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MECHANISMS OF DEFENSE AGAINST DISEASE IN THE AMERICAN LOBSTER

by

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SUMMARY

The lobster's intrinsic defenses against disease include agglutinins, inducible bactericidin(s), precipitin(s) and phagocytosis. This report deals mainly with phagocytosis in which an in vitro procedure was utilized to estimate the normal phagocytic capacity and changes in it induced by treating lobsters with vaccines. Maximum in vitro phagocytosis occurred in 90 minutes at 15C. Lobsters treated with vaccines showed a marked increase in their capacity to phagocytose opsonized A. viridans (var.) homari cells and both opsonized and untreated erythrocytes compared with control lobsters (injected only with artificial sea water). The discussion relates the lobster's substantial phagocytic capacity and its enhancement to the other features of the intrinsic defense mechanisms.

INTRODUCTION

Among lobsters (genus Homarus) held in captivity, high mortalities periodically result from outbreaks of the fatal bacterial infection, gaffkemia. The lack of host defenses against virulent strains of the causative agent, Aerococcus viridans (var.) homari (formerly Gaffkya homari) has been emphasized in recent reviews (Stewart and Rabin, 1970; Sindermann, 1971). Thus the development of some form of resistance through immunization or by the use of chemotherapeutic aids for protection or treatment is highly desirable. Our previous studies on these two subjects have dealt with the hemolymph factors of agglutinin activity (Cornick and Stewart, 1968, 1973) precipitin activity (Stewart and Foley, 1969) bactericidal activity (Cornick and Stewart, 1968 and Stewart and Zwicker, 1972) and the effectiveness of the antibiotic vancomycin prior to and following challenge with the pathogen Aerococcus viridans (var.) homari (Stewart and Arie, in press). Accordingly, this report describes further studies on the lobster defense mechanisms notably phagocytosis by Homarus americanus hemocytes, a factor which it has been possible to enhance and which has a central role in coping with disease.

Before proceeding with this report, however, we believe it would be useful to give a brief review of the changes in the name of this pathogen. The causative agent of gaffkemia, the bacterium formerly known as Gaffkya homari (Hitchner and Snieszko, 1947; Snieszko and Taylor, 1947) is now called Aerococcus viridans (var.) homari after the recommendations of Kelly and Evans (1974) (confirmed by inclusion in Bergey's Manual, Buchanan and Gibbons, in press). During the transition period following the rejection of the generic term Gaffkya Trevisan (Editorial Secretary, 1971; Kocur and Martinec, 1965), confusion existed and the pathogen briefly was called Pediococcus homari following the suggestion of Deibel and Niven (1960). Thus the organism previously known as Gaffkya homari is referred to by us as Aerococcus viridans (var.) homari.

EXPERIMENTAL AND RESULTS

The system developed for maintaining lobster hemocytes (lobster blood cells) in a viable state in vitro (Paterson and Stewart, 1974) was employed to estimate the phagocytic capacity of normal lobsters and those treated with several different vaccines. The test system consisted of hemocytes held as a monolayer in lobster hemolymph medium (LHM) (Table 1) at 15C; bacteria (A. viridans (var.) homari) or sheep erythrocytes (SRBC) either untreated or opsonized with lobster serum were introduced at the same time as the hemocytes. Observations for phagocytosis were made 90 min later using phase contrast microscopy. The dymamics of the process using hemocytes from normal lobsters are illustrated in Fig. 1.

Lobsters were treated with one of the following vaccines: formalin killed Pseudomonas perolens whole cells, an endotoxin prepared from P. perolens or formalin killed A. viridans (var.) homari whole cells. Animals treated with these vaccines showed a marked increase in their capacity to phagocytose opsonized live A. viridans (var.) homari cells and both opsonized and untreated erythrocytes compared with control lobsters which had been injected only with artificial sea water (ASW) (Tables 2, 3 and 4). Both the percentage of hemocytes showing phagocytosis and the number of particles/10^3 hemocytes were higher for the vaccinated animals.

DISCUSSION

Only two percent of the hemocytes from normal lobsters phagocytosed opsonized particles. This level of phagocytosis, however, is considerable when the total numbers of hemocytes is taken into account. A typical market sized lobster will weigh around 500 g and has perhaps 150 ml of hemolymph, giving an approximate total of between 2 and 4 x 10^9 hemocytes/animal. Thus even at the two percent level a normal lobster would have 4 to 8 x 10^7 phagocytic cells which because of their ability to engulf between one and fifty particles/phagocyte provides the lobster with the capacity to cope with "palatable" particles whose numbers exceed 1 x 10^8. This level would appear to be adequate to cope with most bacterial invasions the lobster is likely to experience. Because this already considerable capacity can be enhanced quite readily, the lobster would seem to be well equipped for defense since it also possesses in its hemolymph substantial concentrations of agglutinin, bactericidal and precipitating agents with broad activity ranges.

The lobster, in fact, can ward off infections by all but two bacterial species, one which causes, in a limited number of cases, the external affliction, shell disease, (originally described by Hess (1937)) and gaffkemia. The causative agent of gaffkemia, A. viridans (var.) homari, is uniquely suited to overcome the intrinsic defences of the lobster. Once the pathogen has gained a portal of entry (breaks in the integument) it grows extermely well in the hepatopancreas, heart, muscle and hemolymph (Stewart and Arie, 1973). Virulent strains are unaffected by the bactericidin, and the agglutinin and although the pathogen has been observed recently to be phagocytosed in vivo in the

hemolymph and previous data has shown phagocytosis in the heart and hepatopancreas, the net result has been not elimination of the pathogen but rather a disappearance of the hemocytes (Stewart et al, 1969). Thus the possession of a substantial phagocytic capacity which can be enhanced quantitatively is not sufficient to protect the lobster against this pathogen. Our unpublished results tend to support this by showing that although vaccines prepared from P. perolens cause an enhancement in phagocytic capacity this is not accompanied by increased protection against challenge with A. viridans (var.) homari. Recent evidence (Stewart and Zwicker, 1974; and our unpublished results), however, has shown that by the use of appropriate vaccines prepared from avirulent strains of the pathogen, a limited degree of resistance can be induced; with vaccines prepared from virulent strains of A. viridans (var.) homari we can obtain protection against challenge with high doses of the pathogen (LD_{50} equal to 1.114×10^7). This is in contrast to the situation with untreated lobsters which succumb to challenge from negligible numbers of the pathogen (10 bacteria/kg body wt.).

In summary, P. perolens vaccines enhance phagocytic levels considerably but do not confer protection against gaffkemia while A. viridans (var.) homari vaccines confer protection against this disease, but give a more limited phagocytic enhancement (Tables 2, 3 and 4). This raises the possibility of a qualitative change occurring in phagocytic capacity thus introducing an element of discrimination in addition to quantitative change. Work is continuing on phagocytosis and the induction of resistance for the purpose of developing practical aids for protection of lobsters against disease.

REFERENCES

BUCHANAN, R.E., and N. GIBBONS, EDITORS. Bergey's Manual of Determinative Bacteriology, 8th ed., The Williams and Wilkins Co., Baltimore. In Press.

CORNICK, J.W. and J.E. STEWART. 1968. Interaction of the pathogen Gaffkya homari with natural defense mechanisms of Homarus americanus. J. Fish. Res. Bd. Canada 25: 695-709.

CORNICK, J.W. and J.E. STEWART, 1973. Partial characterization of a natural agglutinin in the hemolymph of the lobster, Homarus americanus. J. Invert. Pathol. 21: 255-262.

DEIBEL, R.H., and G.H. NIVEN, JR. 1960. Comparative study of Gaffkya homari, Aerococcus viridans, tetrad-forming cocci from meat curing brines, and the genus Pediococcus. J. Bacteriol. 79: 175-180.

EDITORIAL SECRETARY (for the Judiciary Commission of the International Committee of Systematic Bacteriology). 1971. Opinion 39, Rejection of the generic name Gaffkya Trevisan, Int. J. System. Bacteriol. 21: 104-105.

HESS, E. 1937. A shell disease in lobsters (Homarus americanus) caused by chitinovorous bacteria. J. Biol. Bd. Canada, 3: 358-362.

HITCHNER, E.R., and S.F. SNIESZKO. 1947. A study of a microorganism causing a bacterial disease of lobsters. J. Bacteriol. 54: 48

KELLY, K.F. and J.B. EVANS. 1974. Deoxyribonucleic acid homology among strains of the lobster pahtogen "Gaffkya homari" and Aerococcus viridans. J. Gen. Microbiol. 81: 257-260.

KOCUR, M. and T. MARTINEC. 1965. Proposal for the rejection of the bacterial generic name Gaffkya. Int. Bull. Bacteriol. Nomen. Taxon. 15(3): 177-179.

PATERSON, W.D. and J.E. STEWART. 1974. In vitro phagocytosis by hemocytes of the American lobster (Homarus americanus). J. Fish. Res. Bd. Canada, 31: 1051-1056.

SINDERMANN, C.J. 1971. Internal defenses of Crustacea: a review. U.S. Fish Wildl. Service Fish. Bull. 69: 455-489.

SNIESZKO, S.F. and C.C. TAYLOR. 1947. A bacterial disease of the lobster (Homarus americanus). Science 105: 500.

STEWART, J.E. and B. ARIE. 1973. Depletion of glycogen and adenosine triphosphate as major factors in the death of lobsters (Homarus americanus) infected with Gaffkya homari. Can. J. Microbiol. 19: 1103-1110.

STEWART, J.E. and B. ARIE. IN PRESS. Effectiveness of vancomycin against gaffkemia, the bacterial disease of lobsters (Genus Homarus). J. Fish. Res. Bd. Canada.

STEWART, J.E., B. ARIE, B.M. ZWICKER and J.R. DINGLE. 1969. Gaffkemia, a bacterial disease of the lobster, Homarus americanus: effects of the pathogen, Gaffkya homari, on the physiology of the host. Can. J. Microbiol. 15: 925-932.

STEWART, J.E. and D.M. FOLEY. 1969. A precipitin-like reaction of the hemolymph of the lobster Homarus americanus. J. Fish. Res. Bd. Canada 26: 1392-1397.

STEWART, J.E. and H. RABIN. 1970. Gaffkemia, a bacterial disease of lobsters (Genus Homarus). In a symposium on diseases of fishes and shellfishes. S.F. Snieszko (Editor) Am. Fish. Soc. Spec. Publ. 5: 431-439.

STEWART, J.E. and B.M. ZWICKER. 1972. Natural and induced bactericidal activities in the hemolymph of the lobster, Homarus americanus: products of hemocyte-plasma interaction. Can. J. Microbiol. 18: 1499-1509.

STEWART, J.E. and B.M. ZWICKER. 1974. Induction of internal defense mechanisms in the lobster, Homarus americanus. In Invertebrate Immunology. E.L. Cooper (Editor), Contemporary Topics in Immunobiology, Vol. 4, pp. 233-239. Plenum Press, New York.

Table 1. Composition of Lobster Hemolymph Medium (LHM)
(From Paterson and Stewart, 1974)

Constituent	g/l
$MgCl_2 \cdot 6H_2O$	1.0
NaCl	28.4
$MgSO_4 \cdot 7H_2O$	2.0
$CaCl_2 \cdot 2H_2O$	2.25
KCl	0.7
Dextrose	0.5
MEM essential amino acids + glutamine (50x)	20 ml/l
MEM Vitamins solution* (100x)	10 ml/l
$Na_2HPO_4 \cdot 7H_2O$	0.125
Phenol Red	1 ml/l
Sodium Bicarbonate	3.0
H_2O (glass distilled)	1.0 liter
Adjust to pH 7.6 with 1 N NaOH	

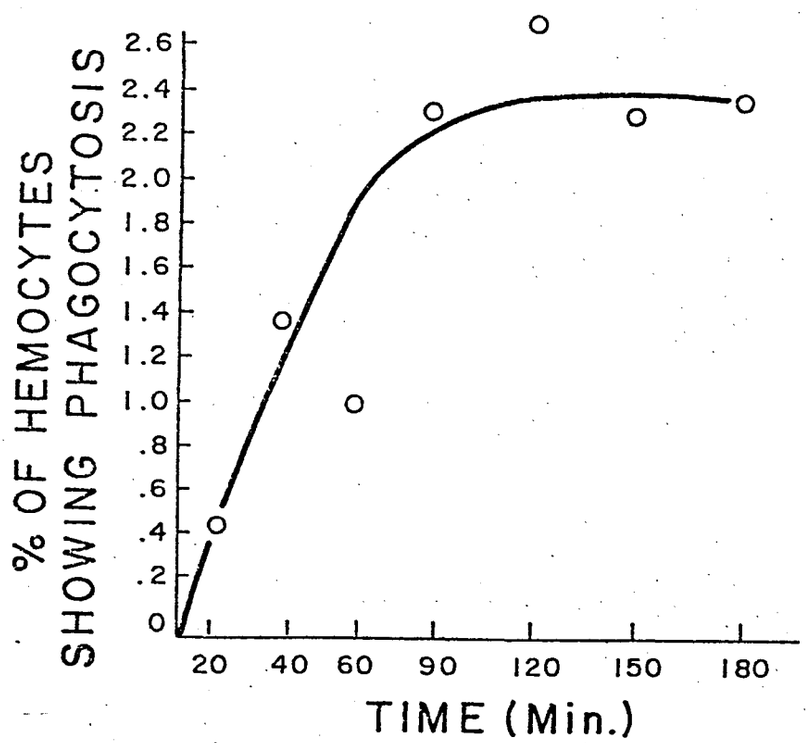

Fig. 1 - Dynamics of phagocytosis of opsonized erythrocytes (SRBC) hemocytes from normal lobsters at 15°C. (From Paterson and Stewart, 1974)

TABLE 2 | EFFECT OF VARIOUS VACCINES* ON THE PHAGOCYTOSIS OF OPSONIZED AND UNTREATED SHEEP ERYTHROCYTES BY HEMOCYTES OF THE AMERICAN LOBSTER

VACCINE ADMINISTERED	PERCENT■ OF HEMOCYTES SHOWING PHAGOCYTOSIS		NUMBER ERYTHROCYTES PHAGOCYTOSED / 10^3 HEMOCYTES		PERCENT□ RESPONSE
	OPSONIZED ERYTHROCYTES	UNTREATED ERYTHROCYTES	OPSONIZED ERYTHROCYTES	UNTREATED ERYTHROCYTES	
CONTROL (ASW)	1.6	0.3	47	3	0 (0/5)
PSEUDOMONAS PEROLENS ENDOTOXIN	4.4	2.6	302	71	60 (3/5)
PSEUDOMONAS PEROLENS (FORMALIN KILLED)	4.9	1.6	119	51	75 (3/4)
AEROCOCCUS VIRIDANS (FORMALIN KILLED)	1.9	0.4	100	6	20 (1/5)

*PHAGOCYTOSIS MEASURED 1 wk AFTER LAST OF 4 WEEKLY INJECTIONS.

■MEAN VALUES FROM 4-6 ANIMALS.

□VALUES GREATER THAN THE ASW CONTROL MEAN + 95% CL CONSTITUTED A POSITIVE RESPONSE.

TABLE 3 EFFECT OF VARIOUS VACCINES* ON THE PHAGOCYTOSIS OF OPSONIZED AEROCOCCUS VIRIDANS VAR. HOMARI BY HEMOCYTES OF THE AMERICAN LOBSTER

VACCINE ADMINISTERED	PERCENT■ OF HEMOCYTES SHOWING PHAGOCYTOSIS	NUMBER OF BACTERIA PHAGOCYTOSED / 10^3 HEMOCYTES	PERCENT□ RESPONSE
CONTROL (ASW)	3.6	393	0 (0/5)
PSEUDOMONAS PEROLENS ENDOTOXIN (200 mg/km)	11.4	1093	83.5 (5/6)
FORMALIN KILLED A. VIRIDANS	7.5	648	75 (3/4)

*PHAGOCYTOSIS MEASURED 1 wk AFTER LAST OF 4 WEEKLY INJECTIONS

■MEAN VALUES FRON 4-6 ANIMALS.

□VALUES GREATER THAN THE ASW CONTROL MEAN + 95% CL CONSTITUTED A POSITIVE RESPONSE.

TABLE 4 EFFECT OF VARIOUS VACCINES* ON PHAGOCYTOSIS OF OPSONIZED SHEEP ERYTHROCYTES BY HEMOCYTES OF THE AMERICAN LOBSTER

VACCINE ADMINISTERED	PERCENT■ OF HEMOCYTES SHOWING PHAGOCYTOSIS	NUMBER OF ERYTHROCYTES PHAGOCYTOSED / 10^3 HEMOCYTES	PERCENT□ RESPONSE
CONTROL (ASW)	1.0	11.6	0 (0/5)
PSEUDOMONAS PEROLENS ENDOTOXIN (200 mg/km)	3.3	75	67 (4/6)
AEROCOCCUS VIRIDANS (FORMALIN KILLED)	1.9	30	75 (3/4)

*PHAGOCYTOSIS IN MEASURED 1 wk AFTER LAST OF 4 WEEKLY INJECTIONS

■MEAN VALUES FROM 4-6 ANIMALS

□VALUES GREATER THAN THE ASW CONTROL MEAN + 95% CL CONSTITUTED A POSITIVE RESPONSE.