RELATIONSHIP BETWEEN EXPERIMENTAL MYCOBACTERIOSIS AND MONogeneAN INFECTIONS ON GILLS OF THE ATEGERNID FISH MELANOTAENIA DUBOULAYI

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Introduction

Interaction between diseases in teleost fish has been suggested by Schaperlaus (1991), Lux (1993), Roberts (1986a), Gelnir (1987), Kamino and Olton (1985) and Pumper (1984), but there is very little experimental evidence to support these suggestions. Reichard (1985) observed a relationship between a monogenean Dactyloopneum volii and Mycobacterium sp. in Capetena tetrazona. The mycobacterial infection produced a generalised granulomatous condition and the most severely affected fish harboured the most severe worm burdens. In a preliminary study I observed that goldfish Carassius auratus with a severe granulomatous pathology of unknown aetiology, harboured more Dactyloopneum sp. on their gills than did fish less severely affected by the same agent. I therefore sought to test the relationship in an experimental model using Melanotaenia duboulayi (Crimson-spotted rainbow fish) with concurrent infections of Mycobacterium marinum and the monogenean Ancycrocephalus sp.

Materials and Methods

The M. marinum (Animal Research Institute ref. 80-0406) used was isolated from the kidney of a naturally infected Melanotaenia splendida splendida and grown on 7H11(Mitchison) selective media. A stock suspension of bacteria was prepared for inoculation by adding 80.0 mg of bacterial mass to 1.6 ml of sterile saline. This produced a stock suspension with a concentration of 50ng bacteria/ml. This was serially diluted and an aliquot (0.1 ml) of each dilution greater than 10^-9 was plated out in duplicate on 7H11 media and incubated at 27°C for 10 days. Colonies visible after this time were counted and the viability of the stock suspension was calculated as 4.6 x 10^9 Colony Forming Units (CFU/ml). One male and one female M. duboulayi, with an average weight of 2.0g, were allocated to one of seven treatments: control (no injection); saline (intraperitoneal IP injection of saline); and IP injection of bacterial stock suspension diluted 10^(-3), 10^(-4), 10^(-5) and 10^(-6). Fish other than controls, were anaesthetized to a surgical plane with benzocaine and treatments were administered via Microfine B-D Lodose (Plasipak, U.S.A.) into the body cavity through the lateral aspect of the abdominal wall at a dose rate of 0.05ml/kg of fish. All fish were then introduced to 60x30x5cm glass aquaria that had been filled with tap water and established, without fish, for four weeks. The aquarium hardware and gravel had been sterilised by chlorine and steam respectively. Fish were fed Wardleys Premium Tropical flake food (Secaucus, N.J., U.S.A.) and held at 27°C for 31 days. Fish were then killed with an overdose of benzocaine. The 1st and 3rd gill arches from both sides of all fish were removed and numbers of Ancycrocephalus sp. present counted using a dissecting microscope. All internal organs were fixed in 10% buffered formalin for histology and then examined under a dissecting microscope and assigned a Pathology Index (PI) score from 0-4 where a score of 0 denoted no granulomas evident and 4 denoted >75% of the organ being replaced by lesions. The scores of all organs observed were totalled and divided by the number of organs observed to give a qualitative assessment of pathology for each fish. For other details see Pyecroft (1992).

Results

The histopathology produced by the mycobacterial infection was typical of that found in natural and experimental infections with Mycobacterium sp. (Timar and Roberts, 1977). Lesions consisted of a central exudative necrotic area of varying shape surrounded by a zone of epitheloid macrophages and an outer fibrous layer with some mononuclear cell infiltration (Fig. 1).

Figure 1. Graded levels of granulomatous pathology produced by inoculation of increasing amounts of M. marinum into M. duboulayi. A) Wet tissue preparation of spleen showing granuloma structure (F) fibrous layer (C) cellular layer (MM) melanin-macrophage centre (x40), B) Normal fish (x4), C) Granulomas in spleen and cranial kidney (x4), D-F) Increasing severity of lesions. Arrows denote granulomas (x4), (Bar =1mm) (CRK) cranial kidney lobe, (CK) caudal kidney lobe, (H) heart, (I) Intestine, (L) liver, (S) spleen, (T) testes, (O) ovary.

Some lesions had coalesced to form larger irregularly shaped nodules. The degree of pathology produced (PI) was proportional to the amount of bacteria injected (Fig. 2). There was a positive correlation between the bacterial challenge and the extent of the fluke infection (Fig. 3) and there was a threshold level of pathology over which the fluke infection was affected i.e. 5x10^(-7) mg.
bacteria/mL. Once this level was reached _Anacystis_ sp. numbers increased greatly. The relationship between PI and _Anacystis_ sp. numbers was highly significant at p<0.05 (i.e. r=0.825, n=14; using Spearman’s Ranked Correlation coefficient [Steel and Torrie, 1960]). The more generalized and extensive the lesions induced the higher the _Anacystis_ sp. burden.

**Fig 2, Effects of mycobacterial infection on the numbers of _Anacystis_ sp. on 1st and 3rd gill arches of _Melanostigma duboulayi_**

**Discussion**

This trial showed that a graded response of pathogenicity could be produced by inoculation of _M. duboulayi_ with variable amounts of _M. marinum_, after 31 days at 27°C. A threshold level of tissue damage was present over which there was a positive effect on the burdens of _Anacystis_ sp. on the gills of inoculated fish. Mycobacteria induce a cellular response in infected fish (Roberts, 1989b), the most commonly affected organs being the kidney, spleen, liver, heart and peritoneal serosa. These are the organs in teleost fish that have the greatest concentration of phagocytic cells, mainly macrophages (Agius, 1985). Monocytes in blood and macrophages in tissue are the major cells stimulated in cell-mediated immunity in fish. Macrophages are the predominant cell type in the chronic inflammatory response where they change to form the epithelioid cells which surround the granulomas characteristic of chronic inflammation in fish. Neutrophils also play a role in this immunity in the early stages (MacArthur & Fletcher, 1985; Fina & Neilson, 1971). The threshold effect of pathogenicity on _Anacystis_ sp. burdens may be due to an exhaustion of the protective response of the fish towards the mycobacteria. The extent of tissue replacement by granulomas and subsequent loss of function, and the extent of macrophage involvement found in some fish represents a drain of immune resources within affected fish. As debility and physiological stress increases within affected fish, their ability to maintain sufficient numbers of immunologically active cells in contact with the mycobacteria decreases and so levels of parasitism rise (MacArthur and Fletcher, 1985; Angelidis et al., 1987; Shihab-Harrison, 1987). This may indicate that cellular immune responses play a role in the regulation of _Anacystis_ sp. populations on _M. duboulayi_.

**Summary**

Experimental infection of the Common-spotted rainbow fish _Melanostigma duboulayi_ by inoculation of _Mycobacterium marinum_, produced granulomas within 31 days. The extent of the response was graded dependent on the amount of bacteria inoculated. The inoculated fish were also naturally infected by the mycobacterium _Anacystis_ sp. on the gills. After 31 days the number of mononuclears were counted and there was a positive correlation between mononuclear numbers and the extent of pathology induced by the bacteria.

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**Bibliography**


