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THE AGGLUTININ RESPONSE IN SOCKEYE SALMON VACCINATED INTRAPERITONEALLY WITH A HEAT-KILLED PREPARATION OF THE BACTERIUM RESPONSIBLE FOR SALMONID KIDNEY DISEASE

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Abstract: Immature sockeye salmon (*Oncorhynchus nerka*) responded to the intraperitoneal injection of heat-killed, adjuvant-suspended cells of the salmonid kidney disease bacterium by producing agglutinins specific for the pathogen. These antibodies were detectable for at least 16 months following a single injection. With water temperatures of 12-15 C which prevailed during the first 100 days following this injection, the response was rather slow to develop, and whether or not antibodies were produced in this period, depended on the dose of antigen given. Under a similar temperature regime, a second injection, given 13 months after the first, elicited a clear-cut anamnestic response. Ninety days following primary and secondary vaccination, maximum agglutinating titres were 1:2,560 and 1:10,240, respectively.

Electrophoregrams of sera from vaccinated fish revealed the presence of one, and sometimes two, fractions of low mobility. These fractions occurred in the gamma and beta regions, and contained antibody. They were most distinct in sera with high titres; they were not observed in sera from non-vaccinated controls (these typically showed three major components of higher mobility); and they were selectively removed when sera were adsorbed with cells of the kidney disease bacterium. Some properties of the antibodies were studied.

INTRODUCTION

All vertebrates, including hagfish,^{1,9,14} are capable of an adaptive immune response. A recent surge of interest in the immune response among the lower vertebrates stems not only from an interest in the phylogeny of the phenomenon,^{3,7,8,10} but also from the practical desire of fish culturists to utilize the response to their advantage.²⁰

In this laboratory, interest in teleostean immunity was sparked, initially, by the need to protect experimental cultures of Pacific salmon against an enzootic bacterial disease. The disease, known in North America as "kidney disease",⁵ and in Europe as "Dee disease",¹⁰ has already been described in fish at this Station by Bell.²

A search of the literature (much of it summarized in an excellent review by Ridgway et al.¹⁸) revealed that parenteral

routes of immunization were more efficacious than immunization by the oral route. Further, it suggested that the use of adjuvants might heighten and prolong the immune response, and thus, the kidney disease bacterium which is grown with some difficulty, would be used with the greatest possible efficacy. Also, since with adjuvant a single injection might suffice to produce immunity, it was possible that the relatively modest numbers of experimental fish held at this Station might all be treated.

This report presents the results of experiments on the immune response of cultured sockeye salmon (*Oncorhynchus nerka*).

MATERIALS AND METHODS

The sockeye salmon used in these experiments ranged in age from 1 to 3 years and had been raised at this Station

from eggs taken and fertilized in the field (Babine Lake, B.C.). The fish were held in running fresh water which ranged from about 4-15 C over the year. However, to facilitate the immune response, vaccinations were timed so that the fish had at least 3 months with water temperatures at 12-15 C following treatment. During experiments, fish which showed signs of maturity were discarded (these were always "precocious" males).

The strain of the kidney disease (KD) bacterium used as antigen was one that had been isolated in 1964 from a cultured sockeye salmon. This fastidious, cysteine-requiring, Gram-positive, rod-shaped bacterium had been maintained by subculture at 15 C on a modified version of Ordal and Earp's medium,¹⁵ and in the course of other experiments, from time to time had been passaged through sockeye salmon which invariably died of typical KD.

Antigen was prepared from bacterial cells that had probably reached the maximum stationary growth phase (they had been grown on the agar medium for 21-28 days at 15 C in a moist chamber). Harvested cells were washed in three changes of cold, sterile, 0.9% saline, and wet, packed (12000 X G, 20 min) cells from the final wash were drained and weighed. Enough sterile saline was then added to the cells to yield a suspension containing about 50.0 mg of cells per ml. The cells were then killed by heating the suspension at 62 C for 45 min in a tightly stoppered tube (by complete submersion of the container in a water bath). The cell suspension was stored at -20 C until needed. To prepare the vaccine, the suspension was thawed and blended on a "tube buzzer"* with an equal volume of adjuvant (complete Freund's adjuvant, or Klearol mineral oil: Arlacel C, 9:1 v/v (Krantz et al.¹²)). The vaccine was immediately administered intraperitoneally, the dosage being adjusted by varying the volume injected. At the highest dosage used (450 mg antigen/kg fish) the volume of vaccine injected amounted to 0.5 ml

per 28-g yearling sockeye salmon. Control fish received a corresponding volume of blended saline-adjuvant, but no cells.

Blood was collected from the severed caudal peduncle and allowed to clot at room temperature for about 1 hour. The clot was then stored overnight at 5 C and the expressed serum was collected, lightly centrifuged, and membrane filter-sterilized (with the smaller fish, serum volumes were sometimes too small to be filtered). Sera were held for up to a week at 5 C during which time they were assayed for agglutinins and examined electrophoretically. After this, they were frozen.

Agglutinin titres were determined in tubes with saline as the diluent. Standard two-fold serial dilutions of serum were used. Heat-killed cells in saline, prepared as already described, served as antigen. In the agglutination tubes, these cells were used at a final concentration yielding a turbidity of 1.25 O.D. at 420 m μ . Titres (expressed as the reciprocal of the highest serum dilution showing macroscopic clumping of the antigen) were read after the reaction had proceeded for 1 hour at 42 C and overnight at 5 C. Sera showing no agglutination at the lowest dilution (1:10) tested, were assigned a titre of zero.

Electrophoretic separation of proteins in serum (and adsorbed serum) was accomplished using Gelman** equipment. Details on the techniques and instruments used are given in the Gelman Manual no. 70176-B, 1966. Procedures were modified only in that 2.5 μ l samples on 6.75 inch cellulose acetate strips were electrophoresed for 1 hour at 180 volts (power supply model 38201) in a chamber (model 51170) containing cold, 0.05 M, pH 8.8, bicine (N; N bis (2-hydroxyethyl) glycine) buffer. Proteins on the strips were then fixed and stained with trichloroacetic acid-Ponceau S reagent. The strips were then cleared in acetic acid-methanol and scanned manually (scanner model 39301).

Sera were adsorbed with heat-killed

*Hallikainen Instruments, Berkeley, California.

**Gelman Instrument Company, Ann Arbor, Michigan.

cells of the type used for vaccination. To minimize dilution of the serum, the serum (about 30 μ l) was stirred into the packed, wet cells (about 15 μ l) and allowed to react for about 5 min at room temperature. The resulting slurry was taken up in a haematocrit tube and centrifuged. The same extraction technique could be repeated on the supernatant about two more times, after which, if all the antibody had not been removed, further extractions were precluded by unavoidable fluid losses (on the walls of containers) and by dilution of the serum with interstitial saline from the packed cells. Each adsorbed serum was compared electrophoretically with the non-adsorbed, homologous serum. The latter was diluted empirically in saline so that the concentration of the albumin-like protein with the highest electrophoretic mobility, approximated that for the corresponding protein in the adsorbed serum.

RESULTS AND DISCUSSION

Following primary vaccination with heat-killed cells of the KD bacterium in adjuvant, sockeye salmon responded by producing antibodies in quantities far exceeding those previously reported for this species¹¹ and in a manner similar to that obtained with trout vaccinated with adjuvant preparations.¹² Although individual animals ranged widely in their response, the data are presented in raw form because the animals used in any given treatment were few. By simple inspection, however, certain trends appear clear. First, even under a temperature regime favouring good growth of the experimental fish, the antibody response was a rather slow process (Table 1). Second, if all of the animals were to respond in a "reasonable" period, it appeared that adequate levels of antigen had to be administered (Table 2).

TABLE 1. Time-course of the antibody response of sockeye salmon held at 12-15 C following primary vaccination.^a

No. days post vaccination	No. fish with antibody per no. fish sampled	No. fish with titre indicated
30	1/10	1 at 10
60	5/10	1 at 80; 2 at 160; 1 at 320; 1 at 1280
90	8/10	1 at 80; 5 at 320; 1 at 1280; 1 at 5120

^a A large dose of antigen (450 mg/kg fish) in mineral oil: Arlacel C was given in a single intraperitoneal injection. Control fish (10 at each sampling) which received adjuvant only, produced no antibodies.

TABLE 2. Effect of vaccine dose size on the primary antibody response (100 days post-vaccination) of sockeye salmon held at 12-15 C.

Dose size mg antigen/kg fish ^a	No. fish with antibody per no. fish tested ^b	No. fish with titre indicated
0	0/5	—
44	2/6	1 at 10; 1 at 2560
88	2/4	1 at 320; 1 at 1280
220	7/7	1 at 80; 3 at 320; 1 at 640; 1 at 1280; 1 at 2560

^a Administered in a single intraperitoneal injection in mineral oil: Arlacel C.

^b Initially, 12 fish were used for each treatment. Missing fish were killed by a water failure.

TABLE 3. Durability of the antibody response following primary vaccination and the effect of secondary vaccination on antibody production in sockeye salmon.

Treatment	Months post vaccination at sampling	No. fish tested	No. fish with titre indicated
Primary	16	12	2 at 0; 2 at 10; 2 at 20; 2 at 160; 1 at 320; 2 at 640; 1 at 1280
Primary (control) ^a	16	12	12 at 0
Primary	3	7	3 at 0; 1 at 320; 1 at 640; 2 at 2560
Secondary ^b	3	8	1 at 320; 1 at 640; 2 at 1280; 1 at 2560; 3 at 10240

^a Control fish received Freund's complete adjuvant only. All other fish received 200 mg antigen/kg fish in Freund's complete adjuvant (intraperitoneally) with each vaccination.

^b Secondary vaccination was given 13 months after primary vaccination.

Slowness of the primary response was partly compensated for by its longevity, and partly by the fact that the animals responded more rapidly on secondary antigenic stimulation — that is, they exhibited an anamnestic response. In Table 3, for instance, antibodies persisted for at least 16 months in the majority of animals tested, and when, 13 months after their first injection, they were vaccinated for a second time, they produced higher antibody titres at 3 months than did animals vaccinated for the first time. Further, in contrast to the latter group which reacted erratically, all of the animals in the former group responded by producing antibodies. Surprisingly, the anamnestic response has apparently not been reported in salmonids to date.

Antibodies produced by the vaccinated sockeye salmon were specific for strains of the KD bacterium. Heat-killed cells of two additional strains of this organism isolated from pink salmon (*O. gorbuscha*) cultured locally and in West Vancouver, B.C., were agglutinated about as strongly as the homologous bacterium by five sera tested for this feature. These sera, however, did not agglutinate heat-killed cells of other Gram-positive (*Sarcina lutea*, *Gaffkya homari*) or Gram-negative (*Aeromonas salmonicida*, *Vibrio anguillarum*) bacteria tested.

Other tests on the properties of the antibodies revealed that they were completely inactivated by heating for 15 min at 56 C, or by exposure to mercapto-ethanol (MSH) (see later), but that they survived cold storage (3 months at 5 C; at least 8 months at -20 C) without appreciable loss of activity. As shall be described shortly, the antibodies varied in their electrophoretic mobilities, but whether they migrated like slow (gamma) or fast (beta-1) antibodies, they were all apparently large molecules (they were not appreciably retarded by passage through Bio-Gel P-300***), and could be precipitated quantitatively from the serum by 22% (w/v) Na₂SO₄.

Sera from vaccinated and non-vaccinated (but adjuvant-injected) sockeye salmon shared three major "high-mobility" components in common. In terms of their relative electrophoretic mobilities, these fractions behaved like albumin, alpha-1 globulin, and alpha-2 globulin of human origin (examples of non-vaccinated sockeye salmon sera are shown in Fig. 1a). In addition to these fractions, sera from vaccinated fish also contained "low-mobility" components which were never observed in sera from adjuvant-injected controls. These fractions were always most distinct in sera with high titres and

***Product of Bio-Rad Laboratories, Richmond, California.

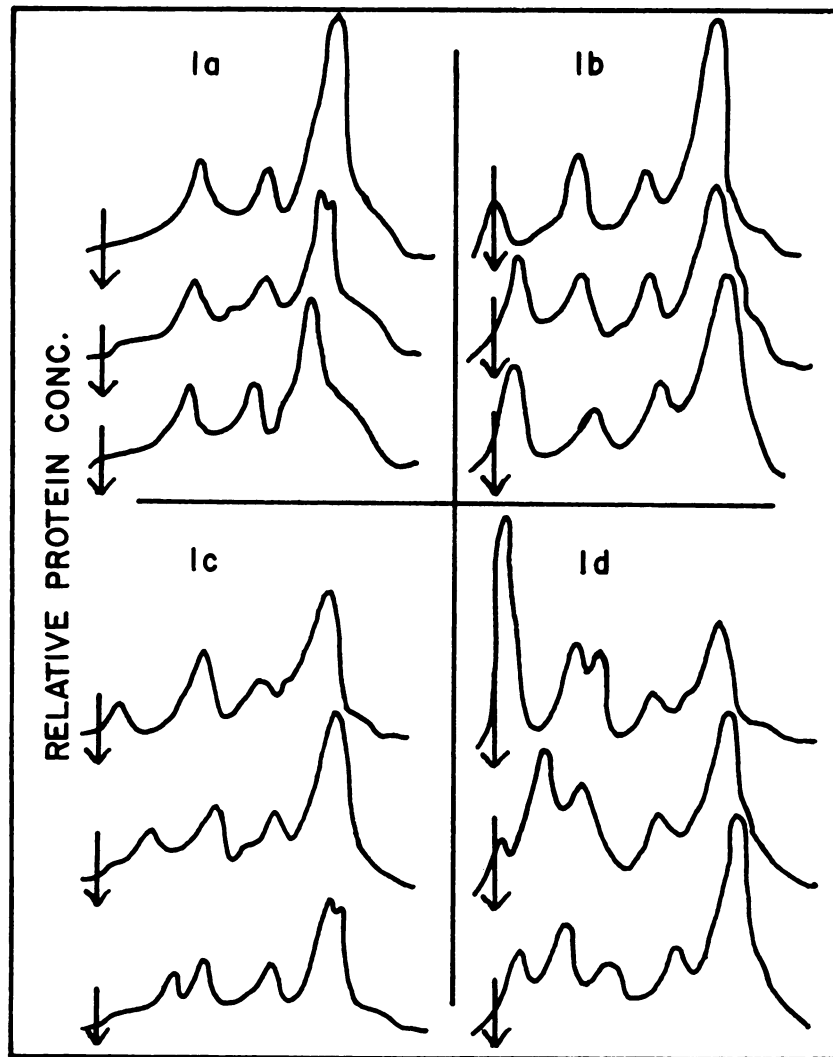


Fig. 1. Cellulose acetate electrophoregrams of sockeye salmon sera. (a) Sera from three control (adjuvant-injected) fish, each serum showing the three "high-mobility" fractions characteristic of non-vaccinated fish; (b) sera from three vaccinated fish, each serum showing a gamma-type antibody peak; (c) sera from three vaccinated fish, each serum showing the antibody peak in a slightly different position; (d) sera from three vaccinated fish, each serum showing two antibody peaks. Arrow denotes point of sample application; anode is at right.

they often accounted for a considerable proportion of the serum protein (48% in one case). They were shown to be antibody because they were selectively removed when sera were adsorbed with heat-killed cells of the KD bacterium.

Interestingly, when antibody was defined in terms of electrophoretic mobility, sockeye salmon appeared to produce a variety of antibody proteins. These antibodies, identified as such by the adsorption technique just mentioned (some adsorption results are given in Fig. 2), ranged in position from the essentially immobile gamma peak (illustrated for three fish in Fig. 1b), to a position just behind the alpha-2 globulin peak (Fig. 1c shows an antibody peak in several positions). Antibody polymorphism was most apparent, however, when a given serum contained more than one antibody-

type. As indicated in Fig. 1d, sera sometimes contained two forms of antibody.

The significance of the antibody polymorphism is not understood. Heterogeneity of low-mobility proteins has already been noted by Krauel and Ridgway¹³ for non-immunized sockeye salmon, and may be characteristic of salmonids in general (see Ridgway et al.¹⁴). In agreement with the findings of Hodgins et al.¹¹ for rainbow trout (*Salmo gairdneri*), the polymorphism of the sockeye salmon antibodies did not appear to be related to the characteristic mammalian antibody-shift from large (MSH-sensitive) to small (MSH-resistant) molecules. For one thing, "early" (3 month) and "late" (8 month) sera from vaccinated sockeye showed no trend in a shift from one antibody form to another, and for another, the antibody, whether of gamma or of beta variety, was always totally inactivated by MSH (0.1 M for 1 hr).

To date, immune antibody activity in salmonids, or more precisely in rainbow trout, has been located in the beta region on electrophoregrams,^{10,11,17,18} but unequivocal evidence for antibody activity in the gamma region has been lacking. In fact, until Krauel and Ridgway,¹³ using sensitive techniques, showed that normal sockeye salmon sera contained traces of proteins in this region, the general view was that salmonids, like certain other teleosts, produced little or no protein with gamma mobility.¹⁴ However, on the basis of the present experiments in which high-titre sera from vaccinated animals were used, there can be little doubt that salmonids can produce proteins of gamma mobility, and that these proteins act as antibodies. In the serum profiles shown in Figs. 1b and 1d, these proteins tended to straddle the "origin" on the cellulose acetate electrophoregrams; they accounted for up to 30% of the serum protein and were selectively removed from the sera by adsorption with homologous antigen.

These experiments demonstrated quite clearly that sockeye salmon are immunologically competent, but whether or not the treatment conferred immunity to the disease still has to be tested.

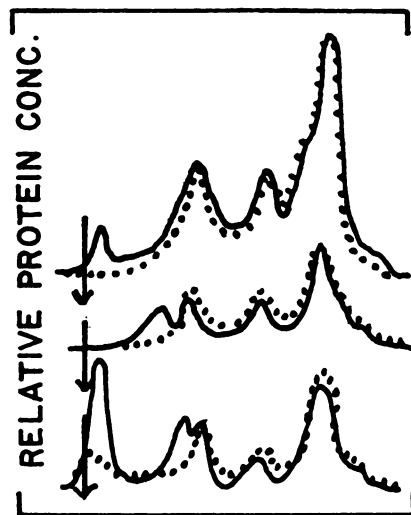


Fig. 2. Cellulose acetate electrophoregrams of adsorbed (dotted lines) and non-adsorbed (solid lines) sera from three vaccinated sockeye salmon. With the adsorption technique used, it was not possible to remove all of the antibody activity from the serum containing the two antibody peaks. Arrow denotes point of sample application; anode is at right.

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LITERATURE CITED

1. ACTON, R. T., P. F. WEINHEIMER, W. H. HILDEMANN, and E. E. EVANS. 1969. Induced bacteriocidal response in hagfish. *J. Bacteriol.* 99: 626-628.
2. BELL, G. R. 1961. Two epidemics of apparent kidney disease in cultured pink salmon (*Oncorhynchus gorbuscha*). *J. Fish. Res. Bd. Canada* 18: 559-562.
3. CLEM, L. W., and M. M. SIGEL. 1966. Immunological and immunochemical studies on holostean and marine teleost fishes immunized with bovine serum albumin, p. 209-217. *In* R. T. Smith, P. A. Miescher, and R. A. Good [ed.] *Phylogeny of Immunity*. Univ. Florida Press, Gainesville, Fla.
4. DEUTSCH, H. F., and W. H. McSHAN. 1949. Biophysical studies of blood plasma proteins. XII. Electrophoretic studies of the blood serum of some lower animals. *J. Biol. Chem.* 180: 219-234.
5. EARP, B. J., C. H. ELLIS, and E. J. ORDAL. 1953. Kidney disease in young salmon. Washington Dept. Fish., Spec. Rep. Ser., No. 1. 74 p.
6. ENGLE, R. L., JR., K. WOODS, E. PAULSEN, and J. PERT. 1958. Plasma cells and serum proteins in marine fish. *Proc. Soc. Exp. Biol. Med.* 98: 905-909.
7. FINSTAD, J., and R. A. GOOD. 1966. Phylogenetic studies of adaptive immune responses in the lower vertebrates, p. 173-188. *In* R. T. Smith, P. A. Miescher, and R. A. Good [ed.] *Phylogeny of Immunity*. Univ. Florida Press, Gainesville, Fla.
8. GOOD, R. A., J. FINSTAD, B. POLLARA, and A. E. GABRIELSEN. 1966. Morphologic studies on the evolution of the lymphoid tissues among the lower vertebrates, p. 149-168. *In* R. T. Smith, P. A. Miescher, and R. A. Good [ed.] *Phylogeny of Immunity*. Univ. Florida Press, Gainesville, Fla.
9. HILDEMANN, W. H., and G. H. THOENES. 1969. Immunological responses of Pacific hagfish. I. Skin transplantation immunity. *Transplantation* 7: 506-521.
10. HODGINS, H. O., G. J. RIDGWAY, and F. M. UTTER. 1965. Electrophoretic mobility of an immune globulin from rainbow trout serum. *Nature* 208: 1106-1107.
11. HODGINS, H. O., R. S. WEISER, and G. J. RIDGWAY. 1967. The nature of antibodies and the immune response in rainbow trout (*Salmo gairdneri*). *J. Immunol.* 99: 534-544.
12. KRANTZ, G. E., J. M. REDDECLIFF, and C. E. HEIST. 1963. Development of antibodies against *Aeromonas salmonicida*. *J. Immunol.* 91: 757-760.
13. KRAUEL, K. K., and G. J. RIDGWAY. 1963. Immunoelectrophoretic studies of red salmon (*Oncorhynchus nerka*) serum. *Int. Arch. Allergy* 23: 246-253.
14. LINTHICUM, D. S., and W. H. HILDEMANN. 1970. Immunologic responses of the Pacific hagfish. III. Serum antibodies to cellular antigens. *J. Immunol.* 105: 912-918.
15. ORDAL, E. J., and B. J. EARP. 1956. Cultivation and transmission of etiological agent of kidney disease in salmonid fishes. *Proc. Soc. Exp. Biol. Med.* 92: 85-88.

16. POLLARA, B., J. FINSTAD, and R. A. GOOD. 1966. The phylogenetic development of immunoglobulins, p. 88-97. In R. T. Smith, P. A. Miescher, and R. A. Good [ed.] *Phylogeny of Immunity*. Univ. Florida Press, Gainesville, Fla.
17. POST, G. 1963. The immune response of rainbow trout (*Salmo gairdnerii*) to *Aeromonas hydrophila*. Utah State Dept. Fish and Game. Departmental Information Bulletin 63-70. 82 p.
18. RIDGWAY, G. J., H. O. HODGINS, and G. W. KLONTZ. 1966. The immune response in teleosts, p. 199-207. In R. T. Smith, P. A. Miescher, and R. A. Good [ed.] *Phylogeny of Immunity*. Univ. Florida Press, Gainesville, Fla.
19. SMITH, I. W. 1964. The occurrence and pathology of Dee disease. Dept. Agric. and Fish. Scotland, Freshwater and Salmon Fish. Res. Series, No. 34. 12 p.
20. SNIESZKO, S. F. 1970. Immunization of fishes: a review. *J. Wildlife Diseases* 6: 24-30.

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