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The Influence of Low Temperature on the Immune System of Teleosts

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ABSTRACT

Due to their status as poikilothermic vertebrates, fish can experience changes in water temperature and, consequently, changes in their body temperature as a result of seasonal changes, migration, or the discharge of significant amounts of effluent into a body of water. Shifting the temperature outside of the ideal temperature range for a specific fish species might have detrimental consequences for the animal's physiology, especially its immune system. Therefore, either acute or chronic exposure to inadequate temperatures can weaken an organism's ability to protect itself against infections, putting the animal's general health at risk. Specifically, the progress gained in understanding the effects of suboptimal temperature on the soluble and cellular mediators of the innate and adaptive immune systems of fishes is the subject of this review paper. Cold stress can have a variety of impacts on different fish species, but in general, both acute and chronic suboptimal temperature exposure have suppressive effects on immunity, especially on adaptive immunity. It is vital to understand the effects of environmental temperature on fish species in order to optimize the management of wild species and to implement the best management techniques for aquaculture species.

1. Introduction

The impact of temperature fluctuations on biological systems is a topic that is becoming increasingly important in light of global climate change and differences in seasonal temperature patterns. Because of rising climatic variability, extreme temperature events such as cold snaps are occurring at a higher frequency and with greater size than previously.

The unprecedented cold weather that occurred in the Gulf of Mexico in 2010 is one example of these extreme temperature events. The drop in air and water temperatures of 12 degrees Celsius and 6 degrees Celsius, respectively, over a two-week period, resulted in widespread mortality in fish populations. Moreover, the variation in seasonal water temperatures experienced by fishes within a given year can be quite large, ranging from below 5 degrees Celsius to 19 degrees Celsius for a cold-water species such as rainbow trout, and from 16 degrees Celsius to 39 degrees Celsius for zebrafish, a warm water species. In the face of these challenges, the poikilothermic nature of fish necessitates special consideration, as changes in water temperature equate to changes in body temperature, and these changes can have an impact on key physiological processes, such as the immune system, and ultimately on the health of the animal. While temperatures that are beyond the physiological range of a fish species might cause a stress reaction that can negatively impair immunological function, temperatures that are below the physiological range of a fish species can have the opposite effect. As an example, when deciding whether to transfer Atlantic salmon smolts to ocean pens, the temperature of the water is a consideration because the prevalence of the parasite Kudoa thyrsites on these fish was highest when water temperatures were above 10 degrees Celsius in the summer and fall and were not detected when water temperatures were below 10 degrees Celsius in the winter and spring. In contrast, there are a number of fish-pathogen model systems that have been shown to have an immunosuppressive impact when exposed to inadequate temperatures. The olive flounder (Paralichthys olivaceus) is sensitive to viral hemorrhagic septicaemia virus (VHSV) at hypothermic temperatures such as 15 degrees Celsius, with mortality rates as high as 24 percent, although mortality rates are negligible when fish are kept at a constant 20 degrees Celsius. When placed in water that was 8 degrees Celsius below (or above) their thermal optimum, tilapia (Oreochromis niloticus) infected with Streptococcus iniae or orange-spotted grouper (Epinephelus coioides) infected with Vibrio alginolyticus died in substantial

numbers. It has been demonstrated that infection of zebrafish with spring viraemia carp virus (SVCV) results in greater mortality rates when the fish are housed at inadequate temperatures in cyprinids. The walleye, Sander vitreus, and walleye dermal sarcoma virus (WDSV) that produces cutaneous mesenchymal neoplasms are two examples of natural model systems that have been shown to demonstrate the impact of temperature on the immune system and illness outcome in fish. It has been shown that tumor progression follows a seasonal cycle, with tumors first emerging in the late fall, continuing until the early spring, and then regressing in the summer, suggesting a relationship with temperature. Once walleye have recovered in the spring and summer, it appears that they have developed immunity to WDSV. Cold stress, it is assumed, has a negative impact on walleye immunity and allows for virus multiplication, tumor formation, and virus transmission. The interactions between fish, infections, and temperature showed above are examples of the intricate interplay that can occur and result in compromised fish health if not addressed. However, it is crucial to note that increases in pathogenesis at low temperatures may also be partially attributable to the effect of temperature on the virulence of the pathogen, as recently reviewed by Guijarro et al. In this paper, we discuss the effects of hypothermia on the innate and adaptive immune systems of teleosts, including teleost fish.

2. Innate Immunity with temperature

2.1. Elements of Innate Immunity

The teleost immune system, like the immune systems of mammals, is composed of innate and adaptive arms (described further below). The innate arm of the immune system is constitutive and consists of germ-line encoded effector molecules (antimicrobial peptides, complement proteins) and cells (macrophages, neutrophils, basophils, eosinophils, cytotoxic cells) that recognize conserved microbial associated molecular patterns (MAMPs) in the presence of bacteria. Macrophages and neutrophils, for example, produce hundreds of bioactive molecules that are crucial to the survival of an organism's immune system. These molecules direct the onset and resolution of an inflammatory response, and they are critical to the survival of an organism. Macrophages in particular are undoubtedly the most important innate immune cells because they recognize, uptake (phagocytosis), and kill pathogens. They also serve as a link between the innate and adaptive arms of the immune system by stimulating T-cell responses through antigen presentation.

2.2. Complement

Complement proteins are the most abundant soluble component of the innate immune system. Complement proteins are composed of roughly 30 proteins that collectively form the classical, lectin, and alternative complement pathways, which have been discussed in detail in. It has been shown that all three pathways result in the creation of the membrane attack complex (MAC), which is cytolytic to target cells/microbes and also results in the release of complement cleavage products, which are implicated in inflammation. Few researchers have studied the effect of suboptimal temperature on complement levels and activity, in spite of the fact that complement is a prominent and critical component of immunity.

One study evaluated the short-term exposure of tilapia (Tilapia zillii) to cold stress (17 degrees Celsius for 30 minutes) and discovered that there was no difference in serum complement activity between the fish exposed to cold stress and control fish kept at 27 degrees Celsius. However, because of the brief duration of heat stress and the single fish species that were studied, it is impossible to determine the applicability of this particular study. Despite the fact that just a few studies have examined the effects of chronic cold stress on fishes, the research that has been done has revealed that different fish species have varying responses to complement activity. It was discovered that rainbow trout acclimated to lower temperatures (5°C) over a period of more than two months had decreased opsonization capability and lytic activity of serum complement when compared to rainbow trout kept at higher temperatures (>10°C), for example. In contrast, when sockeye salmon were bred at 8 degrees Celsius rather than 12 degrees Celsius, their serum complement activity was higher. Both of these fish species, both of which are members of the family Salmonidae, have yet to be discovered to be responsible for complement control. However, this suggests that even within a single family of fish, suboptimal temperatures have varying impacts on complement activity.

There have been few studies conducted on the effects of cold stress on complement molecule regulation in immunostimulated or infected fish, and these studies are needed. Yersinia ruckeri immunized (i.p.) rainbow trout showed upregulation of C5a receptor transcript levels in the spleen and kidney of animals regardless of the temperature (5C, 15C, or 25C) [28], indicating that cold stress may have no effect on the upregulation of the complement system during pathogen challenge in rainbow trout. However, additional research is required in order to determine the effect of temperature on the complement system.

2.3. Numbers of Leukocyte

Animals' immunocompetence can be assessed by examining the availability and proportion of lymphocytes in various organs such as the blood, kidney, and spleen (the key immune organs in teleosts). For example, in Atlantic halibut (Hippoglossus hippoglossus L.) at 8 degrees Celsius rather than 12 to 15 degrees Celsius, or in channel catfish at 10 degrees Celsius rather than 24 degrees Celsius, hypothermic temperatures had no effect on packed cell volume or the percentage of blood leukocytes. In a similar vein, the percentage of monocytes, thrombocytes, and granulocytes in the peripheral blood leukocyte (PBL) population in the spleen

did not alter between rainbow trout kept at 12 degrees Celsius and rainbow trout kept at 15 degrees Celsius. Other fish species, on the other hand, have produced outcomes that are diametrically opposed. After being exposed to acute hypothermic stress, carp (Cyprinius carpio) have increased circulating granulocyte numbers, which coincide with a decrease in kidney granulocyte numbers. Sockeye salmon reared at 8 degrees Celsius versus 12 degrees Celsius have shown a higher percentage of phagocytic kidney macrophages and a decreased percentage of peripheral blood lymphocytes. However, when exposed to suboptimal temperatures, members of the order Perciformes show a decrease in total blood leukocyte numbers, as observed in orange-spotted grouper (Epinephelus coioides), tilapia (Oreochromis mossambicus), and hybrid striped bass (Monrone chrysops Morone saxatilis). The hybrid striped bass also shows a decrease in total blood monocyte numbers. Under non-challenging conditions, these findings suggest that hypothermic temperatures have a different impact on different orders of fish.

In a study of rainbow trout exposed to bacterial and protozoal pathogens at suboptimal temperatures, it was discovered that temperature had an effect on the composition of leukocytes during infection. An increase in the proportion of granulocytes in the PBLs was found in rainbow trout kept at 12 degrees Celsius following infection with A. salmonicida, but no increase was seen in the monocyte or thrombocyte populations. On the other hand, in PBLs isolated from A. salmonicida challenged fish maintained at 15 degrees Celsius, a significantly larger rise in granulocyte and monocyte counts was observed. It was found that fish kept at 15 degrees Celsius rather than 12 degrees Celsius after infection with A. salmonicida had higher numbers of monocytes in their spleens, a finding that was consistent with the previous finding. While the percentage of leukocytes in the peripheral blood of rainbow trout infected with the parasite Tetracapsuloides bryosalmonae grew overtime at both 12°C and 15°C, the growth was significantly greater at the higher temperature at all time periods in all of the experiments. It appears from these findings that, while innate immune cells can be actively mobilized during times of low temperature, their maximal mobilization is diminished when compared to fish maintained at homothermic temperatures, which may predispose these fish to infection.

2.4. Peripheral Blood Leukocyte Function

A broad suppression in activity was observed in PBLs from fish that had been exposed to acute or chronic hypothermic conditions. Orange-spotted grouper (Epinephelus coioides) blood leukocytes were found to have a lower phagocytic index after being exposed to temperatures 8 degrees Celsius below their thermal maximum for 24 hours, 48 hours, and 96 hours [1, 2]. A study with tilapia (Oreochromis mossambicus) yielded results that were similar to those found in this investigation. The phagocytic activity of rainbow trout blood leukocytes was also dramatically reduced in rainbow trout that had been exposed to 5 degrees Celsius for more than 2 months (as opposed to 10 degrees Celsius or 15 degrees Celsius). Channel catfish, orange-spotted grouper, tilapia (Oreochromis mossambicus), and rainbow trout PBLs were found to have decreased generation of reactive oxygen species (ROS) when exposed to hypothermic circumstances. The non-specific immune responses of rainbow trout were inhibited despite pathogen challenge with A. salmonicida when they were kept at a lower temperature (12°C versus 15°C). A. salmonicida-challenged fish maintained at 15 degrees Celsius cleaved tetrazolum salts to a greater extent than fish maintained at 12 degrees Celsius, indicating a reduction in cell activation or metabolism at lower temperatures, according to the findings. As a result, it appears that both acute and chronic cold stress limit phagocytic activity and ROS formation, raising the possibility of impaired innate immune cell function important for pathogen elimination.

2.5. Cytotoxic Cells

It has been demonstrated that fish cytotoxic cells work in a manner similar to that of mammalian natural killer (NK) cells, but they appear to be physically unique. They have been shown to produce cytotoxicity in both mammalian tumor cells and fish protozoal parasites. It has been demonstrated in studies on carp cytotoxic cells that the killing activity of cytotoxic cells derived from fish maintained at hypothermic temperatures is increased. In the first study, carp (Cyprinus carpio) were kept at 25 degrees Celsius but were also acclimated to 10 degrees Celsius for varying lengths of time, up to 112 days. In a cytotoxicity study using human K562 cells as target cells, natural killer-like cells were extracted from the kidney and used in the treatment of the cells.

When the in vitro NK cell killing assay was done at 25 degrees Celsius rather than 10 degrees Celsius, the cytotoxic activity of carp NK-like cells was shown to be greater. The same was true for NK-like cells obtained from fish that had been maintained at 10 degrees Celsius for extended periods of time. When tested in vitro in NK cell killing tests, the cytotoxic activity of the cells was higher at 10 degrees Celsius than at 25 degrees Celsius. According to the findings of this study, fish innate immune cells are capable of adjusting to long-term hypothermic settings in which these new temperature parameters are adapted to as normothermic conditions, allowing NK-like cells to retain their function. In addition, this work demonstrated the necessity of selecting appropriate in vitro assay settings (e.g., temperature) for the evaluation of ex vivo cell functions while performing ex vivo cell function assessments. Another group found a similar increase in cytotoxic cell activity in carp that had been kept at 12 degrees Celsius (as opposed to 20 degrees Celsius) for 28 or 42 days, with similar outcomes. However, 56 days after the fish were transferred from 20°C to 12°C, their cytotoxic activity had recovered to baseline levels. Interestingly, and in contrast to the aforementioned study, there was no evidence of a reduction in cytotoxic cell activity when compared to cytotoxic cells from carp maintained at 20

degrees Celsius at any of the time points tested. Furthermore, when cytotoxic cells from cold-acclimated fish were cultured in vitro at 12 degrees Celsius, the activity of the cells was not increased.

It appears that cytotoxic cells, at least in cyprinids, may adapt to freezing temperatures in vivo and that they may be able to compensate for other components of fish immunity that may be severely influenced by hypothermic temperatures.

2.6. Macrophages and Granulocytes

Microglia, neutrophils, and macrophages are central innate immune cells that are important phagocytic cells as well as generators of reactive oxygen species, and they are part of an extensive antimicrobial arsenal.

Suboptimal temperatures have been shown to have no effect on macrophages and neutrophils (granulocytes) activity in most studies, with the exception of one that found increased inactivity. A considerable variation in the respiratory burst activity of rainbow trout macrophages isolated from the kidney in response to a macrophage activating factor (MAF) in response to low-temperature conditions in vitro was found, however, the difference was not statistically significant. Meanwhile, rainbow trout neutrophils that were cultured at lower in vitro temperatures produced less reactive oxygen species in response to phagocytic stimuli. However, because the aforementioned investigations were carried out in vitro, they may not be representative of in vivo circumstances in all cases. During in vivo tests, pronephros macrophages isolated from carp (Cyprinus carpio) were kept at 12 degrees Celsius (as opposed to 20 degrees Celsius) for 28 days and showed a stronger respiratory burst response as well as a higher phagocytic index. Similarly, blood granulocytes from tench (Tinca tinca L.) fish kept at 12 degrees Celsius (winter temperatures) demonstrated stronger phagocytic capacity and generation of superoxide anions than blood granulocytes from fish kept at 22 degrees Celsius. These studies demonstrate that long-term exposure to hypothermic temperatures may strengthen the cellular components of the innate immune system, allowing for compensation in areas of immunological deficiency in other parts of the body.

2.7 The Expression of Genes Involved in the Proinflammatory Response

When it comes to fish development, the effects of hypothermic temperatures on the expression of numerous immune genes, such as proinflammatory cytokines, antiviral pathway proteins, and Toll-like receptors (TLRs), have been investigated most extensively in zebrafish. Studies on zebrafish embryogenesis have revealed that suboptimal temperatures (15 degrees Celsius compared to 28 degrees Celsius) inhibit the expression of il1b, tnfa, ifn1, ifng, inos, irf3, mda5, and mx, although the expression patterns of tlr3, tlr21, and tlr22 remained intact. Additionally, in Atlantic salmon parr, gene expression of mx was delayed at 6 degrees Celsius compared to 14 degrees Celsius following poly I: C injection, but it was longer lasting at 6 degrees Celsius, suggesting that lower temperatures slow the kinetics of this particular response and perhaps others, but do not completely eliminate the response. Furthermore, the development of zebrafish suggested that they could be used as a temperature-dependent model of anemia in the future. Zebrafish grown at lower temperatures (17 degrees Celsius versus 26.5 degrees Celsius) for up to 7 months exhibit a selective decline in erythrocytes but not myeloid cells, resulting in an anemic condition.

Using gene expression analysis to examine the expression of key growth factors involved in hematopoiesis in kidney marrow, it was discovered that the expression of erythropoietin (epo) and erythropoietin receptor (epor) in kidney marrow, which are important for erythropoiesis, decreased, but not the expression of colony-stimulating factor-1a (csf1a) or colony-stimulating

Interestingly, while macrophages and granulocytes (neutrophils) continue to be produced at normal levels during fish ontogeny, the expression of key proinflammatory cytokines and proteins involved in viral recognition may be suppressed or delayed, at least at the transcript level, when the temperature is lowered to hypothermic levels during the fish's development.

The production of important innate immune components in response to pathogen mimics or pathogens themselves appears to be suppressed or delayed in adult fish when exposed to hypothermic conditions, suggesting a halt in the induction of proinflammatory or antiviral response. When rainbow trout head kidney leukocytes were treated with lipopolysaccharide (LPS) in vitro, the il1b mRNA levels were higher at 22 degrees Celsius than at 14 degrees Celsius, and transcription of il1b mRNA was entirely stopped at 4 degrees Celsius. In vivo tests tend to follow a pattern that is similar to this. mx transcripts in the head kidney of seven-band grouper (Epinephelus septemfasciatus) injected with poly I:C were found to be lower in fish maintained at 15 and 20 degrees Celsius compared to fish maintained at 25 degrees Celsius. The proinflammatory transcripts il1b and ifng were elevated in the spleen and kidney of rainbow trout that had been inoculated (i.p.) with Yersinia ruckeri at either 5°C or 15°C; however, the upregulation of the proinflammatory transcripts occurred slightly faster at the ideal temperature. According to the results of a study conducted on rainbow trout maintained at 15 degrees Celsius but not at 5 degrees Celsius, only il10 transcripts were induced following Y. ruckeri vaccination, whereas no changes in tgfb transcript levels were found in fish maintained at either temperature. In contrast, the route through which the rainbow trout were vaccinated with Y. ruckeri had an impact on whether or not temperature-dependent effects on proinflammatory cytokine expression were observed – bath vaccination of rainbow trout did not produce the same results as intraperitoneal vaccination did. Despite varying outcomes in the modulation of proinflammatory

transcript levels, fish that was bath vaccinated with Y. ruckeri and maintained at 15 degrees Celsius was able to survive a subsequent homologous challenge infection, whereas fish that were maintained at 5 degrees Celsius were unable. These findings imply that the resolution of Y. ruckeri infection in trout is temperature-dependent, and that, while the exact mechanism of protection is still being investigated, it appears that the generation of pro-inflammatory cytokines plays a role.

2.8. Antigen Presentation Pathway

Antigen processing and loading for the major histocompatibility complex (MHC) I and II occurs through a complex process that is led by accessory molecules, according to research in mammals.

A portion of the endogenous pathway is comprised of the proteasome, which digests proteins and converts them into peptides. These peptides are then transported into the lumen of the endoplasmic reticulum (ER) by the transporter associated with antigen processing (TAP), where they will be loaded into the peptide-binding groove of MHC I. MHC class I heavy chain is produced by ribosomes along with the rough ER and secreted into the ER by the endoplasmic reticulum. It is necessary to attach to the chaperone calreticulin in order for the MHC I heavy chain to be stabilized. The beta-2 microglobulin (2M) subunit of the receptor is recruited to this complex, and the calreticulin is replaced by the calnexin subunit of the receptor. The chaperones ERp57 and tapasin are responsible for recruiting this complex to TAP. Tapasin is a protein that aids in the loading of peptides from TAP into the peptide-binding groove of the MHC I protein. Once the right peptide has been loaded, the MHC I heavy chain: beta-2 microglobulin: peptide trimer is stable and may shed the chaperones and migrate to the cell surface, where the peptides can be presented to CD8+ T lymphocytes, as shown in Figure 1.

The exogenous pathway also involves the formation of nascent MHC II alpha and beta chains, which are folded together in the lumen with the assistance of the MHC II Associated Invariant Chain (Ii), which also serves to block the peptide-binding groove of MHC II, thereby preventing the binding of endogenous peptides in the ER lumen. As a result of this, the MHC II is transported into endosomes, where it is fused with exogenous proteins that have been ingested by the APC, either through phagocytosis, pinocytosis, or receptor-mediated internalization. Another chaperone, DM, aids in the replacement of Class II-associated Invariant Peptide (CLIP) in the peptide-binding groove, allowing the exogenously produced peptides to replace CLIP. A stable trimer composed of the two MHC polypeptide chains as well as a peptide is generated, and the complex remains stable as it travels to the cell surface to present antigen to CD4+ T cells for the second time. The presentation of antigen to T cells initiates and directs the sort of adaptive immune response that results, which can be either a cell-mediated response or an antibody-mediated response. Without a doubt, the presentation of antigen by phagocytes to initiate an adaptive immune response for the purpose of protecting the body against infections is critical.

Cold stress has been demonstrated to have an effect on antigen presentation in fish, according to research. Cell surface MHC I was downregulated in carp when kept at 6 degrees Celsius (as opposed to 12 degrees Celsius), which appeared to be attributable to lower mRNA transcription of the 2M gene. Although 2M is not synthesized by cells in cold-adapted fish species such as rainbow trout and Atlantic salmon at 2C, it is still trafficked to the surface along with MHC I. This suggests that these fish species, in contrast to mammals and other fish species, may be adapted for virus detection at low temperatures. The findings of subsequent research demonstrate that cells cultivated at 2C, whether stimulated or unstimulated, show no accumulation of 2M in the media, suggesting that while the MHC I receptor is present on the cell surface, it is not functional. In contrast, MHC II expression in rainbow trout cells is downregulated at 2 degrees Celsius, but not at 5 degrees Celsius, suggesting that the fish may be more susceptible to bacterial infections when exposed to cold stress. Many rainbow trout genes implicated in the antigen processing pathway (APP) have been found, described, and produced polyclonal antibodies in recent investigations. These genes include MHC I and 2M, TAP1/2 tapasin, calreticulin, ERp57, and Calnexin, among others. Collectively, these investigations suggest that the APP in rainbow trout is a highly conserved gene that has been passed down through generations. Other studies investigated the modulation of APP in response to viral infection with the virus of hemorrhagic septicemia (VHSV) in the presence of a cold or in the absence of a virus infection. The protein levels of MHC I, 2M, and tapasin in rainbow trout cells that have been infected with VHSV at 14 degrees Celsius have increased, as demonstrated here. As previously observed, the levels of MHC I and 2M proteins do not change in response to cold stress. After infection at 2 degrees Celsius (cold stress), the VHSV-infected cells failed to upregulate protein levels of MHC I, 2M, and tapasin. This suggests that cold stress has an adverse effect on antigen presentation and results in an impaired immune response when cells are challenged with a pathogen, as previously demonstrated.

Using a newly generated Arctic char cell line, Semple et al. discovered that there was no difference in the protein levels of MHC I and 2M at 1, 4, or 14 degrees Celsius in nonstimulated cells when the temperature was 1, 4, or 14 degrees Celsius. Arctic charr is particularly well-adapted to extremely cold temperatures, and it is possible that they have evolved strategies to keep their antigen presentation routes operational in extremely cold conditions. This cell line, however, did not show an increase in MHC I, 2M, or ERp57 protein levels after being treated with the poly I:C solution at 14°C, indicating that the regulation of the Arctic charr antigen presentation pathway differs from that of other salmonids in response to temperature, possibly due to their adaptation to colder temperatures. The absence of MHC I, 2M, and ERp57 protein level upregulation in the APP was specific to the APP and did not

indicate a general impairment of immunity, as Mx protein was induced by poly I:C at concentrations as low as 10 g/mL in 24 h at 14°C, and in cells exposed to 1°C or 4°C within 7 days at a concentration of 50 g/mL in 24 h at 14°C. Even while there are some general tendencies in the effects of temperature on immunity in teleost fish, and notably in salmonids, there are certain species-specific variances that have most likely developed as adaptations to the environments in which these species live. As a result, while some preliminary studies have been conducted, the effects of thermal stress on APP function and MHC trafficking remain largely unknown. This represents a knowledge gap in terms of how antigen presentation is impacted in fish species that are subjected to increased magnitude and variability in environmental temperatures.

3. Adaptive Immunity and Temperature

3.1. Elements of Adaptive Immunity

Cellular (B cells and T cells) and humoral (antibodies) components of the adaptive arm of the vertebrate immune system are capable of being induced, are pathogen-specific, and are often associated with immunological memory. After recognizing antigens presented by cells expressing MHC I and II, T lymphocytes can induce particular cytotoxicity or release cytokines that operate on other lymphocytes and innate immune cells to direct a specific response against disease, depending on the antigen. B lymphocytes release antibodies in response to antigen identification or activation, and these antibodies go on to perform a variety of tasks, including opsonization, neutralization, agglutination, and activation of the complement system.

3.2. B Lymphocytes

Igs are produced by B lymphocytes, which are responsible for the production of B-cell receptors and antibodies, both in their membrane and secretory forms. In order to better understand the impact of suboptimal temperatures on these cell populations, the majority of the research to date has been conducted using channel catfish as the model. PBL proliferation in channel catfish was found to be relatively unaffected by the temperatures studied in vitro. However, peak proliferation was found to be delayed as the assay temperature decreased, with the greatest delay observed at 17 degrees Celsius compared to 22 degrees Celsius, 27 degrees Celsius, and 32 degrees Celsius. However, when PBLs were isolated from fish that were kept at 11 degrees Celsius rather than 24 degrees Celsius, proliferation was suppressed at both assay temperatures, 17 degrees Celsius and 27 degrees Celsius. The number of B cells in the blood decreased after exposure to 11 degrees Celsius and did not recover until 5 weeks later, indicating that channel catfish may decrease the number of circulating B cells in response to large temperature decreases, but that they may be able to acclimate to lower water temperatures over time. The authors speculated that the increase in unsaturated fatty acids in the plasma membrane of B cells from fish that were acclimated to 17°C versus 24°C in vivo had an effect on lymphocyte function. While the exact nature of the effect on lymphocyte function has not been determined, they speculated that it stiffened cell membranes, decreasing cell-to-cell interactions and, therefore, immune responses. Studies on rainbow trout have also revealed that inadequate temperatures have a negative impact on the function of B lymphocytes in the fish. After infection with Tetracapsuloides bryosalmonae, an increase in transcript expression was observed in the anterior kidney for all B cell markers studied (secretory IgM, membrane-bound IgT, pax5, and blimp1) at 15°C, and for all but IgT in the posterior kidney, with blimp1, the B cell differentiation marker, being the most strongly up-regulated. At 12 degrees Celsius, however, no substantial up-regulation was seen, showing that B cell activation in response to pathogens may be inhibited at low-temperature levels. Additionally, a statistically significant increase in posterior kidney IgM+ B cells was seen at seven weeks post-infection when the temperature was raised to 15 degrees Celsius rather than 12 degrees Celsius. Similarly, findings from another study revealed that when exposed to Aeromonas salmonicida, the percentage of B cells in the spleen and blood increased more significantly and occurred more quickly at 16 degrees Celsius than at 11 degrees Celsius, suggesting that B lymphocyte proliferation may be impaired under these conditions.

The possible impact of quick, substantial temperature drops on B lymphocytes and the teleost immune system as a whole should be taken into consideration. When the temperature was dropped from 25 degrees Celsius to 16 degrees Celsius during three hours, the percentage of B cells in the spleen and blood of the common carp decreased significantly, but this was reversed after 24 hours. When exposed to acute stress, researchers detected an increase in the number of annexin V-positive B cells in the blood immediately following the temperature shock, suggesting that there may be a drop in the number of circulating B cells. It is vital to be mindful of the possibility that big temperature drops can operate as short-term stressors and immunosuppressors in both in vivo and in vitro research, despite the fact that temperature swings in the wild do not occur as quickly as the changes employed in these experiments.

3.3. T Lymphocytes

The effects of suboptimal temperature on T cells have been examined the most extensively in channel catfish, which is a small animal. T cell growth in response to the T cell mitogen concanavalin A was suppressed, and the quantity of T cells in the blood was decreased when PBLs were isolated from fish maintained at 11 degrees Celsius as opposed to 24 degrees Celsius. This is similar to the findings in B cell investigations. However, in contrast to B cells, peak T-cell proliferation in PBLs induced with concanavalin A was related to temperature and was significantly reduced at 22 degrees Celsius when compared to 27 degrees Celsius and 32

degrees Celsius. More importantly, temperature-dependent time course mixed leukocyte reactions utilizing PBLs from 24C-acclimated fish revealed that the fastest reaction occurred at 27 degrees Celsius and the slowest reaction occurred at 17 degrees Celsius. Following this, researchers discovered that pulsed antigen-stimulated monocyte cell lines at 11°C, 17°C, or 27°C could stimulate the proliferation of autologous responder T cells from PBLs, but peak proliferation was delayed at the lower temperatures. Across all temperatures tested, there was an increase in radioactivity associated with antigen-presenting cells as a result of uptake of radiolabelled antigen, indicating that the observed suppression of T cell responses at suboptimal temperatures was not due to impaired antigen presentation by the antigen-presenting cells. Further research attempted to explain the differences in the degree of channel catfish T and B cell proliferation in response to low temperature by examining the fatty acids present in these cells in subsequent experiments. Unsaturated fatty acid levels in the plasma membrane of T cells rose at 17 degrees Celsius compared to 24 degrees Celsius, similar to what was observed in B cells.

T cells, on the other hand, do not appear to be able to generate oleic acid endogenously from stearic acid, although B cells do. As a result, B cells accumulate stearic acid in their membranes, which reduces the fluidity of the membrane. This discrepancy could explain why low temperatures have varying impacts on lymphocyte proliferation in different people.

The addition of exogenous oleic acid to T cells resulted in the recovery of 60 percent of the proliferative response to concanavalin A at a lower temperature, further confirming this hypothesis. Other fishes have also been reported to experience the inhibitory effects of suboptimal temperatures, which frequently result in a decrease in T cell activation and activity. For example, in the common carp, T lymphocyte proliferation in PBLs appears to be proportional to temperature, with proliferation increasing from 12 degrees Celsius to 20 degrees Celsius to 28 degrees Celsius both in vivo and in vitro. Cell-mediated cytotoxicity, thought to be caused by specific cytotoxic T cells in the ginbuna crucian carp, was found to be more efficient when cells were cultured at 25 degrees Celsius rather than 20 degrees Celsius or 15 degrees Celsius, suggesting that this process may also be temperature-dependent. However, whereas particular cell-mediated cytotoxicity in the common carp was downregulated at 9 degrees Celsius compared to 18 degrees Celsius, a drop in activity was also detected at 26 degrees Celsius, which may represent a nonpermissive higher temperature for carp. T cells in rainbow trout head-kidney leukocytes produced less macrophage activating factor, now known as interferon-gamma (IFN-gamma) when the cells were kept at 6 degrees Celsius compared to 10 degrees Celsius and 18 degrees Celsius, although supernatants from each temperature condition were still able to significantly affect the respiratory burst activity of macrophages. Conclusions

Some IFN- activity was regained when leucocytes were obtained from fish that had been acclimated to 6 degrees Celsius and then allowed to adapt for 48 hours to the higher assay temperatures, demonstrating that the harmful effects of inadequate temperatures on T cells may be reversible. Overall, our findings appear to indicate that suboptimal temperatures have a deleterious influence on T cell proliferation and activity in fish, which may have an adverse effect on their ability to control the spread of infection and mediate particular responses against pathogens in the fish population. The polarization of helper T (TH) cells results in the induction of TH1 and TH2 cells, which are responsible for the transmission of type I and type II immunity. Th1 cells mediate macrophage activation and increased cytotoxic T cell activity, which are important for responding to intracellular bacteria, whereas Th2 cells are important effector cells against extracellular parasites because they have the ability to activate mast cells and eosinophils, which are important for responding to extracellular parasites.

The polarization process in rainbow trout has been shown to be affected by inadequate temperatures, according to some experimental findings. At the cold-stress temperature of 12 degrees Celsius, TH1 associated genes in rainbow trout kidney leukocytes were modestly elevated, however, TH2 associated genes were not. This was observed following exposure to Tetracapsuloides bryosalmonae. Both sets of genes, on the other hand, remained elevated for 2 weeks after exposure at the control temperature of 15 degrees Celsius. Only the TH2 related gene expression remained elevated six weeks after the exposure, demonstrating that suboptimal temperatures may be able to influence immune response polarization.

3.4. Antibodies and the Humoral Response

The suboptimal temperature has been shown to have negative effects on antibody production in a variety of fish species, with B cell responses to T-dependent antigens being the most severely affected. T-dependent antigens such as dinitrophenol (DNP) conjugated to keyhole limpet hemocyanin (KLH) elicited a temperature-dependent response in channel catfish, whereas the magnitude of the peak response of IgM secreting cells isolated from PBLs for the T-independent antigen trinitrophenol-LPS elicited a temperature-independent response. Additionally, the peak reaction for both antigens was delayed as the temperature decreased, with the largest delay happening at 17 degrees Celsius vs 22 degrees Celsius, 27 degrees Celsius, or 32 degrees Celsius.

When DNP-KLH was used to stimulate antibody synthesis in carp, researchers discovered that the response was proportional to temperature, with antibody levels at 12 degrees Celsius much lower than those at 20 degrees Celsius or 28 degrees Celsius. This pattern of temperature impacts antibody production, on the other hand, is not always found in all cases. It was shown that decreasing antibody production in response to DNP-KLH and trinitrophenol-LPS over a three-hour period following an in vivo

temperature drop from 25 degrees Celsius to 16 degrees Celsius was associated with a decrease in temperature. The production of mucosal immunoglobulins in channel catfish after bath vaccination with DNP-KLH was inhibited at 30 degrees Celsius as compared to 23 degrees Celsius and 15 degrees Celsius, in contrast to the serum immunoglobulin response. As a result of these studies, it appears that low temperatures have a greater impact on the ability of fish to produce antibodies against T-dependent antigens than they do on the ability of fish to produce antibodies against T-independent antigens, except in the case of rapid, large temperature reductions or mucosal responses.

A significant concentration of natural antibodies may be discovered in fish serum, which serves as an important mediator of nonspecific immunity and assists in the provision of protection against infections. Low temperatures appear to have a different effect on the basal blood levels of these antibodies in various fish species than they do in humans. In rainbow trout greater than 1 kg from the same fish farm, serum immunoglobulin levels were higher in the summer than in the winter in fish larger than 1 kg, despite the fact that the mean seasonal temperatures were 19°C and 7°C, respectively.

In contrast, no variations in basal plasma IgM levels were seen in tilapia kept at 25 degrees Celsius or 12 degrees Celsius throughout a 15-day period, but it is possible that differences in basal plasma IgM levels might have been observed over a longer time interval. A greater amount of data is needed to evaluate the influence of environmental temperature drops on basal immunoglobulin levels, while current findings indicate that the outcome varies depending on the species.

The influence of suboptimal temperature on antibody formation in response to live or inactivated pathogens and vaccinations is likewise variable, albeit it is largely suppressive in most cases. When channel catfish were infected with Ichthyophthirius multifiliis, serum antibody levels were much greater at 25 degrees Celsius and 30 degrees Celsius than they were at 15 degrees Celsius and 20 degrees Celsius, with no detectable specific antibodies after 21 days at 15 degrees Celsius. In addition, serum antibody titres following injection of inactivated Yersinia ruckeri or phosphate-buffered saline were found to be temperature-dependent, with the lowest titres observed at 5°C versus 15°C and 25°C, indicating that humoral responses against inactivated pathogens, as well as responses to injection, are suppressed by cold temperatures. Similar results were achieved in rainbow trout when a DNA vaccination against VHSV was used in conjunction with the experiment. At 15 degrees Celsius, a higher serum antibody titre was reported than at 10 degrees Celsius, and no detectable antibodies were found at 5 degrees Celsius. Furthermore, the percentage of the population that survived after being exposed to the virus was shown to be temperature-dependent, suggesting that vaccination efficacy may be reduced at lower temperatures.

However, in rainbow trout, production of specific antibodies to inactivated Aeromonas salmonicida appeared to be delayed at the normal temperature of 15 degrees Celsius compared to 12 degrees Celsius, with both reaching similar levels by 90 days post-stimulation, demonstrating that the effects of suboptimal temperatures are not universally similar. Overall, our findings indicate that inadequate temperatures have a suppressive effect on antibody production in response to live or inactivated pathogens and vaccinations, thus impairing the fish's capacity to clear illnesses successfully when exposed to suboptimal temperature conditions.

4. Concluding Remarks and Prospects for the Future

Because teleosts have adapted to a wide range of environmental temperatures, the influence of environmental temperature on their immune systems varies depending on the duration and size of the temperature shift as well as the fish species under investigation. Overall, lower temperatures cause immune response pathways to shut down or slow down, which is often reversible upon return to higher temperatures, suggesting that overwintering techniques may be employed in these conditions. Cold stress had a variety of effects on different components of the innate immune system, with the enhancement of innate cellular components potentially compensating for deficiencies in adaptive immunity, as there was a consistent suppression of the adaptive immune system in response to colder temperatures, as previously reported.

Because low temperatures are required to produce adaptive immunity, it is possible that this suppression is linked to some of the negative effects of low temperatures on other parts of the innate immune system. It is possible that these impacts could result in a diminished ability of fishes to respond to diseases during the winter months or in response to temperature reductions, which will have a severe influence on their overall health. However, at low temperatures, this tradeoff may be energetically advantageous to the fish host, similar to the reduced immune responses observed in hibernating mammals, and it is possible that this is why the effects of cold temperatures on the immune system appear to be universally conserved.

When attempting to investigate the effects of inadequate temperatures on the teleost immune system, there are numerous difficulties to overcome. Because different model systems have different thermal optimums and preferences, it is difficult to compare the magnitude of temperature decreases across different experiments. For example, a temperature change as small as 3 degrees Celsius may negatively impact one fish species while having no effect on many other fish species, and vice versa. Aside from that, acclimatization circumstances can vary substantially, ranging from fast huge temperature reductions to keeping fish at a specific temperature for several months at a time.

Therefore, it is not unexpected that, whereas sudden temperature drops appear to be extremely damaging, fish that have been subjected to long-term acclimatization tend to be slightly better equipped to cope with cold temperatures. One other significant factor to consider is the temperature of the assay used. A lot of studies focused on different immunological parameters have found that peak response activity is observed when the assay temperature is equal to the temperature of the fish acclimation period. However, because this component is frequently overlooked in the literature, it might be difficult to appreciate the full implications of such studies' findings.

While some of these disparities are more difficult to reconcile on a practical level than others, it is critical that future research make an effort to account for them in order to make it easier to compare results from different experimental studies.

The last point to consider for future studies is the importance of employing more comprehensive experimental methodologies. When it comes to dealing with initial pathogen interaction, basal levels of immune components are significant; nevertheless, inducible elements are equally important and necessary for the clearance of infectious agents. These two sides of the immune system are not often addressed together in the same study. Aside from that, the temperature has an impact on the pathogenicity of infections, but it can be difficult to distinguish these effects from those of the host immune system at times. Despite this, it is important to recognize the potential value of conducting more intensive and comprehensive studies that can help us gain a better understanding of the impact of low environmental temperatures on host-pathogen systems, specifically in elucidating the underlying immune mechanisms that result in host mortality when fishes are exposed to acute or chronic cold stress, as well as the potential value of conducting more intensive and comprehensive studies that can help us gain a better understanding of the impact of low environmental temperatures on host-pathogen systems.

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