Immune Responses of Club Cells in Fish: A Review

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Abstract

The primary line of defense against pathogens from the environment is often fish epidermis tissue. Nevertheless, little is understood about the physiological mechanisms that underlie the non-specific and/or specific protection that these cells can offer. The exact nature of the relationship between the evolution of ostariophysan fish club cells and chemical warning signals is still unknown and controversial. Fish epidermis layer cells comprise mucus cells, lymphocytes, macrophage cells, cuboidal and squamous epithelial cells, and cells specific to certain fish species. Club cells, also called "alarm cells," are chemical alarms that sound in the event of a potentially hazardous scenario. These cells will burst in the presence of a predator, releasing pheromones that, if the skin is physically damaged, trigger an avoidance and terror reaction. In sturgeon larvae, mucus cells were visible in week 1, but club cells did not appear until week 4. Due to their later development during ontogenesis and after wounding, club cells may not have as much of a protective effect during wound healing as filament or mucus cells. Club cells are mostly found in the epidermis of the skin, and it is thought that when they work in tandem with mucus and goblet cells to fight infections, they serve as the body's first line of defense.

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INTRODUCTION

Fish depend on their innate immune system to survive because they are free-living creatures from early embryonic development. Fish have a basic defense system called nonspecific immunity (Zhu *et al*., 2013). Furthermore, immunity is essential for homeostasis and acquired immunological responses through the receptor protein system. Furthermore, immune system modulation plays a role in gonadal function regulation (Segner *et al*., 2017).

Fish epidermal tissue frequently serves as the main defense against environmental infections. Specialized cells like macrophages, granulocytes, and natural killer cells, as well as physical barriers like mucous layers and skin epithelial tissue lines, are examples of the cell-mediated innate processes of epidermal tissues (Mustafa and AL-Taee, 2020). Many fish in the superorder Ostariophysi feature epidermal club cells on their skin, which have predominantly evolved as part of the immune system. However, little is known about the functional mechanisms underlying these cells' ability to provide non-specific and/or specific protection. Because the evolution of ostariophysan fish club cells has been connected to chemical alarm signals in the past, it is still unclear and contentious. Such data would lend credence to the theory that fish club cells are preserved by natural selection as a result of the advantages they provide in the altercation against infections (Pandey *et al*., 2021).

Significantly more fish species have been examined for alarm reactions to certain skin, and we now know that most fish species typically show antipredator responses to substances produced from damaged species (Chivers *et al*., 2012). The majority of aquatic animals exhibit comparable behaviors, including mollusks, amphibians, platyhelminthes, and arthropods (Delroisse *et al*., 2023). A requirement for specialized structures for the production of alarm cues is undermined by the fact that only a small number of these other species of aquatic creatures have specialized structures similar to epidermal club cells (Pandey *et al*., 2021). Data demonstrating no decrease in cue potency when club cells are suppressed or absent further erode the case for an alarm function for epidermal club cells in ostariophysan fishes, implying that club cells may be a component but not the only source of the alarm cue. This review study integrates several recent studies that have been conducted previously and reveals the immune response initiated by club cells in fish.

MATERIALS AND METHODS

This study retrieved papers documented in Scopus, Pubmed, and SpringerLink databases using the keywords "club cells", "immune response", "fish", and "fish epidermis". Excluded papers were those that did not discuss the immune response, were not available in full text, were not in English, were case studies, and had irrelevant data.

RESULTS AND DISCUSSION

Fish skin is composed of up of layers known as the epidermis, dermis, and hypodermis histologically (Damasceno *et al*., 2012). Mucus cells, club cells, pigment cells, and stratified squamous epithelial cells comprise the epidermal layer. The stratum germinativum is known as the basic layer of the epidermis. Basal cells comprise the epithelium that constitutes this layer. The epidermal layer lacks blood arteries, thus diffusion is used to meet its nutritional requirements (Khairani *et al*., 2021).

The epidermal layer's thickness varies significantly according to the body part, age, sex, time of the reproductive cycle, and surroundings. Fish epidermis layer cells include cuboidal and squamous epithelial cells, mucus cells, lymphocytes, macrophage cells, and cells peculiar to certain fish species (Henrikson and Matoltsy, 1967). Fish skin epithelium cells are living, breathing tissues. Fish skin's outer epithelium proliferates quite vigorously, causing it to constantly reorganize and strike a balance between differentiation and proliferation (Päkk *et al*., 2011).

In addition to serving as a lubricant for the skin's surface, mucus cells can prevent several infections from entering the epidermis and from forming colonies there. Mucus facilitates swimming, protects the body from abrasion when making nests, and functions as a lubricant to lessen body surface friction in the water (Kenconojati *et al*., 2023). Fish that live in hiding places or muddy habitats secrete an excessive amount of mucus. Mucous cells are differentiated from the basal epidermis and migrate toward the surface to discharge their fluid as they get larger (Quatrini *et al*., 2021).

The dorsal side of pigment cells is blackish-brown, while the abdominal side is brown. Pigment cells not only give fish skin its color pattern, but they also serve to absorb and reflect radiation, which helps fish maintain a healthy body temperature. The connective tissue, blood vessels, pigment cells, and fat tissue make up the dermis layer of the skin on both fish species. The stratum spongiosum on the exterior and the stratum compactum on the interior make up the two sections of the dermis. The stratum spongiosum, which gives rise to the comparatively loose portion of the epidermis, is made up of collagen connective tissue, adipose tissue, and blood vessels. Collagenous connective tissue that is thicker and arranged regularly is found in the stratum compactum (Khairani *et al*., 2021).

Collagen fibers are dispersed throughout the skin's dermis layer; they are more loosely distributed in the stratum spongiosum and more closely packed in the stratum compactum (Rani *et al*., 2022). Because of the thickness of the stratum compactum, the collagen fibers on the dorsal skin are thicker than those in the abdomen (Safira *et al*., 2022). Fish scales form in the dermis layer, which is also high in collagen. Known as the dermis skeleton, the scales are formed in the dermis and are often coated in epidermal tissue (Sire and Huysseune, 2003).

Club cells, sometimes known as "alarm cells," serve as chemical alarms that sound when there is a potentially dangerous situation. When a predator poses a threat, these cells will rupture and release pheromones that, when the skin is physically harmed, cause a fear response and an avoidance response towards the predator. Club cells aid in the repair of mucosal and epithelial cells that have been harmed by pathogenic agent-induced injury (Chivers *et al*., 2012).

Fish oesophagi and skin are common places to find club cells. Club cells have a cytoplasm that is abundant in non-vesicular secretions but relatively deficient in organelles. The perinuclear region contains the few organelles that have been discovered (endoplasmic reticulum, Golgi complex, polyribosomes, and mitochondria), whereas filamentous substances occupy the remaining cytoplasm (Forbes and Ferguson, 2001). As a result, the cytoplasmic content can be divided into two regions: the plentiful and electron-dense zone that makes up nearly the whole volume of the cytoplasm, and the bright, clear region around the nucleus. The whole length of the plasma membrane exhibits invaginations, resulting in an uneven cell surface that marginally intersects the epidermal cells (Bogataj *et al*., 2019).

As demonstrated by the absence of glycoprotein content in the cytoplasm of club cells, negative responses to PAS and toluidine blue staining are present. Due to positive bromophenol blue staining, club cells of some Cyprinidae species, including red-tailed sharks, produce proteins (Abd-Elhafeez and Soliman, 2016). In phylogenetically related animals, club cells are linked to the synthesis, storage, and release of alarm chemicals that cause alarm reactions. Alarm reactions, which are brought on by chemicals secreted from wounded skin, occur when the fish is in danger. The club cell's plasma membrane is disrupted as a result of this occurrence, exposing and releasing the cytoplasmic contents into the water. There have also been suggestions that these cells contain phagocytic and anti-pathogenic properties. Certain fishes' club cell cytoplasm has also been shown to include chondroitin and keratin, which may have a therapeutic purpose and help heal injured tissues. Serotonin immunoreactivity has been detected in the club cells of *Carapus acus* and *Heteropneustes fossilis*. Club cells contain serotonin, which may affect pheromones or other potential uses for these cells (Mokhtar *et al*., 2023).

Club cells are certain epidermal cells seen in teleosts that are different from mucous and filament-containing cells. The club cells of the Ostariophysians Carassius and Corydoras have been named Schreckstoffzellen (Mokhtar and Abdelhafez, 2021). If a catfish, goldfish, or related species gets injured in nature, it is assumed that the club cells (SchreckstoffzeIlen) burst and discharge their contents into the intercellular space and surrounding water. Undefined chemical composition in the dub cell content causes other fish in the vicinity to smell it and respond fearfully, swimming quickly and suddenly and then diving into a hiding place. There are interspecific and interfamilial responses, but fish that are triggered by a fear chemical released by a different member of their species exhibit the strongest reactivity. Additionally, there is a positive association between a fish's ability to respond to fright substances and the proportion of club cells it possesses. Fish without unique club cells do not react to fear material (Jesuthasan and Mathuru, 2008).

The previous investigation revealed that the plasma membranes of nearby filament-containing cells were continuous and undamaged, whereas the club cells' plasma membranes in catfish and goldfish occasionally appeared disrupted. Poor fixation makes it impossible to rule out artifacts, but it's plausible that fixation is enough trauma—roughly like when a fish is wounded—to induce these cells to release their fibril into the epidermis' intercellular space. Under electron microscopy, club-shaped cells from the eel's epidermis were shown to differ significantly from those of the goldfish and catfish. The eel club cell's cytoplasm is full of structures that look like coiled filaments but are invisible under a light microscope. These coils appear to be arranged in bundles; a plane passing through a single cell would display multiple bundles with varying orientations (Henrikson and Matoltsy, 1967).

Fish immune systems have received significant attention recently for the following reasons: (1) The majority of immunological research that is pertinent to human health is conducted on mice or humans. Because of their genetic and physiological similarities to humans and the advancement of optical, genetic, and chemical investigative tools, fish serve as an invaluable comparative group for evolutionary biologists studying the evolution of the immune system. Moreover, knowledge of fish disease is important for managing fish populations that are raised for food, sport, or commercial purposes, as well as for the health of ecosystems (Pandey *et al*., 2021).

The most researched immune system in fish is that of teleosts, which have components of both the innate and adaptive immune systems. Distinguishing between the self and the non-altered self is a crucial immune system function. Generally speaking, the immune system in multicellular animals is made up of both innate and adaptive components. It is a networked and interacting system that includes a wide variety of macromolecules, cells, tissues, and organs (Henrikson and Matoltsy, 1967).

Since teleost skin lacks keratinization, skin cells—including club cells found in the mid-epidermis—are frequently in contact with the water and its surroundings. They are probably going to be innate immune cells in the fish immune system because of where they are structurally. Numerous environmental stressors and immunomodulators have been studied concerning club cells. Alarm cues (skin extracts from the same or different fish species), hypoxanthine-3-N-oxide, cortisol, parasites, bacterial and mold infections, UV radiation, white-blue light, water pollutants like cadmium, lead, and copper, detergents, azo dye, heavy metals like cadmium, copper, and cadmium, mechanical injury, testosterone, and food rations are all included in this (Mokhtar *et al*., 2023). Contradictory results have been revealed by several of these stressors; for instance, an infestation of parasites does not always translate into a higher density of club cells. Although cercariae that parasitize turtles cause an increase in the number of club cells in minnows, parasites that have evolved to avoid the immune system of minnows do not cause the proliferation of club cells (Pandey *et al*., 2021). When infected with water mold, club cell density increased; when infected with *Aeromonas hydrophila*, club cell density decreased (Fikri *et al*., 2022). In a similar vein, club cells in fathead minnows showed a decrease in density and sensitivity to high UV radiation levels, possibly due to a brief cortisol response. However, in razorback suckers (*Xyrauchen texanus*), club cells underwent hyperplasia and hypertrophy and were discovered to be photoprotective (Pandey *et al*., 2021). According to a previous study, club cells' elevated production and colocalization of the proteins calbindin and calretinin upon exposure to blue and white light may be related to the cells' potential photoprotective function. Finally, it was discovered that dissolved organic carbon shielded club cells from UV light damage (Wilske *et al*., 2020). Club cells are the final of the filament cells that differentiate during larval ontogeny. Club cells first show up in fathead minnows 28–37 days after hatch (Pandey *et al*., 2021). Club cells did not emerge in sturgeon larvae until week 4, although mucus cells were seen in week 1. When compared to filament or mucus cells, club cells may have a less protective effect during wound healing due to their later development during ontogenesis and wounding (Khairani *et al*., 2021).

CONCLUSION

As the skin's first line of defense against infections and parasites, club cells are ideally situated at the middle-epidermal layer, which is subject to several environmental stresses. They react to a wide range of environmental stressors, including as UV light, heavy metals, cortisol, infections, parasites, mechanical injuries, testosterone, and food rations. It is believed that club cells, which predominate in the skin's epidermis, function best as the body's first barrier when they are paired with mucus and goblet cells to combat infections.

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AUTHORS' CONTRIBUTIONS

YD, MB, and SS: Collecting and searching references. HKK and EZ: Selecting papers based on study inclusion. YD, HKK, EZ, MB, and SS: Writing and revising draft manuscripts. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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