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Specific inhibitors of mitogen-activated protein kinase and PI3-K pathways impair immune responses by hemocytes of trematode intermediate host snails

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Abstract

To characterize molecular mechanisms regulating snail cellular immune responses, the contributions of mitogen-activated protein kinases (MAPKs) and phosphatidylinositol 3-kinase (PI3-K) were examined in hemocytes of the trematode intermediate host snails *Biomphalaria glabrata* and *Lymnaea stagnalis*. Simultaneous measurement of phagocytosis/encapsulation and H_2O_2 production by hemocytes in the presence or absence of specific signal transduction inhibitors was used to assess the role of extracellular-signal regulated kinases 1 and 2 (ERK1/2), p38, JNK and PI3-K. Hemocyte spreading was significantly reduced in a dose-dependent manner by the ERK inhibitor, PD098059, and by wortmannin, a potent PI3-K inhibitor. The JNK inhibitor, SP600125, and the p38 kinase inhibitor, SB203580, had no effect on hemocyte spreading. Sheep red blood cell phagocytosis was significantly impaired by PD098059, SP600125, and SB203580. Hydrogen peroxide production during phagocytosis was severely inhibited by PD098059. Additionally, PD098059, but not the other inhibitors, significantly impaired the cellular encapsulation of trematode larvae and H_2O_2 production during encapsulation. These results suggest that MAPK and PI3-K signal transduction pathways play a pivotal role in the immune responses of snail hemocytes. PI3-K and ERK appear to strongly regulate cell motility. ERK, JNK and p38 contribute to phagocytosis-mediated signal transduction. ERK also play a major role in oxidative burst activation and the encapsulation of trematode larvae by snail hemocytes.

Keywords: *Lymnaea stagnalis*; *Biomphalaria glabrata*; Hemocyte; Extracellular regulated kinase (ERK); c-jun N-terminal kinase (JNK); p38 kinase (p38); Phosphatidylinositol 3-kinase (PI3-K); Phagocytosis; Encapsulation; Signal transduction

1. Introduction

The life cycle of trematode parasites requires a molluscan intermediate host. The outcome of parasite exposure depends on the genetic specificity of the host–parasite interaction, induction of host defenses and parasite immune evasion strategies. Circulating blood cells, hemocytes, are the principal effectors of the host defense as they

Abbreviations: MAPK, mitogen-activated protein kinase; ERK, extracellular-signal regulated kinase; JNK, c-jun N-terminal kinase; PI3-K, phosphatidylinositol 3-kinase; CBSS, Chernin's balanced salt solution; SRBC, sheep red blood cells; PD, PD98059; SB, SB203580; SP, SP600125; wo, wortmannin; BgS, *Biomphalaria glabrata*: *Schistosoma mansoni*-susceptible strain; BgR, *Biomphalaria glabrata*: *Schistosoma mansoni*-resistant strain; Ly, *Lymnaea stagnalis*

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phagocytose small target particles or encapsulate larger ones.

Phagocytosis is a membrane-directed process driven by the actin cytoskeleton of the host cell. Internalization of the target requires phagosome formation by invagination of the hemocyte plasma membrane. Actin filaments are assembled from actin monomers and oligomers localized underneath the plasma membrane at sites where contact is made with the phagocytosed particle. This step is controlled by various small GTPases of the Rho/Rac family [1,2]. Phosphatidylinositol 3-kinase (PI3-K) is also recruited to the plasma membrane where it plays a role in pseudopod extension and phagosome formation [3]. Most phagocytosed particles are killed in the phagolysosome by lysosomal enzymes and toxic oxygen metabolites produced during the oxidative burst. Upon attack, reactive oxygen species (ROS) can induce killing in active effector cells or serve as a signal in activating defense responses in distant cells. Thus, ROS act both as direct antipathogenic agents as well as potential participants in signal transduction pathways.

Activation of oxidative burst enzymes and subsequent release of ROS have been implicated in the mechanisms of parasite encapsulation and killing by snail hemocytes [4]. Encapsulation is a process in which hemocytes surround larger invaders in multiple cell layers. For encapsulation to occur, hemocytes must first migrate and attach to the invader, then spread and adhere to each other [5,6]. Inter- and intracellular signaling is necessary for the coordination of hemocytes during all steps of the immune processes: cell migration, adhesion and spreading, cytoskeleton remodeling, phagocytosis, encapsulation and the final toxic response.

At present, little is known of the signal transduction pathways that govern activation of invertebrate hemocytes. This is in contrast to mammalian phagocytes, where studies have suggested the participation of phospholipases, protein kinase C, PI3-K and tyrosine kinase-dependent pathways leading to mitogen-activated protein kinase (MAPK) stimulation [7,8]. MAPKs are a family of serine/threonine kinases with a two-lobed structure that mediate signal transduction for cellular processes, including stress, cell cycle, and growth control in all eukaryotes. They perform their functions as part of protein kinase modules, which in addition to other components are composed of MAPKs, dual-specificity protein kinase MAPK kinases (MAPKKs), and MAPKK kinases (MAPKKKs) [9]. At present, the three most characterized MAPKs families are the extracellular regulated kinases 1 and 2 (ERK1/2 or p42/p44), the c-jun N-terminal kinases 46 and 54 (JNK46/JNK54) and the p38 kinases.

Previous gene expression profiling analyses of hemocytes from uninfected and *Schistosoma mansoni* infected *Biomphalaria glabrata* showed differential expression of several MAPK-transcripts (Zelck et al. unpublished results). The aim of the current investigation was to follow-up these findings and to delineate signal transduction pathways induced by specific agents stimulating immune responses by snail hemocytes. Phagocytosis or encapsulation by snail hemocytes leads to oxidative burst activation and target killing, and possibly also to oxidative stress in the effector cells themselves. Our model system comprises two strains of the schistosome intermediate host snail, *B. glabrata*: susceptible snails in which the parasite develops successfully and resistant snails in which the immune system actively responds to the invading parasite resulting in encapsulation and killing. Additionally, the second snail, *Lymnaea stagnalis* (Ly), represents the intermediate host of *Trichobilharzia* spp., which cause swimmer's itch in humans. These snails are susceptible to *Trichobilharzia* spp. but resistant to *S. mansoni*, which is successfully encapsulated and killed by lymnaean hemocytes. Using sheep red blood cells (SRBCs) or *S. mansoni* sporocysts to induce immune responses by snail hemocytes, we demonstrate that MAPK and PI3-K signal transduction pathways play a pivotal role in these processes. PI3-K and ERK appear to strongly regulate cell motility. ERK, JNK and p38 contribute to phagocytosis-mediated signal transduction while ERK signaling also contributes to oxidative burst activation and to the encapsulation of trematodes.

2. Material and methods

2.1. Snails

B. glabrata hemolymph of resistant (13-16-R1) and susceptible (M-line) laboratory strains was isolated by heart puncture as previously described [10]. *L. stagnalis* were collected in a natural pond (Goldersbach, Bebenhausen), cleaned and screened for trematode infections before being maintained in freshwater tanks at room temperature in the laboratory for at least 14 days. Only snails that