JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2010. Vol. 4(2), p. 911-913

Immune Response in Earthworm (*Perionyx excavatus* Perrier) to the Wound Pathogens

N. Packialakshmi

Department of Microbiology, Jamal Mohamed College, Tiruchirappalli - 620 020, India.

(Received: 20 February 2010; accepted: 28 March 2010)

The earthworm *perionyx excavatus* exhibits a rudimentary immune system. For this study young earthworms were injected with 0.05ml of 1.0×10^6 cfu /ml of *Staphylococcus aureus* .The coelomic fluid were collected and noted the total cell counting of *Perionyx excavatus* before and after the pathogen infection. The data indicated a marked proliferation in total cell counts in comparison to the control worms. This trend of increasing total cell counts continued over the five days. The pathogen would be inhibited through the cellular activity of coelomic fluid.

Key words: Immune responses, Pathogens, Cellular activity.

Inflammation is a chemical and cellular response to irritants like bacteria, viruses, and foreign objects. In mammals inflammation involves both innate and Acquired immune defenses. Innate responses are immediate nonspecific defenses like skin. Chemicals in blood and cells that attack anything foreign to the body (Goldsby, 2000). One aspect of this study focused on innate responses to inflammation, without the interactions of the adaptive immune system by examining the responses in earthworms to bacteria. The earthworm model was for several reasons chosen for analysis. They have a simple system, in that they lack adaptive immunity (Fischer,1977) and they display an innate immune response (Dhainaut, 2001). They are also successful in a highly contaminated world (Dhainaut, 2001). The earthworm lives a relatively long time and must have a functional defense system against microbes. Earthworms also have a well studied life cycle. *Perionyx excavatus* is one earthworm species that is an easily maintained and can be used in continuing studies. This study focused mainly on the coelomic cells, which are also called coelomocytes (Dhainaut, 2001).The coelomocytes are found in the coelomic cavity of the earthworm. Earthworms have pores that connect with the coelomic cavity to the exterior, through which cells are exuded following stress. Coelomocytes are similar in appearance and function to some human white blood cells (Dhainaut, 2001; Affar, 1998).

MATERIAL AND METHODS

Collection of *Perionyx excavatus*

Adult (sexually mature) *Perionyx excavatus* were obtained from Annamalai University, Annamalai Nagar. The earthworms were maintained at 15ºC in the moist soil and used for experiments.This experiment was used

* To whom all correspondence should be addressed.
E-mail: packia_lakshmi_1977@yahoo.com

mature earthworms about 8cm long. Each experiment earthworms were separated into groups of 3 worms and housed in glass jars with sterile damp paper towel bedding. The bedding was kept damp with bottled spring water and food was provided once a week.

Bacterial Inoculation

The gram positive and gram negative bacterium of *Staphylococcus aureus* and *Klebsiella pneumoniae,* was grown in nutrient broth (NB) overnight. The bacteria were washed in phosphate buffered saline (PBS) and then suspended in PBS to 10^6cfu/ml. The worms were anesthetized in 5ml of 5% ethanol and PBS for 5-10 minutes (Affar, 1988) and injected in between the 4^{th} and 6^{th} segment behind the clitellum. Next an amount of 0.05ml of bacteria was inoculated into the experimental group of anesthetized worms was inoculated with 0.05ml of PBS. The experimental worms were separated into five jars, with each glass jar containing three bacterially inoculated worms. The control jars, with each glass jar containing three bacterially inoculated worms. The control group was then separated into five jars, with each control jar containing three PBS inoculated worms. The same experiment was done in *Staphyloccus aureus* and *Klebsiella pneumoniae*

Total coelomic cell count

To extract the coelomic cells the worms had to be prepared. This was achieved by taking the three worms out of the jar and placing those 5ml of PBS in a petridish. The worms were shocked with a 9 volt battery for 1-2 seconds. After 5minutes the exuded coelomic cells were collected and centrifuge in micro-centrifuge tubes. The micro-centrifuge tubes were decanted and the coelomic cell pellets were pooled into one centrifuge tube and diluted with 0.5ml of PBS. The cells were counted using a Nikon phase contrast microscope and a haemocytometer. This procedure of collecting and counting cells was performed on the experimental and the control group for each day of the experiment.

Determination of phagcytotic activity of coelomocytes

The coelomic fluid was incubated with bacteria *Staphylococcus aureus* and *Klebsiella pneumoniae* suspension of a concentration of 10^6 cells per 1ml, temperature of 23ºC during 5 hrs, according to modified method of Toupin *et al.* (1977). The slides were analysed in photo Nikon microscope at 1 hr intervals, calculating the percentage of coelomocytes containing phagocyted bacterial cells according to the formula (Fornusek *et al*., 1985).

$$\text{Phagocytotic activity (\%)} = \frac{\text{Number of coelomocytes with bacterial cells}}{\text{Total number of coelomocytes}} \times 100$$

The results were statistically analyzed with student t-test for independent data. Differences of $p<0.05$ (OKTABA and NIEDOKOS, 1980) were adopted as statistically significant.

RESULTS AND DISCUSSION

The present investigation based on the phase contrast microscopy study indicates that the coelomic fluid of the *Perionyx excavatus* contains four types of cells such as basophils , acidophils , neutrophils and chlorogocytes.The previous report showed that the clear differentiation in the structure of oligochaete coelomocytes was associated with their functions with phagocytosis (Dales and Kalac, 1992) encapsulation, coagulation of systemic fluids (Valembois *et al.,*

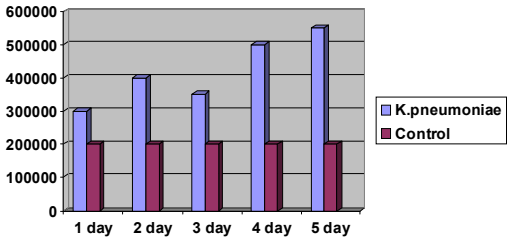

Fig. 1. Total coelomic counts of *K.pneumoniae* injected into the *Perionyx excavatus*

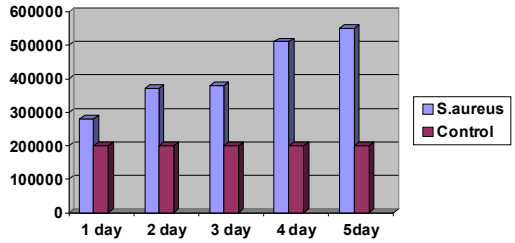

Fig. 2. Total coelomic counts of *S.aureus* injected into the *Perionyx excavatus*

1988) wound healing (Parry,1975), immune reaction.

In the present Investigation (Fig 1, 2) showed that after injection of pathogen the coelomic cell counts should be increased the pathogen level totally decreased in the 5th day of the cell counting. The previous report Valembois *et al.,* (1985) reported that the granular amoebocytes of earthworms are capable of phagocytosis and encapsulation.

CONCLUSIONS

The purpose of this experiment was to establish methods to quantity the innate responses in *Perionyx excavatus*. The data in this experiment indicates that there are four cell types basophils, acidophils, neutrophils and chlorogocytes t can be analysed in the coelomic fluid. That the inoculation of *Staphylococcus aureus* and *Klebsiella pneumoniae* increase the number of exudates cells but does not alter the cell types. Therefore *Perionyx excavatus* responds to bacterial challenges by increasing the number of coelomic cells.

REFERENCES

1. Affar, E.B., Dufour,M., Poirier, G.G. and Nadeau, D. Isolation, Purification and partial Characterization of Cholorogocytes from Earthworm species *Lumbricus terrestis*. *Molecular and cellular biochemistry.,*1998; **185**: 123-133
2. Cooper E.L, Earthworm immunity. In: Rincevich, B., and Muller, W.E.G. (eds.). Invertebrate immunology. Springer- Verlag, Berlin-Heidelberg 1996.
3. Dhainaut, A. and Scaps, P. Immune defense and biological responses induced by toxics in Annelida. *Canadian Journal of Zooloogy.,* 2001; **79**: 233-253
4. Dales, R.P. and Kalac, Y. Phagocytic defense by the earthworm Eisenia *foetida* against certain pathogenic bacteria. Comp. *Biochem. Physiol.*, 1992; **101**A**:** 487-490.
5. Fisher, E. and Horvath, I . *In vivo* cumulation and discharge of Azine, Thiazine, and Xanthene dyes and their effects on the chlorogen cells of *Lumbricidae* (Oligochaeta). *Acta biological academiae scientiarum Hungaricae.,*1977; **28**: 33-47
6. Goldby, A., Kindt, T.and Osborne, B, Overview of the Immune system. Immunology, 4th ed. N.Y. Freeman & Company 2000.
7. Lange,S., Kauschke,E., Mohrig,W. and Cooper,E. Biochemical characteristics of eiseniapore, a pore-forming protein in the coelomic fluid of Earthworms. *European journal of Biochemistry.,*1999; **263**: 547-556.
8. Licata, A., Ainis, L., Martella, S., Ricca, M., Licata, B., Lauriano, E. and Zaccone, G. Immunohistochemical localization of n NOS in the skin and nerve fibres of the Earthworm *Lumbricus terrestis L.* (*Annelida oligochaeta*). *Acta Histochem.,* 2002; **104**(3): 289-295.
9. Parry, M.J. Evidence of mitotic division of coelomocytes in the normal wounded and grafted earthworm *Eisenia foetida*. *Experientia.,*1975; **32**: 449-451.
10. Quaglino, D., Cooper, E.L., Salvioli, S., Capri,M., Suzuki, M.M., Ronnchetti, I,P., Franceschi, C. and Cossarizza, A. Earthworm coelomocytes *in vitro*: cellular features and "granuloma" formation during cytotoxic activity against the mammalian tumor cell target K562. *Eur. J. Cell. Immuno.,* 1996; **70**: 278-228.
11. Stein, E. and Copper, E. The role of opsonins in phagocytosis by coelomocytes of the earthworm, *lumbricus terrestis*. *Dev. Comp. Immunol*.,1981; **5**: 415-425.
12. Valembois, P., Roch, P and Lassegues, M. Evidence of plasma clotting system in earthworms. *J.Invertebr.Patho.,* 1988; **51**: 221-228.
13. Valembois, P., Lassegues, M., Roch, P.and Vaillier,J. Scanning electron microscopic study on the involvement of coelomic cells in earthworm antibacterial defense cell. *Tiss. Res.,* 1985; **240**: 479-484.