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# Clinical trial results on the use of a recombinant feline interferon-ω to protect Japanese pearl oysters *Pinctada fucata martensii* from akoya-virus infection

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ABSTRACT: Japanese pearl oysters (akoya oyster: *Pinctada fucata martensii*) are currently suffering mass mortalities from akoya-virus infection. In the present study, akoya oysters were injected with an anti-viral, recombinant feline interferon-ω (rFeIFN-ω) in an attempt to confer resistance to this virus. In infectivity experiments, oysters were twice injected with rFeIFN-ω at 1 mega unit $kg^{-1}$ of the meat weight. They were challenged with a single inoculation of cultured akoya-virus and held for 20 to 30 d at 25°C. Control oysters received only the viral challenge without rFeIFN-ω administration. In prophylactic experiments, oysters that were given the akoya-virus on Days 1 to 5 after rFeIFN-ω administration showed lower mortalities. Furthermore, the survivors had fewer muscular lesions resulting from the virus infection than the controls. In treatment experiments, the virus was inoculated on Days 1 to 3 before rFeIFN-ω administration. None of the treated oysters died within a 30 d experimental period. Survivors displayed repaired lesions with fibrous tissues that were produced by enhanced agranulocytes in the body musculature. Moreover, rFeIFN-ω was not toxic to akoya oysters. Thus, rFeIFN-ω administration is efficacious in preventing mortality of akoya oysters with akoya-virus infection.

KEY WORDS: Japanese pearl oyster · Akoya-virus infection · Recombinant feline interferon-ω · Efficacy in enhancement · Agranulocyte · Collagen fiber production



## INTRODUCTION

Since 1994 mass mortalities have been occurring in cultured Japanese pearl oysters (akoya oyster: *Pinctada fucata martensii*) in all western regions of Japan. In recent years, millions of Chinese pearl oysters *P. fucata* ssp. have been imported from Hainan Island to replace the akoya oysters lost in the mass mortalities. As a matter of fact, large numbers of Chinese pearl oysters were illegally imported in 1994 (Wada 1997). Shortly afterwards, great numbers of akoya and Chinese pearl oyster hybrids were produced because the hybrids are resistant to the present mortality. Chinese pearl oysters produce pearls of a poor quality even when they are implanted in pieces of the akoya oyster mantle lobe with pearl cores and reared in Japanese bays. Similarly, it has been found that pearls produced by the hybrids are also of low quality. Nevertheless, Japanese pearl farmers use Chinese pearl oysters and the hybrids for pearl cultures because of a shortage of akoya oysters. This illegal importation of Chinese pearl oysters and production of the hybrids have been tacitly permitted by the Japanese Fisheries Agency. DNA differences have not been examined but each oyster possesses different shell morphologies; thus, hybridization suggests that akoya and Chinese pearl oysters differ at

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the subspecies level (if they are different at all). Chinese pearl oysters and hybrids are freely placed in many bays that contain both cultured and wild akoya oysters, which readily permits natural hybridization. Because Chinese pearl oysters that were imported several years ago have matured, it is likely that wild hybrids are already inhabiting some bays in Japan (Wada pers. comm.). Such natural hybridization will continue in the future as long as Chinese pearl oysters are illegally imported. Because hatcheries sometimes introduce wild oysters for spawners, wild hybrids will likely be used for spawning. Oyster farmers rear both artificial oyster seeds from hatcheries and those derived from wild oyster larvae to produce pearls. This practice in the culture of pearls will increase both artificial and wild hybrids and will decrease pearl quality. A major concern is that if such natural hybridization is allowed to continue purebred akoya oysters that produce high quality pearls will be completely lost in Japan.

As we recently showed, a previously undescribed virus (akoya-virus) is the etiological agent of the present mass mortality. The virus infects smooth muscle fibers in the adductor, pallial and peduncle musculatures as well as the cardiac muscle, causing atrophy, various kinds of degeneration and necrosis (Miyazaki et al. 1999). Infected akoya oysters die of a combination of physiological changes that affect feeding, respiration, glucose metabolism and cardiac function. Artificial infection with viral cultures (Miyazaki et al. 1999) revealed the virus to be pathogenic to akoya oysters and replicated the pathological changes and high mortality that occurred in the natural outbreaks. Therefore, in the present study, we attempted to prevent the mortality of akoya oysters suffering from akoya-virus infection and introduced an anti-viral, recombinant feline interferon-ω (rFeIFN-ω) for clinical use. rFeIFN-ω is produced in silkworms by a recombinant baculovirus (Sakurai et al. 1992, Ueda et al. 1993) and has anti-viral activity for feline calicivirus and herpesvirus (Yamamoto et al. 1990, Uchino1997) as well as canine parvovirus (Uchino 1997, Ishiwata et al. 1998). Thus, rFeIFN-ω has a cross-species anti-viral activity. We did not expect this cross-species protection to extend to akoya oysters. However, rFeIFN-ω is produced by silkworms and the silkworms, like the akoya oyster, is an invertebrate. So, we considered it likely that some 3-dimensional structure of rFeIFN-ω could effectively stimulate akoya oyster cells that are involved in the defense system. The present paper reports the efficacy of rFeIFN-ω administration in preventing mortality in infectivity experiments and describes the defensive reactions of the treated akoya oysters. This is the first report of the efficacy of IFN administration in a mollusc viral disease.

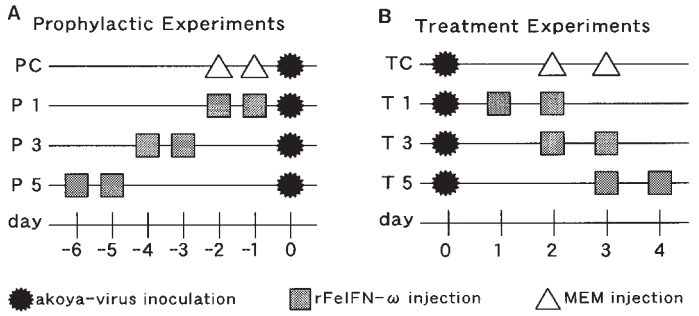
## MATERIALS AND METHODS

**Akoya oysters, and inoculations of IFNs and akoya-virus.** Healthy akoya oysters were selected from oyster groups collected from culturing areas that experienced few mortalities. The mean wet weight of the meat of experimental oysters was approximately 15 g. Ten mega units (MU) of rFeIFN-ω (Toray, Japan) was dissolved in 65 ml of Eagle's minimum essential medium (MEM: Nissui, Japan). The rFeIFN-ω solution (0.1 ml), which corresponded to a dose of approximately 1 MU $kg^{-1}$ wet weight of the oyster meat, was immediately injected into the mantle lobe of the oysters. The injected volume of rFeIFN-ω solution was adjusted slightly depending on the size of the oysters. rFeIFN-ω was injected twice into each oyster at a 24 h interval. For comparison, an anti-viral interferon IFN-α (Serotec, England) which was produced by human lymphocytes was also injected in the same manner and concentration as rFeIFN-ω. Akoya-virus was cultured in EPC (epithelioma papilosum cyprini) cells from different diseased oysters and the primary isolates of akoya-virus were mixed and inoculated by a single injection into the mantle lobe of the oysters in accordance with to Miyazaki et al. (1999). As shown by Miyazaki et al. (1999), the virus never grew in a small volume of medium inside a small well of a 96-well plate (microtiter plate), so the virus titer of inocula was not measured in the present studies. After the injections, all oysters were held without feeding in aquaria with a circulatory system using artificial seawater at 25°C for 20 to 30 d.

**Prophylactic experiments.** In a first set of experiments, 3 experimental groups of oysters were inoculated once with akoya-virus on Days 1, 3 and 5 after the second rFeIFN-ω injection. The control group was injected with MEM instead of rFeIFN-ω and was inoculated with the virus on Day 1 after the second MEM injection (Fig. 1A). Another negative control group was established to examine the toxicity of rFeIFN-ω and received only a couple of rFeIFN-ω injections. Each of these 5 groups contained 11 oysters.

In a second set of experiments, 1 experimental group was established to reevaluate the prophylactic efficacy of rFeIFN-ω administration. Viral challenge was performed on Day 1 after the second rFeIFN-ω injection. A control group was established and treated in the same way as the first part of the first experiment. Each of these 2 groups contained 10 oysters.

**Treatment experiments.** In a first set of experiments, 2 experimental groups were established. One group received rFeIFN-ω injections on Days 2 and 3 after the akoya-viral inoculation, and the other group received rFeIFN-ω injections on Days 3 and 4 after the viral inoculation. A control group received MEM injections



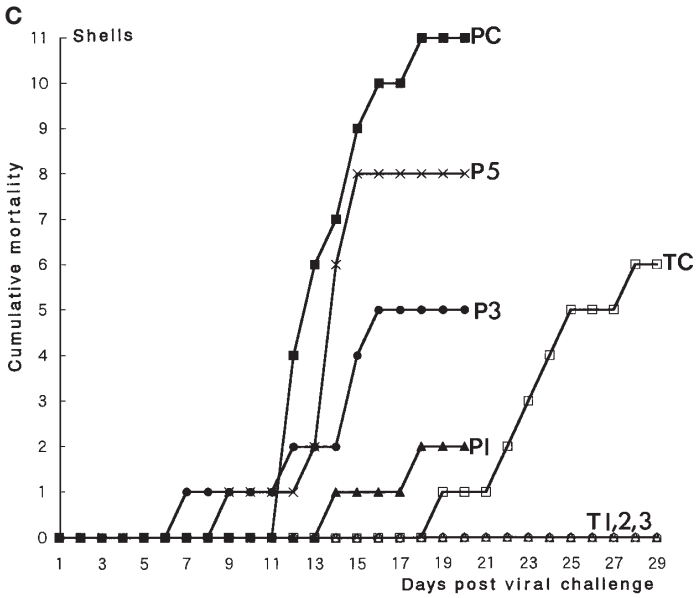




Fig. 1. (A) Protocol of prophylactic experiments. (B) Protocol of treatment experiments. (C) Mortality graphs in prophylactic and treatment experiments with rFeIFN-ω administration. Ps indicate prophylactic experimental groups (20 d, 11 oysters). PC: a control received only viral challenge; P1: viral challenge on Day 1 after a second rFeIFN-ω injection; P3: viral challenge on Day 3 after rFeIFN-ω administration; P5: viral challenge on Day 5 after rFeIFN-ω administration. Ts indicate treatment experimental groups (30 d, 10 to 11 oysters). TC: a control received only viral challenge; T1: viral challenge on Day 1 before a first rFeIFN-ω injection; T2: viral challenge on Day 2 before rFeIFN-ω administration; T3: viral challenge on Day 3 before rFeIFN-ω administration

on Days 2 and 3 after the viral inoculation. Each of these 3 groups contained 10 oysters (Fig. 1B).

The second treatment experiment was simultaneous with the second prophylactic experiment. One experimental group was injected with rFeIFN-ω on Days 1 and 2 after the viral inoculation. Another group was injected on Days 1 and 2 after the viral inoculation with human IFN-α at the same dose as that of rFeIFN-ω to compare the efficacy between 2 IFNs. Each of these 2 groups contained 11 oysters. The control was the same one that was set up in the second prophylactic experiment.

**Histopathological and electron microscopic examinations and viral reisolation.** All moribund oysters and recently dead oysters were removed from the aquaria and processed for histopathological and electron microscopic examinations and viral reisolation according to Miyazaki et al. (1999). At the end of the experimental period, all surviving oysters were removed from the aquaria and processed in the same way as the moribund and dead oysters.

## RESULTS

### Prophylactic efficacy

In the first prophylactic experiments, the control group that was injected with akoya-virus but not administered rFeIFN-ω showed 100% mortality by Day 17. The group that was challenged with akoya-virus on Day 1 after rFeIFN-ω administration had 18% mortality by Day 20 of the experimental period. However, higher mortalities (45 and 73%) occurred by Day 16 in groups that were challenged on Day 3 or Day 5 respectively after rFeIFN-ω administration (Fig. 1C). In the second set of experiments, the group that was challenged on Day 1 after rFeIFN-ω administration had 30% mortality while the control group showed 60% mortality between Days 23 and 30.

Most oysters that died or were moribund by Day 17 after the viral challenge in both rFeIFN-ω-administered and the control groups displayed only slight atrophy in the body and the adductor muscle. However, oysters that died or were moribund at the later (after Day 21) experimental period displayed an atrophied body and adductor muscle with a yellowish coloration. Oysters that were alive at the end of experimental period did not show any distinct change except for slight atrophy in the body. In both the control groups and the groups which received prophylactic rFeIFN-ω administration, oysters that died or were moribund late in the experimental period displayed extensive virally infected lesions in the musculatures of the adductor, mantle lobes, peduncle and heart. These muscular lesions displayed necrosis, vacuolization or atrophy of muscle fibers and infiltration by many agranulocytes and granulocytes (Fig. 2A). These signs are the same as those observed in the natural outbreak (Miyazaki et al. 1999). Granulocytes could be distinguished by the presence of periodic acid-Schiff reaction (PAS)-positive material within the cytoplasm while agranulocytes had low level of PAS-

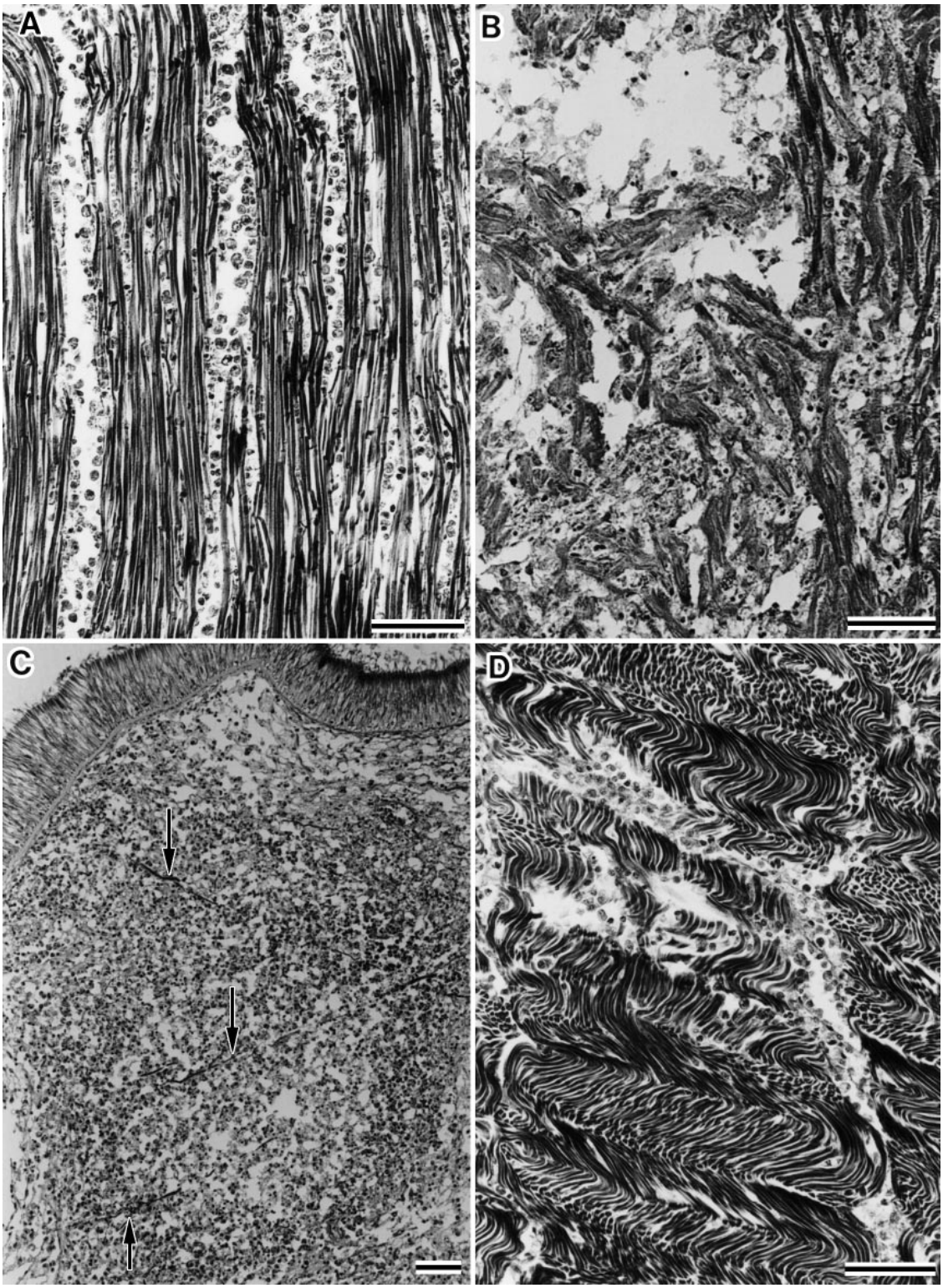



Fig. 2. *Pinctada fucata martensii.* (A) Adductor muscle of a moribund oyster in a control group. Muscle fibers are extensively necrotized, atrophied and have been penetrated by many hemocytes. (B) Heart of a moribund oyster in a P3 group. Cardiac muscle fibers are extensively necrotized, destroyed and have been penetrated by many hemocytes. (C) Visceral connective tissue of a moribund oyster in a P3 group. Many hemocytes accumulate around the necrotized muscle fibers (arrows). (D) Adductor muscle of a survivor in a P1 group. Muscle fibers are partially necrotized and have been penetrated by hemocytes. Azan stain. Scale bars = 50 µm

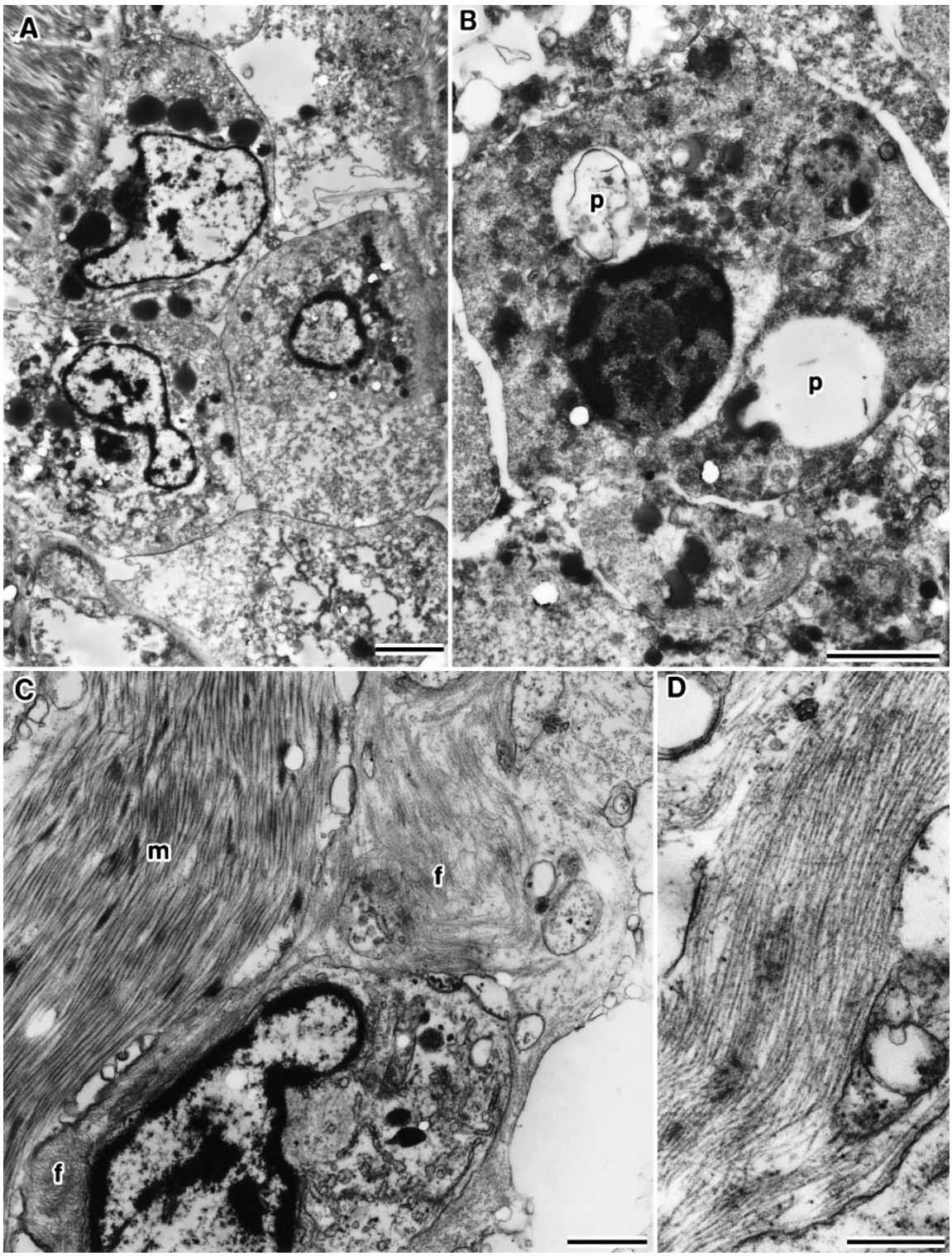



Fig. 3. *Pinctada fucata martensii.* Electron micrographs of lesions in the adductor muscles of control oysters. (A) In a moribund oyster, agranulocytes possessing many lysosomes penetrate necrotized muscle fibers. Scale bar = 2 µm. (B) High power view of an agranulocyte. It possesses many lysosomes, and forms phagosomes but no fibril. Scale bar = 2 µm. (C) A myofibroblast appeared in a healing lesion in a survivor. The cell actively produces many collagen fibrils within the cytoplasm. Abundant ribosomes, rough endoplasmic reticula and mitochondria are enclosed around a nucleus within a membrane-like structure. Scale bar = 1 µM. (D) High power view of collagen fibrils in a myofibroblast. They have prominent banding patterns. Scale bar = 500 nm. f: collagen fibrils; m: myofibrils of a smooth muscle fiber; p: phagosome

positive granules. Agranulocytes possessing many lysosomes (Fig. 3A,B) predominantly migrated into infected lesions but their phagocytic activity within the necrotized muscle fibers was not so prominent. The number of nutrient-storing cells (NS cells) had decreased among digestive caeca which had markedly flattened epithelia due to the long-term suspension of feeding. Many hemocytes accumulated focally around the necrotized muscle fibers in the connective tissues located around the digestive caeca and gonadal follicles, beside the intestine, and in the lips of mantle lobe. On the other hand, moribund and dead oysters which appeared by Day 17 in both the rFeIFN-ω-administered and the control groups displayed more extensive necrotic lesions in the cardiac muscle (Fig. 2B) and/or marked hemocyte accumulations around necrotized muscle fibers in the connective tissues (Fig. 2C), whereas necrotic lesions were less severe in the adductor and peduncle musculatures. The number of NS cells was only slightly decreased.

In contrast with the dead or moribund oysters, oysters that were administered rFeIFN-ω and survived (hereafter survivors) also possessed virally infected, muscular lesions but the lesions were very small in size, occurred in only a few areas, and did not occur systemically. In these lesions, muscle fibers were partially necrotized and accompanied by hemocyte infiltration in the musculatures of the adductor (Fig. 2D), mantle lobes, peduncle or heart (Fig. 4A). The number of NS cells had decreased among the digestive caeca that had flattened epithelia and expanded lumens.

The putative virus was reisolated from the adductor muscles and hearts of all surviving and moribund oysters that were examined.

### Treatment efficacy

In the 2 treatment experiments, both control groups showed 60% mortality within Days 21 to 30 (Fig. 1C), and dead or moribund oysters displayed an atrophied body and adductor muscle with a yellowish coloration. These dead or moribund oysters displayed extensive necrotic lesions with hemocyte infiltration in the musculatures of the adductor, mantle lobes, peduncle and heart. In contrast, the survivors had small-sized necrotic lesions that had healed and were replaced by loose fibrous tissue composed of thin collagen fibers in the musculatures of the adductor (Fig. 4B) and peduncle. EM of the healed lesion in the adductor muscle revealed cells that actively produced many microfibrils within the cytoplasm which possessed many ribosomes, rough endoplasmic reticula (rER) and mitochondria (Fig. 3C). The features of these cells were similar to those of mammalian myofibroblasts (Ross et al. 1989). In the putative myofibroblasts, organelles were located around a nucleus and enclosed by a thin membranous structure, and outside the membrane, many microfibrils were present with swollen mitochondria and endoplasmic reticula. Newly produced microfibrils were mostly thin and short, but thickened fibrils were long and possessed the same prominent banding pattern as collagen fibers (Fig. 3D). In the heart, a meshwork of thin collagen fibers formed around the cardiac muscle fibers that had atrophied and necrotized as result of the viral infection (Fig. 4C). Similar fibrous tissue was produced in the foci of massively accumulated hemocytes in the visceral connective tissues. These changes suggest a natural healing of the akoya-virus-infected lesions because similar lesions were observed in some oysters that were collected at the end of the natural outbreaks in winter (Miyazaki unpubl. data).

On the other hand, none of the 3 rFeIFN-ω-administered groups had any dead oysters by the end of experimental period, and all of the survivors showed slight atrophy in the body and the adductor muscle. The survivors usually had repaired lesions that extensively replaced the necrotic lesions in the musculatures of the adductor, mantle lobes and peduncle. In the advanced state of the repair process, dense connective tissue consisting of thick collagen fibers and large numbers of hemocytes penetrated the necrotized muscle fibers and developed between the atrophied muscle fibers, but no regenerating muscle fibers appeared (Fig. 4D). EM of the repaired lesion revealed that many agranulocytes phagocytized and produced microfibrils within the cytoplasm on/in necrotized muscle fibers (Fig. 5A,B). In the early state of repair, agranulocytes possessing many lysosomes formed large phagosomes containing cellular debris and also produced many short microfibrils within the cytoplasm (Fig. 5A). Another agranulocyte possessing few lysosomes but abundant ribosomes and rER, and large numbers of mitochondria, actively produced many microfibrils in the entire cytoplasm (Fig. 5 B). In these agranulocytes, the organelles were not so distinctly separated from microfibrils in the cytoplasm. In the advanced state of repair, elongated and bundled collagen fibrils extended around phagosome-like vacuoles and a nucleus of agranulocytes whose organelles and plasma membrane had disappeared (Fig. 5C). In different locations, many thick bundles of collagen fibrils were densely arrayed (Fig. 5D). However, the collagen fibrils that were produced by agranulocytes did not possess the same banding pattern as those of the putative myofibroblasts that were found in healing lesions of control oysters. The putative myofibroblasts were almost absent in these lesions. The muscle fibers

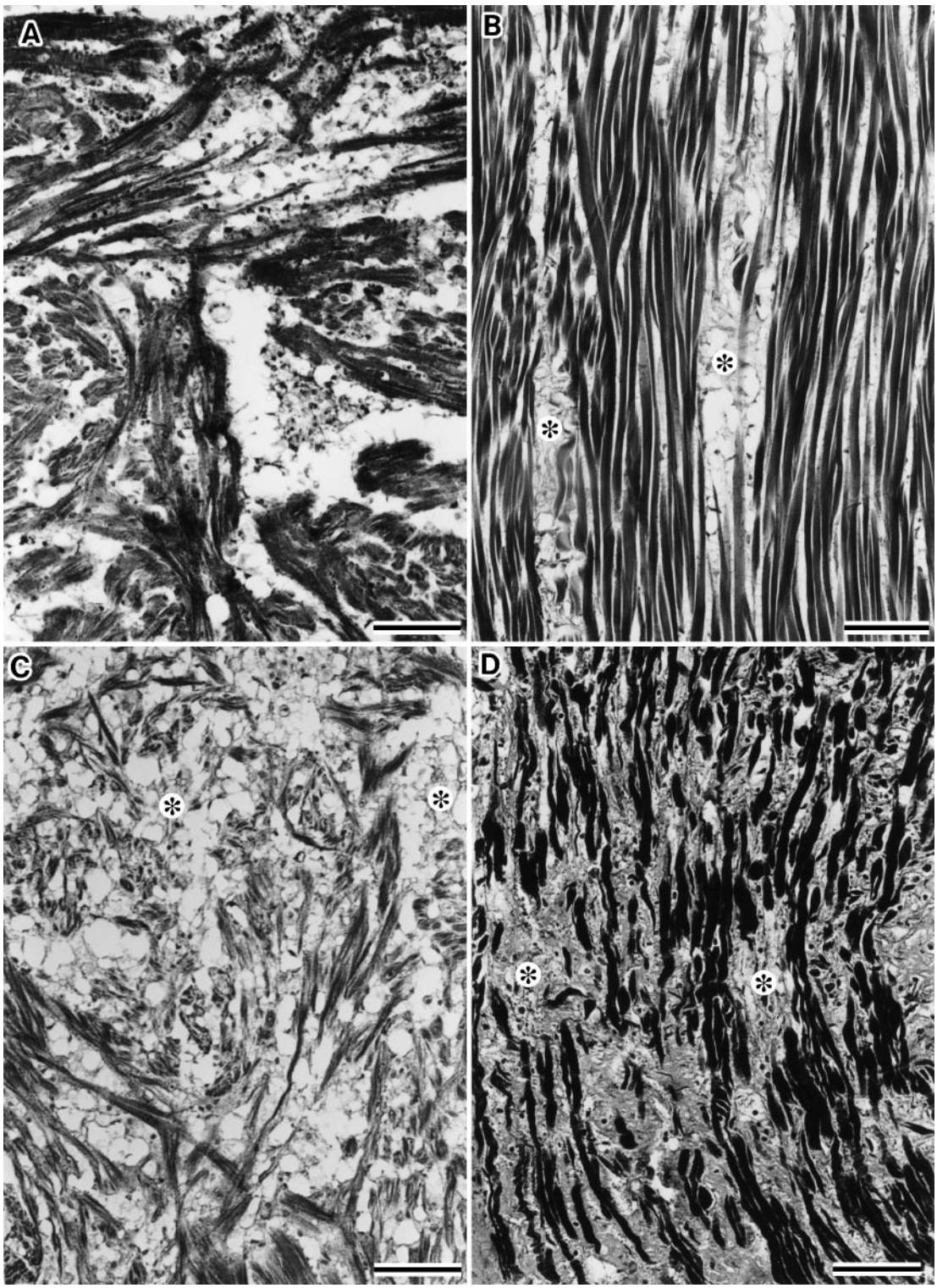



Fig. 4. *Pinctada fucata martensii.* (A) Heart of a survivor in a P1 group. Cardiac muscle fibers are partially necrotized and have been penetrated by hemocytes. (B) Adductor muscle of a survivor in a TC group. Affected muscle fibers have been replaced by fibrous connective tissue consisting of thin collagen fibers. (C) Heart of a surviror in a TC group. Meshwork of thin collagen fibers has formed among thinned and fragmented cardiac muscle fibers. (D) Repaired lesion in the adductor muscle of a survivor in a T3 group. Dense fibrous tissues have replaced necrotized muscle fibers and developed between the thinned muscle fibers which remained in the lesion. Azan stain. Scale bars = 50 µm. ✻: Representative areas of fibrous tissues

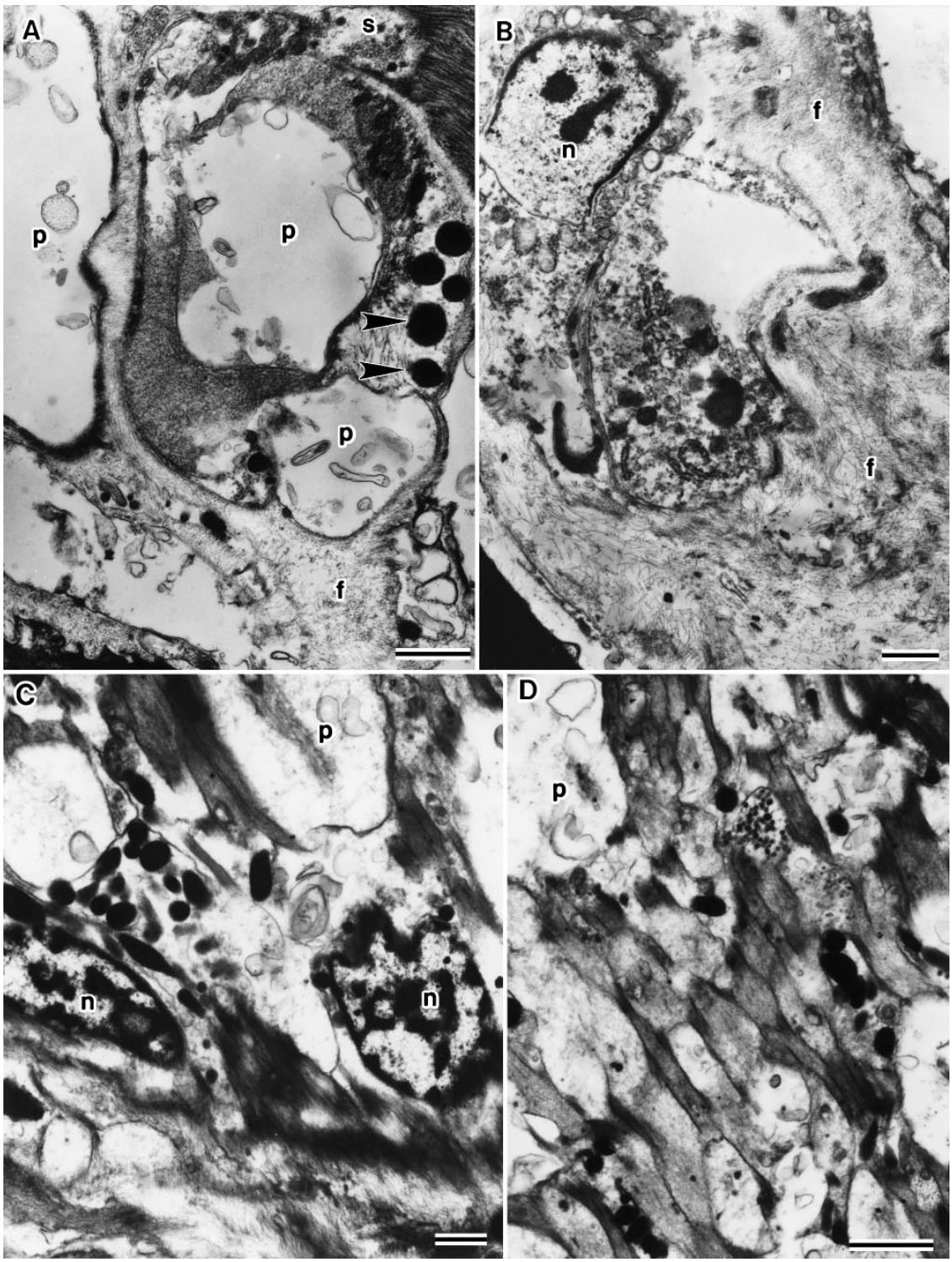



Fig. 5. *Pinctada fucata martensii.* Electron micrographs of repairing lesions in the adductor muscles of survived oysters in treatment experiments. (A) Agranulocyte having many lysosomes (arrowheads show 2 of them) forms large phagosomes and produces microfibrils in the cytoplasm. Scale bar = 1 µm. (B) Agranulocyte possessing abundant rER and ribosomes produces numerous microfibrils. Scale bar = 1 µm. (C) Advanced stage of fiber production by agranulocytes. Thick collagen fibrils densely develop around nuclei and phagosomes. High electron-dense bodies may be natural pearl cores. Scale bar = 1 µm. (D) In the advanced state of repair, thick bundles of collagen fibrils are densely arrayed. These collagen fibrils that are produced by agranulocytes show no banding pattern. Scale bar = 2 µm. f: microfibrils; n: nucleus; p: phagosomes; s: synapsis

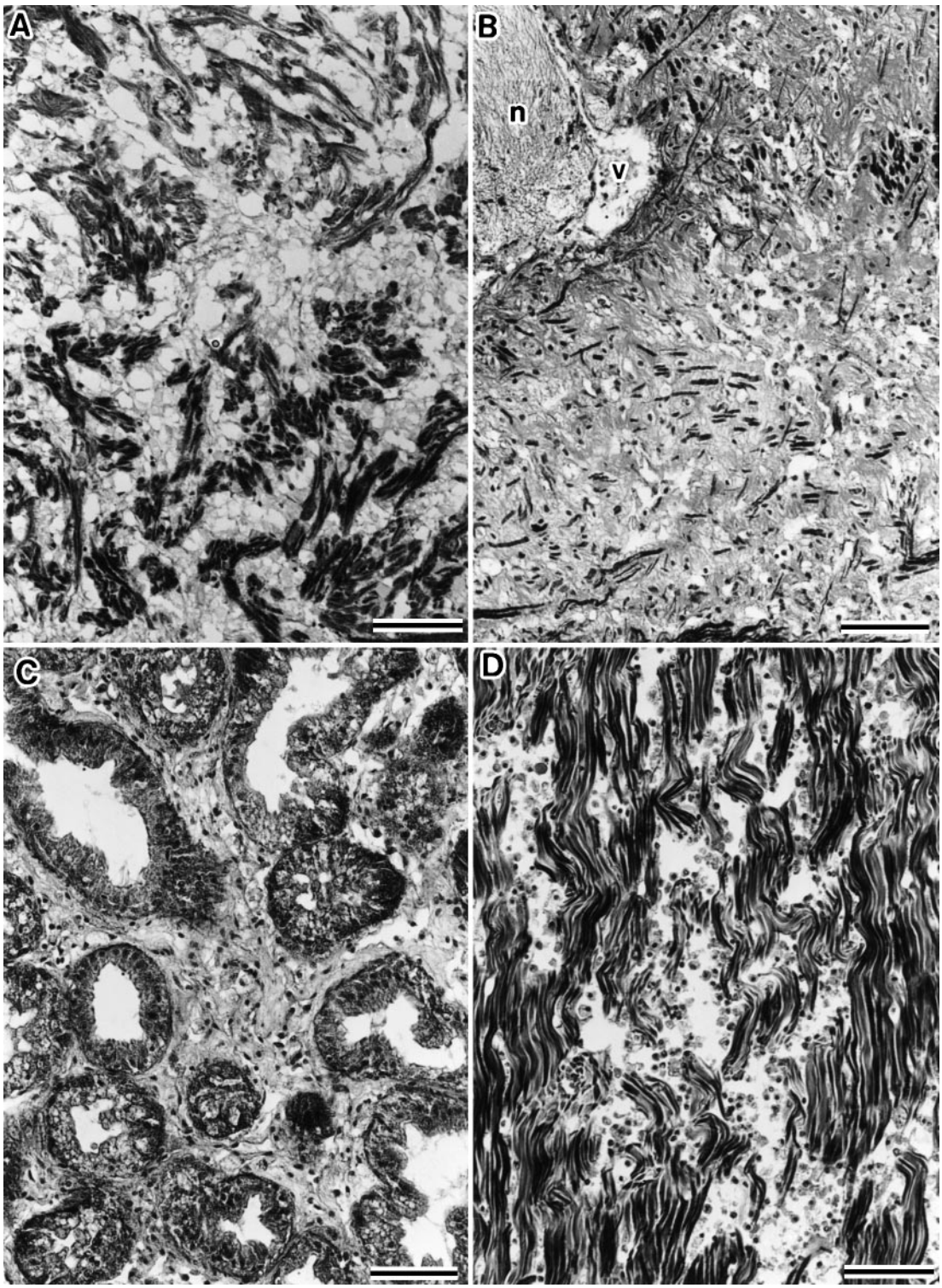



Fig. 6. *Pinctada fucata martensii.* (A to C) Tissues of survivors in a T2 group. (A) In the heart, meshwork of thick collagen fibers has formed among atrophied and fragmented cardiac muscle fibers. (B) In lip of mantle lobe, thick collagen fibers are produced in the connective tissue containing atrophied and fragmented muscle fibers. (C) Digestive caeca have flattened epithelia with a decreased number of phagosomes. The surrounding connective tissue contains thickened collagen fibers. Nutrient-storing cells had disappeared among digestive caeca. (D) Adductor muscle of a moribund oyster in a IFN-α-administered group. Muscle fibers are extensively necrotized and infiltrated with many hemocytes. Azan stain. Scale bars = 50 µm. n: pallial nerve fiber, v: pallial vessel

that remained in the repaired lesions had fine myofibrils while glycogen granules were markedly decreased in the myoplasm. It was difficult to determine whether akoya-virus was replicating in these affected muscle fibers. A similar production of thick collagen fibers was markedly observed in myocardial lesions (Fig. 6A). Thick collagen fibers were formed also in connective tissues in the lips of the mantle lobe (Fig. 6B) and around digestive caeca which had flattened epithelia (Fig. 6C). NS cells had almost disappeared (Fig. 6C).

The putative virus was not reisolated from the adductor muscles of any survivor in the rFeIFN-ω-administered groups.

### rFeIFN-ω toxicity and IFN-α efficacy

The group examined for rFeIFN-ω toxicity had no mortality by the end of the 30 d experiment. None of oysters showed any remarkable change except for a slight atrophy in the body and lesions in the musculatures or visceral organs. However, the connective tissues contained thick collagen fibers.

The group that was administered IFN-α instead of rFeIFN-ω showed 82% mortality between Days 21 and 30. Moribund and dead oysters that appeared in the late experimental period displayed an atrophied body and adductor muscle with a yellowish coloration. A total of 9 moribund and dead oysters displayed extensive necrosis with hemocyte infiltration in the musculatures of the adductor (Fig. 6D), peduncle and mantle lobe. The other 2 surviving oysters had limited necrotic lesions in these musculatures.

## DISCUSSION

In the present study, we attempted to prevent or decrease the mortality of akoya oysters suffering from akoya-virus infection by injecting them with anti-viral rFeIFN-ω. The results of this study revealed that rFeIFN-ω administration was efficacious in limiting akoya-virus-infected lesions or in repairing the virally caused lesions in the musculatures of the adductor, mantle lobes, peduncle and heart. The treatment appears to depend on the repair of virally caused lesions in the musculatures and was very effective in preventing mortality of infected oysters. The repairing reaction was characterized by marked production of fibrous tissues consisting of thick collagen fibers in addition to phagocytosis by agranulocytes in/around virally affected muscle fibers. Collagen synthesis in repairing lesions was different between oysters in the rFeIFN-ω-administered and control groups. In the case of natural healing of the muscular lesions in control oysters that did not receive rFeIFN-ω, myofibroblasts produced collagen fibrils with the banding pattern inside the cytoplasm. As shown in the 'Results', the state of collagen production of oyster myofibroblasts resembled that of mammalian myofibroblasts (Ross et al. 1989). Thus, in natural healing, muscular lesions were replaced by fibrous tissues produced by myofibroblasts.

In contrast, in repaired lesions of the rFeIFN-ω-administered oysters, agranulocytes performed both phagocytosis and production of the collagen fibrils as shown in Fig. 5A,B. Previous studies of pearl sac formation and wound healing in akoya oysters demonstrated that agranulocytes, as well as granulocytes, have phagocytic activity (Suzuki et al. 1991, Wada 1991, Suzuki & Awaji 1995), which has also been shown in other mollusca (Reade & Reade 1972, Moore & Lowe 1977, Takahashi & Mori 1995). Moreover, according to these studies, agranulocytes have been proposed to produce a collagen matrix in the healing lesions of akoya-oysters (Suzuki et al. 1991, Suzuki & Funakoshi 1992, Suzuki & Awaji 1995). Collagen fibrils that were produced by agranulocytes did not possess banding patterns different from those produced by myofibroblasts.

It was very interesting that agranulocytes performed both phagocytosis and marked production of collagen fibrils in the repaired lesions of rFeIFN-ω-administered oysters, while in non-rFeIFN-ω-administered oysters, agranulocytes only performed phagocytosis and myofibroblasts produced collagen fibrils to repair the virally caused lesions. Therefore, it is considered likely that rFeIFN-ω administration enhances the potential of agranulocytes to produce collagen fibrils in addition to phagocytosis. As a result, muscle fibers that were necrotized and destroyed due to akoya-virus infection were phagocytized by enhanced agranulocytes and replaced with the fibrous tissues produced by enhanced agranulocytes. These agranulocytes activities might remove the akoya-virus from necrotized muscle fibers and lead to the repair of the virally caused lesions. In addition to the repaired muscular lesions, thickened collagen fibers were observed in the connective tissues in treated oysters. Relatively thickened collagen fibers were also observed in the connective tissues of oysters that were administered only rFeIFN-ω. These findings suggest that rFeIFN-ω also stimulates cells in the connective tissue (possibly agranulocytes and fibroblasts) to produce collagen fibers.

The prophylactic rFeIFN-ω administration was not completely effective in preventing mortality. In the survivors, the virally infected lesions were prevented from occurring systemically and were only allowed to form on a small scale in a few areas in the muscula-

tures. It is well known that mammalian interferons (IFNs) stimulate vertebrate cells to produce an antiviral protein that inhibits viral replication (Imanishi 1997). It is likely that rFeIFN-ω administered prior to akoya-virus inoculation stimulates granulocytes and agranulocytes to attack injected akoya-virus, and as a result, limits the extent of muscular lesions. Thus, rFeIFN-ω stimulates a non-specific host response and rFeIFN-ω-administered oysters remained alive longer than the non-treated controls. rFeIFN-ω administration was effective as a prophylactic when it was administered soon before an akoya-virus inoculation. However, the prophylactic was not completely efficacious, and it was less efficacious when viral invasions occurred several days after rFeIFN-ω administration. This lower efficacy appears to be due to the short-life of rFeIFN-ω (Ishiwata et al. 1998) and probably to the short-term period of enhancement of hemocyte antiviral activities.

In contrast to administration of rFeIFN-ω, administration of human IFN-α was not efficacious in akoya-virus-infected oysters. It is likely that all IFNs are not efficacious in preventing this mollusc viral disease. We suspect that rFeIFN-ω might have a 3-dimensional structure that allows it to bind to some receptor of akoya oyster hemocytes and enhance the potential of these cells. We are presently attempting to determine whether such receptors are present on hemocytes and how rFeIFN-ω activates hemocytes.

The results of this study demonstrate that rFeIFN-ω administration is effective in both the prophylaxis and treatment of akoya-virus infection. In natural outbreaks, it is impossible to determine when the akoya-virus will infect the oysters. Therefore, the prophylactic administration of rFeIFN-ω is not feasible in field use. It is effective as a prophylactic only when it is administered soon before an akoya-virus infection. Pearl farmers perform an operation to insert pearl cores into the body of the mother oysters. During this operation, the farmers can simultaneously and easily inject the mother oysters with rFeIFN-ω. Thus, field use of rFeIFN-ω injections for treatment of existing akoya-virus infections appears to be feasible during this operation. It is expected that if rFeIFN-ω is injected into the mother oysters during the operation, it will be efficacious in repairing their virally caused lesions, if they have any, and in reducing mortality of the operated oysters. On the other hand, injecting larval and young oysters with rFeIFN-ωi is not feasible because of their large numbers and small sizes. Severe mortality among larval and young oysters has usually occurred when they were exposed to high temperatures (over 25°C) for long periods. The severity of mortality due to akoya-virus infection will be higher when higher temperatures continue over a longer period of time. Our infectivity experiments, therefore, were performed at 25°C (Miyazaki et al. 1999). Some data indicate that mortalities are less severe in some pearl culturing areas when the period that the water temperature is over 25°C, is about 1 mo or less (a pearl farmer pers. comm.). This suggests that short durations of high temperatures during summer result in lower mortality of oysters with akoya-virus infection. Rearing young oysters in such areas appears to be effective in decreasing mortality although these areas are not plentiful in Japan. Thus, if a system of management of akoya oyster farming can be devised to prevent mass mortality due to akoya-virus, it will contribute toward restoring pearl culture.

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