Bull. Environ. Contam. Toxicol. (1985) 34:106–108


Environmental Contamination and Toxicology

# Alcohol-Induced Suppression of the Humoral Immune Response

J. S. Stolen[1] S. Draxler,[1] and J. J. Nagle[2]

[1]National Marine Fisheries Service, Northeast Fisheries Center, Sandy Hook Laboratory, Highlands, NJ 07732 and [2]Drew University, Madison, NJ 07940

Alteration of immune responsiveness, whether by drugs or environmental pollutants, is an area of interest both medically and epidemiologically. We report here that summer flounder (Paralichthys dentatus) pretreated by injection of a small amount of the common alcohol, ethanol, or ethanol containing PCB, showed complete suppression of the immune response to a formalin-killed sewage sludge isolate of the human enteric bacteria, E. coli. The unresponsiveness persisted for up to 42 days after injection of bacteria. Demonstrable agglutinating antibody to E. coli was seen in unpretreated fish after seven days. A second injection of bacteria 42 days after the first in pretreated fish, now resulted in immune responsiveness although the magnitude of the response resembled a primary response, indicating that memory to the first injection was also suppressed. Slightly lower responses were seen in the groups of fish injected with ethanol containing the PCB Aroclor 1254 when compared with those given ethanol alone, but the major suppression was alcohol-induced.

There is much interest in agents which modulate the immune response. Sublethal doses of toxicants can have an effect on immune responsiveness which may ultimately be as harmful as toxic doses because of the changes in susceptibility to disease agents. In some cases, such as in allergic responses or the survival of transplants and grafts, it is desirable to suppress immune responsiveness. A limited body of literature exists on the modulation of fish immune systems. Fish are exposed to a wide variety of toxic agents and are capable of accumulating high levels of these toxicants (Zeeman and Brindley 1981). The aim of these experiments was to examine the effect of PCBs on fish immune systems, but it became evident that the main culprit was the diluent which others reported to use for PCBs (Johansson et al. 1972).

## MATERIALS AND METHODS

Summer flounder between 300-400 g were maintained at 18-20°C in 250 gallon flow-through salt water tanks. Fish were pretreated either with ethanol, or ethanol containing Aroclor 1254 and then injected with E. coli (Table 1). They were bled weekly and tested

for agglutinating antibody to E. coli by the microtitration method.

Table 1. Experimental protocol.

| Group | # of fish | Treatment on Day 0 | 7 | 14 | 42 |
|---|---|---|---|---|---|
| 1 | 8 | ETOH* | ETOH | E. coli | E. coli |
| 2 | 8 | ETOH-PCB# | E. coli[a] | | E. coli |
| 3 | 8 | ETOH-PCB | | E. coli | E. coli |
| 4 | 8 | ETOH-PCB | ETOH-PCB | E. coli | E. coli |
| 5 | 8 | | E. coli | | E. coli |

*0.1 ml ethanol (95%)/100 g body weight was injected intraperitoneally (IP).
#PCB (Aroclor 1254) of concentration 5 µg/g body weight was injected IP.
[a]0.15 ml of a 20% suspension of formalin-killed E. coli (Stolen et al. 1983) in Freund's complete adjuvant was injected intramuscularly.

RESULTS AND DISCUSSION

None of the fish pretreated with ethanol, or ethanol containing PCB showed any response to E. coli, whether injected one week after pretreatment as in group 2, or two weeks after pretreatment as in group 3. No agglutinating antibody to E. coli was seen in weekly bleedings up to 42 days. Control fish had a mean $\log_2$ titer of 11.5 at 42 days, while detectable titers appeared as early as 7 days (Figure 1). Agglutinating titers developed after a second injection of E. coli at 42 days in the pretreated fish, but were well below those of the control fish given a second injection at the same time. The pretreated fish had titers roughly equivalent to the primary response demonstrated by untreated fish. If the first injection had elicited memory formation it would be expected that a second injection would have initiated a burst or a heightened response. Slightly lower responses were seen in the groups pretreated with PCBs in ethanol when compared with just ethanol pretreatments, indicating that PCBs have some suppressive effect.

The mode of action of ethanol cannot be deduced from these experiments. Studies done with cannabinoids in 95% ethanol solutions in human cell cultures showed that ethanol inhibits blastogenesis at a concentration greater than 1% (Desoize et al. 1981).. In fish, studies have been done on turpentine-induced inflammation (Weinreb 1958). We observed severe inflammatory responses in 20% of the fish treated with ethanol. Of the 20%, about 1/2 died, the others recovered from the inflammation within a two month period, and during this period responded to the second

injection of E. coli so it is not likely that the immunosuppression was directly related to the inflammation.

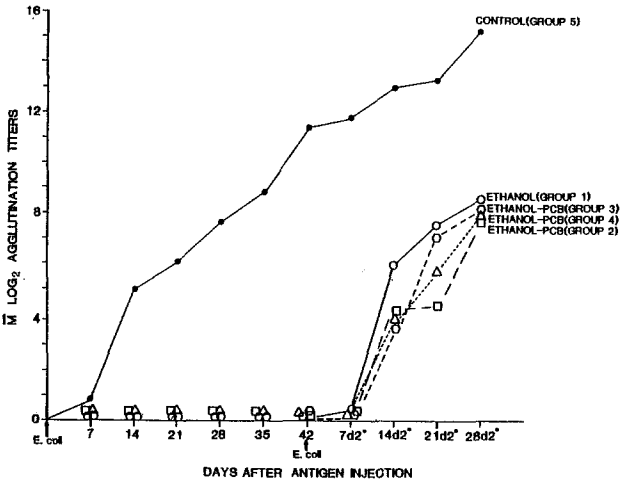


Figure 1. The effect of ethanol and ethanol containing PCB on the immune response of summer flounder (Paralichthys dentatus) to E. coli. For treatment refer to experimental protocol.

Acknowledgments. This study was funded by contract no. NA 80-FAC-2050 from the U.S. Department of Commerce, National Marine Fisheries Service to Drew University. Our thanks to Wendy Kaplan for her technical assistance, and to Drs. John Pearce and Carl Sindermann for their support.

REFERENCES

Desoize B, Nahas GG, Leger C, Bancheveau J (1981) Cannabinoids and the immunity system. In: Sharma RP (ed) Immunol consid in toxicol II. CRC Press, Boca Raton, FL, USA, p 61-82.

Johansson N, Larsson A, Lewander K (1972) Metabolic effects of PCB (polychlorinated biphenyls) on the brown trout (Salmo trutta). Comp Gen Pharmac 3:310-314.

Stolen JS, Kasper V, Gahn T, Lipcon V, Nagle JJ, Adams WN (1983) Monitoring environmental pollution in marine fishes by immunological techniques. Biotech 1:66-68.

Weinreb EL (1958) Studies on the histology and histopathology of the rainbow trout, Salmo gairdneri irideus, I. Hematology under normal and experimental conditions of inflammation. Zoologica 43:145-147.

Zeeman MG, Brindley WA (1981) Effects of toxic agents on fish immune systems: A review. In: Sharma RP (ed) Immunol consid toxicol II. CRC Press, Boca Raton, FL, USA, p 1-60.

Received February 27, 1984; accepted March 18, 1984