Immune Defense Mechanisms in Fish to Protozoan and Helminth Infections

DONALD L. EVANS AND JOHN B. GRATZEK
Department of Medical Microbiology, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602

SYNOPSIS. Fish respond to parasite infections (and infestations) by the production of antigen specific IgM-like antibodies as well as by the elaboration of nonspecific soluble factors and phagocytic cells. Fish infected with the hemoflagellates *Trypanosoma* and *Cryptobia* generally elicit antibody and complement dependent responses. The levels of these responses vary depending on ambient temperature fluctuations. Below 10-15°C there is an almost complete depression of immune responsiveness. The protozoan that has received the greatest emphasis regarding studies of immunity is *Ichthyophthirius multifiliis*. Both primary and secondary antibody responses are produced in fish to this parasite. Cellular responses are also produced against "Ich." These cells (nonspecific cytotoxic cells) may provide an important (but previously not described) component of anti-parasite resistance.

The second major group of parasites considered in this review are categorized as helminths. Among these, the cestodes, trematodes (mono- and digenetic), and Acanthocephala have been studied for elicitation of immune responses in fishes. For virtually all organisms studied, the host response was mediated via antibodies (plus complement in most cases). Cellular responses (neither antigen specific nor phagocytic activities) have not been shown to mediate any type of anti-helminth response in fishes.

INTRODUCTION

Substances participating in responses to parasite infections

Non-specific immunity. Several different substances have been described which may participate in innate or nonacquired defense responses in fishes. Substances involved in these types of responses may be categorized as either soluble or cellular components of nonspecific immunity.

Non-specific soluble factors. Soluble mediators of resistance (nonspecific immunity) may be found in the serum, or mucus membranes, or as secretory products of activated cells. Certain of these soluble factors that have been described previously are: components of a system which appear to have complement-like activity (Harrell et al., 1976); lysozyme, a low molecular weight component which lyases certain microorganisms (Fletcher and White, 1973; Flange et al., 1976; Murray and Fletcher, 1976); C-reactive protein, a normal component of serum that may act as a bacteriolytic substance (Fletcher and Baldo, 1977); transferrin, an iron binding glycoprotein that may play an important role in nonspecific resistance (Suzumoto et al., 1977; Buller et al., 1978); and some have suggested that interferon-like molecules may be effective in certain antiviral responses in fish (De Sena and Ria, 1975; Dorson et al., 1975).

Non-specific cellular immunity. Nonspecific cellular immunity in fishes may be expressed as either phagocytes or cytotoxic cells. Differing reports have been published regarding the phagocytic ability of fish reticuloendothelial cells (Klontz et al., 1965; Klontz and Yasutake, 1966; Ellis et al., 1976; McKinney et al., 1977). Macrophages (Finn, 1970; Finn and Nielsen, 1971) and their roles as phagocytes in inflammation and infectious disease have only been partially identified. Perhaps better characterized is the system of cytolytically active cells referred to as nonspecific cytotoxic cells (Evans et al., 1984a; Evans et al., 1984b; Evans et al., 1984c; Evans et al., 1984d; Graves et al., 1984; Carlson et al., 1985; Evans et al., 1987). These in addition to other nonantigen specific cells (Hinuma et al., 1980; Petey and McKinney, 1981) may be the fish equivalent of mammalian NK cells.

Antigen-specific immune responses. All vertebrates so far examined for the presence

of surface membrane immunoglobulin bearing cells have been found to have this type of cell. Channel catfish leukocytes have been examined for surface antigen phenotype using monoclonal antibodies (Lobb and Clem, 1982; Lobb et al., 1984; Ellsaser et al., 1985). Cytofluorographic examination of these labeled cells revealed that 40% of catfish peripheral blood leukocytes have surface immunoglobulin. Other studies (Sizemore et al., 1984; Miller et al., 1985) have shown that peripheral blood from fish can be divided into 3 functionally different subpopulations based on the presence of surface immunoglobulin. Functional heterogeneity of fish cells was also shown using monoclonals generated against slgM (De Luca et al., 1983). In this study, slgM+ cells were responsive to LPS but not Con-A, indicating that in fish, B-cells were present and they exhibited surface immunoglobulin. Immunoglobulins (IgM) are also found in the mucus in plaice and channel catfish (Bradshaw et al., 1969; Fletcher and Grant, 1969; Ourth, 1980). Others (Simonsen et al., 1987; van Ginkel et al., 1987) have described the presence of T-cell responses in teleost fishes. A monoclonal antibody designated 13C10 detected a T-helper subset in catfish, and additional evidence for T-lymphocyte heterogeneity was provided by experiments where mixed leukocyte responses were generated in rainbow trout.

These studies have shown that, when considered as a group, teleost fish have progressed to an immunologic stage of development where antigen specific antibody and T-cell mediated responses are functional. In addition, considerable evidence has shown that nonspecific mediators of resistance (including phagocytic and cytotoxic cells) should be considered as principal mediators of parasite resistance.

**Immunity to Protozoa**

Trypanosoma and Cryptobia (Mastigophora). The members of the genera Trypanosoma and Cryptobia represent the two groups of hemoflagellates that have been most frequently reported to cause disease and mortality in fishes. Trypanosome infections have been reported in salmonids, carp, goldfish, and flounder as well as in a number of other freshwater, marine, and estuarine bottom feeding (demersal) fishes.

The host range of the genus Cryptobia appears to be somewhat restricted. This hemoflagellate has been found in 28 species of fishes (Backer and Katz, 1965) including 8 species of salmonids (Jones et al., 1986). Many species of freshwater fishes are however naturally refractive to infection by this highly species-specific hemoflagellate (Bower and Woo, 1977). In addition to inherent nonsusceptibility of certain fishes (presumably based on some measure of nonspecific immunity in addition to genetic insusceptibility) factors such as environmental temperature drastically affect morbidity (parasitemia) and Cryptobia associated mortality (Woo et al., 1983; Bower and Margolis, 1985). In fish refractory to infection by this hemoflagellate, the explanation for resistance to C. catostomi (Bower and Woo, 1977) was via the alternate pathway for activation of complement mediated lysis. A similar result using the "plasma incubation test" (where nonheat inactivated serum decreased the viability of the parasite following coincubation for different lengths of time) was not observed using infected rainbow trout (Salmo gairdneri) plasma in the presence of C. salmositica. Perhaps this lack of plasmacidal activity of infected trout is caused by an apparent immunosuppressive effect of C. salmositica (Jones et al., 1986). Rainbow trout infected with this hemoflagellate had significantly suppressed antibody responses to sheep red blood cells and to Yersinia ruckeri. The authors of this study proposed that immunosuppression was nonspecific, probably caused by antigenic competition (Jones et al., 1986).

The second hemoflagellate of importance regarding studies of immune mechanisms belongs to the genus Trypanosoma. Trypanoplasma bullocki (Strout) is found in marine (Burreson, 1982) and estuarine fishes; Trypanoplasma salmositica is a hemoflagellate found in coho and Pacific salmon (Oncorhynchus kisutch) (Woo, 1979) and in many species of freshwater teleosts; and Trypanosoma danilewskyi and T. borelli infect
many different species of freshwater teleosts including carp and goldfish (Dykova and Lom, 1979; Woo, 1981). An additional factor which affects susceptibility to infection by hemoflagellates is seasonal temperature fluctuations. At temperatures below 10–15°C there is a near-complete depression of immune response capability in fish (Rijkevs et al., 1980a; Rijkers et al., 1980b). Lower temperatures not only prolong the latent periods for generation of specific immunity, but also increase susceptibility to pathogenic and opportunistic parasites (Cottrell, 1977). This relationship was more recently shown by investigating the relationship between infection of summer flounder with *T. bullocki* and immune responsiveness (Sypek and Burreson, 1983). In this study, using the in vitro “plasma incubation test,” increased “trypanoplasmacidal” activity occurred over a range of 10–22°C. Lysis was not observed by plasma from flounder maintained at 5°C. Trypanosomes were completely lysed by plasma from flounder maintained at 22–24°C. Interestingly, once the trypanosomes were cleared from the infected flounder (22–24°C) plasmacidal reactivity could no longer be detected. In addition, because depletion of complement from the plasma completely removed the lytic activity, an implied antibody and complement mediated mechanism of immunity was suggested. One other study (Woo, 1981) had previously shown a similar relationship between environmental temperature and the lytic activity of immune plasma obtained from *T. daniilewskyi* infected goldfish. In this study, it was also shown that immune fish could produce protective secondary responses, and that plasma from these immune goldfish could passively protect naive goldfish from infection by this hemoflagellate. The production of secondary antibody responses has also been reported (Burreson and Frizzell, 1986) in summer flounder against *T. bullocki*.

Evidence to support the involvement of cellular mechanisms of resistance to hemoflagellate infections by *T. salmonis* was provided by a study (Woo, 1979; Sypek and Burreson, 1983) showing the phagocytic uptake of hemoflagellates by monocytes and macrophages in ascitic fluid. Phagocytosis appeared to be less dependent on temperature because cells obtained from flounder kept at 5–10°C had approximately the same numbers of mononuclear phagocytes as samples taken from flounder kept at higher temperatures.

Ichthyophthirius (*Holotrichia*). The ciliated protozoan of greatest importance regarding studies of antiparasite immunity in freshwater studies is *Ichthyophthirius multifiliis*. This organism has a relatively simple life cycle. The parasite resides in the skin and gills (as trophozoites) where it parasitizes host tissue. It then detaches after a period of growth, enters the aquatic environment and undergoes several cycles of cell division with the production of new infective stages (theronts). These infective stages then elicit antibody responses that in some cases produce protective immunity (Hines and Spira, 1974). Both primary and secondary anti-“Ich” responses have been reported in black mollies (*Poecilia latipinna*) (McCollum, 1986), rainbow trout (*Salmo gairdneri*) (Wahl et al., 1986), and carp (*Cyprinus carpio* L.) (Houghton and Matthews, 1986). Although these investigators and others (Wahl and Meier, 1985; Clark et al., 1987) have shown that serum from “immune” fish can immobilize the parasite or induce tomite agglutination, direct evidence has not been produced that these activities are mediated by immunoglobulins. One study (Clark et al., personal communication) however has shown good evidence of immunoglobulin mediated responses of channel catfish to ciliary antigens of “Ich” using an enzyme linked immunosassay. Because direct studies of the host-parasite immune interaction in ichthyophthiriasis is very difficult (“Ich” is an obligatory parasite and large quantities of viable organisms are difficult to obtain in vitro), *Tetrahymena pyriformis* has been extensively used as a model system for “Ich” studies (Goven et al., 1980a, b). These protozoans have similar morphological and antigenic characteristics (Corlis, 1979). Antigenic relatedness has more recently been shown (Dickerson et al., 1986) between “Ich” and *Tetrahymena*. Antigen prepared from whole “Ich” tomites sig-
nificantly reduced the antibody activity of antiserum generated against *Tetrahymena* cilia using a passive hemagglutination assay. In other studies (Dickerson *et al.*, 1984; Goven *et al.*, 1980a, b) it has been shown that immunization of catfish with cilia from *T. pyriformis* elicits a protective "immune" response against "Ich" challenge.

Perhaps more convincing evidence of specific immune response mechanisms involved in anti-"Ich" reactions comes from studies of nonspecific cytotoxic cells (NCC). Extensive work has been carried out studying the properties and functions of NCC (Evans *et al.*, 1984c; Evans *et al.*, 1984d; Graves *et al.*, 1984; Carlson *et al.*, 1985; Graves *et al.*, 1985a, b; Evans *et al.*, 1987). NCC have been shown to bind to *Tetrahymena pyriformis*; *Tetrahymena* can competitively inhibit NCC lysis of NC-37 target cells; and *I. multifiliis* can likewise competitively inhibit NCC lysis of *Tetrahymena* (Graves *et al.*, 1985b). These results clearly demonstrate that NCC recognize antigenic determinants found on both "Ich" and *Tetrahymena*; further, these protozoan determinants are similar (or the same) as those recognized by NCC on other eukaryotic cells. Preliminary studies (Friedmann *et al.*, personal communication) demonstrate that these target cell determinants consist of a 42 kD molecule which may exist as a homodimer of 80 kD.

Further evidence to support the hypothesis that cellular immunity plays a critical role in anti/protozoan immunity was shown by investigations of the levels of NCC in direct response to "Ich" infections (Graves *et al.*, 1985a). In channel catfish heavily infected with "Ich" (e.g., moribund fish) a shift in NCC activity occurred from low to high levels in the peripheral blood. Immune fish had lower peripheral blood NCC activity. Moribund fish also had lower levels of NCC activity in anterior kidney tissue compared to levels found in the peripheral circulation. These tissue differences in levels of activity indicated that during an acute "Ich" infection, NCC may be mobilized from the anterior kidney to the peripheral blood and as such may populate peripheral sites of parasite invasion (e.g., gills and fins). This recruitment notion was further supported by the observation by others (Hines and Spira, 1973) of a shift into the peripheral circulation in "Ich" infected carp of increased numbers of immature neutrophils, blast cells, and "fine reticular cells."

To further support the relationship between parasite infection and changes in NCC activity, the percent active NCC, the NCC killing efficiency, and the binding affinity of NCC were compared in normal, "Ich" immune and moribund (Ich infected) fish (Graves *et al.*, 1985b). Moribund "Ich" infected catfish had approximately twice the number of active NCC in the peripheral circulation compared to uninfected fish. The largest difference between NCC in moribund compared to uninfected fish was that the binding affinity of NCC from moribund fish was 36 times higher than controls. The results of this study indicated that the NCC that are recruited into the peripheral blood during parasite infections have increased binding affinity and increased killing efficiency compared to uninfected controls.

These data suggest that cellular immunity may play a crucial role in mediating anti-parasite resistance. Perhaps these responses may be more critical for host survival than the role played by humoral immunity.

*Sporozoa.* These organisms are Protozoa (Hoffman, 1967) that do not have cilia or flagella, and their mobility is not obtained by means of pseudopodia. All organisms in this group develop spores. Asexual cell division is known as schizogony, the sexual reproduction process is referred to as sporogony.

*Glugea (Microsporidia).* These parasites are intracellular cyst forming sporozoans usually found in marine and brackish water fish. The life cycle is transmission from fish to fish either directly or via a crustacean vector (McVicar, 1975). Humoral immunity assessed during infection by *Glugea* spores of winter flounder (*Pseudopleuronectes americanus*) (Laudan *et al.*, 1986a, b; Dykova and Lorn, 1978; Dykova *et al.*, 1980), or following injection with solubilized *Glugea* antigen demonstrated a generalized immunosuppression response. This was indicated by showing decreased
Table 1. Summarized list of different types of immune responses to protozoan (including sporozoan) parasites.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Hemoflagellates</td>
<td>Complement dependent “plasma incubation test”</td>
<td>Bower and Woo, 1977</td>
</tr>
<tr>
<td>A. Cryptobia</td>
<td>Immunosuppression of antibody production</td>
<td>Jones et al., 1986</td>
</tr>
<tr>
<td>B. Trypanosoma</td>
<td>Complement dependent “plasma incubation test”</td>
<td>Sysek and Burreson, 1983</td>
</tr>
<tr>
<td></td>
<td>Primary and secondary antibody responses</td>
<td>Woo, 1981; Burreson and Frizzell, 1986</td>
</tr>
<tr>
<td></td>
<td>Phagocytosis</td>
<td>Woo, 1979</td>
</tr>
<tr>
<td>II. Ciliated protozoans</td>
<td>Primary and secondary antibody responses</td>
<td>Hines and Spira, 1974; McCollum, 1986; Webb et al., 1986; Houghton and Matthews, 1986</td>
</tr>
<tr>
<td>A. Ichthyophthirius</td>
<td>Parasite immobilization</td>
<td>Clark et al., 1987; Wahl and Meier, 1989</td>
</tr>
<tr>
<td></td>
<td>Inhibition of passive hemagglutination</td>
<td>Dickerson (personal communication)</td>
</tr>
<tr>
<td></td>
<td>Nonspecific cytotoxic cell lysis</td>
<td>Evans et al., 1984a-d, 1987</td>
</tr>
<tr>
<td>III. Sporozoans</td>
<td>Immunosuppression of antibody responses</td>
<td>Laudan et al., 1986a; b; Dykova et al., 1987</td>
</tr>
<tr>
<td>A. Glugea</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

levels of antibody against *Klebsiella pneumonia* and horse red blood cells (Laudan et al., 1986a). Specific suppression occurred at the level of decreased serum IgM (Laudan et al., 1986b).

Summary

Immune responses to protozoan parasites in teleost fishes have been described for two major groups, the hemoflagellates and ciliated protozoans (Table 1). Types of immunity generated against these organisms consist of both nonspecific and antigen specific responses. *Cryptobia* and *Trypanosoma* generally elicit antibody responses which may result in complement dependent lytic parasiticidal activities. Similar immune reactivities have been reported for ciliated protozoans. For this group of organisms, *Ichthyophthirius* represents the model parasite system studied. Primary and secondary antibody responses (including agglutination or parasite immobilization) have been described. Perhaps more important, a new cell type has been described (nonspecific cytotoxic cells) which mediates direct lysis of certain ciliates. Evidence has been provided that this cell may be an important (but previously undescribed) component of anti-parasite resistance.

Immunity to helminths

The term “helminths” is used to designate organisms occurring in five phyla: Platyhelminthes; Nematoda; Nematomorpha; Acanthocephala; and Annelida. Of these phyla, investigations involving studies of some level of immune reactivity in fishes have been reported for cestodes and trematodes (Platyhelminthes) and for Acanthocephala.

Cestodes. Indirect evidence shown by the demonstration of the toxic effects of *Raja radiata* serum on the viability of the tapeworm *Acanthobothrium quadripartitum* (McVicar and Fletcher, 1970) indicated the possibility of some sort of immune reaction against this parasite. The parasiticidal effect of this serum was obliterated by heat treatment and by dilution. Serum obtained from the parasite susceptible species *R. naevus* demonstrated no toxic effects. In another study (Kennedy and Walker, 1969) an indirect approach (percent recovery of viable parasites after injection), was used to show
some association between survival and immunity. However there was no evidence of antibody present in fish from which parasites were not recovered.

**Trematodes.** Species that are naturally found in fishes have been reported for both the monogenetic and digenetic groups. Monogenetic trematodes complete their life cycle in one host (Hoffman, 1967). Usually the immature worms are morphologically similar to the mature worms. Certain members of the monogenetic group may cause serious disease in cultured trout, goldfish and bluegills (Hoffman, 1967). Evidence that certain fish may produce some type of immunity to monogenetic trematode infections has been shown using challenge studies. Finding of decreased numbers of *Gyrodactylus bullatarudis* in infected guppies (*Poecilia reticulata*) and the occurrence of a decreased duration of secondary infection indicated some type of resistance response (Scott and Robinson, 1984). A similar relationship between *Gyrodactylus* infection and resistance to challenge was also reported for sticklebacks (Lester and Adams, 1974). Exact mechanisms of resistance were not determined in either of these studies; however it was suggested that "resistance" might be associated with mucus secretions.

For digenetic trematodes sexual reproduction in the adult is followed by asexual multiplication in the larval stages (e.g., digenetic life cycle). Generally the life cycle of the digenetic trematode involves a first intermediate host. In this host (snails, clams, etc.) the trematodes develop to produce cercariae and metacercariae. The metacercariae are free swimming forms which attach to and penetrate fish tissues. It is at this stage of the life cycle where organisms are capable of elicitation of some type of host immunologic response. Rainbow trout (*Salmo gairdneri*) were either immunized with sonicated metacercariae (*Diplodictyum spathecercum*) or were injected with live organisms (Bortz *et al.*, 1984). Antibody responses were measured using a standard ELISA test using metacercarial protein coated plates. Peak primary metacercarial antibody responses occurred by 21 days post-inoculation. Using this detection system, serum from naturally infected rainbow trout also was found to contain metacercarial antigen binding antibodies. These experiments were unique in that the responses were measured using a rabbit anti-trout IgM conjugate, thus true antibody mediated responses were verifiable.

In an earlier study (Cottrell, 1977) antibodies against *Rhipidocotyle johnstonei* and against *Cryptocotyle lingua* were detected in plaice serum. Some contained precipitating antibodies to *C. lingua* detected by the Ouchterlony technique. These antibodies were sensitive to 2-mercaptoethanol (2-ME) treatment and the responses were dependent on the temperature at which the plaice were maintained. As previously reported (Rijkers *et al.*, 1980a; Rijkers *et al.*, 1980b) at 5°C antibody responses were not detected, whereas at 20–25°C antibody responses were produced by 10–20 days post-injection. This study also supported previous data (Bortz *et al.*, 1984) that the substances responsible for the precipitin reactions were (IgM) immunoglobulins.

A similar approach was taken to determine the existence of antibody responses to *Telogaster opisthorchis* in the serum from infected eels (*Anguilla australis schmidtii* Phillips) and in gut mucus from infected *Anguilla dieffenbachii* (McArthur, 1978). As previously reported by others, the activity resembled that produced by IgM-like immunoglobulins. All anti-trematode (anti-T) serum antibodies were 2-ME sensitive and a fraction of serum activity was apparently reactive to a determinant(s) on sheep red blood cells (referred to as anti-S antibodies). Because both anti-T and anti-S reactivity was sensitive to 2-ME, and because there appeared to be a molecular mass difference in fractions exhibiting these activities, these data suggested that in reality the antibodies are probably against heterophile antigens and that these antibodies could exist as stable aggregates. More recently this group (McArthur and Sen Gupta, 1982) of investigators reported the development of an ELISA test to detect antibodies in eel serum against *Telogaster opisthorchis*. This work provided more con-
TABLE 2. Summarized list of types of immune responses in fishes produced against different helminths.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Cestodes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Acanthobothrium</td>
<td>Complement dependent lysis</td>
<td>McVicar and Fletcher, 1970</td>
</tr>
<tr>
<td>II. Trematodes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Monogenetic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Gyrodactylus)</td>
<td>Generalized “resistance”</td>
<td>Scott and Robinson, 1984; Lester and Adams, 1974</td>
</tr>
<tr>
<td>B. Digenetic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Diplostomum)</td>
<td>Primary antibody response</td>
<td>Bortz et al., 1984</td>
</tr>
<tr>
<td>(Rhipidocystyle)</td>
<td>Antibody (IgM)</td>
<td>Cottrell, 1977a, Rijkers et al., 1980a, b</td>
</tr>
<tr>
<td>(Anguilla)</td>
<td>Antibody</td>
<td>McArthur, 1978</td>
</tr>
<tr>
<td>(Telogaster)</td>
<td>Antibody</td>
<td>McArthur and Sengupta, 1982</td>
</tr>
<tr>
<td>C. Acanthocephala</td>
<td>Antibody</td>
<td>Harris, 1972</td>
</tr>
</tbody>
</table>

Vincing evidence for the presence of antibodies specific for this digenean because the antiserum (e.g., enzyme conjugate) used was generated against partially purified eel serum Ig.

Acanthocephala. Helminths belonging to this phylum are common parasites of fishes. Fish become infected by eating copepods (intermediate hosts) containing encysted larvae. In the host (fish), the adult worm develops in the intestine and begins to shed eggs into the aquatic environment. In chub (Leuciscus cephalus) naturally infected with Pomphorhynchus laevis, serum antibody-like activity was detected by Ouchterlony technique (Harris, 1972). However, no correlation was described between worm burden and the presence or absence of antibody. Precipitin activity was also detected in concentrated mucus obtained from infected chub intestine. This activity was sensitive to 2-ME treatment.

Summary

Studies characterizing immune responses of fishes to helminth parasites are summarized in Table 2. Humoral antibody responses appear to be the only mechanism yet described for helminth mediated immunity. These responses can be collectively characterized as consisting of IgM-like antibodies (sensitive to 2-ME treatment) that elicit either a complement dependent or indirect opsonin-like (agglutinin) activity. These antibody responses also varied depending on the ambient temperature at which the fish were maintained post-infection.

REFERENCES


