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In Vitro Exposure of Seal Peripheral Blood Leukocytes to Different Metals Reveal a Sex-Dependent Effect of Zinc on Phagocytic Activity

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Although the immunotoxicity of heavy metals is well established, evaluation of their potential immunotoxicity in wildlife species is complicated by variables that could modulate the immune response to xenobiotics under field conditions. Phagocytosis plays a key role in the mammalian immune response. The objectives of our study were to develop a method for measuring the phagocytic activity of seal peripheral blood granulocytes, and to determine the effects of both zinc chloride ($ZnCl_2$), cadmium chloride ($CdCl_2$) and mercuric chloride ($HgCl_2$) on this immune function of peripheral blood granulocytes *in vitro*. Peripheral blood leukocytes (PBLs) were isolated from the peripheral blood of either harbour seals (*Phoca vitulina*) or grey seals (*Halichoerus grypus*) captured in the St Lawrence Estuary. Cells exposed for 4 h to 10^{-4} M and 10^{-3} M $HgCl_2$ displayed lower phagocytic activity but this was related to a general cytotoxic effect of $HgCl_2$ as opposed to a specific effect on phagocytosis. Exposure of PBLs from either male or female PBLs to $CdCl_2$ had no effect on phagocytic activity at the concentrations tested except a significant decrease in cells from male harbour seals exposed to 10^{-4} M. Exposure to $ZnCl_2$ at physiologically relevant concentrations enhanced the phagocytic activity of PBLs from mature females of both species, whereas no effect was observed in cells from either males or immature females. In conclusion, we have developed an *in vitro* assay to test the effects of environmental contaminants on the phagocytic activity of seal PBLs. Our data indicate that exposure of PBLs to $ZnCl_2$ results in a sex-dependent response and that PBLs from mature female seals are more sensitive to $ZnCl_2$ than either male or immature seals. This sex-dependent response to $ZnCl_2$ could lead to a differential sensitivity to heavy metal exposures for males and females seals in the field.

Keywords: seals; zinc; cadmium; mercury; phagocytosis; gender effect.

Introduction

Heavy metals are present in the marine environment where they bioaccumulate along the food chain. Since seals are at the top of the marine food web, they accumulate high levels of stable pollutants in their tissues (Wagemann, 1989; Skaare *et al*., 1990). Many laboratories and epidemiological studies have demonstrated the immunotoxicity of heavy metals in a variety of animal species. Using both *in vitro* and *in vivo* experiments, several studies have demonstrated that the immune system as a whole is affected by exposure to these contaminants (Lawrence, 1985; Descotes, 1988; Bernier *et al*., 1995; Zelikoff and Thomas, 1998). However, the extent of immune dysfunction is dependent on the metal, type of exposure, species, and even the strain of animal used. Furthermore, many of these studies have tested exposures to concentrations that are unrealistic under field conditions.

By the end of the 1980s, populations of north-western European harbour seal (*Phoca vitulina*) had been decimated by a morbillivirus closely related to the canine distemper virus. Although this strain of morbillivirus is known to be lethal, the impact of xenobiotics on the resistance of marine mammals to such epizootic episodes cannot be excluded. Particularly when considering the high burden of xenobiotics found in the dead animals, and the immunotoxic potential of these compounds (Brouwer *et al*., 1989; Ross *et al*., 1996).

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Necropsies of stranded beluga whales (*Delphinapterus leucas*) in the St Lawrence Estuary indicate a high prevalence of tumours and infections with mildly pathogenic bacteria. These pathologies appear to be immunodeficiency-related and their occurrence has led to the speculation of an immunomodulation related to the whales body burden of both inorganic and organic contaminants (De Guise *et al*., 1995).

Harbour seals experimentally fed for two years with contaminated fish from the Baltic Sea displayed altered immune responses when compared to seals fed with less polluted fish from the Atlantic Ocean. Natural killer cell activity, mitogen-induced T-lymphocyte proliferative responses, delayed-type hypersensitivity, mixed lymphocyte reactions and antigen-specific proliferative responses to rabies virus and *tetanus toxoid* antigens were all impaired. These results reflected an impact on the whole immune response of seals (De Swart *et al*., 1994; Ross *et al*., 1996). Despite these facts, an unequivocal relationship between immunomodulation and xenobiotics has not been yet established for seals under field conditions. This may result from the number of parameters that may affect the extent of mass mortalities (Thompson and Hall, 1993) as well as the number of factors that may modulate the immunotoxic effects of contaminants.

Considering these difficulties in establishing a direct relationship between the exposure to xenobiotics and immunodeficiency in marine mammals, *in vitro* exposures of immune cells may constitute a useful approach for identifying mechanisms by which xenobiotics have immunotoxic effects. This may also help in the identification of parameters that could modulate these effects.

Phagocytosis plays a key role in both non-specific and specific immune responses of mammals and represents the first line of defence of the immune system against invading agents, especially bacteria (Van Oss, 1987). The objective of the present study was to develop an *in vitro* assay to measure the effects of marine contaminants on the phagocytic activity and viability of granulocytes from seal peripheral blood. In order to compare the effects of essential and non-essential metals present in the marine environment, peripheral blood leukocytes (PBLs) of two species of seals from the St Lawrence Estuary were exposed to concentrations ranging from 10^{-9} to 10^{-3} M of either zinc chloride ($ZnCl_2$), cadmium chloride ($CdCl_2$) or mercuric chloride ($HgCl_2$).

Zinc (Zn) is one of the most important trace elements in the body and plays a role in many biological processes (Mills, 1989). In contrast, mercury (Hg) and cadmium (Cd) are environmental contaminants that have been found in high concentrations in the liver and kidney of marine mammals (Wagemann *et al*., 1990; Dietz *et al*., 1998). These metals have been shown to cause immunomodulation in other animal species (Lawrence, 1985; Descotes, 1988; Bernier *et al*., 1995; Zelikoff and Thomas, 1998).

Materials and Methods

Isolation of PBLs

Blood samples were collected from 11 harbour seals and 6 grey seals captured in the St Lawrence Estuary, Quebec, Canada (between 47°56′ N and 48°41′ N). The animals included four adult male, four adult female and three first year female harbour seals, as well as three adult male and three adult female grey seals. Animals were caught using a gill net and handled on a boat for blood sampling and identification. Blood samples were drawn from the epidural vein into heparinized tubes (Vacutainer©, Becton Dickinson, Rutherford, NJ, USA). Blood was kept at 4°C until the time of analysis, 6–8 h after sampling.

Erythrocytes were lysed by osmotic shock using a 1/17 dilution of blood sample with an aqueous solution containing 9 g/l NH_4Cl (Sigma Chemicals, St Louis, MO), 1 g/l $KHCO_3$ (Sigma Chemicals) and 37 mg/l tetrasodium salt EDTA (Sigma Chemicals) at 37°C for 7 min. After three washes with Hank's Balanced Salt Solution (HBSS) (Gibco BRL, Mississauga, ON), the ratio of live/dead cells was assessed using acridine orange/ethidium bromide dye uptake and visual examination under a fluorescent microscope. This ratio was always greater than 80%. The cell concentration was adjusted to 1×10^6 viable PBLs in phenol free-RPMI 1640 (Gibco BRL) containing 100 U ml^{-1} of penicillin (Gibco BRL) and 100 μg ml^{-1} of streptomycin (Gibco BRL) (RPMI medium).

In vitro exposures and phagocytosis

Seal PBLs were incubated for 3 h in polystyrene tubes at 37°C under agitation with or without metals at concentrations ranging from 10^{-9} to 10^{-3} M for $HgCl_2$ and $ZnCl_2$, and from 10^{-9} to 10^{-4} M for $CdCl_2$ (Sigma Chemicals). Following these first 3 h of incubation, phagocytic activity was measured, based on the protocol of Brousseau *et al*. (1999). Briefly, 100×10^6 yellow-green fluorescent latex beads of 1.58 μm diameter (Molecular Probe Inc., Eugene, OR) were added to each cell suspension and incubated at 37°C with agitation for 1 h. A subsample of cells were incubated on ice with sodium azide (0.2% w/v; NaN_3) (Sigma Chemicals). These cells served as negative controls since the NaN_3 and low temperature inhibit phagocytosis. This allows for the determination of non-specific binding of the fluorescent latex beads to the plasma membrane of the cells. The fluorescent response of lymphocytes was also used as negative controls to evaluate the surface attachment of beads that were not processed by phagocytic activity (non-specific attachment). Cells were separated from non-phagocytosed beads by centrifugation (8 min at 150 ***g***, 4°C) on a cushion of 3% bovine serum albumin (BSA, Fraction V; INC Biomedicals, Ohio, USA) plus 10% foetal calf serum (Gibco BRL) in RPMI-1640 (Gibco BRL). Supernatant was discarded and cells were suspended in Hematall (Fisher, Montreal,

Que.). Samples were analysed with a FACScan flow cytometer equipped with an argon laser providing excitation at 488 nm (Becton Dickinson, San José, CA). For each sample, the fluorescence of 10 000 events was read. Results were analysed with LYSYS-II software (Becton Dickinson) to determine the percentage of PBLs that engulfed more than two beads. Parallel duplicate control tests for phagocytic activity of cells in the absence of metals were performed for each seal. The phagocytic activity of cells exposed to metal was expressed as a percentage of the mean response observed with non-exposed cells (average of duplicates).

After measuring phagocytic activity, the percentage of dead cells was evaluated in the same cell population by flow cytometry using propidium iodide (Sigma Chemicals) labelled PBLs as described by Brousseau *et al.* (1999).

Statistical analyses

Statistical analysis consisted of a parametric Student's *t*-test or ANOVA test to compare groups when data were normally distributed. When data were not normally distributed, a non-parametric Wilcoxon–Mann Whitney *U* test or Kruskal–Wallis test was used.

Results

Phagocytosis of pinniped granulocytes

Phagocytic activity of pinniped granulocytes has not been previously documented. The flow cytometric method which was used in the present study allowed us to identify different cell subpopulations on the basis of their size and complexity while at the same time excluding cellular debris and non-lysed erythrocytes (Fig. 1). Using the LYSIS II software analyses (Becton Dickinson), we were able to draw up analytical gates in order to assess the fluorescence of these cell subpopulations. The fluorescence of the cells is proportional to the number of phagocytosed fluorescent beads, which provides a measurement of phagocytic activity. The differential fluorescence response of lymphocytes and granulocytes is shown in Figs. 2a and 2b respectively. The absence of fluorescence observed in lymphocytes corresponds with their inability to perform phagocytosis and represents an appropriate negative control. Henceforth, all subsequent assays performed with the total leukocyte population (PBLs as indicated) reflect phagocytosis by granulocytes. As expected, incubation with sodium azide resulted in a 50% decrease of phagocytic activity (data not shown).

Cytotoxicity

PBLs isolated from both harbour and grey seals were exposed, *in vitro*, to different doses of $ZnCl_2$, $HgCl_2$ (10^{-9} to 10^{-3} M), or $CdCl_2$ (10^{-9} to 10^{-4} M). We did not observed any significant differences between species, sexes or sexual maturity with respect to the cytotoxicity of the metals tested (Fig. 3, Tables 1 and 2). Exposures to metals at doses ranging from 10^{-9} to 10^{-5} M did not result in increased cytotoxicity (Fig. 3, Tables 1 and 2). However, the extent of cytotoxicity at higher doses was metal-dependant. Cells exposed to 10^{-4} M $HgCl_2$ resulted in 70% mortality (Table 1) whereas similar doses of $CdCl_2$ and $ZnCl_2$ did not cause a significant increase in cell death (Fig. 3, Table 2).

Phagocytic activity

In vitro exposures to $HgCl_2$. At the lowest $HgCl_2$ concentrations (10^{-9} to 10^{-5} M) there was no significant effect on phagocytic activity of seal PBLs. At 10^{-4} and

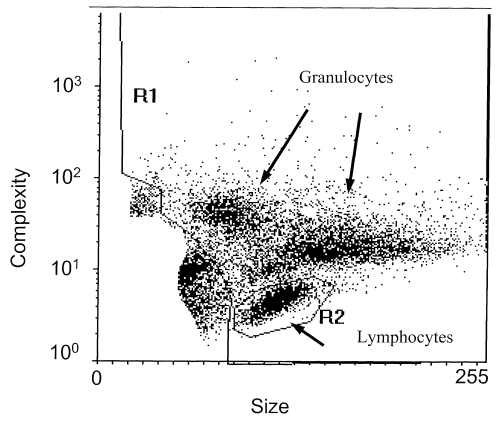

Fig. 1 Scattergram of the flow cytometric profile of blood cells from harbour seal after red blood cells lysis and incubation with fluorescent beads. We exclude the most part of cell debris and unlysed erythrocytes during acquisition data process (blind zone). A part of the granulocyte population is more complex (upper left of the main cell population) as a result of the presence of latex beads inside the cells. The analytical gate R1 excludes the remainder of the cell debris and unlysed erythrocytes. The analytical gate R2 is draw up to isolate the lymphocytes from the total PBLs.

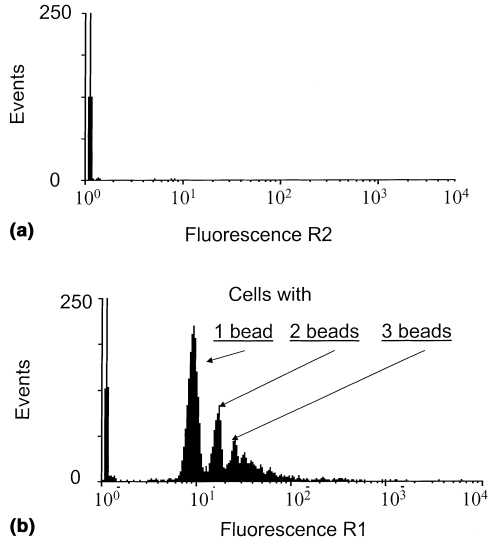

Fig. 2 Fluorescence of the PBLs. We have excluded the remainder of the cell debris and unlysed erythrocytes and isolated the lymphocyte population (as described in Fig. 1). Lymphocytes (gated in R2 as a part of R1) displayed no fluorescence signal (a) with respect to the entire cell population as a whole (b).

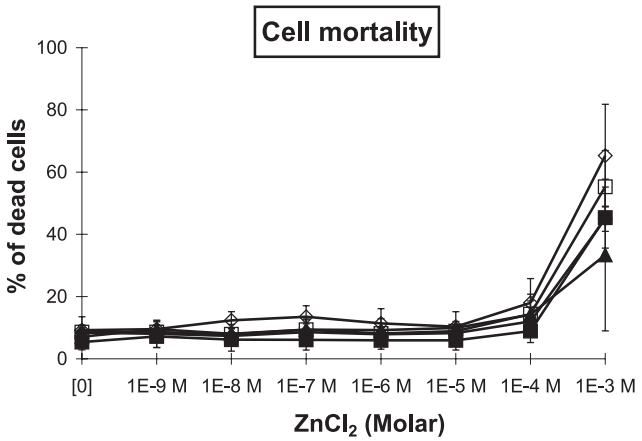

Fig. 3 Mortality of cells following exposures to zinc chloride. Comparison between cells from female harbour seals (◆), ($n = 4$); male harbor seals (■), ($n = 4$); immature female harbor seals (▲), ($n = 3$), male (□) and female (◇) grey seals ($n = 3$). Results are expressed as a % of dead cells and are presented as the mean ± SD.

10^{-3} M $HgCl_2$, the phagocytic activity of cells was significantly reduced as compared to unexposed cells ($p \leqslant 0.05$). However, at these concentrations, $HgCl_2$ was cytotoxic and resulted in more than 70% cell death. We observed the same dose-response pattern with cells from all groups of seals tested (Table 1).

In vitro exposures to $CdCl_2$. $CdCl_2$ caused a small but significant reduction on phagocytic activity of blood granulocytes from male harbour seals at the highest dose tested (Table 2). While the small, but non-significant decrease in cell viability contributed to this observation, we cannot discount a direct effect of $CdCl_2$ on phagocytosis by these cells. No effect was observed on cells isolated from the other seals (Table 2).

In vitro exposures to $ZnCl_2$. As mentioned above, Zn did not cause significant cytotoxicity to PBLs. In addition, there were no differences among species, gender or with sexual maturity in $ZnCl_2$-induced cell mortality (Fig. 3). However, our results show a sex-dependent effect of $ZnCl_2$ on the phagocytic activity of seal peripheral blood granulocytes. Exposure to 10^{-5} M $ZnCl_2$ has a significant ($p \leqslant 0.05$) immunopotentiative effect on cells from adult female harbour seals (124 ± 15% of response control) but no effect was observed on cells from male or immature females (Fig. 4). We also observed the same sex-related differences with cells from grey seals. Phagocytic activity of cells from females increased significantly following exposure to 10^{-5} M and 10^{-4} M $ZnCl_2$ when compared to that in control cells (123± 7.5% and 128 ± 10% of response control respectively), whereas no significant effect was observed in cells from males. Furthermore, difference in cell response between male and female grey seals was significant ($p \leqslant 0.05$) after exposure to 10^{-4} M $ZnCl_2$ (Fig. 5).

Discussion

This is the first study on the phagocytic activity of granulocytes from seal peripheral blood. The scattergrams of the flow cytometric profile of blood cells after the lysis of erythrocytes show a large population of granulocytes. In agreement with data from Bossart and Dierauf (1990) this cell subpopulation could represent between 50 and 85% of total seal PBLs. We observed

TABLE 1

Phagocytic activities of PBLs from seals from St Lawrence Estuary following *in vitro* exposures to mercuric chloride.[a]

Hg		10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M
Harbor seals (M)	$n = 4$							
Phagocytosis		106.6 ± 25.5	102.9 ± 10.8	109.6 ± 10.1	105.3 ± 13.1	80.9 ± 30.6	39.1 ± 29.2*	15.6 ± 14.2*
Mortality		5.9 ± 3.1	5.4 ± 3.2	6.0 ± 2.9	6.9 ± 4.2	8.1 ± 5.4	73.7 ± 8.7	72.6 ± 24.3
Harbor seals (F)	$n = 4$							
Phagocytosis		121.6 ± 19.8	120.5 ± 21.5	112.1 ± 13.8	116.8 ± 11.7	84.8 ± 28.3	63.8 ± 21.3*	29.0 ± 22.6*
Mortality	$n = 3$	7.9 ± 3.7	7.9 ± 3.1	8.1 ± 2.9	8.5 ± 2.6	10.5 ± 5.1	75.1 ± 8.0	88.7 ± 2.5
Harbor seals (Im)	$n = 3$							
Phagocytosis		134.0 ± 33.4	104.3 ± 5.9	106.2 ± 29.6	93.2 ± 11.5	85.5 ± 30.2	64.9 ± 26.3	15.1 ± 5.1*
Mortality	$n = 2$	12.0–11.3	9.9–9.9	8.2–12.3	10.2–13.6	7.2–13.2	77.6–69.9	90.2–87.0
Grey seals (M)	$n = 3$							
Phagocytosis		99.1 ± 20.1	102.7 ± 10.7	107.8 ± 33.2	111.0 ± 36.6	110.6 ± 14.7	57.6 ± 7.0*	24.6 ± 20.3*
Mortality		8.7 ± 1.6	9.2 ± 1.8	9.2 ± 1.9	9.3 ± 1.2	27.1 ± 29.7	66.1 ± 9.1	76.5 ± 9.8
Grey seals (F)	$n = 3$							
Phagocytosis		117.0 ± 2.1	106.3 ± 35.4	111.8 ± 27.4	123.9 ± 29.6	100.4 ± 12.9	66.3–75.7–(285.7)[b]	13.5 ± 7.6*
Mortality		8.5 ± 5.4	8.5 ± 5.0	9.4 ± 5.8	8.9 ± 6.0	11.8 ± 4.9	77.3–71.2–67.9	92.7 ± 3.2

[a] (M) = adult males, (F) = adult females, (Im) = first year females. Phagocytosis is measured as the % of cells that engulfed more than two beads. Results (mean ± standard deviation) are expressed as a % of the control response (unexposed cells). Mortality results (mean ± SD) are expressed as the % of dead cells in the same cell population. When $n = 2$, each value is given.

[b] At this concentration, an apparatus artifact result in a disjointed measure of 285.7% of phagocytosis activity with more than 60 % cell death.

* Significantly ($p \leqslant 0.05$) different from control (100%).

TABLE 2

Phagocytic activities of PBLs from seals from St Lawrence Estuary following *in vitro* exposures to cadmium chloride.[a]

$CdCl_2$		10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
Harbor seals (M)	$n = 4$						
Phagocytosis		98.3 ± 8.3	112.0 ± 12.3	98.8 ± 17.6	115.9 ± 10.7	100.6 ± 12.7	71.6 ± 14.9*
Mortality		5.5 ± 2.7	5.6 ± 2.8	6.0 ± 2.9	6.7 ± 3.9	5.9 ± 3.2	13.9 ± 6.9
Harbor seals (F)	$n = 4$						
Phagocytosis		129.6 ± 22.0	126.6 ± 20.7	111.6 ± 20.1	126.2 ± 17.5	113.3 ± 22.5	88, 7 ± 50.0
Mortality	$n = 3$	9.0 ± 2.8	7.6 ± 3.3	7.2 ± 3.2	8.4 ± 2.3	9.7 ± 1.6	21.6 ± 6.5
Harbor seals (Im)	$n = 3$						
Phagocytosis		109.4 ± 13.8	124.2 ± 20.7	124.4 ± 29.1	114.4 ± 49.1	99.2 ± 13.8	124.2 ± 16.5
Mortality	$n = 2$	6.2–12.1	6.7–11.6	5.8–11.7	8.3–13.7	8.0–12.9	15.0–23.8
Grey seals (M)	$n = 3$						
Phagocytosis		115.6 ± 11.5	82.5 ± 21.8	120.4 ± 17.2	120.8 ± 41.6	107.7 ± 24.0	101.3 ± 30.1
Mortality		10.0 ± 3.2	8.9 ± 1.3	8.7 ± 2.1	9.0 ± 2.2	10.2 ± 2.4	17.6 ± 9.1
Grey seals (F)	$n = 3$						
Phagocytosis		107.4 ± 24.1	108.1 ± 20.1	117.0 ± 5.0	100.3 ± 27.1	103.4 ± 29.0	94.1 ± 36.1
Mortality		11.6 ± 8.1	8.7 ± 4.8	9.6 ± 5.3	8.9 ± 4.5	9.1 ± 4.1	17.2 ± 10.8

[a] (M) = adult males, (F) = adult females, (Im) = first year females. Phagocytosis is measured as the % of cells that engulfed more than two beads. Results (mean ± standard deviation) are expressed as a % of the control response (unexposed cells). Mortality results (mean ± SD) are expressed as the % of dead cells in the same cell population. When $n = 2$, each value is given.
* Significantly ($p \leqslant 0.05$) different from control (100%).

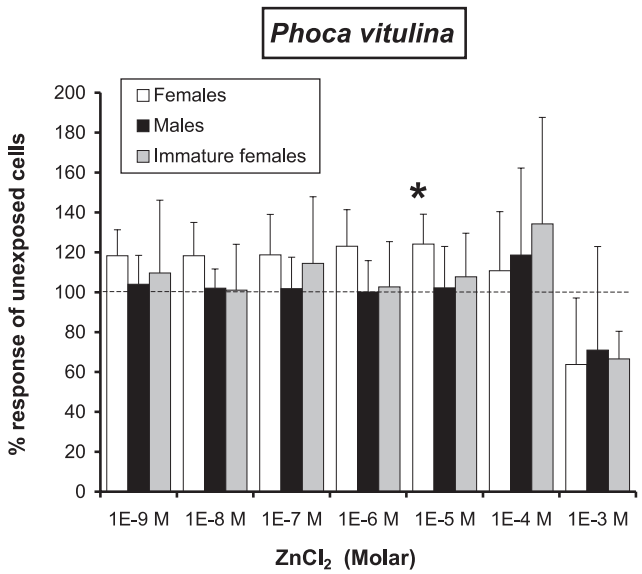

Fig. 4 Phagocytic activities of PBLs from female (□), immature female (▨) and male (■) harbor seals (*P. vitulina*) following in vitro exposures to zinc chloride. Phagocytosis is measured as the % of cells that engulfed more than two beads. Results are expressed as a % of the control response (unexposed cells) and are presented as the mean ± standard deviation ($n = 4$; except for immature females, $n = 3$). Dotted line represent the level of control response. * Significantly different from control (100%) ($p \leqslant 0.05$).

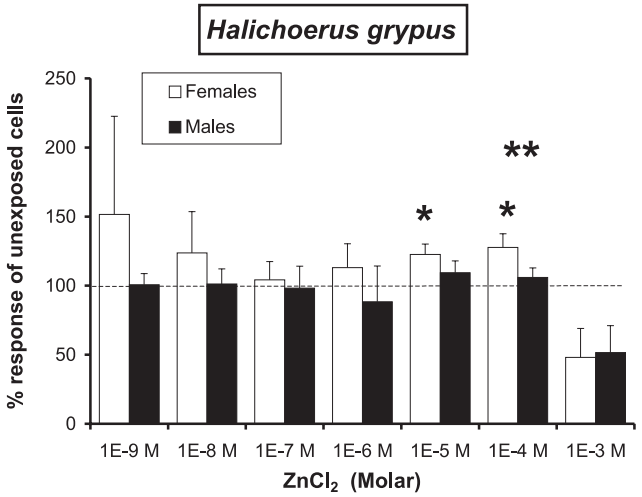

Fig. 5 Phagocytic activities of PBLs from female (□) and male (■) grey seals (*H. grypus*) following in vitro exposures to zinc chloride. Phagocytosis is measured as the % of cells that engulfed more than two beads. Results are expressed as a % of the control response (unexposed cells) and are presented as the mean ± standard deviation ($n = 3$). Dotted line represent the level of control response. * Significantly different from control (100%) ($p \leqslant 0.05$). ** Significant difference between male and female cell responses ($p \leqslant 0.05$) at this concentration.

typical histogram of fluorescence emitted by beads engulfed by phagocytosis after 1-h incubation with fluorescent latex beads. This has also been reported for other mammalian species (Brousseau *et al.*, 1999). The absence of fluorescence in the lymphocyte subpopulation indicates lack of phagocytosis by the lymphocytes and confirms the validity of our methodological approach.

PBLs from the two species of seals sampled in the St Lawrence were exposed *in vitro* to $HgCl_2$, $CdCl_2$ and $ZnCl_2$. Zn has been chosen because of its importance as a trace element and its role in various biological processes (Mills, 1989). In contrast, Hg and Cd are immunotoxic contaminants that have been reported to be present at high concentrations in the liver and kidney of marine mammals such as beluga whales from St Lawrence Estuary or seals from Arctic region (Wagemann *et al.*, 1990; Dietz *et al.*, 1998). Our results show that the dose-response to $HgCl_2$ followed the same general

pattern for every group of seals tested, with *in vitro* exposure to 10^{-4} M being highly cytotoxic. The percentage of dead cells observed following an exposure to this concentration of $HgCl_2$ appears responsible for the significant reduction in phagocytic activity measurements. In contrast, *in vitro* exposure of seal PBLs to $ZnCl_2$ or $CdCl_2$ did not significantly reduce the viability of cells from any of the groups tested. These observations concur with previous results from studies comparing the toxicity of Hg, Cd and Zn on human immune cells (Steffensen *et al.*, 1994).

Observed differences in cytotoxicity following *in vitro* exposures to the three metals may be related to the different ability of these metals to induce metallothioneins (MTs). MTs are small cysteine-rich proteins (6–8 kDa) that bind heavy metals. These proteins can actively sequester intracellular essential metals, thereby regulating their bioavailability. They also play an important role in the detoxification of heavy metals such as Cd. MTs are produced mainly in the liver and kidney but can also be synthesized by leukocytes as well as other cell types (Kimura, 1991). Yamada and Koizumi (1991) have shown that the concentration of metal required to reach the maximal thionien induction in human peripheral blood lymphocytes is 20-fold higher for Zn than it is for Hg or Cd, but Zn was able to induce more than twice the amount of MTs.

The effects of Zn on the non-specific immunity have been demonstrated in laboratory experiments. Wirth and collaborators (1989) have shown that in mice fed a zinc-deficient diet, peritoneal macrophages displayed a reduced capacity to phagocytose *Trypanosoma cruzi*. Treatment of cells with Zn prior to incubation with the parasite restored phagocytic function. Although the mechanisms by which zinc modulates phagocytosis remain unknown, MTs could play a role in the modulation of phagocytosis. As previously mentioned, MTs regulate the bioavailability of cations inside the cells. In this way, they control free Zn^{2+} ion concentration, which in turn modulates the degree of saturation of Zn binding sites for both catalytic and structural actions. MTs have also been shown to interact with certain immune processes such as monocyte activation and superoxide production by macrophages (Leibbrandt *et al.*, 1994; Youn *et al.*, 1995). Therefore, Zn and MTs are two of the major components in a complex pathway by which the toxicity of heavy metals, especially Cd, is decreased (Mishima *et al.*, 1997). As a consequence, their regulation and potential effect on immune cells are particularly important.

There is no information on Zn levels in the plasma of seals. Zn concentrations in the plasma of beluga whales from the Churchill River Estuary were in the order of 10^{-5} M (18.1 ± 4.1 μmol/l) (De Guise *et al.*, submitted) and is similar to what has been reported for other mammalian species.

We observed that *in vitro* exposure to this physiological concentration of Zn increased phagocytic activity of PBLs from adult females of both species of seals. It should be pointed out that we did not observe a significant effect of Zn on the phagocytosis by PBLs from either males or immature females at any of the concentrations tested. A 10^{-4} M exposure to $ZnCl_2$ had similar effects on cells from female grey seals, resulting in a significant difference in the phagocytic response between males and females of this species.

The relationship between the immune and endocrine systems is well documented for human and rodent species. Sex hormones interact with immune cells, thereby modulating immune functions (for review see Grossman, 1985, 1989; Schuurs and Verheul, 1990). Studies have demonstrated altered neutrophil functions in periparturient bovines (Kehrli *et al.*, 1989), and phagocytosis has been shown to be modulated both *in vitro* and *in vivo* by oestradiol in porcine granulocytes (Magnusson and Einarsson, 1990; Magnusson, 1991). In addition, Zn is involved in several processes of hormone regulation and could interact with the hormonal modulation of the immune system. As regulators of Zn bioavaibility, MTs can act as a powerful modulators of these particularly complex pathways.

The fact that we observed an immunopotential effect *in vitro* in a hormone-free medium suggests that PBLs from female seals may be differently preconditioned by exposure to female sex hormones *in vivo*. The mechanism responsible for this is presently unknown but may be related to differences in gene expression in these cells and caused by sex hormones, leading to different protein pool in these cells.

In vivo modulation of phagocytic activity could have serious consequences on the health of seals. Phagocytosis plays a key role in the network that constitutes the complex mammalian immune response. Any disruption of phagocytic activity may result in increased susceptibility to bacterial infections (Gainer, 1977; Descotes, 1988). If females are more sensitive to heavy metals, as suggested by the present study, this may have significant consequences on seal population and recruitment.

In conclusion, an *in vitro* approach has been developed to test the effects of environmental contaminants on phagocytosis of peripheral blood granulocytes from pinnipeds. Using this assay, we were able to demonstrate a sex-dependent effect of Zn on phagocytosis. Such an effect may provide crucial information on the immunotoxic sensitivity of seals and the potential consequences on seal populations exposed in the wild to a variety of xenobiotics, suggesting that female seals may be more sensitive to the immunodulatory effects of heavy metals than either mature males or immature female seals.

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