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Reaction of Hypersensitivity in Tilapia

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ABSTRACT

Delayed hypersensitivity reaction in tilapia was not marked by visible and localized skin responses, characteristic of this reaction in mammals and birds. The tilapias were injected intraperitoneally with a suspension of heat-killed, freeze-dried *Mycobacterium tuberculosis* bacilli and tested 4 and 6 weeks after sensitization by intra dermal inoculation of purified protein derivative (PPD) of *M. tuberculosis*. They were examined 24, 48, 72, and 96 hours post-inoculation. However, lymphocytes from tilapia in which delayed hypersensitivity has been induced, when exposed in vitro to the sensitization antigen inhibited response suggest that tilapia like other vertebrates is capable of exhibiting delayed hypersensitivity reaction which is a T cell – mediated immune response.

1. Introduction

Cell-mediated immune (CMI) response is one of the immune protective mechanisms of the body to infectious and non-infectious agents. It is fundamental to allogeneic- and synergeneic-graft rejection, graft-versus-host reactions and the delayed hypersensitivity reactions (Finstad et al., 1964; Roselynn and Bonnie, 1990). Delayed hypersensitivity has been studied extensively in mammals (Legendre et al., 1977; Chambers et al., 1983; Kobayashi et al, 2001; Martin et al., 2006) and to a lesser .extent, in avian species (Zwilling et al., 1972). However, there have been limited studies on CMI response in the lower vertebrates including fish (Sin et al., 1996; Thomas and Woo, 1990). Antigen-dependent macrophage migration inhibition (MI) was the first assay proposed as an *in vitro* correlate of delayed hypersensitivity reaction (Rich and Lewis, 1932) which is induced by lymphocytes.

Lymphocytes from an animal in which delayed hypersensitivity reaction has been induced when exposed in vitro to the sensitizing antigen, release biological effector molecules. Some of these defector molecules, migration inhibition factor (MIF) a lymphokine, inhibit the migration of macrophages in the same culture medium (George and Vaughan, 1962). The MI technique has therefore, been accepted as a valid *in vitro* for detecting CMI, and also typified by tuberculin skin test reactions.

Preliminary skin tests of tilapia, a teleost, sensitized with heat-killed, freeze-dried *Mycobacterium tuberculosis* failed to demonstrate an obvious and measurable mammalian or avian type of delayed hypersensitivity reaction. Histological features of the reaction were therefore, combined with the capillary tube technique (Bloom and Bennet, 1966) to detect the development of T-cell dependent CMI of the delayed hypersensitivity reaction which the main focus of this study.

2. Materials and methods

2.1 Fish and Tuberculin test:

Oreochromis niloticus weighing between 150g and 187g were obtained from tropical Aquaculture Products Limited, Moniya, Ibadan and maintained at in the aquarium facilities of the Department of Veterinary, Public Health and Prevention Medicine, University of Ibadan, Nigeria. Twelve (12) experimental and eight (8) control fish were used. Heat- killed, freeze-dried *Mycobacterium tuberculosis* bacilli, (Central Veterinary Laboratory, Weybridge, England) were ground to a fine- powder in a mortar before a suspension in liquid paraffin containing 4 mg/ml of the tubercle bacilli was prepared.

Each experimental fish was injected intraperitoneally with 1.0ml of the suspension. The fish were maintained for 4 and 6 weeks before the first and second tuberculin tests respectively and the migration-inhibition assay. Skin testing of both experimental and control fish was performed using

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O.lml PPD solution containing 500 um/ml while for the *in vitro* test, a twofold dilution of the 0.5 mg/ml PPD was prepared immediately before use. Fish were examined at 24, 48, 72 and 96 hours post injection.

2.2 Histopathology:

After 56 weeks the experimental fish were bled for M1 test and then killed by pithing. Skin samples from injection sites and the lymphoid organs (spleen and kidneys) were fixed in 10% neutral buffered formalin and processed in an automatic tissue processor (Roberts, 1978). 5mm paraffin sections cut on a rotary microtome were stained with Haematoxylin and Eosin - for light microscopy.

2.3 Migration-Inhibition Test:

Lymphoid cells were separated from blood over Ficoll-paqueR (phamacia Fine Chemicals, Upsalla, Sweden) and the lymphoid organs by teasing in sterile - phosphate buffered saline (PBS) supplemented with 5% by volume of fetal calf serum (FCS) using a sterile pair of forceps. Washed lymphoid cell suspension containing approximately 1 x 106 cells/ml were aspirated into capillary tubes which were then sealed at one end with cristosseaI[R]. All tubes were centrifuged at 500 x g for 2 minutes in a haematocrit centrifuge to obtain a cell pellet. Each capillary tube was carefully broken at the cell-fluid interface and the piece containing the cell pellet-fixed to the-base of a double-chambered petri dish-with a dab of sterile silicone grease. 2 ml L-15 tissue culture medium containing 10% FCS and antibiotics were added to each chamber.

One chamber of each petri dish served as the experimental culture while the second chamber served as the control culture, 1ml PPD solution containing 62.5ug/ml was added to each experimental culture chamber. The concentration of PPD used, 62.5ug/ml was chosen as an optimum value because of similar studies, previous worker used different concentrations of PPD as follows: 10ug/ml PPD (Thor, 1967); 50ug/ml old tuberculin (Fauser et al., 1973); 100ug/ml PPD (Clausen and Soborg, 1969) and high doses of 100 - 300 ug/ml PPD were recommended by Bloom et al., (1973).

To each control chamber was added 1 ml sterile PBS which was used as diluents for PPD. Lymphoid cell cultures obtained from normal (control) fish were similarly treated. The migration culture dishes were incubated at 28°C for 24 hours. Migration or migration-inhibition was confirmed on a qualitative basis only.

3. Results and discussions

3.1 Histopathology:

The partial intradermal injection of mammalian tuberculin into sensitized tilapia did not produced visible and measurable localized skin response. However, histopathology revealed a - slight swelling of the skin at the site of injection infiltrated by lymphocytes (Fig. 1) similar in structure to those identified by Peleteiro and Richards (Peleteiro and Richards, 1985) together with some mono-nuclear cells. The spleen and the kidney were also examined microscopically for any changes resulting from both sensitization and tuberculin testing but only the spleen exhibited a massive increase both in number and density of melanomacrophage centers. These centers were observed to be in close apposition with the ellipsoids (Fig. 2).

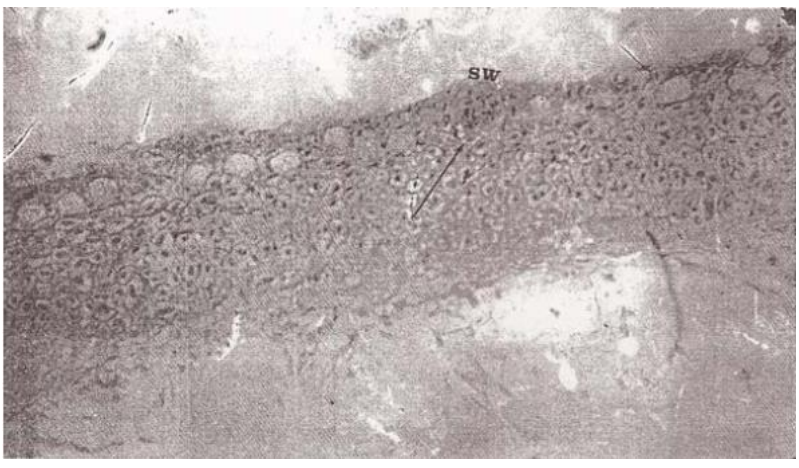

Fig.1. Slight skin reaction (SW) at the inoculation site appeared invaded by lymphocytes (arrow) H & E X 100.

3.2 Migration-Inhibition of Lymphoid Cells and PPD:

PPD inhibited the migration of lymphoid cells obtained from sensitized tilapia but these cells grew in- culture in the absence of the PPD (Fig. 3).

Lymphoid cells obtained from normal (control) fish grew both in the presence and in the absence of PPD. It was apparent that migration-inhibition was complete in the culture without PPD resulting in zero migration indexes.

Delayed hypersensitivity was demonstrated by the in *vitro* migration inhibition by specific antigen (PPD) of lymphoid cells obtained from sensitized tilapia. However, correlative tuberculin skin tests did not produce the obvious skin responses usually observed in mammalian and avian species. This could be due to the insensitivity of tilapia to mammalian type mycobacteria, tuberculin or the use of killed organisms instead of viable *Mycobacterium tuberculosis* (BCG) organisms generally employed by other workers such as Legendre et al., (1977) and Bartros and Sommer, (1981). BCG has also be used in immunotherapy (Boorjian et al., 2010; Lockyer and David, 2001).

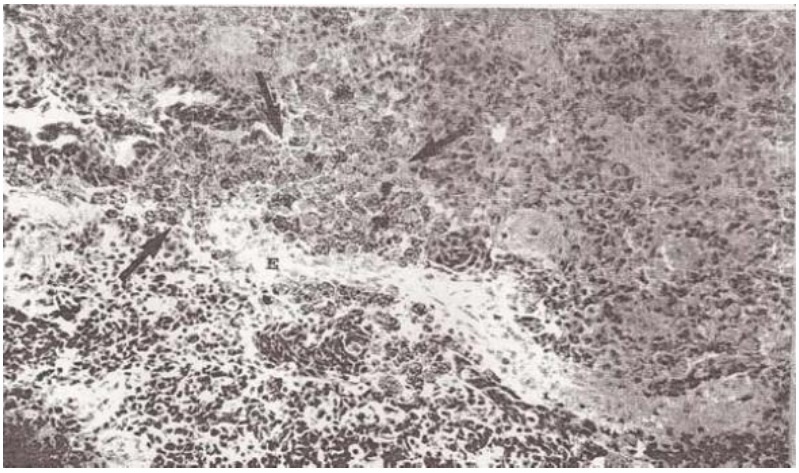

Fig. 2. Spleen from sensitized tilapia sacrificed 6 weeks post-sensitization and 24 hours post-tuberculin test. Prominent melanomacrophage centers were found throughout the stromata, mostly in close apposition with the ellipsoids. In this figure, the melanomacrophage centre (arrow) is at the base of an ellipsoid (E) cut longitudinally. H & E X 250

Slight but transient microscopic inflammatory responses occurred at the injection sites, when skin sections were examined. The transient nature could be due to the high regenerating capability of the teleost living skin cells which could have led to the quick resolution of the response (Roberts, 1978) or, as found with the cat, the transient nature of the response if the skin testing was not done at optimal times. Positive skin reactions may occur during maximal in vivo response. This hypothesis could be evaluated in future experiments. Thus, it can be tentatively inferred that histopathologic evaluation may not be a reliable index of hypersensitivity in tilapias. In different mammalian species, there is surprisingly great variation of the delayed hypersensitivity response to tuberculin: man, guinea pigs and rabbits exhibit strong responses (Clausen and Soborg, 1969), while rats, mice and cats display 'much weaker reactions. Reports of direct demonstration of delayed hypersensitivity reactions in lower vertebrates are generally sparse; Barters and Sommer (1981) and Sin et al., (1996) have however, demonstrated delayed hypersensitivity reactions in rainbow trout (*Salmo gairdneri*) and gold fish (*Carassius auratus*), respectively.

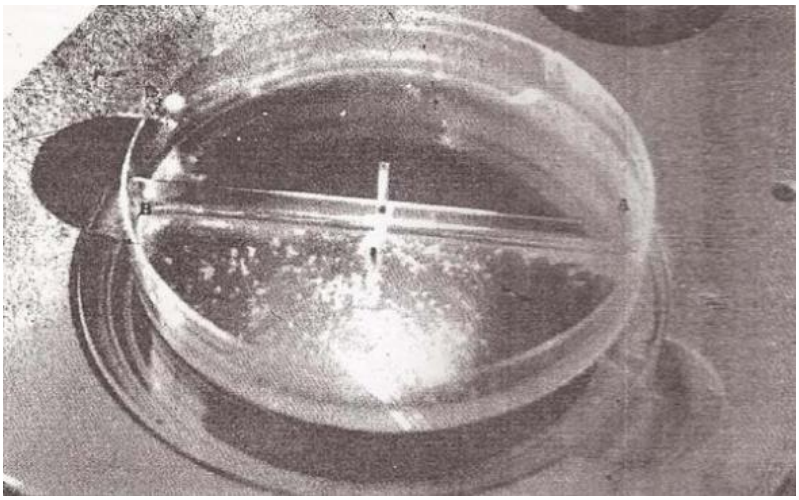

Fig. 3. Antigen-specific migration inhibition. Cells from PPD-sensitive tilapia challenged with PPD did not migrate (Chamber A) while some cells without PPD (Chamber B) migrated.

The migration-inhibition of lymphoid cells observed in the present study is similar to that of exudates cells obtained from hypersensitive guinea pigs in the presence (in the medium) of specific

antigen. This inhibition of migration seems characteristic of cells from animals with delayed hypersensitivity since exudates cells obtained from non-hypersensitive animals immunized to produce only circulating antibody are not inhibited by antigen. The results obtained from this *in vitro* assay correlate well in other respects with observations of delayed hypersensitivity *in vitro,* in that killed cells, cell extracts, or living cells whose protein synthesizing capacity have been inhibited, failed to effect the reaction (Bloom and Bennet, 1966).

Further, these results indicate that inhibition of migration of lymphoid cells (macrophages) *in vitro* is mediated by sensitized lymphocytes through the possible elaboration of a soluble material – a lymphokine- produced only in the presence of specific antigen. Bloom and Bennet (1996) showed that the migration of macrophages separated from peritoneal exudates cells from hypersensitive animals was not inhibited by the sensitizing antigen. The conclusion of their studies was that two types of cell migration were required to produce inhibition of cell migration by an antigen. One cell type should be sensitive lymphocytes, the other macrophages which need not of necessity originate from a hypersensitive organism. In experiments with peripheral human leucocytes, Thor (1967) also showed that sensitive lymphocytes separated from the blood could not be inhibited by addition of antigen, but that the presence of granulocytes and monocytes were necessary for the inhibition of migration.

Macrophages are responsive to a number of lymphokines that induce their growth differentiation and activation; since lymphokines are released by primed lymphocytes, produced chiefly by Tlymphocytes, on contact with an antigen (Roselynn and Bonnie, 1990), and the demonstration of a lymphookine mediated reaction in this study strongly supported the existence of T like lymphocytes in Tilapias and that the fish is capable of delayed hypersensitivity reactions.

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