocytes
H_2O_2	Hydrogen peroxide
HC	Hypocellularity
HOCI	Hypochlorous acid

IDS	Internal defense system
iNOS	Inducible Nitric oxide synthase
L-NAME	NG-nitro-L-arginine methyl ester
L-NMMA	NG-monomethyl-L-arginine
L-NNA	NG-nitro-L-arginine
LOHA	L-hydroxyarginine
M	Mitochondria
MMP	Mitochondrial membrane potential
NADPH	Nicotinamide adenine dinucleotide phosphate
NO	Nitric oxide
NOS	Nitric oxide synthase
ODC	Ornithine decarboxylase
ONOO	Peroxynitrite
PDGF	Platelet-derived growth factor
PI	Propidium Iodide
PKC	Protein kinase C
PL	Phagolysosomes
PP	Pseudopodia
PRRs	Pattern recognition receptors
PS	Phosphatidyl serine
ROS	Reactive oxygen species
S. mansoni	*Schistosoma mansoni*
SDS	Sodium dodecideyle sulphate
SNO	S-nitrosothiols
SSC	Side scatter

TGF	Transforming growth factor
WHO	World Health Organization

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INTRODUCTION

Schistosomiasis is an important health problem that affects over 200 million people worldwide. Among the schistosomes species that infect human beings; *Schistosoma mansoni* is transmitted by *Biomphalaria alexandrina* snails and causes intestinal and hepatic schistosomiasis in Africa, Arabian Peninsula, and South America **(Gryseels *et al*., 2006)**.

The pathogenic trematodes that cause schistosomiasis, is endemic over 70 tropical and subtropical countries **(Hotez *et al*., 2008 and Montresor *et al*., 2012)**. *B. alexandrina* is the common aquatic snails that serve as an intermediate host for *S. mansoni* in Egypt. The fates of *Schistosoma* miracidia that penetrate different species of *Biomphalaria* vary from destruction within hours to productive infections that yield human-infective cercariae several weeks later. To understand the mechanisms used by the resistant snails to destroy the parasites, or those used by the parasites to ensure their survival in their suitable hosts, one must comprehend the internal defense mechanisms that are available to the host **(Bayne, 2009).**

The internal defense system (IDS) is one of the factors that influence the susceptibility pattern of the snails. This system is stimulated by the excretory/ secretory products of the penetrating miracidia **(Zahoor *et al*., 2010)**.

The innate immune system represents an ancient first line of defence against a variety of invading organisms **(Hoffmann *et al*., 1999)**.

In mollusks, innate immunity comprises humoral and cellular elements, with the cellular component considered to be more important for defense cells called haemocytes, which are functionally analogous to mammalian monocytes and macrophages (**van der Knaap *et al.*, 1992)**.

Molluscan haemocytes can recognize and subsequently eliminate, or sequester, invading pathogens through processes that include phagocytosis, encapsulation and the production of lysosomal enzymes and bacteriostatic substances (**Yoshino and Vasta, 1996).** The haemocytes may be circulating in haemolymph or fixed in tissues (**Da Silva and Villalba, 2004)**.

Apoptosis is important for the functioning of the molluscan immune system as indicated by the high baseline apoptosis rates observed in circulating and resident haemocytes. Apoptosis, also, plays a role in host protection against parasites by limiting the spread of the pathogen while preventing inflammatory damage of surrounding tissues **(Böttger *et al.*, 2008)**. In molluscs, interaction between immune cells and parasites or pathogens usually triggers apoptosis; however, some pathogens (especially obligatory intracellular parasites that depend on the host cell for their survival and proliferation) can inhibit this response and prevent host cell death (**Terahara and Takahashi, 2008**).

Nitric oxide (NO) is an important signaling molecule that acts in many tissues to regulate a diverse range of physiological processes including vasodilation, neuronal function, inflammation and immune function and has now been demonstrated to play a role in a variety of biological processes including

neurotransmission, immune defence, the regulation of cell death (apoptosis) and cell motility **(Bruckdorfer, 2005)**.

Electron microscopy has proved to be a successful tool for studying of haemocyte in *B. alexandrina* snails against the exposure to *S. mansoni* miracidia. Classification of molluscan haemocytes commonly has been based upon morphological and biochemical features analysed by light and electron microscopy **(Adamowicz and Bolaczek, 2003)**.

Flow cytometry (FCM) is a powerful tool that allows analysis of thousands of cells in a few seconds, at the single cell level. In the last 15 years, researchers have used FCM to investigate the cellular machinery of invertebrates. Analyses have focused on functions linked to innate immunity **(Donaghy *et al.*, 2009)**.

AIM OF THE WORK

The present study was designed to characterize haemocyte subsets of *Biomphalaria alexandrina* snails in order to detect their immunological role during *Schistosoma mansoni* infection in Egypt, by the following steps:

1- Characterization and identification of cell shape of haemocytes after infection

2- Ultrastructural examination of haemocytes by electron microscopy

3- Assess of cellular responses of susceptible and resistant snails to schistosomal infection

REVIEW OF LITERATURE

SCHISTOSOMIASIS

Schistosomiasis, a parasitic disease caused by a trematode of the genus *Schistosoma,* is pointed out as one of the most serious and outstanding public health problems worldwide, with 200 million infected individuals and 600 million living in risk areas in 76 countries, mainly in tropical and subtropical regions **(WHO, 2008)**.

B. alexandrina snails are the most common freshwater intermediate host snail prevailing in developing countries and have been recognized as the intermediate host for *S. mansoni,* which responsible for wide spread schistosomiasis infections of *S. mansoni* in Egypt and are considered to be potential hosts of the schistosome parasite **(El-Khoby *et al.*, 2000)**.

Schistosoma mansoni infection in *Biomphalaria alexandrina* snails

S. mansoni infects *B. alexandrina* snails by means of active penetration of the parasite´s ciliated larvae; miracidia, at any site of the snail's exposed parts, frequently the base of the antennae and cephalopodal mass. In the process of penetration, the parasite undergoes morphological and physiological changes, being transformed into primary sporocyst that remains in the fibro-muscular tissue of the host´s cephalopodal region near the penetration site **(Pan, 1965)**.

Pereira *et al.* (1984) reported that, the primary sporocysts generate the secondary ones, which migrate from the cephalopodal musculature to the digestive glands or hepatopancreas of the mollusc, where they undergo profound anatomic

changes and their germinative cells can generate the cercariae. **Mohamed *et al.* (2011)** stated typical capsules were observed 72 hour post miracidial exposure and a number of up to four layers of accumulating haemocytes surrounded the mother sporocysts.

Molluscan Immunity

1. Molluscan Immune System

The immune system of molluscs, as all other invertebrates, consists only of innate immunity and is lacking the adaptive immunity components. While the innate immune system is often regarded as an evolutionary more ancient and hence more primitive form of immunity than the adaptive responses seen in vertebrates, it is actually a surprisingly complex and efficient form of protection against many parasites and pathogens encountered by mollusks.

External barriers (such as shells, mucus and epithelia) are the first line of defense against pathogens and parasites in mollusks, when these barriers are breached, the second, internal line of defense involving cellular and soluble (humoral) haemlymph components come into play **(Sokolova, 2009)**.

2. Internal defense system of the Mollusk

The internal defense system (IDS) of snails is composed of cellular elements; haemocytes and soluble factors present in haemlymph. The haemocytes may be