PERSPECTIVE

DIFFERENTIAL EFFECTS OF TEMPERATURE ON SPECIFIC AND NONSPECIFIC IMMUNE DEFENCES IN FISH

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Summary

The susceptibility of fish to disease is partly dependent on their environment, in particular on water temperature. It is generally accepted that lower temperatures adversely affect specific immune responses mediated by T helper cells. The probable mechanisms involved in such suppression in teleost fish are reviewed. Furthermore, the effects of temperature on nonspecific defences, such as phagocytosis and cytotoxicity, are described and total immune competence in teleosts at low environmental temperatures is discussed.

Key words: temperature, ectotherm, teleost, immunity, fish.

The close relationship that exists between teleost fish and their environment is the basis of a wide variety of studies. In particular, fish have a body temperature that is essentially the temperature of the surrounding water (Fry, 1967) so that their entire physiology, including immune functions, is influenced by environmental temperature. Most infectious diseases, such as the spring viraemia of carp (*Cyprinus carpio*), occur at lower environmental temperatures (Baudouy *et al.* 1980). Thus, the pathological situation in fish depends both on temperature-dependent immune system regulation and on pathogen growth. The proposal that low environmental (and hence body) temperatures can be immunosuppressive has been supported by studies such as that of Bly and Clem (1992). This observation is the conclusion of numerous studies concerning the thermosensitivity of specific immune responses, whereas few data are available on the effects of temperature on nonspecific defences in fish. In this article, we attempt to review these two fields of immunomodulation induced by environmental temperature and the efforts that have been made to understand the mechanism(s) involved.

The effects of temperature on specific immune responses, especially the humoral response, have been studied (Avtalion, 1969; Avtalion *et al.* 1970). These experiments, conducted on carp immunized against bovine serum albumin, revealed that the primary antibody response is suppressed at low temperatures. In contrast, the secondary response can be elicited at low temperatures if immunological memory is established at high temperatures (Avtalion *et al.* 1972). In contrast, some results showed that lower temperatures induce a delay in the peak of the primary response but have no effect on the magnitude of the response (Rijkers *et al.* 1980). On the basis of these observations, the existence of one or more thermosensitive step(s) during the humoural response was postulated. Consequently, it was suggested that temperature can influence discrete events during the maturation and/or cooperation of immunocompetent cells (Cone and Marchalonis, 1972; Avtalion *et al.* 1973). Furthermore, lymphoproliferative responses to concanavalin A (ConA) and phytohaemagglutinin were reduced at low temperatures (<22 °C) in catfish (*Ictalurus punctatus*) (Cuchens and Clem, 1977). Subsequently, similar effects of temperature were observed on the specific cytotoxicity of carp kidney lymphocytes against trinitrophenyl (TNP)-modified autologous cells (Verlhac *et al.* 1990). Lower environmental temperatures adversely affected both cellular and humoural specific immune responses in various fish species. The immunologically 'permissive' and 'non-permissive' temperatures depend on fish species, and the non-permissive temperature has been established as 4 °C in salmonids, 14 °C in carp and 22 °C in catfish (Bly and Clem, 1992).

The first approach undertaken to determine the mechanism(s) by which lower temperatures reduces specific immune responses was based on work using hapten–carrier conjugates (Weiss and Avtalion, 1977). Antibody production against hapten, normally suppressed at low temperature, occurs when the carrier preimmunisation is performed at high temperature. Avtalion and collaborators suggested that the low-temperature-sensitive step in antibody production was the generation of helper function. To confirm this hypothesis, leukocyte separation methods were required: *in vitro* culture and cell partitioning experiments were carried out with channel catfish cells and served to define further the cellular basis for

low temperature immunosuppression in this fish (Clem *et al.* 1991). Initial studies reported that the lymphoproliferative response of T-cells to ConA was diminished at low temperatures, while that of B-cells to lipopolysaccharide was not affected (Clem *et al.* 1984). Mixed leukocyte reactions (mediated by T-cells) were also shown to be sensitive to low temperatures (Miller *et al.* 1986). Moreover, experiments using both thymus-dependent (TD) and thymus-independent hapten–carrier conjugates discriminated between lymphocyte functions that are targeted by temperature (Miller and Clem, 1984). Only the generation of T helper, and not T memory or B-cell, function, was impaired by lower temperatures. Futhermore, it was reported that the previously observed suppression of primary T-cell responses at low temperatures was not due to a failure of antigen-presenting cells to process TD antigens (Vallejo *et al.* 1992) or to produce interleukin-1 (IL-1) (Bly and Clem, 1992). Taken together, these data have clearly defined the affected subset of cells in the specific immunosuppression observed at low temperature.

In an attempt to locate the low thermosensitive step during catfish T helper cell activation, proliferative responses to ConA were subjected to abrupt changes of temperature *in vitro* (Clem *et al.* 1984). This step was found to occur relatively early in cell activation (during the first 8 h following stimulation). Experiments using phorbol ester ionophore stimulation specified the critical point, which was located before protein kinase C activation (Ellsaesser *et al.* 1988; Lin *et al.* 1992). This thermosensitive step can be a very early event, such as T-cell receptor–CD3 transmembrane signalling and/or G-protein activation. Low temperatures would exert their influence at the level of the T helper plasma membrane.

Consequently, studies were conducted to identify the event preceding protein kinase C activation that is sensitive to low temperatures. T-cell culture supernatants, containing IL-2, were able to rescue ConA-induced lymphoproliferation, which involves the presence of IL-2 receptors on T-cells at low temperature (Clem *et al.* 1991; Lin *et al.* 1992). Moreover, T-cells were able to patch and cap receptor–ligand complexes at low temperatures to the same extent as B-cells (Bly *et al.* 1987, 1988). Futhermore, appropriate acclimation of fish to low temperature restored the T helper responses (Clem *et al.* 1984; Miller and Clem, 1984). It was postulated that catfish T-cells may be unable to develop homeoviscous adaptations to compensate for the rigidifying effect of lower temperatures on cell membranes. This hypothesis has been partially supported by results obtained from fluorescence polarisation studies in catfish (Abruzzini *et al.* 1982; Bly and Clem, 1988). T-cells required more time than B-cells to achieve homeoviscous adaptation of their plasma membrane, but were ultimately able to do so. Further investigations concerning variations in plasma membrane composition at low temperature showed that the ability to develop T-cell functions was correlated with an increase in the level of oleic acid (18:1) and a decrease in the level of stearic acid (18:0) (Bly *et al.* 1986). Moreover, the addition of stearic acid induced suppression of the T-cell response to ConA and the addition of oleic acid induced stimulation of the T-cell response to ConA (Bly *et al.* 1990). Catfish B-cells were able to desaturate stearic acid to oleic acid, whereas T-cells were not. These observations suggest that major qualitative, but minor quantitative, changes in fatty acid composition occur in lymphocyte plasma membranes. According to these authors, these processes of homeoviscous adaptation of lymphocyte plasma membranes may be an important aspect of teleost immunity, but are probably not sufficient to explain immunomodulation induced by low temperature.

Other plasma membrane components, such as the proteins and glucides known to be involved in various cell recognition processes, can also be affected by environmental temperature (Sharon and Lis, 1989). In an attempt to assess this hypothesis, studies have been undertaken in our laboratory on carp (*Cyprinus carpio*) leukocytes (Le Morvan *et al.* 1996). Protein profiles prepared using electrophoresis and electrofocusing showed no qualitative modifications at low environmental temperature. In contrast, glucides seem to be thermosensitive. In particular, we noted that sialic acid levels decreased with temperature reduction. Thus, the uncovering of other terminal sugar residues or a different arrangement of glycans induced by desialylation of glycoconjugates correlates with a modification of plasma membrane structure and function. Consequently, lower environmental temperatures may act on the immune response in part by changing the cell surface carbohydrates.

Low environmental temperature can also act through other mechanisms, such as stress. In catfish, blood serum chemistry and lymphocyte and neutrophil counts were differentially affected by low temperature compared with transport-induced stress (Ellsaesser and Clem, 1986; Bly and Clem, 1991). However, our results in carp showed an increase in the level of plasma cortisol 2 h after an abrupt change in water temperature from 20 to 12 °C (Le Morvan *et al.* 1995). Taken together, our results reveal a direct effect of low temperature on cellular immune function by modulation of plasma membrane structure and probably, during the first hours following temperature decrease, by stress induction. Nevertheless, we can only conclude with certainty that lower temperatures modulate specific immune response in various ways.

In order to continue this investigation, it would be of value to determine the mechanism(s) by which optimal temperature enhances specific immunity. However, this phenomenon has been ignored, probably because of the interest in suppression induced by lower temperatures in winter (Bly and Clem, 1992). Similarly, it would be interesting to analyse the effect of environmental temperature on nonspecific immunity since little is known about thermal influences on nonspecific defences in fish. Most studies have been conducted on the first important immunological process to become active during resistance to infection, namely phagocytosis. It was noted in channel catfish (*Ictalurus punctatus*) that the effect of temperature on phagocytosis was a function of the assay temperature, although phagocytes appeared to be more resistant to low temperature than were lymphocytes (Scott *et*

al. 1985; Ainsworth *et al.* 1991). However, adaptation to low temperature did lead to an increase in the respiratory burst activity, which would imply improved bactericidal ability (Dexiang and Ainsworth, 1991). This greater effectiveness of phagocytosis at low environmental temperature was also reported in tench (*Tinca tinca*) (Collazos *et al.* 1994*b*). Lastly, macrophages isolated from rainbow trout (*Oncorhynchus mykiss*) cultured at low temperatures were responsive to macrophage activating factor and showed a higher relative increase in respiratory burst activity compared with their counterparts cultured at higher temperatures (Hardie *et al.* 1994). Results obtained in carp in our laboratory (Le Morvan *et al.* 1997) showed that macrophage respiratory burst activity and yeast phagocytosis are increased with a reduction of environmental temperature. Moreover, the effects of temperature on a potential opsonin, phosphorylcholine-reactive protein (PRP), have been studied in channel catfish (Szalai *et al.* 1994). It appears that during the winter months, while PRP levels are low, the binding with its ligand occurs with high avidity.

Since they may be of great importance for resistance to disease, the effects of temperature on nonspecific immune functions in fish need to be definitively addressed. For this reason, we studied the effect of temperature on nonspecific cytotoxic cell activity. Nonspecific cytotoxic cells (NCCs) from teleost fish are known to be the phylogenetic precursor of mammalian natural killer (NKs) cells and lyse a wide variety of human and mouse tumour target cells (Evans and McKinney, 1991) and certain fish protozoan parasites (Graves *et al.* 1985). It appears that NCCs are thermosensitive, in particular to lower temperatures. We have noticed in carp that lytic activity mediated by NCCs against murine mastocytoma cells is markedly increased by low environmental temperature. Our results reveal that low environmental temperatures have a direct effect on NCCs, perhaps by acting on their plasma membrane (Le Morvan *et al.* 1995). This phenomenon occurs as early as 24 h after exposure of the fish to low temperature and disappears 8 weeks later, with a maximum effect after 4 weeks (Le Morvan *et al.* 1996). Nevertheless, we cannot rule out a possible indirect effect of low temperature on NCCs through stress induction (Kurata *et al.* 1995). Collectively, our results are in agreement with recent studies in cyprinid fish on the alternative and classical complement pathway activities which were found, respectively, to be predominant and depressed during cold periods (Yano *et al.* 1984; Hayman *et al.* 1992; Collazos *et al.* 1994*a*). These data indicate that teleost fish subjected to lower environmental temperatures are not fully immunocompromised and emphasize the important role of nonspecific defences in winter when the specific immune response is depressed.

In conclusion, nonspecific defences in teleost fish tend to offset specific immune suppression at low environmental temperatures until the specific immune system adapts. The study of mechanism(s) by which lower temperatures modulate nonspecific immunity may reveal those involved in the modulation of specific immunity.

Suboptimal *in vitro* temperatures appear to suppress murine T helper cell, but not B-cell, responses (Yang *et al.* 1990), and oleic acid can rescue this suppression (Buttke *et al.* 1991). These results obtained with mice are remarkably similar to those reported previously for channel catfish. These similarities argue that low temperatures affect the immune functions of ectothermic and endothermic lymphocytes in a seemingly phylogenetically conserved fashion. This effect of low temperature may prove useful in dissecting the cellular events required for the activation of T helper cells in fish as well as in mammals.

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