BARD

FINAL REPORT

PROJECT NO. US-3030-98

Importance of Endobiotics in the Defense Against Protozoan Ectoparasites of Fish

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2003

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Date of Submission of the report: 5 Sept 03

BARD Project Number: US-3030-98

Project Title: Importance of endobiotics in the defense against protozoan ectoparasites of fish

Investigators

Institutions

Principal Investigator (PI):

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North Carolina State University

Co-Principal Investigator (Co-PI):

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Collaborating Investigators:

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Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Piscidins, histone-like proteins, stress, innate immunity, antibiotics

Abbreviations commonly used in the report, in alphabetical order:

HLP: histone-like protein

Budget: IS: \$135,000

US: \$164,000

Total: \$299,000

Signature

Principal Investigator

Signature

Authorizing Official, Principal Institution

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Publication Summary (numbers)

	Joint IS/US Authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)		5		5
Submitted, in review, in preparation	1	1		1
Invited review papers		3		3
Book chapters				
Books				
Master theses				
Ph. D. theses		2		2
Abstracts		7		
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits and Meetings	1	1		
Longer Visits (Sabbaticals)				

Description of Cooperation:

Patent Summary (numbers)

	Israeli inventor (s) only	US inventor (s) only	Joint IS/US inventors	Total
Submitted		2		2
Issued (allowed)				
Licensed				

Abstract

Infectious disease is one of the most serious causes of economic loss in all sectors of aquaculture. There is a critical need to understand the molecular basis for protection against infectious disease so that safer, more reliable and more cost-effective strategies can be designed for their control. As part of this effort, the major goal of our BARD project was to determine the importance of endobiotics as a defense against protozoan ectoparasites in fish. Endobiotics, or antimicrobial polypeptides, are peptides and small proteins that are increasingly recognized as having a vital role in the innate defense of virtually all animals.

One objective of our BARD project was to determine the antiparasitic potency of one specific group of endobiotics that were isolated from hybrid striped bass (*Morone saxatilis* x *M. chrysops*). We found that these endobiotics, which we had previously named histone-like proteins (HLPs), exhibited potent activity against *Amyloodinium* and that the putative levels of HLPs in the skin were well within the levels that we found to be lethal to the parasite in vitro. We also found evidence for the presence of similar antibiotics in sea bream (*Sparus aurata*) and Mediterranean sea bass (*Dicentrarchus labrax*).

We also examined the effect of chronic stress on the expression of HLP in fish and found that HLP levels were dramatically decreased after only one week of a crowding/high ammonia sublethal stress. We also began to explore the feasibility of upregulating endobiotics via immunostimulation. However, we did not pursue this objective as fully as we originally intended because we spent a much larger effort than originally anticipated on the last objective, the attempted isolation of novel endobiotics from hybrid striped bass. In this regard, we purified and identified four new peptide endobiotics. These endobiotics, which we have named piscidins (from "Pisces" meaning fish), have potent, broad-spectrum activity against a number of both fish and human pathogens. This includes not only parasites but also bacteria. We also demonstrated that these peptides are present in the mast cell. This was the first time that the mast cell, the most common tissue granulocyte in vertebrates, was shown to possess any type of endobiotic. This finding has important implications in explaining the possible function of mast cells in the immune response of vertebrates.

In summary, the research we have accomplished in this BARD project has demonstrated that endobiotics in fish have potent activity against many serious pathogens in aquaculture and that there is considerable potential to use these compounds as stress indicators in aquaculture. There is also considerable potential to use some of these compounds in other areas of medicine, including treatment of serious infectious diseases of humans and animals.

Achievements

Significance of the main achievements

1) Demonstration of potent anti-*Amyloodinium* activity of HLPs at levels that are well within concentrations present in fish tissues: These data suggest that HLPs may play a critical role in defending against *Amyloodinium* and probably other important fish pathogens. Furthermore, the discovery that HLPs can kill *Amyloodinium* trophonts has never been reported for any drug, as the trophont stage is typically very resistant to any treatment. Thus, these data provide new insights into novel avenues of control of this pathogen.

2) Demonstration of significant reduction of HLP levels in tissues of chronically stressed fish: These data showed that HLP levels declined dramatically in chronically stressed fish before they showed any signs of disease. This suggests that HLP might be very useful as a biomarker of chronic stress. This biomarker is directly relevant to fish health as HLPs are directly toxic to many fish pathogens. Other features of HLP also make it very attractive as a means to assess health in fish populations, including the potential to cheaply and easily measure it on-site (pond-side).

3) Discovery of a novel, potent, broad-spectrum family of endobiotics (piscidins) in mast cells of fish: Piscidins are highly lethal to both fish and human pathogens (bacteria and parasites). This includes multi-antibiotic resistant bacteria, which are causing a major crisis in human medicine. The quite dramatic activity of these peptides in physiological salt concentrations also make them attractive as templates for the design of clinically useful antibiotics. The demonstration of piscidins in mast cells provides the first evidence that this highly common immune cell might directly participate in killing pathogens. This has important implications for the pathogenesis of a number of vertebrate (fish, human, etc.) diseases. Our demonstration of piscidin-like factors in other unrelated fish species (e.g., spot [*Leiostomus xanthurus*], croaker [*Micropogonias undulatus*] and others) also suggests that piscidins might be widespread defenses in fish.

Agricultural and/or economic impacts of the research

There are several potential benefits to society from our research:

- * Improved health, welfare and productivity in aquaculture (food, pet, lab animal)
- * Improved means of assessing environmental and fisheries health
- * Development of new antimicrobial-based solutions for emerging and re-emerging infections in humans and other animals

Some of the technology developed in this project was used to spin off a new biotechnology startup company (Norcarex Bio Corporation) from NCSU that is aimed at utilizing endobiotic defenses as tools for health assessment and therapy. These products are planned to be made widely available to end users in Israel, the U.S., and worldwide.

Details of cooperation and how objectives were promoted as a result of the cooperation

The investigators of this team have worked together on several projects and have a long-running collaboration, which was established nearly 15 years ago during a previous BARD project. This close collaboration has continued under the current BARD project. In October 1999, Drs. Noga and Levy visited Dr. Colomi's and Dr. Avtalion's laboratories to make plans and to assist with implementation of future experiments. At this time, they also shared the ELISA reagents that were used to initiate these studies in Israel and transferred details on the ELISA technology to Dr. Avtalion.

Dr. Colomi also visited NCSU in Fall 2001 to standardize methods for quantitatively sampling HLPs from fish tissues and to discuss future directions for completion of the project. Using the technology transferred by the Noga laboratory, Dr. Colomi proceeded to sample sea bream (*Sparus aurata*) and Mediterranean sea bass (*Dicentrarchus labrax*) for HLP. As a result, the presence of HLP in these fish's tissues was detected by the Noga laboratory via ELISA.

For anti-parasitic studies, Dr. Colomi isolated the Israeli *Amyloodinium* strains that were eventually used for anti-parasitic testing of piscidin. Dr. Noga's laboratory provided purified piscidin that was used in these studies, and lead to the discovery by Dr. Colomi of highly potent anti-parasitic activity against several important marine fish pathogens.

Publications

Noga, E.J. 2000. Skin ulcers in fish: *Pfiesteria* and other etiologies. *Toxicological Pathology* 28:807-823 (cover article for this issue).

Noga, E.J. 2001. Nonspecific immunity: Applications to aquaculture. Proceedings of the U.S.D.A. Agricultural Research Service/Oceanic Institute Conference on Biotechnology-Aquaculture Interface: Site of Maximum Impact, Shepherdstown, WV, March 2001, pp. 31-38.

Robinette, D.R. and E.J. Noga. 2001. Histone-Like Protein (HLP): A novel method for assessing chronic stress in fish. *Diseases of Aquatic Organisms* 44:97-107.

Noga, E.J., Z. Fan and U. Silphaduang. 2001. Histone-like proteins from fish are lethal to the parasitic dinoflagellate *Amyloodinium ocellatum*. *Parasitology* 123:57-65.

Silphaduang, U. and E.J. Noga. 2001. Peptide antibiotics in mast cells of fish. *Nature* 414:268-269.

Noga, E.J. and P. Udomkusonsri. 2002. Use of fluorescein as a biomarker of skin ulceration in fish. *Veterinary Pathology* 39:726-731 (cover article for this issue)

Noga, E.J., Z. Fan and U. Silphaduang. 2002. Host site of activity and cytological effects of histone-like proteins against the parasitic dinoflagellate *Amyloodinium ocellatum*. *Diseases of Aquatic Organisms* 52:207-215.

Noga, E.J. and U. Silphaduang, U. 2003. Piscidins: A novel family of peptide antibiotics from fish. *Drug News and Perspectives* 16:87-92.

Appendix

Published papers - see attached (3 copies of all papers that have not yet been submitted to BARD)

In press, accepted, or submitted papers – None

Unpublished data - None

Other relevant material - None