IMPAIRED IMMUNITY IN HARBOUR SEALS (*PHOCA VITULINA*) FED ENVIRONMENTALLY CONTAMINATED HERRING

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SUMMARY

In recent years, mass mortalities among seals and dolphins have been attributed to infections with different morbilliviruses. In all cases, these marine top predators were exposed to high levels of persistent lipophilic environmental contaminants accumulated through the food chain. This observation led to the hypothesis that a contaminant-related suppression of the immune system might have contributed to the severity of the virus outbreaks. We conducted a semi-field feeding experiment, in which we fed two groups of harbour seals (*Phoca vitulina*) fish with different levels of contaminants. During a period of 2 1/2 years, blood samples were taken at regular intervals, and the functioning of different compartments of the immune system was monitored and compared. We found impaired natural killer (NK) and specific T cell responses in the seals fed contaminated fish. This is the first demonstration of immunosuppression in mammals following chronic exposure to environmental contaminants at ambient environmental levels.

MORBILLIVIRUS INFECTIONS IN MARINE MAMMALS

In 1988, an apparently infectious disease caused the deaths of approximately 20000 harbour seals in northwestern Europe. The primary cause proved to be a previously unrecognized member of the genus *Morbillivirus* of the family *Paramyxoviridae*, and was named phocine distemper virus (PDV) (1). In subsequent years, mass mortalities among Baikal seals (*Phoca sibirica*) in Lake Baikal (Russia) and striped dolphins (*Stenella coeruleoalba*) in the Mediterranean Sea were also attributed to infections with morbilliviruses, in these cases canine distemper virus (CDV) and the newly identified dolphin morbillivirus (DMV), respectively. The characterization of these three different morbillivirus isolates from marine mammals suggested that there was no epidemiological link between the outbreaks. Serological studies indicated that antibodies against PDV and DMV were prevalent in many phocid and cetacean populations worldwide (1).

ENVIRONMENTAL CONTAMINATION: TOXIC EFFECTS IN MARINE MAMMALS?

In all three morbillivirus epizootics the populations affected inhabited highly contaminated coastal waters. Marine top-predators are exposed to high levels of bioaccumulated persistent lipophilic contaminants, such as PCBs and organochlorine pesticides. This exposure has been associated with a number of biological abnormalities, including reproductive toxicity, hormonal alterations and developmental irregularities (2). Laboratory animal studies have shown that a number of these contaminants can be toxic to the mammalian immune system, in some cases leading to increased susceptibility to infectious diseases (3). The high contaminant levels found in diseased seals and dolphins therefore led to the hypothesis that a contaminant-related immunosuppression might have contributed to the severity and extent of the outbreaks.

IMMUNOTOXICOLOGICAL FEEDING STUDY

We carried out a prospective feeding study under semi-field conditions, in which two groups of 11 juvenile harbour seals each were fed herring from the relatively uncontaminated Atlantic Ocean or from the highly polluted Baltic Sea for a period of 2 1/2 years. Blood samples were taken every 6-9 weeks, and toxicological, haematological and immunological parameters were evaluated. Results of the study have been reviewed elsewhere (4,5) and are summarized in table 1. Briefly, we found impaired NK cell and mitogen- and antigen-induced T cell responses in peripheral blood mononuclear cells from seals fed Baltic herring (6-8). The latter finding could be confirmed *in vivo*, when the seals fed polluted herring also had suppressed delayed type hypersensitivity

Table 1. Summary of differences in immunological parameters between two groups of harbour seals after chronic exposure to environmentally contaminated herring (table adapted from De Swart *et al.*, Environ Health Perspect 104 Supplement 4, July 1996).

parameter	assay	effect	reference
NK cell	^{51}Cr release assay	↓[1]	6,7
T lymphocyte	mitogen-induced proliferation	↓	6,8
	antigen-induced proliferation		8
	mixed lymphocyte reaction	↓	8
	delayed type hypersensitivity skin test	↓	9
B lymphocyte	mitogen-induced proliferation	-[2]	6,8
	Specific serum antibody responses	-/↓	8,9
	Ex vivo/in vitro immunoglobulin production	-	8
haematology	Lymphocyte counts in peripheral blood	-	6,10
	Neutrophil counts in peripheral blood	↑[3]	6,10

1: significantly lower responses in the seals fed highly contaminated Baltic herring, as compared to the seals fed relatively uncontaminated Atlantic herring; 2: no significant differences over time between the two groups of seals; 3: significantly higher responses in the seals fed Baltic herring, as compared to the seals fed Atlantic herring

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(DTH) responses (9). The serum antibody response to vaccination with primary antigens proved to be unaffected for most antigen/adjuvant combinations, but was suppressed in response to vaccination with ovalbumin/DDA (7,9). Measurement of routine haematology and clinical chemistry parameters demonstrated an increase in numbers of circulating neutrophils in the seals fed Baltic herring (10).

FASTING EXPERIMENT

In their natural environment seals periodically fast, during which metabolization of blubber lipids supplies most of their energy- and water requirements (11). During these periods, the mobilization of lipophilic contaminants from the blubber might pose an additional immunotoxic risk. Contaminant residue analysis of blubber samples taken from the 22 seals near the end of our study showed that the seals fed Baltic herring had accumulated higher levels of organochlorine pollutants than the seals fed Atlantic herring (9,12). We subjected the animals to a 15-day fast, and measured the same parameters assayed during the chronic exposure study. In assays carried out with blood samples taken at days 8 and 15 of the experiment, we could not find an aggravation of the immunotoxic effects observed prior to the fasting period (12).

CONCLUSIONS

We conclude that chronic exposure to persistent contaminants accumulated through the Baltic Sea food chain has led to an impairment of immune function in the seals of our study. Levels of organochlorines measured in these seals were lower than those observed in many European seal populations. This may reflect the relatively short exposure time in captivity, and their birth in a relatively uncontaminated region. Since immunotoxic effects in laboratory animals are most severe following perinatal exposure, we speculate that free-ranging animals in contaminated regions suffer from at least similar immunotoxic effects as the seals in our study. Since NK cells and T cells are of critical importance in the immune response to virus infections, this immunosuppression may well have facilitated the emergence and aggravated the course of the recent morbillivirus epizootics.

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