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GENERAL BIOLOGY

Phagocytic Activity of Leukocytes in Harp Seals

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Abstract—Phagocytic and cytochemical parameters of peripheral blood leukocytes in adult and 1.5-month-old harp seal pups were studied. Phagocytic number, proportion of phagocytic leukocytes, and phagocytic activity, average number of absorbed latex particles per leukocyte, were determined. Differences between groups of seals by average values of these indicators have not been revealed. A correlation analysis revealed a significant relationship between the phagocytic activity of leukocytes and their glycogen content in adult seals ($r = 0.89$). Content of cationic bactericidal protein (CP) in leukocytes of adult animals was 10–12 times higher than in pups. Correlation of CP and eosinophil count in adults was high ($r = 0.88$). Adult had significantly more eosinophils than pups. The noted features can provide higher efficiency of phagocytosis and destruction of absorbed bacteria in adult seals as compared to pups, despite the high level of indicators of the first, absorbing, phase of phagocytosis in the latter.

Keywords: marine mammals, immunity, harp seal, phagocytosis

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INTRODUCTION

Among the problems of modern evolutionary immunology, one of the most significant is the study of the relationship between nonspecific (innate) and specific (acquired) immunity [1]. The problem of assessing the effectiveness of the mechanisms of resistance to adverse factors is relevant not only for captive-held and rare species, but also for commercial species of marine mammals, the number of which is significantly reduced due to intoxication and epizootics [2, 3]. The hypothesis that was formulated a long time ago [4] about the existence of specific features of the immune system in cetaceans and pinnipeds, caused by evolution in a clean oceanic environment, not containing pathogenic land microorganisms, providing insufficient immunological resistance under modern conditions, has not been confirmed to date. The high level of morbidity of marine mammals is associated with the impact of pollution of their habitat on health, including the immune system.

Phagocytosis is the most ancient cellular mechanism providing, along with humoral factors, nonspecific protection against pathogenic microorganisms. Nonspecific immunological reactions develop much faster than specific reactions. This is essential for aquatic animals, in constant contact with the environment where pathogenic microorganisms and xenobiotics may be present [5].

Information on the bactericidal functions of marine mammalian leukocytes is limited. Thus, a direct dependence of the phagocytic activity of blood leukocytes in Northern fur seal pups on the intensity of invasion by *Uncinaria lucasi* has been established [6]. For dolphins, it is known that the stress developing after catching animals and placing them on a ship for transportation is accompanied by a decrease in the phagocytic functions of leukocytes, the restoration of which occurs only after four to six months [7]. The total number of lysosomes and number of lysosomes containing cationic bactericidal proteins decreases after capturing, transportation, and in the first days of the stay of dolphins in the basin [8]. Some indicators of phagocytosis of leukocytes of beluga whales [9] and common, gray, and harp seals have been determined [10].

The main part of innate cellular immunity is phagocytic leukocytes. They act as the inflammatory mediators and have cytotoxic and antitumor effects [11]. The bactericidal activity of leukocytes is provided by an oxygen-dependent system (including myeloperoxidase, singlet oxygen, superoxide anion, hydroxyl radical, hydrogen peroxide, halogens) and an oxygen-independent nonenzymatic system (including cationic proteins, transferrin, lactoferrin, and lactic acid). Cationic proteins (CP) are an important component of the antimicrobial defense of the body, and their deficiency leads to a sharp decrease in nonspecific resis-

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Table 1. WBC differential count and cytochemical parameters of harp seal leukocytes (M ± SE)

Harp seal group	Types of leukocytes,%					GL		CP	
	N	E	B	M	L	ACC	GL+	ACC	CP+
Adult, $n = 10$	53.8 ± 3.6	19.3 ± 2.6	0.1 ± 0.1	2.7 ± 0.4	24.1 ± 4.2	1.57 ± 0.08	90.0 ± 2.2	0.45 ± 0.07	21.7 ± 2.9
Normal pups, $n = 10$	60.8 ± 5.8	1.6 ± 0.6	0.2 ± 0.1	9.0 ± 0.1	28.4 ± 6.6	1.33 ± 0.09	86.9 ± 1.9	0.06 ± 0.01	2.7 ± 0.5
Undernourished, $n = 10$	61.3 ± 4.0	2.0 ± 0.4	0.2 ± 0.1	10.1 ± 1.1	26.4 ± 4.4	1.26 ± 0.10	82.4 ± 3.6	0.03 ± 0.01	1.7 ± 0.5

GL, glycogen; CP, cationic protein; ACC, average cytochemical coefficient; GL+, percentage of leukocytes containing glycogen; CP+, percentage of leukocytes containing CP. N, neutrophils; E, eosinophils; B, basophils; M, monocytes; L, lymphocytes.

tance [12]. CP exhibit bactericidal activity under anaerobic conditions, which is essential for aquatic mammals exposed to hypoxia during diving.

The relationship and interaction of reactions of specific and nonspecific immunity can be estimated by studying their development in the ontogenesis of animals. Nonspecific resistance plays an important role in providing the survival of seal pups, since the specific immunity system in mammals is still being formed after birth. In this study, we determined the parameters of phagocytosis and some cytochemical parameters of leukocytes in harp seals of different ages.

MATERIALS AND METHODS

The material for research was obtained at the seal fisheries in the village of Koida, Arkhangelsk region, from pups after juvenile molting (aged 1–1.5 months), young harp seals, and adult animals. The animals were divided into two groups: "normal", well-fed pups weighing 30–35 kg; and undernourished or "starveling" ones weighing 10–15 kg.

The study of phagocytic reactions was carried out using reagent kits and techniques used in human clinical studies. Latex particles 0.5–0.6 μm in diameter were used as the object of phagocytosis. Suspension of particles 0.05 mL (2.5×10^7) in Hank's balanced salt solution was mixed with 0.1 mL of heparinized blood and 0.05 mL of Hank's solution or 0.05 mL of 0.00025% prodigiosan. The samples were incubated for 1 h at 37°C with shaking tubes every 5 min. This method determines the effectiveness of the initial, absorption phase of phagocytosis, since latex is not digested by the cell. The number of latex particles was counted in 100 leukocytes. The phagocytic number (PN), the percentage of phagocytic cells; and phagocytic activity (PA), the average number of absorbed latex particles per one leukocyte were determined. The reserve phagocytic capacity was assessed based on the value of the stimulation index (SI) of phagocytic reactions by prodigiosan (lipopolysaccharide of *Bacillus prodigiosum*). The Romanowsky-Giemsa staining of blood film and cytochemical methods: detection of glycogen, bactericidal cationic proteins were also used [13]. The average cytochemical coefficient (ACC) was calculated. ACC = (A1 × 1 + A2 × 2 + A3 × 3)/100, where A is the number of leukocytes with different amounts of the detected substance. The preparations were examined with oil immersion (microscope objective lens, 100×; ocular lens, 10×).

RESULTS AND DISCUSSION

The leukocyte grouping of seals using the Romanowsky–Giemsa method was usually unambiguous. Neutrophils predominated in the studied blood samples of seals, as in many species of land mammals, including representatives of the order of carnivores, phylogenetically closest to true seals. Adult animals differed from pups by a significantly higher number of eosinophilic leukocytes. The content of glycogen in leukocytes of adult seals and pups was the same, and the level of cationic protein was much higher (Table 1).

As evidenced by the data shown in Table 2, the differences between the mean values for the groups of seals for all parameters were statistically nonsignificant ($p > 0.05$). This is consistent with the results of a published study of common, grey, and harp seals: the phagocytic activity of leukocytes in newborn animals and adult females is the same, at the end of the lactation period in pups it is higher than in their mothers and then it gradually decreases [10]. However, individual fluctuations of phagocytosis indices in harp seals are significant, especially in the degree of increase in PA in underweight pups after stimulation with prodigiosan.

The correlation analysis revealed significant links between some cytochemical and phagocytic indices. A high correlation has been demonstrated between the glycogen content and the phagocytic activity of leukocytes in adult seals, in contrast to pups (Fig. 1). A study of leukocytes stimulated by prodigiosan provided a similar result: $r = 0.90$, $r = 0.14$, $r = 0.41$ (adult, normal pups, and undernourished pups, respectively).

In addition, a significant correlation was found between the reserve of phagocytic activity (SI_2) and the relative amount of neutrophil glycogen in normal pups ($r = 0.68$, $p < 0.05$), in contrast to the undernourished pups ($r = 0.15$) and adults ($r = 0.32$). Phagocytosis is an energy-dependent process. Different content of glycogen (and features of its metabolism) provides a different energy base for phagocytosis and other cell

Table 2. Indicators of phagocytic activity of leukocytes of harp seals (M±SE; in brackets, the variation limits of indicators). PN, phagocytic number, percentage of phagocytizing cells; PA, phagocytic activity, average number of absorbed latex particles per leukocyte

Harp seal	PN			PA		
	spontan.	stimul.	SI_1, %	spontan.	stimul.	SI_2, %
Adult, $n = 6$	58.5 ± 6.7 (32–74)	68.34 ± 5.8 (41–80)	17.7 (0–25.4)	18.2 ± 1.4 (14.2–23.5)	22.7 ± 2.3 (14.2–31.6)	25.0 (0–54.1)
Normal pups, $n = 10$	61.3 ± 4.4 (36–86)	70.6 ± 4.5 (58–86)	15.1 (0–72.6)	13.7 ± 1.6 (8.7–26.5)	16.2 ± 1.8 (10.0–25.5)	18.3 (0–31.2)
Undernourished, $n = 10$	49.5 ± 5.0 (30–68)	56.2 ± 5.4 (29–79)	13.5 (0–45.7)	16.0 ± 1.6 (10.4–24.7)	20.6 ± 2.0 (13.5–31.5)	28.2 (0–110)

functions. In undernourished, long-term starved seal pups, a high glycogen level is probably not a prerequisite for effective phagocytosis. Their glycogen storage is the last-consumed reserve, and energy obtained from subcutaneous fat and proteins is used for the provision of vital functions.

In adult seals, a high correlation between the number of eosinophils and leukocytes in which the bactericidal cationic protein is present was found (Fig. 2). Probably, the phagocytic function in aquatic mammals is associated with eosinophils. The high level of phagocytic activity of leukocytes of harp seal pups was provided by neutrophilic leukocytes. However, the content of the bactericidal cationic protein in these cells was extremely low, and, therefore, the destruction of absorbed bacteria was apparently insufficiently effective. In seals that started self-feeding (at an age of 3–3.5 months), the number of eosinophils increased to 7–40% [14]. At the same time, the content of the bactericidal cationic protein increased to the level of adult animals. Probably, the formation of the phagocytic system occurs during this stage of ontogenesis of harp seals.

For the entire group of studied harp seals, significant correlations between PN after stimulation of leukocytes with prodigiosan and the number of eosinophils and cells containing cationic protein were found ($r = 0.94$ and $r = 0.99$, respectively). This, together with eosinophilia in adult harp seals, suggests the leading role of eosinophils in phagocytic and, probably, other reactions of nonspecific immunity.

The harp seal is the most abundant pinniped species in the northern hemisphere, playing a significant role in the ecosystems of the Arctic seas. The state of the populations of marine mammals is largely determined by the pollution level of the habitat, which affects the functioning of the cellular and humoral mechanisms of the immune resistance of organisms. Baby seals can be particularly vulnerable to various pathogens. It is advisable to supplement the results obtained in this study by investigating both specific

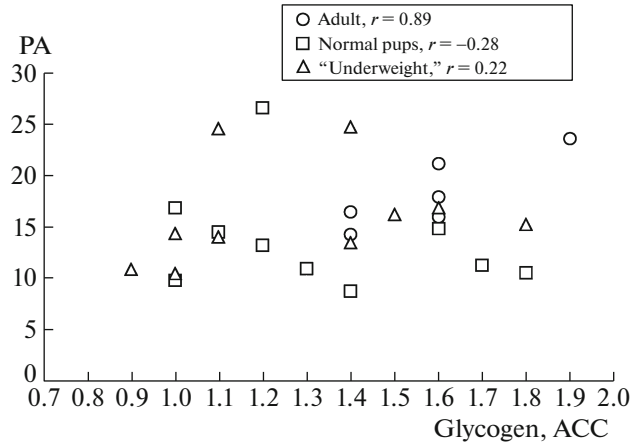

Fig. 1. Correlation of phagocytic activity (PA) and glycogen content in leukocytes of harp seals (ACC is the average cytochemical coefficient).

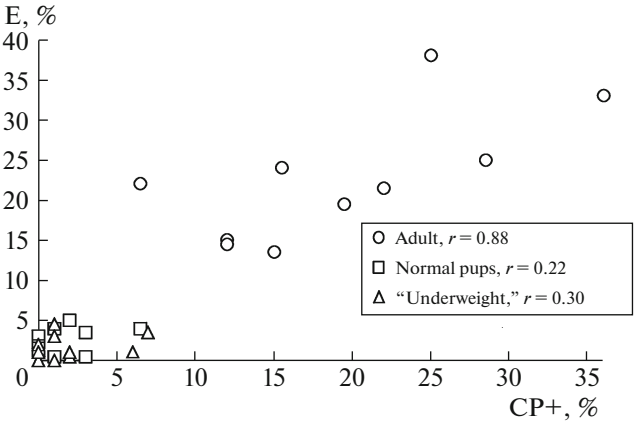

Fig. 2. The ratio of the number of eosinophils (E) and leukocytes containing bactericidal cationic protein (CP+).

and nonspecific immune reactions of seals of different ages.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

REFERENCES

1. Galaktionov, V.G., *Med. Immunol.,* 2004, vol. 6, nos. 3–5, pp. 159–170.
2. Harwood, J. and Reijnders, F., *New Sci.,* 1988, vol. 119, no. 1634, pp. 28–29.
3. Khuras'kin, L.S., Zakharova, N.A., Kuznetsov, V.V., et al., in *Morskie mlekopitayushchie Golarktiki* (Sea Mammals of the Holarctic), Moscow, 2002, pp. 276–278.
4. Cavagnolo, R., *Dev. Comp. Immunol.,* 1979, vol. 3, no. 2, pp. 245–257.
5. Desforges, W., Sonne, C., Levin, M., et al., *Env. Int.,* 2016, vol. 86, pp. 126–139.
6. Bukina, L.A., in *IX Vsesoyuz. soveshch. po izucheniyu, okhrane i ratsional'nomu ispol'zovaniyu morskikh mlekopitayushchikh: Tez. dokl.* (Abstr. IX All-Union Conf. on the Study, Protection, and Rational Use of Sea Mammals), Arkhangel'sk, 1986, pp. 60–61.
7. Matisheva, S.K., and Shapunov, V.M., in *X Vsesoyuz. soveshch. po izucheniyu, okhrane i ratsional'nomu ispol'zovaniyu morskikh mlekopitayushchikh: Tez. dokl.* (Abstr. X All-Union Conf. on the Study, Protection, and Rational Use of Sea Mammals), Moscow, 1990, pp. 187–188.
8. Shvatskii, A.B., Radygin, G.Yu., Zhivkova, E.P., et al., in *X Vsesoyuz. soveshch. po izucheniyu, okhrane i ratsional'nomu ispol'zovaniyu morskikh mlekopitayushchikh: Tez. dokl.* (Abstr. X All-Union Conf. on the Study, Protection, and Rational Use of Sea Mammals), Moscow, 1990, pp. 328–329.
9. De Guise, S., Flipo, D., Boehm, J.R., et al., *Vet. Immunol. Immunopathol.,* 1995, vol. 47, nos. 3–4, pp. 351–362.
10. Frouin, H., Lebeuf, M., Hammill, M., et al., *Vet. Immunol. Immunopathol.,* 2010, vol. 134, nos. 3–4, pp. 160–168.
11. Zaichik, A.Sh, and Churilov, L.P., *Mekhanizmy razvitiya boleznei i sindromov* (Mechanisms of Diseases and Syndromes), St. Petersburg: ELBI-SPb, 2002.
12. Pigarevskii, V.E., *Zernistye leikotsity i ikh svoistva* (Granular Leucocytes and Their Properties), Moscow: Meditsina, 1978.
13. Butenko, Z.A., Gluzman, D.F., Zak, K.P., et al., *Tsitokhimiya i elektronnaya mikroskopiya kletok krovi i krovetvornykh organov* (Cytochemistry and Electron Microscopy of the Cells of Blood and Hematopoietic Organs), Kiev: Naukova Dumka, 1974.
14. Kavtsevich, N.N., Morphological and cytochemical characteristics of marine mammals as related to adaptation to the environment, *Doctoral (Biol.) Dissertation*, Murmansk, 2011.

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