Parasitology Research (2019) 118:3561–3564
https://doi.org/10.1007/s00436-019-06494-5

IMMUNOLOGY AND HOST-PARASITE INTERACTIONS - SHORT COMMUNICATION

The effect of trematode infection on the markers of oxidative stress in the offspring of the freshwater snail *Lymnaea stagnalis*

Yana L. Vorontsova[1] · Irina A. Slepneva[2] · Natalia I. Yurlova[1] · Natalia M. Ponomareva[1] · Viktor V. Glupov[1]

Received: 25 March 2019 / Accepted: 30 September 2019 / Published online: 14 November 2019

Abstract

Most invertebrate species exhibit immunological responses that can inactivate and eliminate penetrating parasites. Such immune responses in particular involve the formation of potentially toxic reactive oxygen species (ROS). We explored the immune capabilities of the first-generation (F1) offspring of naturally infected freshwater snails, *Lymnaea stagnalis*, in response to infection by trematode cercariae under laboratory conditions. The rates of ROS formation and peroxidase activity in the hemolymph of the F1 offspring of *L. stagnalis* parents infected by an asexual stage of trematodes were significantly higher than in F1 offspring of uninfected parents. Compared to offspring from uninfected parents, the growth rate of F1 snails from infected parents was higher, but survival was lower. After infection of F1 snails by trematode cercariae of *Echinoparyphium aconiatum* under laboratory conditions, the rate of ROS formation and peroxidase activity in the hemolymph of F1 offspring of uninfected parents increased compared to control snails. This pattern persisted throughout the entire 3-week observation period. In contrast, the rate of ROS formation in the hemolymph of F1 snails from infected parents after experimental infection by *E. aconiatum* cercariae did not differ from controls, and peroxidase activity even decreased. Thus, trematode parthenitae infection of parents could alter the immune response of their offspring.

Keywords Oxidative stress · Peroxidase · Reactive oxygen species · Snails · Trematode

Introduction

Lymnaea stagnalis (Gastropoda, Pulmonata) is a freshwater gastropod snail often used as a model organism to investigate immunological defense mechanisms (van der Knaap et al. 1993). Two stages of the life cycle of the trematode *Echinoparyphium aconiatum* (Trematoda, Echinostomatidae) (the asexual stages of sporocysts and rediae, and metacercariae) are associated with *L. stagnalis* snails (Yurlova et al. 2006). Reproduction of parthenitae in snails is completed by the formation of cercariae, which are free-swimming larvae of hermaphroditic generation. Subsequently, the cercariae transform into metacercariae within the second intermediate host and encyst to later be ingested by the definitive host (Galaktionov and Dobrovolskij 2003).

Snails, similar to other invertebrates, have developed a system of innate immunity to inactivate and eliminate penetrating parasites. For example, the formation of potentially toxic reactive oxygen species (ROS) increases during the defense reactions of invertebrates (Moné et al. 2011). Because of their high reactivity, ROS can participate in the destruction of parasites.

Peroxidase is present in the hemolymph of *L. stagnalis* and is also involved in snail immunity (Dikkeboom et al. 1984). Dordrecht Gornowicz et al. (2013) reported a significant increase of enzyme activity in the hemolymph of *L. stagnalis* naturally infected with digenean trematodes. Peroxidase activity may be important for the formation of cytotoxic molecules that can either be involved in cytotoxic reactions (Nappi and Ottaviani 2000) or lead to the production of other ROS (Arakawa 1994). Thus, peroxidase activity and ROS

Yana L. Vorontsova, Irina A. Slepneva and Natalia I. Yurlova conributed equally to this work.

Handling Editor: Julia Walochnik

✉ Yana L. Vorontsova
yavoronts@yandex.ru

[1] Laboratory of Insect Pathology, Institute of Systematics and Ecology of Animals, Siberian Branch of Russian Academy of Sciences, Frunze Str., 11, Novosibirsk, Russia 630091

[2] Voevodsky Institute of Chemical Kinetics and Combustion, Siberian Branch of Russian Academy of Sciences, Institutskaya Str., 3, Novosibirsk, Russia 630090

generation can be considered markers of an oxidative stress in response to parasite infection of a host (Moné et al. 2011).

Parasite can activate the host immune system and trigger changes in the resistance of surviving specimens to future infections (Little et al. 2003). Interactions during the coexistence of the host and parasite are assumed to result in genetic changes in both, including reciprocal epigenetic changes (Vilcinskas 2016). Similar changes can be manifested in host offspring of the next generation. However, little information exists regarding the effect of parasites on the offspring of previously infected parents (Milutinovic and Kurtz 2016).

The objective of the present study was to examine the effect of infection by cercariae of the trematode *E. aconiatum* on peroxidase activity and ROS generation in the hemolymph of F1 offspring of *L. stagnalis* parents infected with parthenogenetic stages of the trematode.

Materials and methods

Snails and parasites

Lymnaea stagnalis snails were collected from the coastal zone of the Kargat River in the Chany Lake area, Russia (54°30′–55°09′ N, 76°48′–78°12′ E). All collected snails were examined for cercarial shedding by exposure to artificial light. The identification of digenean species (cercariae) was based on previously published information on morphological features (Krasnolobova 1987). Snails infected with parthenogenetic (asexual) stages of *Plagiorchis mutationis* (Trematoda, Plagiorchiidae) and uninfected snails were used as the parental generation to produce F1 offspring. Infected and uninfected

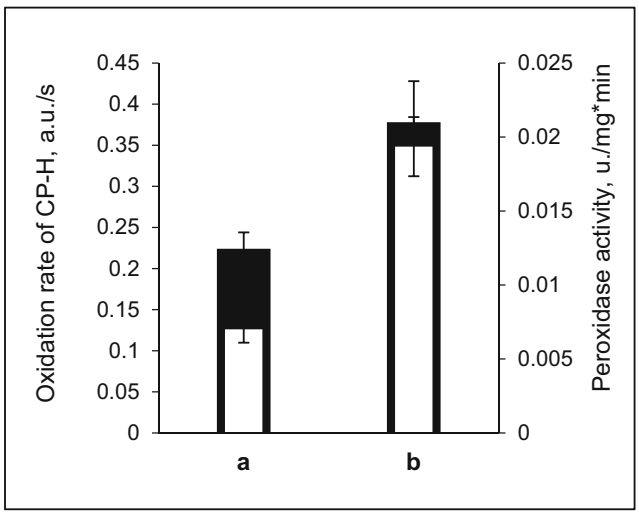

Fig. 1 ROS generation (black bars) and peroxidase activity (white bars) in hemolymph of *L. stagnalis* offspring of uninfected (**a**) and parthenitae-infected (**b**) parents .*Data labeled with "**a**" differ significantly from data labeled with "**b**" ($P \leq 0.05$)

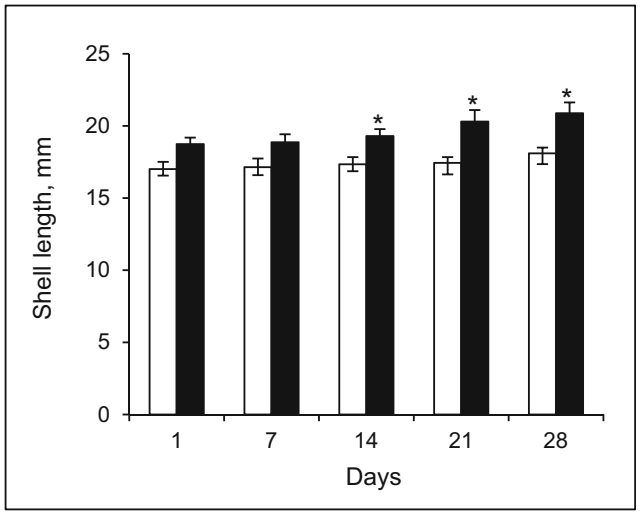

Fig. 2 Growth rate of the offspring of *L. stagnalis* parent snails that were either uninfected (white bars) or infected with trematode parthenitae (black bars). *Significant difference from offspring of uninfected parents at $P \leq 0.05$

snails were maintained separately under laboratory conditions .

After the getting of the offspring, the infection state of snail-parents collected in the field was confirmed by autopsy (according Yurlova et al. 2006).

The cercariae of the trematode *E. aconiatum* were shed by naturally infected snails collected in the coastal zone of the Kargat River. The F1 offspring were infected individually. Five just released cercariae of *E. aconiatum* were added to one snail (shell length: 15 ± 3 mm) in a vessel containing 10 mL of filtered water. If no cercariae were found in the vessel 30 min later, the infection process was considered successful.

Experimental design

In the experiments, we used F1 snail offspring from parents that were either infected or uninfected by the parthenitae trematodes. Snails within each group were divided into two subgroups: one remained uninfected (control), while the other was exposed to cercariae that transformed into metacercariae within snails. Hemolymph samples were taken from the same snails in both groups (n = 8–12) at 1, 7, 14, and 21 days after cercarial infection for detection of ROS generation and peroxidase activity.

The growth rate and survival of snails were determined for each group over the same period.

Hemolymph collection

Hemolymph was collected by stimulation of the foot sole, as described by Sminia (1972). The collected hemolymph was mixed (1:1) with phosphate-buffered saline (50 mM phosphate buffer, 150 mM NaCl, 50 μM DTPA, pH 7.2) and

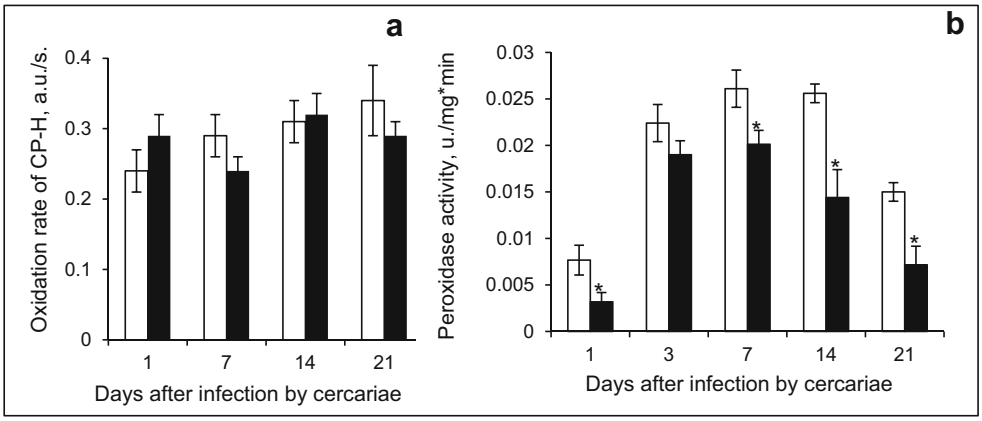

Fig. 3 ROS generation (**a**) and peroxidase activity (**b**) in hemolymph of *L. stagnalis* offspring of trematode parthenitae-infected parents (white bars, control) after infection by *E. aconiatum* cercariae (black bars). *Significant difference from the control ($P \leq 0.05$)

centrifuged at 500×*g* for 5 min at 4 °C to remove hemocytes. Supernatant was used for assays of ROS generation and peroxidase activity.

Determination of ROS generation rate

A CP-H (1-hydroxy-3-carboxy-pyrrolidine) spin trap (Slepneva et al. 1999) was used to measure rates of ROS formation. CP-H is oxidized non-specifically by highly oxidizing metabolites, which results in the formation of the stable nitroxyl radical, CP. A mixture of tested hemolymph (50 μL) with 1 mM CP-H and 0.2 mg/mL dopamine was placed in a glass capillary, and the time-depended accumulation of CP was measured by monitoring the amplitude of the low field component of the electron paramagnetic resonance (EPR) spectrum.

EPR measurements were performed at room temperature using an ER 200-D SRC X-band ESR spectrometer (Bruker).

Peroxidase assay

Peroxidase activity in the hemolymph was assayed spectrophotometrically using 4-aminoantipyrine as a substrate (Nicell and Wright 1997). Samples were placed in a plate cuvette, and the absorption at 510 nm for 5 min was recorded using the Multiscan Ascent plate reader (Thermo). Peroxidase activity was expressed in units of optical density of the incubation mixture per 1 min and 1 mg of protein.

Statistical analysis

Data were analyzed using the software SigmaPlot for Windows, version 9.0 (Systat Software, Inc.). Data were expressed as the means ± standard error ($n \geq 5$). Significant differences in the data were analyzed by Student's *t* test ($P \leq 0.05$) using the Origin 6.0 program.

Results and discussion

Peroxidase is a key enzyme in the immune response of the pond snail *L. stagnalis* (Dordrecht Gornowicz et al. 2013). Vorontsova et al. (2015) demonstrated that peroxidase activity in the hemolymph of *L. stagnalis* can result in the generation of active metabolites, such as o-semiquinones. By considering peroxidase activity and ROS generation as markers of the

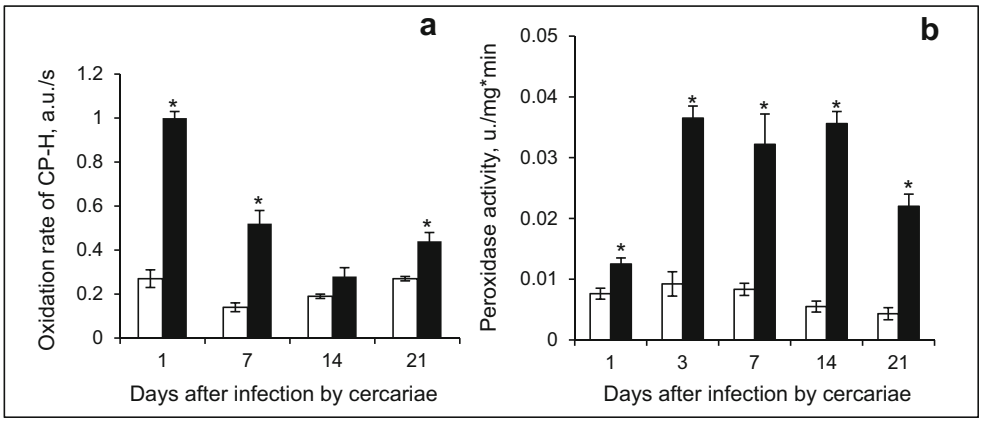

Fig. 4 ROS generation (**a**) and peroxidase activity (**b**) in hemolymph of *L. stagnalis* offspring of uninfected parents (white bars, control) after infection by *E. aconiatum* cercariae (black bars). *Significant difference from the control ($P \leq 0.05$)

oxidative stress of *L. stagnalis*, we assessed how these two factors changed in the hemolymph of the F1 offspring from parents naturally infected with trematode parthenitae, in response to subsequent infection with cercariae of *E. aconiatum*. Cercariae quickly transform into metacercariae in snails (Ataev 2010); therefore, we examined the effect of cercariae penetration and metacercariae infection on the second intermediate host.

Both peroxidase activity and ROS generation in the hemolymph of offspring of infected parents were clearly higher than values in offspring produced by uninfected parents (Fig. 1). These results indicate that the status of trematode infection of parents influences the filial generation of snails. This response can be considered the manifestation of an "adaptive immunity" of invertebrates, a concept that has been recently debated in the literature (Moret and Schmid-Hempel 2001; Ottaviani 2015). Indeed, we were unable to experimentally verify the exhaustion of offspring produced by the infected parents. In contrast, growth rates were actually higher (Fig. 2) and reproductive sizes were reached earlier in these offspring compared to the offspring of uninfected parents (Yurlova, unpublished data). These unexpected results could represent compensatory mechanisms for the successful reproduction of the offspring. The increased markers of oxidative stress of the offspring of infected parents may accelerate their physiological development, although at the cost of decreased survival (30% compared to 70% for the offspring of uninfected parents).

The laboratory infection of the offspring of infected parents with *E. aconiatum* cercariae did not result in an additional increase in the markers of oxidative stress compared to the control (Fig. 3). The level of ROS generation was still comparable to the control during the entire observation period, and peroxidase activity actually decreased. Furthermore, the survival of offspring in this group increased significantly (by 2.3-fold compare with the uninfected snails) after laboratory infection.

A 1.3-fold increase in survival after cercarial infection was also observed in the offspring of the uninfected parents. However, the markers of oxidative stress of these snails were substantially higher after cercarial infection compared to the controls (Fig. 4). These results indicate that the offspring of uninfected parents activate their own response to trematode infection, while the offspring of infected parents exploit the immune potential provided by their parents. Thus, trematode parthenitae infection in parents could alter the immune response of their offspring.

Similar effects have been demonstrated following viral, bacterial, and fungal infections, suggesting a broadly functioning immune response in invertebrates (Chambers and Schneider 2012).

Funding information The study was supported by The Federal Fundamental Scientific Research Programme for 2013-2020 (AAAA-A16-116121410124-8) and AAAA-A18-1181222190010-8.

References

Arakawa T (1994) Superoxide generation in vitro in Lepidopteran larval haemolymph. J Insect Physiol 40:165–117

Ataev GL (2010) The influence of *Echinostoma caproni* metacercariae (Trematoda) on the survival of biomphalaria molluscs (Pulmonata). Parasitologiia 44:481–495

Chambers MC, Schneider DS (2012) Pioneering immunology: insect style. Curr Opin Immunol 24:10–14

Dikkeboom R, van der Knaap WPW, Mueleman EA, Sminia T (1984) Differences between blood cells of juvenile and adult specimens of the pond snail *Lymnaea stagnalis*. Cell Tissue Res 238:43–47

Dordrecht Gornowicz D, Dmochowska K, Zbikowska E, Zoltowska K (2013) Total antioxidative status and the activity of peroxidase and superoxide dismutase in the haemolymph of *Lymnaea stagnalis* (L.) naturally infected with digenean trematodes. J Mollusc Stud 79: 225–229

Galaktionov KV, Dobrovolskij AA (2003) The biology and evolution of trematodes. Kluwer Academic Publishers

Krasnolobova TA (1987) Trematodes of fauna (animals) of USSR. Plagiorchis genus. Nauka Press, Moscow

Little TJ, O'Connor B, Colegrave N, Watt K, Read AF (2003) Maternal transfer of strain-specific immunity in an invertebrate. Curr Biol 13: 489–492

Milutinovic B, Kurtz J (2016) Immune memory in invertebrates. Semin Immunol 28:328–342

Moné Y, Ribou A-C, Cosseau C, Duval D, Théron A, Mitta G, Gourbal B (2011) An example of molecular co-evolution: reactive oxygen species (ROS) and ROS scavenger levels in *Schistosoma mansoni/ Biomphalaria glabrata* interactions. Int J Parasitol 41:721–730

Moret Y, Schmid-Hempel P (2001) Immune defence in bumble-bee offspring. Nature 414:506

Nappi AJ, Ottaviani E (2000) Cytotoxicity and cytotoxic molecules in invertebrates. BioEssays 22:469–480

Nicell JA, Wright A (1997) A model of peroxidase activity with inhibition by hydrogen peroxide. Enzym Microb Technol 21:302–310

Ottaviani E (2015) Invertebrate immunological memory: could the epigenetic changes play the part of lymphocytes? Invertebr Surviv J 12: 1–4

Slepneva IA, Glupov VV, Sergeeva SV, Khramtsov VV (1999) EPR detection of reactive oxygen species in haemolymph of *Galleria mellonella* and *Dendrolimus superans sibiricus* (Lepidoptera) larvae. Biochem Biophys Res Commun 264:212–215

Sminia T (1972) Structure and function of blood and connective tissue cells of the freshwater pulmonate *Lymnaea stagnalis* studied by electron microscopy and enzyme histochemistry. Z Zellforsch Mikrosk Anat 130:497–526

van der Knaap WPW, Adema CM, Sminia T (1993) Invertebrate blood cells: morphological and functional aspects of the haemocytes in the pond snail *Lymnaea stagnalis*. Comp Haematol Int 3:20–26

Vilcinskas A (2016) The role of epigenetics in host–parasite coevolution: lessons from the model host insects *Galleria mellonella* and *Tribolium castaneum*. Zoology 119:273–280

Vorontsova YL, Slepneva IA, Yurlova NI, Glupov VV (2015) Do snails *Lymnaea stagnalis* have phenoloxidase activity in hemolymph? Invertebr Surviv J 12:5–12

Yurlova NI, Vodyanitskaya SN, Serbina EA, Biserkov VY, Georgiev BB, Chipev NH (2006) Temporal variation in prevalence and abundance of metacercariae in the pulmonate snail *Lymnaea stagnalis* in Chany Lake, West Siberia, Russia: long-term patterns and environmental covariates. J Parasitol 92:249–259