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Bacterial kidney disease: the potential role of soluble protein antigen(s)

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Soluble protein antigens, isolated from the supernatants of *Renibacterium salmoninarum* cultures, have been found to suppress the *in vitro* antibody reponses of coho salmon, *Oncorhynchus kisutch*, lymphocytes and to be associated with decreasing haematocrit values *in vivo*.

I. INTRODUCTION

Renibacterium salmoninarum is responsible for one of the most devastating salmonid bacterial diseases in the world (Fryer & Sanders, 1981), bacterial kidney disease (BKD). Of major epidemiologic importance is the fact that this disease is not only a big problem in hatchery-reared salmonids, but also in wild populations (Mitchum *et al.*, 1979).

Unfortunately, little information has been forthcoming as to the possible molecular mechanisms of the pathogenesis of BKD. This study, therefore, focuses on the possible toxigenic role(s) that *R. salmoninarum* soluble proteins (SP) may have in BKD pathology.

II. MATERIALS AND METHODS

Renibacterium salmoninarum soluble antigens were prepared as described by Getchell *et al.* (1985). The effects of SP on the *in vitro* antibody responses of normal and infected coho salmon to trinitrophenylated-lipopolysaccharide (TNP-LPS; Jacobs & Morrison, 1975) were examined using *in vitro* tissue culture medium (TCM) and techniques (Kaattari & Yui, 1987). Prior to culture addition, SP was diluted in tissue culture media and filter sterilized (0·45 μm). Haematocrit values were assessed using heparinized blood samples taken from 5 fish at 10-day intervals after intraperitoneal injection of 0·1 ml of 1 O.D. (500 nm) live *R. salmoninarum*. The plasma portions of these samples were analyzed for the concentration of SP by use of an ELISA procedure (P. Turaga *et al.*, in prep.).

III. RESULTS

In vitro cultures of anterior kidney lymphocytes, stimulated with an optimal concentration of TNP-LPS, were suppressed by 10 and 100 μg ml^{-1} SP (Fig. 1). Comparable concentrations of the control protein, chicken ovalbumin, were not suppressive. This suppression was not due to a toxic effect, since control and suppressed cultures expressed equivalent cellular viability as assessed by trypan blue exclusion staining.

Anterior kidney lymphocytes from normal and infected fish were cultured with TNP-LPS (Fig. 2). Cultures of lymphocytes from infected fish (possessing 3–80 μg ml^{-1} serum SP) demonstrated a marked suppression as compared

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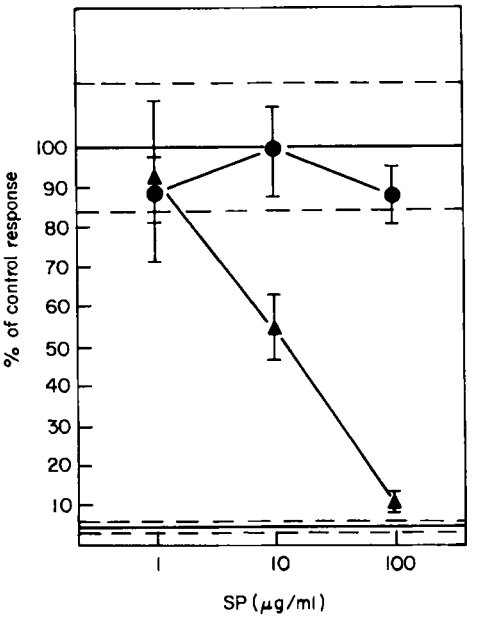

FIG. 1. Lymphocytes from anterior kidney were cultured with TNP-LPS in the presence of 1, 10, 100 μg SP (▲) or ovalbumin (●). The control response (without protein addition) was equal to 431 ± 21 antibody-producing cells per 10^6 lymphocytes. Each point represents the mean of triplicate cultures and bars ± 1 S.E.

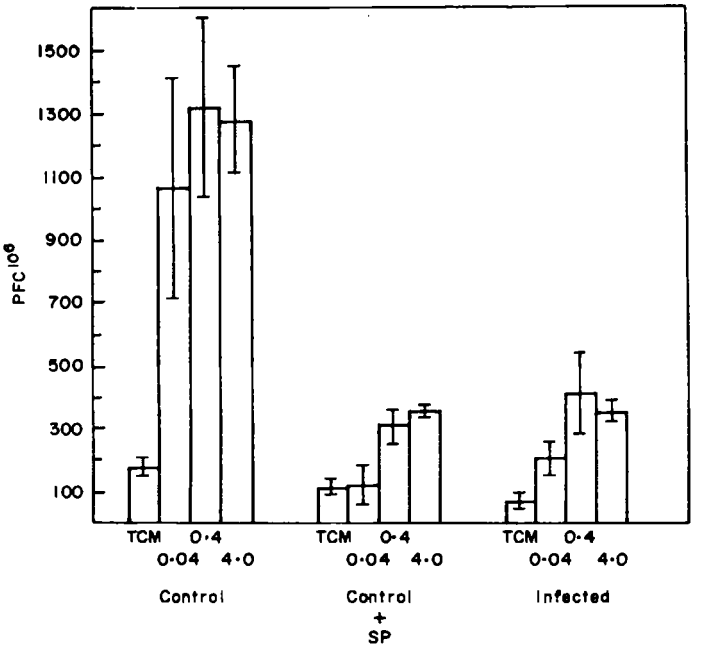

FIG. 2. Lymphocytes from the anterior kidneys of normal and infected salmon were cultured with 0·04, 0·4, and 4·0 μg ml^{-1} of TNP-LPS concentrations. A portion of the normal lymphocytes were also cultured with 100 μg ml^{-1} SP (a concentration comparable to that seen in infected fish). Each histogram represents the mean of triplicate cultures and ± 1 S.E. are indicated.

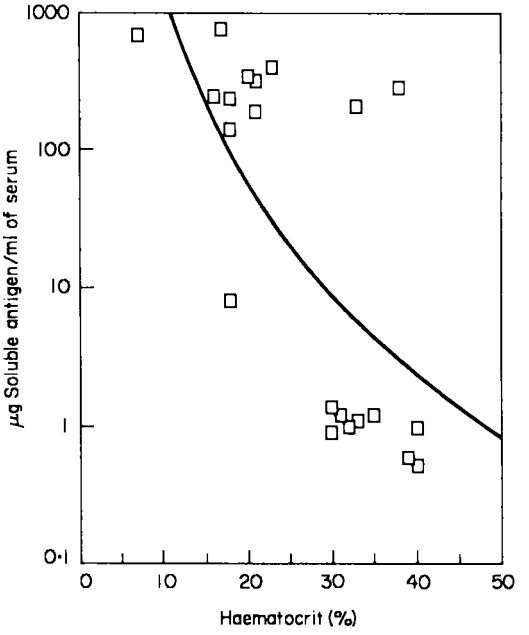

FIG. 3. Haematocrit values, expressed as percentage packed red cell volume, plotted versus the corresponding SP serum concentration for individuals sampled at various stages of infection.

to lymphocytes from normal fish. This suppression was comparable to the suppression seen when normal lymphocytes are co-cultured with 100 μg ml^{-1} SP.

Examination of experimentally infected salmon at various times post-injection revealed a distinct association of decreasing haematocrit with increasing levels of SP antigen in the serum (Fig. 3).

IV. DISCUSSION

These studies demonstrated that soluble antigens produced by *R. salmoninarum* are capable of suppressing the *in vitro* antibody response, and are associated *in vivo* with decreasing haematocrit values.

Studies concerning the mechanisms of pathogenesis for *R. salmoninarum* have primarily been limited to the analysis of the histopathology (Wood & Yasutake, 1956; Hendricks & Leek, 1975; Lester & Budd, 1979; Young & Chapman, 1978) and the appearance of abnormal clinical indices (Hunn, 1964). Although the initial focus of the infection appears to be the kidney (Wood & Yasutake, 1956) with subsequent haematological dysfunction (Hunn, 1964), the disease eventually becomes systemic, with lesions occurring in many organs and tissues.

The identification of *R. salmoninarum* toxins has not been forthcoming, except for the detection of an haemolysin-like activity found in formalinized cells (Bruno & Munro, 1986). Those authors have suggested that a putative toxin may be responsible for the decreases in haematocrit values and for indices of splenomegaly associated with the disease. Our observations of increasing serum SP levels associated with decreasing haematocrits lend support for that pathogenic mechanism.

The *in vitro* antibody assay revealed that a non-cytotoxic antigen(s) was capable of suppressing the production of the antibody response. Of particular interest was the observation in the present study of a decrease in the number of adherent (e.g. macrophage) cells upon culture with SP. Recent studies with catfish (Miller *et al.*, 1985) and with coho salmon lymphocytes (Tripp & Kaattari, in prep.) reveal that antibody responses to TNP-LPS require adherent cell function. Since antibody responses from lymphocytes from infected fish appear suppressed (Fig. 2), it may be possible that immune dysfunction *in vivo* could be mediated by the elaboration of these antigens. Further studies, however, will be required to determine the role of alternative *in vivo* mechanisms (e.g. bacterial destruction of tissues, or contamination due to live *R. salmoninarum*) involved in the reduction of antibody-producing cells from infected fish.

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