Identification of squalamine in the plasma membrane of white blood cells in the sea lamprey, *Petromyzon marinus*

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Abstract **It is well established that innate mechanisms play an important role in the immunity of fish. Antimicrobial peptides have been isolated and characterized from several species of teleosts. Here, we report the isolation of an antimicrobial compound from the blood of bacterially challenged sea lamprey, *Petromyzon marinus*. An acetic acid extract from the blood cells of challenged fish was subjected to solid-phase extraction, cation-exchange chromatography, gel-filtration chromatography, and reverse-phase high-performance liquid chromatography, with the purified fractions assayed for antimicrobial activity. Surprisingly, antimicrobial activity in these fractions originated from squalamine, an aminosterol previously identified in the dogfish shark, *Squalus acanthias*. Further chromatographic and mass spectrometric analyses confirmed the identity of squalamine, an antimicrobial and antiangiogenic agent, in the active fraction from the sea lamprey blood cells. Immunocytochemical analysis localized squalamine to the plasma membrane of white blood cells. Therefore, we postulate that squalamine has an important role in the innate immunity that defends the lamprey against microbial invasion. The full biochemical and immunological roles of squalamine in the white blood cell membrane remain to be investigated.**—Yun, S-S. and W. Li. **Identification of squalamine in the plasma membrane of white blood cells in the sea lamprey, *Petromyzon marinus*. *J. Lipid Res.* 2007.** 48: **2579–2586.**

Supplementary key words aminosterol • innate immunity • neutrophils

It is well established that innate immunity plays an important role in the defense against bacterial and viral invasion and can be mediated by cellular and humoral elements (1). The cellular components of innate immunity include macrophages and neutrophils, whereas the humoral elements are represented by complement, lysozyme, lactoferrin, and low molecular weight antibiotic substances such as peptides, lipids, and alkaloids (1–4). There have been numerous reports of effector molecules associated with innate immunity and antimicrobial peptides in several well-known fish species. These molecules include pardaxin from the Moses sole *Pardachirus marmoratus* (5), chrysophsin from the red sea bream *Chrysophrys major* (6), pleurocidin from the winter flounder *Pleuronectes americanus* (7), parasin I from the catfish *Parasilurus asotus* (8), misgurin from the loach *Misgurnus anguillicaudatus* (9), hipposin from the Atlantic halibut *Hippoglossus hippoglossus* (10), and bass hepsidin, piscidins, and moronecidin from the hybrid striped bass (11–13).

The sea lamprey, *Petromyzon marinus*, is an ancestral jawless (Agnathan) fish with unique parasitic and migratory life stages (14). It is well established that Agnathans lack conventional adaptive immune components, including the major histocompatibility complex, immunoglobulins, and T-cell receptors, that are present in all jawed vertebrates (15). Even though lymphocyte-like cells have been described in lampreys (16, 17) and shown to transcribe novel variable lymphocyte receptors, consisting of leucine-rich repeats that can generate diversity through a somatic rearrangement process (18), the involvement of these receptors in adaptive immunity has not been established in the lamprey. Consequently, it is likely that the lampreys rely on an innate immune response as well as specific molecular compounds with properties that defend against pathogenic invasion. However, very little is known about the effector molecules involved in innate immunity in the sea lamprey. The purpose of this study was to identify antimicrobial substances that may play a role in innate immune defense.

In this study, we combined a series of separation techniques with standard antimicrobial assays to identify antimicrobial substances in the sea lamprey. We discovered the antimicrobial substance squalamine (an aminosterol) in the lamprey blood cells. Immunocytochemical localization of squalamine to the plasma membrane of the white blood cells indicates a possible function as an immune effector. The precise immunological role of this aminosterol in the white blood cells of the sea lamprey remains to be investigated.

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MATERIALS AND METHODS

Chemicals and equipment

The reagents ammonium acetate (NH_4OAc), acetic acid, *o*-phthalaldehyde (OPA), ninhydrin, mercaptoethanol, MES, Histopaque 1077, Freund's incomplete and complete adjuvants, ethyl acetate, and sodium chloride were purchased from Sigma (St. Louis, MO). The HPLC-grade solvents acetonitrile (ACN), methanol, and trifluoroacetic acid (TFA) were from EMD Chemicals (Gibbstown, NJ), Fisher Scientific (Pittsburgh, PA), and Pierce (Rockford, IL), respectively. The secondary antibody conjugated with Alexa Fluor 568 was purchased from Invitrogen (Carlsbad, CA). 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) was obtained from Pierce (Rockford, IL). Ion-exchange, gel-filtration, and reverse-phase liquid chromatography were performed on a Waters 600 delivery system equipped with a 996 PDA detector and a fraction collector (Waters, Milford, MA). Polycarbonate filter papers of 1 and 0.45 μm pore size were from Millipore (Bellerica, MA). Fluorescence was detected using a 474 detector (Waters). The centrifuges used were Allegra 6R (Beckman Coulter, Fullerton, CA) and KR 22i (Jouan, Inc., Winchester, VA). The bacterial strains *Micrococcus luteus* and *Escherichia coli* D31 were a kind gift from Dr. Xavier Lauth (University of California, San Diego). Squalamine lactate was a generous gift from Dr. Michael Zasloff (Georgetown University).

Experimental animals

Parasitic and adult sea lamprey were obtained from Lake Huron by the staff of the Hammond Bay Biological Station, United States Geological Survey. The lamprey were transported to the wet lab facility at Michigan State University (MSU) and kept in a 1,000 liter flow-through tank at 12°C. After acclimation, the lamprey were injected with 10^7 cells of log-phase *M. luteus* and *E. coli*. Eighteen hours after the bacterial challenge, blood was drawn using a heparinated syringe. The pooled blood sample was centrifuged at 3,000 rpm at 4°C for 20 min. The pellet was frozen in liquid nitrogen and kept at −80°C until extraction.

Extraction of blood cells

The pellet of blood cells was homogenized under liquid nitrogen, extracted in a 10% acetic acid solution with a protease inhibitor cocktail (Roche, Indianapolis, IN), and incubated on ice for 3 h with shaking. After centrifugation at 20,000 *g*, the supernatant was filtered through 1 and 0.45 μm filter papers before solid-phase extraction. The filtered extract was loaded onto a 35 CC C18 Sep-Pak (Waters) that was primed with 50 ml of methanol and 10% acetic acid. After washing with water, the trapped material was eluted with 30, 50, and 80% ACN in 0.1% TFA. The eluates were lyophilized using a freeze-dryer (Labconco, Kansas City, MO) and tested using a bacterial lawn assay to determine microbial growth inhibition.

Cation-exchange chromatography

Positive eluate (50% ACN eluate) was further separated using a HiPrep Sepharose SP column, a sulfopropyl strong cation-exchange column (16 × 100 mm; Amersham, Piscataway, NJ). The 50% ACN eluate was reconstituted in solvent A (20 mM NH_4OAc, pH 5.5) and loaded onto the cation-exchange column. The elution was performed with a linear gradient of 0–100% solvent B (2 N NaCl in 20 mM NH_4OAc) for 120 min at a flow rate of 1 ml/min. Fractions (4 ml) were collected using a fraction collector (Waters), and the individual fractions were desalted using a Sep-Pak Plus cartridge (Waters) by eluting with 5 ml of 70% ACN and 0.1% TFA. Ultraviolet light absorption was monitored at 280 nm using the 996 PDA detector. The desalted fractions were freeze-dried and tested for antimicrobial activity using the bacterial lawn assay.

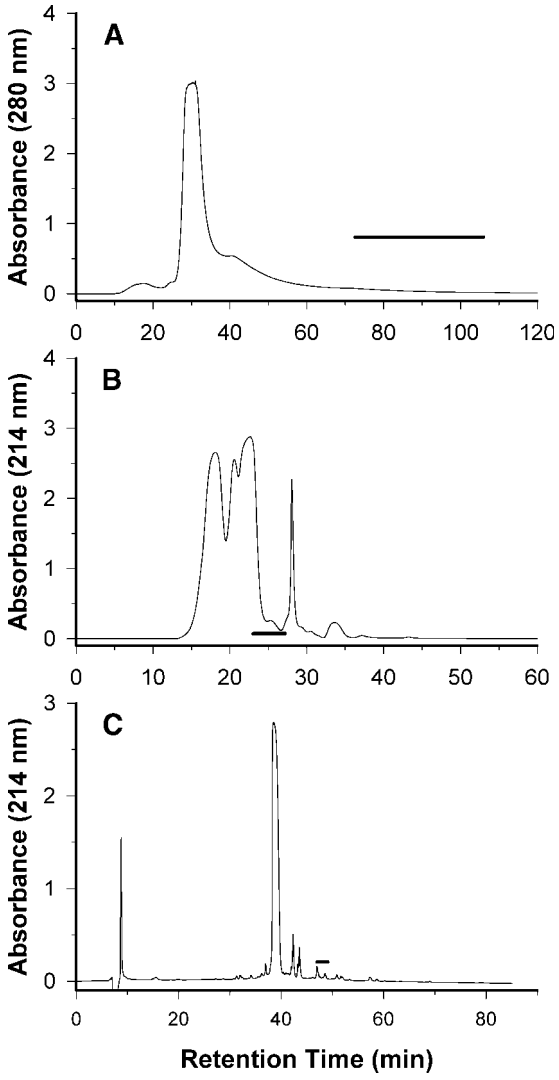

Fig. 1. Purification of squalamine from blood cell extracts of sea lamprey was performed using a series of separation methods, including ion-exchange chromatography, gel-filtration chromatography, and high-performance liquid chromatography. A: Cation-exchange chromatography of solid-phase extract from sea lamprey blood cells. An eluate of 50% acetonitrile (ACN) and 0.1% trifluoroacetic acid (TFA) from the C18 Sep-Pak was loaded onto a HiPrep Sepharose SP column and eluted with 0–2 N NaCl over 120 min. Five minute factions were collected and desalted using Sep-Paks, and antimicrobial activity was assayed. Positive fractions are indicated by the solid line. B: Gel-filtration chromatography of pooled fractions from cation-exchange chromatography. Isocratic elution was performed with 30% ACN and 0.1% TFA for 60 min. Two minute fractions were collected. Fractions eluting at 23–26 min showed strong antimicrobial activity, as indicated by the solid line. C: Reverse-phase high-performance liquid chromatography of the pooled fractions from gel-filtration chromatography. Pooled fractions were loaded onto a C18 Jupiter column and eluted with a linear gradient of 20–50% ACN and 0.1% TFA for 50 min. Most antimicrobial activity was found in the fractions eluting at 47–50 min (solid line).

Gel-filtration chromatography

Fractions with antimicrobial activities from the cation-exchange chromatography were further separated on a TSK gel G2000 SW column (300 × 7.5 mm; Tosoh, Tokyo, Japan). The antimicrobial substance was eluted with 30% ACN and 0.1% TFA at a flow rate of 0.5 ml/min for 60 min, with ultraviolet light absorption monitored at 214 nm.

Reverse-phase HPLC

The final step of purification was done by reverse-phase HPLC. The positive fractions from gel-filtration chromatography was loaded onto a C18 Jupiter column (4.6 × 250 mm; Phenomenex, Macclesfield, Cheshire, UK) and eluted with a linear gradient of 20–50% ACN and 0.1% TFA for 50 min.

Derivatization of squalamine with OPA

Derivatization of the active fraction and squalamine standard with OPA was performed according to Joseph and Davies (19). Briefly, 27 mg of OPA was dissolved in 0.5 ml of ethanol and 5 ml of 0.4 M boric acid, pH 9.5, followed by 20 μl of mercaptoethanol. The reaction was performed by mixing 50 μl of sample solution with 4 volumes of OPA reagent for 2 min. This reaction mixture was then analyzed on a C18 column (20 μl aliquot). Elution of the derivatized squalamine was monitored using the 474 fluorescence detector (excitation at 340 nm and emission at 455 nm).

Thin-layer chromatography

Thin-layer chromatography was performed on a silica plate (5 × 20 cm; Whatman, Clifton, NJ) by loading the reference and active fractions. The plate was developed with a mixture of ethyl acetate-methanol-ammonium hydroxide (5:1:1, v/v) for 1 h. After baking the plate at 90°C for 30 min, the reaction products were visualized by spraying 0.3% ninhydrin solution in ethanol and heating at 90°C.

Assay of antibacterial activity

The antimicrobial assay was performed as described by Moore et al. (20). Fractions were desalted or lyophilized and then resuspended in distilled water. An aliquot of each fraction was spotted on a lawn of *E. coli* D31 and *M. luteus* on a Luria-Bertani plate, then incubated at 37°C for 18–24 h. Fractions displaying a zone of clearing were pooled and subjected to the next separation step.

Mass spectrometry

Fast-atom bombardment (FAB) mass spectrometry was performed on the active fraction. Mass spectrometry analysis was done at the Mass Spectrometry Facility, Research Technology Support Facility, MSU. Mass spectra were obtained using a JEOL HX-110 double-focusing FAB mass spectrometer (JEOL, Peabody, MA), operable in the positive ion mode. Ions were produced by bombardment with a beam of Xe atoms (6 keV). The accelerating voltage was 10 kV, and the resolution was set at 3,000. For FAB-MS/MS, helium was used as the collision gas in a cell located in the first field-free region. The helium pressure was adjusted to reduce the abundance of the parent ion by 50%. The Shrader TSS 2000 data system generated linked scans at a constant ratio of magnetic to electrical fields. High-resolution mass spectrometry was performed by peak matching with a resolution of 10,000.

Separation of white blood cells

White blood cells were separated according to Ourth and Chung (21). Briefly, sea lamprey blood was collected using heparinated syringes. The peripheral blood was diluted with HBSS at 1:2. Five milliliters of diluted blood was layered over 3 ml of Histopaque 1077 (Sigma) in a 15 ml centrifuge tube. White blood cells were separated by centrifuging at 400 *g* for 30 min. White blood cell layers were removed to a separate tube and pooled together. The white blood cells were washed three times with PBS before use in extraction and immunocytochemistry.

Antibody production

The squalamine antibody was raised using a BSA-squalamine conjugate, produced by conjugating squalamine (2 mg) to 10 mg of BSA through reaction with EDC in 0.1 M MES buffer (pH 5.0). This BSA-squalamine conjugate was purified using a PD-10 column (Amersham) and lyophilized. Immunization of rabbits was performed by injecting 1 mg of the emulsified conjugate with 0.5 ml of Freund's complete adjuvant (Sigma), followed by boosting with the conjugate in Freund's incomplete adjuvant.

Immunocytochemistry

Immunocytochemical studies were performed on separated white blood cells, liver, and intestine from sea lamprey. The white blood cells were fixed in 4% paraformaldehyde in 0.1 M PBS (pH 7.4) and placed on glass slides. Liver and intestine tis-

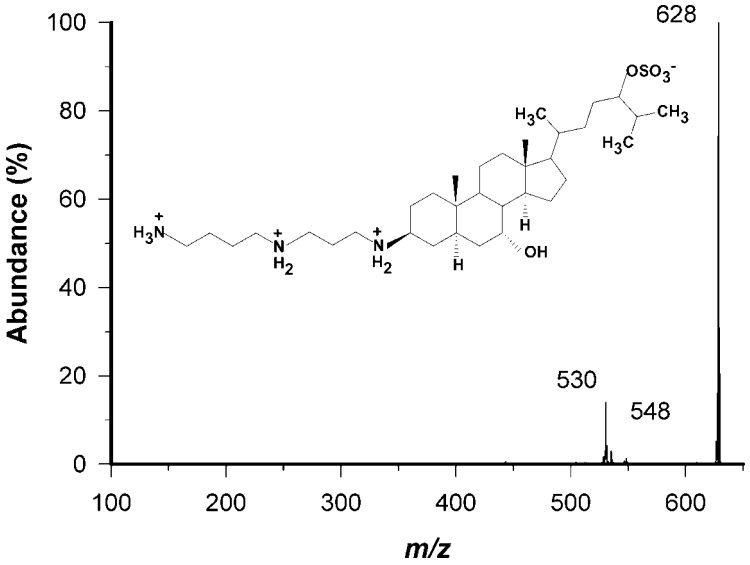

Fig. 2. Fast-atom bombardment (FAB)-MS/MS analysis of the antimicrobial factor from sea lamprey blood cells. An aliquot of the pooled fractions showing strong antimicrobial activity was subjected to FAB-MS and FAB-MS/MS. Major daughter ions were found at *m/z* 548 and 530, indicating fragmentation of sulfate (−80) and additional water (−18). A database search revealed that the antimicrobial substance found in the sea lamprey is squalamine. The chemical structure of squalamine is presented in the inset. Squalamine is a spermidine conjugate of a sulfated bile acid with 5α configuration. This antimicrobial compound shares some chemical structures with previously known sea lamprey bile acids.

sues were fixed in 4% paraformaldehyde followed by 20% sucrose treatment. The sucrose-treated tissues were sectioned 12 μm thick using a Cryostat (Leica, Wetzlar, Germany) and then placed on glass slides and incubated with squalamine antibody (LY 338; dilution, 1:100) in PBS containing 0.03% Triton X-100 (Sigma) overnight at 4°C. Control experiments were performed by incubating the slides with PBS or preimmune serum (LY 338pre). The next day, the slides were washed three times with PBS and incubated with secondary antibody conjugated with Alexa Fluor 568 for 2 h at room temperature. After washing three times with PBS, the slides were mounted with Vectashield mounting medium containing 4′,6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA). The slides were examined using a Zeiss Axioskop II fluorescence microscope (Carl Zeiss, Thornwood, NY).

Confocal laser scanning microscopy

The white blood cells stained with squalamine antibody were examined with a Zeiss 5 Pascal confocal laser scanning microscope (Carl Zeiss) under a 63× objective. The fluorochrome was excited with 543 nm, and red emission was collected using a 560 nm long-pass barrier filter. The image is a single confocal plane at maximum intensity. Projection images were created from Z series collected through 10 μm distance using a 0.7 μm step. Confocal laser scanning microscopy was done at the Center for Advanced Microscopy, MSU.

RESULTS

Purification of the antimicrobial substance from blood cells

The acid-extracted homogenates of blood cells were subjected to solid-phase extraction using C18 Sep-Pak. Antimicrobial assay of the eluates from solid-phase extraction revealed that the fraction eluted with 50% ACN and 0.1% TFA was most active at inhibiting the growth of bacterial strains *E. coli* D31 and *M. luteus*. This fraction was further fractionated on a cation-exchange column, and antimicrobial activity was found in the fractions eluting between 75 and 105 min (**Fig. 1A**). These active fractions (75–105) were pooled, loaded onto a gel-filtration chromatography column, and eluted with 30% ACN and 0.1% TFA. Fractions eluting between 23 and 27 min showed strong antimicrobial activity (Fig. 1B). As a final purification step, reverse-phase HPLC was performed. Fractions eluted at 47–50 min demonstrated growth inhibition in *M. luteus* (Fig. 1C).

Mass spectrometry analyses of the active fraction

FAB-MS analyses of the active fraction from the final HPLC step revealed an ionized peak at *m/z* 628 (positive mode). Further MS/MS analyses of the ionized peak at *m/z* 628 resulted in a fragmentation pattern, as shown in **Fig. 2**. Major daughter ions were seen at *m/z* 548 and 530, indicating fragmentation of sulfate (−80) and water (−18). A database search for the compound matching the mass and fragmentation pattern identified squalamine with a molecular formula $C_{34}H_{65}O_5N_3S$, an aminosterol originally found in the dogfish shark, *Squalus acanthias* (20). This formula was further confirmed by high-resolution mass spectrometry analysis at the 0.1 ppm level, with measured mass of *m/z* 628.4724 compared with calculated mass of *m/z* 628.4723 in the positive mode (molecular structure shown in Fig. 2).

Confirmation of the chemical structure

A series of analyses were performed to verify the chemical structure of the antimicrobial factor from the sea lamprey as squalamine. Squalamine lactate (from Prof. M. Zasloff) and the active HPLC fraction were subjected to OPA derivatization followed by separation on a C18 column. Identical chromatographic properties of both compounds were seen by coelution (**Fig. 3**) and by comigration by thin-layer chromatography on a silica gel plate (data not shown).

Identification of squalamine in white blood cells

Both chemical and immunohistological analyses revealed that squalamine is present in sea lamprey white blood cells

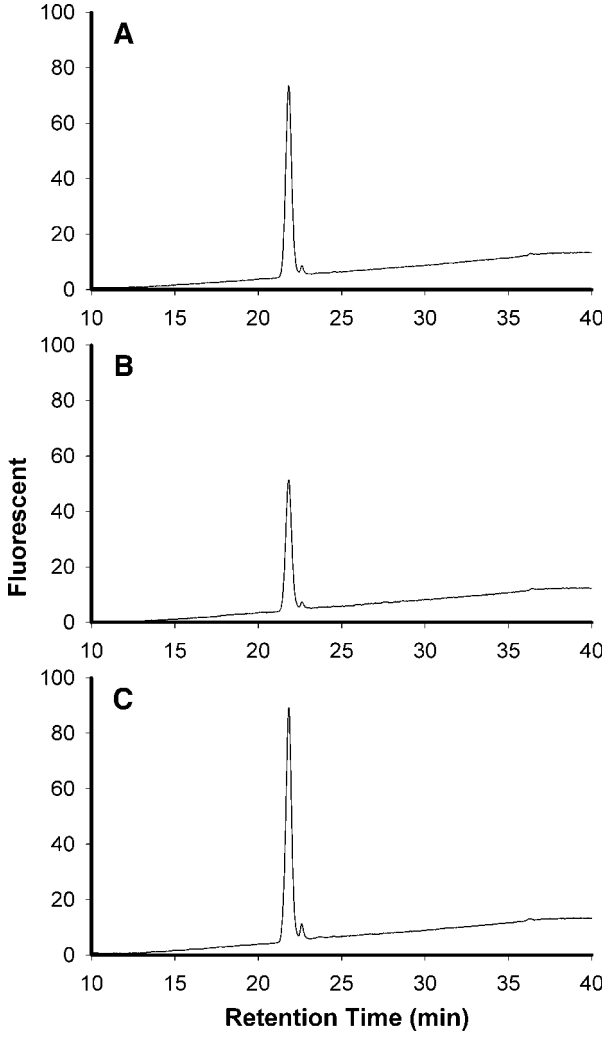

Fig. 3. Confirmation of squalamine as the antimicrobial factor in the sea lamprey. Both squalamine lactate and an aliquot of the pooled active fractions were derivatized using *o*-phthalaldehyde and separated on a C18 column. Note that squalamine lactate (A) and the aliquot of active fractions (B) elute at the same time in separate HPLC runs. Furthermore, coelution of squalamine lactate and the aliquot of active fractions is observed (C), indicating that the chromatographic properties of both compounds are identical.

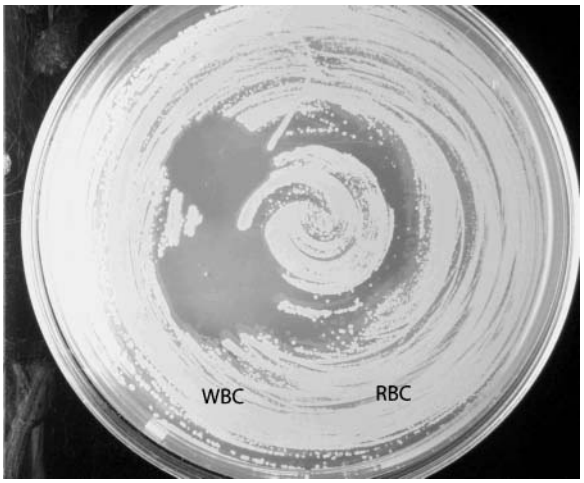

Fig. 4. Identification of white blood cells (WBC) as the source of squalamine. An extract from white blood cells was able to inhibit *E. coli* growth on Luria-Bertani medium, whereas no inhibition of bacterial growth was observed with the extract of red blood cells (RBC). Two preparations were tested on the bacterial lawn.

(**Figs. 4**, **5**). Acidic extracts of separated white and red blood cells subjected to FAB-MS analysis indicated that squalamine is present in the white blood cells (data not shown). Further evidence was obtained by testing acidic extracts on bacterial lawn, as shown in Fig. 4. Extract from white blood cells inhibited the growth of *E. coli* D31, whereas that of red blood cells had no effect on bacterial growth. Immunocytochemical study using antibodies raised against squalamine demonstrated that squalamine-like immunoreactivity was present in the white blood cells and not in other tissues examined, such as liver, intestine, and kidney (Fig. 5).

Localization of squalamine in the plasma membrane of white blood cells

Confocal laser scanning microscopy analysis of squalamine-immunolabeled white blood cells revealed that squalamine is embedded in the plasma membrane, as shown by the spatial distribution of red staining in the Z series (**Fig. 6**).

DISCUSSION

We have identified squalamine, an antimicrobial aminosterol, in the sea lamprey. A series of chromatographic separations combined with antimicrobial assays led to the discovery of squalamine in white blood cells of sea lamprey. Although squalamine has also been reported in the dogfish shark (20), the identity of this antimicrobial factor in the sea lamprey was confirmed by mass spectrometric analysis as well as by fluorescence HPLC and TLC. To our knowledge, sea lamprey is the second animal species found to possess squalamine. The discovery of squalamine in the sea lamprey is very intriguing in the context of the

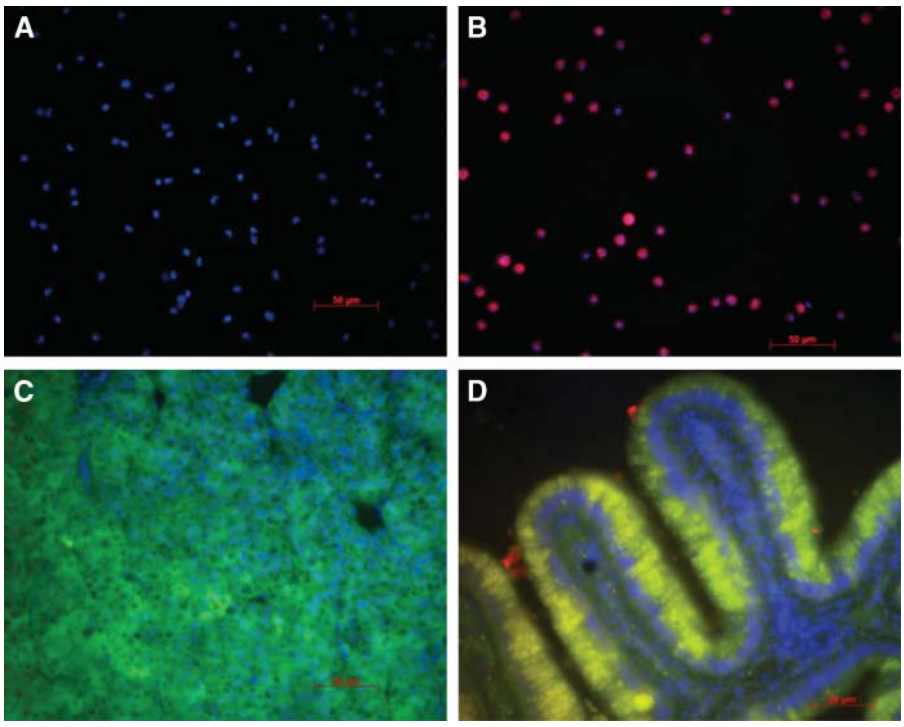

Fig. 5. Localization of squalamine-like immunoreactivity using anti-squalamine antibody. Staining can be viewed as red color from secondary antibody conjugated with Alexa Fluor 568. A: No staining of white blood cells was found with preimmune serum. B: Positive staining of white blood cells with anti-squalamine antibody (LY 338I). C: Positive staining of liver tissue with anti-squalamine antibody. D: Negative staining of intestine with anti-squalamine antibody. Bars = 50 μm.

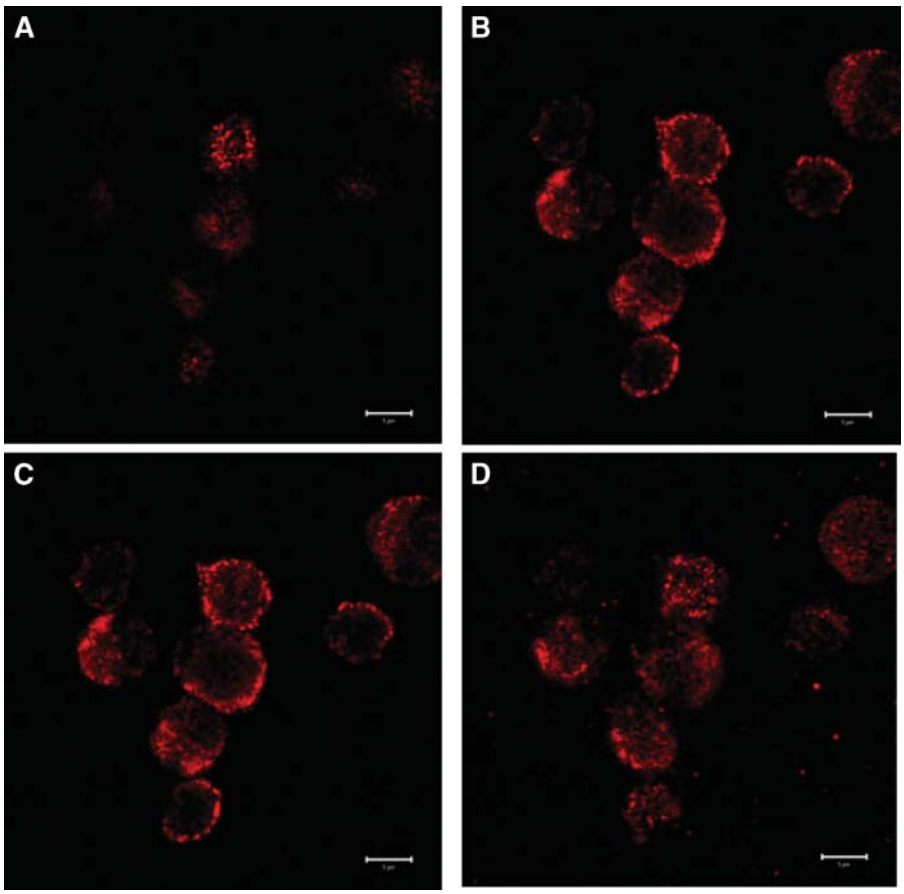

Fig. 6. Confocal laser scanning microscopy of stained white blood cells. Z sectioning of stained white blood cells strongly suggests that squalamine-like immunoreactivity is found only in the plasma membrane. A: Top view. B: Center view 1. C: Center view 2. D: Bottom view. Bars = 5 μm.

evolution of immunity, as both lampreys and sharks belong to ancestral vertebrate lineages.

It was found that squalamine is carried by the white blood cell population, which consists of >90% neutrophils (22), and that this aminosterol is a component of the plasma membrane. The presence of squalamine in these immune cells makes it reasonable to speculate that squalamine evolved in lower vertebrates as an immune effector molecule. Considering the distinct differences in adaptive immunity between Agnathans and cartilaginous fish species (1), the presence of squalamine in both species suggests a conserved role in innate immunity. The restriction of squalamine to the white blood cells of the sea lamprey is contrary to its ubiquitous distribution in the shark. This suggests that the mechanisms and roles of immune functions mediated by squalamine may be distinct between the two vertebrate classes.

The localization of squalamine immunoreactivity in the plasma membrane is intriguing because most immune effectors in leukocytes, such as antimicrobial peptides, are known to be stored in granules as propeptides, from which mature peptides are released into the microbes engulfed within the phagosome (23–26). This difference in the site of storage between squalamine and antimicrobial peptides suggests that squalamine has evolved as an immune effector with distinct modes of action. The white blood cell-specific squalamine in the sea lamprey strongly indicates that this molecule is more likely to be involved in immune mechanisms mediated by white blood cells.

Squalamine is a cationic aminosterol, the spermidine conjugate of a sulfated C27 bile acid. The molecular structure is very similar to those of bile acids known as migratory and sex pheromones in the sea lamprey (27–30). A recent study by Sorensen et al. (29) identified an aminosterol, petromyzonamine disulfate (PADS), as part of the migratory pheromone system that guides migrating sea lamprey to their spawning streams. Primary amine oxidation and 7-OH sulfation of squalamine can produce PADS, suggesting that both compounds share biosynthetic pathways. The biosynthetic strategy that evolved in sea lamprey to produce an array of closely related aminosterol compounds with different biological functions seems to be similar to that found in sharks, in which squalamine and its analogs are produced (31, 32).

Bile acids are known to play an important role in digestion and lipid metabolism, especially for the removal of cholesterol (33). The majority of bile acids identified in the sea lamprey are involved in chemical communication between conspecific individuals. For example, larval sea lampreys use petromyzonol sulfate, petromyzosterol disul-

fate, and PADS to guide migrating adults to their spawning streams (27, 29, 34). In adults, 3-keto petromyzonol sulfate and 3-keto allocholic acid are intraspecific signaling molecules during spawning (28, 30). Squalamine shares many of its chemical features with PADS, and it will be interesting to determine whether squalamine acts as a chemosensory cue in the sea lamprey.

Although these experiments do not demonstrate whether squalamine is produced in response to bacterial challenge, our results indicate that this compound plays an important role in inhibiting the growth of Gram-negative and Gram-positive bacterial strains. Previous studies demonstrated that squalamine is more potent than known antibiotic substances, with minimal inhibitory concentration values of 1–10 μg/ml for the microorganisms tested (20). Squalamine is believed to disrupt the bacterial membrane by forming a channel and causing the leakage of cellular material [similar to other cationic antimicrobial peptides (35)]. The squalamine-triggered release of fluorescent dyes trapped within phospholipid vesicles observed by Selinsky et al. (36) indicates a possible membrane-associated antimicrobial action for this compound. In this study of the sea lamprey, squalamine did not seem to disrupt the white blood cell membranes. The aminosterol may interact only with anionic lipids in prokaryotic membranes rather than with zwitterionic lipids in eukaryotic membranes (35).

In addition to its antimicrobial activity, squalamine is antiangiogenic (37–39) and has been developed as an anticancer agent (40, 41). Squalamine's mechanism of action may involve inhibition of the mammalian brush-border Na/H exchanger isoform NHE3 (42). Sills et al. (38) found that squalamine can inhibit rat brain endothelial cell proliferation and migration induced by some mitogens. However, the pharmacological mechanisms whereby squalamine exerts its antiangiogenic effects do not provide clues about how squalamine plays an immunological role in the sea lamprey. Biochemical interactions of squalamine with other cellular components may offer some insights on the actual modes of action in the fish.

It has been suggested that squalamine is synthesized in the liver, a well-known site of bile acid biosynthesis (20). However, the present immunolabeling study revealed that liver is not a biosynthesis site for squalamine. It is possible that squalamine is produced in either white blood cells or the hematopoietic tissues.

There have been a few nonpeptide antimicrobial factors identified in fishes, in addition to squalamine and squalamine-related aminosterols from the sharks (32). A low molecular weight antimicrobial factor has been identified from channel catfish leukocytes (21). These are believed to play a role in innate immune responses, along with other effector molecules such as complement, antimicrobial peptides, and lysozyme. In the jawless fishes, innate immune components have been identified and characterized, including complement protein and mannose binding lectins in lamprey (43, 44) and cathelicidin homologs in hagfish (45). The lack of antimicrobial peptides from lamprey may explain why squalamine is such a dominant antimicrobial factor in the sea lamprey. Therefore, we speculate that squalamine plays an important role as an innate immune effector in the lamprey.

In summary, we have isolated an aminosterol, squalamine, from the white blood cells of the sea lamprey and have identified the white blood cells as the source of the compound. Immunocytochemical studies indicate that squalamine is located in the plasma membrane. These results show that squalamine is a novel immune effector in the sea lamprey. Discovery of squalamine in the sea lamprey may offer a model system to better understand squalamine biosynthesis, its immunological roles, and innate immune responses mediated by aminosterols in lower vertebrates.

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