6 Conclusions

1) Earthworms respond to antigenic stimulation by formation of antigen-binding protein (ABP), the increase in ABPs level being independent of the size or extent of glycosylation of proteins. Formed ABPs bind preferably the same protein as that used for stimulation, suggesting the presence of partially specific adaptive immunity in annelids. Nevertheless, the specificity of antigen recognition is considerably lower as compared to immunoglobulin since ABP reacts not only with the protein used for stimulation but also to a lesser extent with related proteins.

2) *E. fetida* earthworms respond to antigenic stimulation, body injury or stress conditions by a marked non-specific increase in coelomic fluid protein concentration, but in contrast to antigenic stimulation, the increased level of the protein concentration caused by body injury as a result of wounding returns back to the level of non-injected animals after 2 days post injection. Stimulation of earthworms by microbial antigens leads to distinct changes: lysozyme-like activity in the coelomic fluid of earthworms challenged with bacteria and glucan increases within the first 4 days; hemolytic activity of the coelomic fluid of earthworms challenged with Gram-negative bacteria decreases 12 hours and 2 days after the infection, with Gram-positive bacteria 2 days after the infection; protein level of the coelomic cytolytic factor (CCF) in the coelomic fluid increases within the first 24 hours, most likely due to the release of CCF from intracellular stocks after the infection with bacteria and glucan. Expression of CCF in coelomocytes significantly increases from 2 to 40 hours upon experimental administration of microbial compound, with the maximum level at 17 hours post-injection, while the expression of fetidin is not affected in *E. fetida* earthworms.

3) Two *E. fetida* hemolytic proteins, fetidin and lysenin, are encoded by two separate genes with a high homology and their level of expression in coelomocytes differs from individual to individual. The two hemolytic proteins, fetidin and lysenin, are most probably unique for *E. fetida* earthworms.
4) Earthworm species *D. veneta*, *L. rubellus*, *A. icterica*, *A. rosea*, *A. caliginosa*, *L. terrestris*, *A. longa* contain in their coelomic fluid CCF-like molecules having 80-90% homology with *E. fetida* CCF, and the monoclonal antibody against *E. fetida* CCF reacts with CCF-like proteins in coelomic fluids of all tested species. CCF from *E. fetida* is unique in that it has a broader saccharide binding specificity and is the only one recognizing N,N'-diacetylchitobiose and peptidoglycan constituents as well as having the ability to lyse tumor TNF-sensitive cell line and *Trypanosoma* parasites. Phylogenetic relationship between the original *E. fetida* CCF and the CCF-like molecules of other Lumbricid species shows that earthworms of the genus *Lumbricus* belong to a group having the same progenitor, whereas the CCF-like molecules of the genus *Aporrectodea* from a paraphyletic group, and *E. fetida* CCF is closely related to the CCF-like molecule of *D. veneta*.

5) *E. fetida* coelomic fluid displays phenoloxidase activity; however, the level of PO activity is lower than in other invertebrates. ProPO activation is also slower as compared to other invertebrate species, suggesting that the proPO cascade does not represent the main defense system in earthworms.