

The innate immunity of wild Eurasian beaver from Poland – present knowledge and the need for research

ANDRZEJ POMIANOWSKI¹, ALEKSANDRA GIŹEJEWSKA², JOANNA MAŁACZEWSKA³,
PATRYCJA SCHULZ³, ANDRZEJ K. SIWICKI³

¹Department of Internal Medicine and Clinic, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland

²Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland

³Department of Microbiology and Clinical Immunology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland

Abstract

For the first time we tried to examine the basic parameters of innate immunity in beavers for developing the new methods of prevention against infectious diseases in different reintroduction and translocation programmes in Poland. The aim of the present study was to determine the selected innate immunity parameters in Eurasian beavers living in natural conditions. The analyses of the results showed that the phagocytic ability (RBA) and potential killing activity (PKA) of blood phagocytes were higher in adult beavers compared to the young animals. The similar pattern was observed in proliferative response of blood lymphocytes stimulated by mitogens Concanavalin A (Con A) and lipopolysaccharides (LPS). The highest proliferative response in adult Eurasian beavers was observed. The ceruloplasmin activity in plasma was on similar levels in adult and young Eurasian beavers. The results of humoral mediated immunity showed that the lysozyme activity, total protein and gamma-globulin levels in serum were higher in adult beavers compared to the young beavers. The results of this preliminary study are important in comparative and clinical immunology.

Key words: *Castor fiber, Eurasian beaver, immune system, natural environment.*

(Centr Eur J Immunology 2014; 39 (4): 485-487)

Introduction

The immune system is a highly evolved system that functions to provide organisms with the ability to resist pathogenic agents and is characterised by two pathways: innate and acquired. The innate immune system can be exposed to a variety of physical and chemical stressors. The interactions of xenobiotics with the immune system could finally induce immunosuppression with development of viral, bacterial or fungal diseases. The innate immune defence consists of a large number of humoral and cellular factors, which play an important role as the first line of defence against a wide range of infective agents. Important responses involve changes in the hepatic, neuroendocrine, haematopoietic and immune systems to re-establish homeostasis in the organism.

The Eurasian beaver is a semi-aquatic, herbivorous mammal. Beavers can occupy the same territory of Poland for numerous years. In Poland, the Eurasian beavers are subject to partial protection [1] and that is why in our country we have beaver overpopulation [2]. Because of such large

quantities it colonizes new places. It is also approaching human habitats where is exposed to a wider range of both chemical and stress factors. This species has been the subject of many reintroduction and translocation programmes with varying success [3, 4]. The beaver is a wildlife animal which can be a good indicator of environment pollution [5] and effect of this pollution on cells mediated and humoral mediated immunity. Actually it is a good mammalian model for analysing heavy metal accumulation in the wild life organisms [6]. Populations, which have undergone bottlenecks or re-introduction may exhibit the poliethiological stress and in consequence reduced disease resistance.

There is no information in the literature about cellular and humoral defence mechanisms and protection against diseases of Eurasian beavers. For the first time we tried to examine the basic parameters of innate immunity of this species for developing new methods of prevention against infectious diseases in different reintroduction and translocation programmes in Poland. The aim of the present study

Correspondence: Patrycja Schulz, Department of Microbiology and Clinical Immunology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland, e-mail: patrycja.schulz@uwm.edu.pl

was to determine the selected innate immunity parameters in Eurasian beavers inhabiting natural ecosystems.

Material and methods

Beavers were captured in the north-east territory of Poland (Wiżajny) in November 2012. The animals were collected upon the approval of the Regional Director of Environmental Protection in Olsztyn (decision no. RDOŚ-28-OOP-6631-0007-638/09/10/pj) and Resolution of the Local Ethics Committee for Animal Experiments No. 11/2010.

Beavers were captured by a specialized team from the Polish Hunting Association during daytime. The age of animals was assessed based on the work of Rossell et al. [7], which allowed to divide individuals into two age groups: juveniles (< 3 years old) and adults (> 3 years old). Basic veterinary examination as well as autopsy showed that all animals were healthy and in good physical condition at the time of capture.

The blood was collected from 8 free-ranging beavers, of which 4 were young (2 male and 2 female) and 4 adult (2 male and 2 female), from the caudal vein by the Vacutainer system to the two tubes: heparinized and non-heparinized tubes (Vacutainer set – Vacuette Greiner Labortechnik; 50 IU/ml of heparin).

The blood leucocytes were isolated by centrifugation at 2000 g for 30 min at 4°C on the Gradisol G gradient (Polfa) or lymphoprep (Sigma), washed three time in PBS and re-suspended in RPMI 1640 medium (Sigma) supplemented with 10% FCS (Foetal Calf Serum, Gibco-BRL) at a stock concentration of 2×10^5 cells/ml of medium. Viability of cells was checked by supravital staining with 0.1% w/v trypan blue (1 : 1 mixture of cell suspension and trypan blue solution). Two hundred cells were counted and only samples containing at least 90% of viable cells were used for experiments.

The metabolic activity of blood phagocytes was determined based on the measurement of intracellular respira-

tory burst (RBA) after stimulation by PMA (phorbol myristate acetate, Sigma), as described by Siwicki et al. [8].

The potential killing activity (PKA) of blood phagocytic cells was determined according to the method presented by Siwicki et al. [8].

The proliferative response of the blood lymphocytes (LP) stimulated by mitogen concanavalin A (ConA, Sigma) or lipopolysaccharide (LPS, Sigma) were determined by MTT assay previously described by Wagner et al. [9].

The lysozyme activity in plasma was measured by a turbidimetric assay presented by Siwicki and Anderson [10]. The assay is based upon the lysis of the lysozyme-sensitive Gram-positive bacterium *Micrococcus lysodeikticus* (Sigma) which is obtained freeze-dried from major chemical suppliers. The standard was hen egg white lysozyme (Sigma).

The ceruloplasmin activity in the plasma was determined spectrophotometrically with modifications for micro-methods [11].

Analysis of total protein and γ -globulin levels in serum was based on the Lowry micro method (Sigma, Diagnostic Kits). The total γ -globulin level was measured using Lowry micro method presented by Siwicki and Anderson [10]. This method requires first precipitating the γ -globulin out of the serum with polyethylene glycol (10 000 kDa).

Data were analysed statistically by one-way analysis of variance (ANOVA). Bonferroni's post test was used to determine differences between groups. Statistical evaluation of results was performed using GraphPad-Prism software package. The table illustrates the means, standard deviation and levels of significance $p < 0.05$.

Results

The innate cellular and humoral defence mechanisms in young and adult Eurasian beavers are presented in Table 1. The analyses of the results showed that phagocytic ability (RBA) and potential killing activity (PKA) of blood

Table 1. The innate cellular and humoral immunity in healthy Eurasian beavers from natural conditions (mean \pm SD)

Immunological parameters	Young Eurasian beavers (n = 4)	Adult Eurasian beaver (n = 4)
metabolic activity of blood phagocytes RBA (OD 620 nm)	0.37 \pm 0.01	0.47 \pm 0.02*
potential killing activity of blood phagocytes PKA (OD 620 nm)	0.30 \pm 0.01	0.37 \pm 0.02*
blood lymphocyte proliferation stimulated by ConA (OD 620 nm)	0.43 \pm 0.03	0.58 \pm 0.04*
blood lymphocyte proliferation stimulated by LPS (OD 620 nm)	0.34 \pm 0.02	0.41 \pm 0.02*
lysozyme activity in plasma (mg/l)	6.50 \pm 0.44	14.06 \pm 1.55*
ceruloplasmin activity in plasma (IU)	45.27 \pm 2.63	46.52 \pm 1.29
total protein level in serum (g/l)	52.60 \pm 0.95	68.46 \pm 4.68*
total γ -globulin level in serum (g/l)	4.87 \pm 0.35	13.36 \pm 0.69*

*statistically significant $p < 0.05$

phagocytes were statistically significantly higher ($p < 0.05$) in adult animals compared to the young ones. A similar pattern was observed in proliferative response of blood lymphocytes stimulated by mitogens ConA or LPS. The results showed that the proliferative response of lymphocytes was statistically significantly higher ($p < 0.05$) in adult beavers. The results showed that in adult Eurasian beavers higher cell mediated immunity was observed and suggested that they has a solid and longer time contact with different pathogens and intensively activate the innate cellular immune response, important line of defence mechanisms and protection against diseases.

The results of the studies of the humoral innate immunity indicate that the lysozyme activity in plasma and gamma-globulin levels in serum were statistically significantly higher ($p < 0.05$) in adult beavers compared to the young animals. Also statistically significantly higher ($p < 0.05$) levels of total protein in adult beavers were observed. But the ceruloplasmin activity in plasma was on the similar levels in adult and young Eurasian beavers.

Discussion

The objective of the present study was to determine the values of the innate cellular and humoral defence mechanisms in Eurasian beavers, which are bred in natural conditions. This basic examination provides very important information about physiological levels of nonspecific humoral and cellular protection against pathogens in different environmental conditions. In this study we present for the first time innate defence mechanisms in adult and young Eurasian beavers living in natural conditions. The basic information regarding innate cellular and humoral defence mechanisms in healthy animals gives the possibility to monitor health of beavers by immunological parameters. These studies are very important to develop effective methods of prevention of infectious diseases and to reduce mortality in different reintroduction and translocation programmes in Poland.

The authors declare no conflict of interest.

References

1. Regulation of Polish Minister of the Environment of 12 October 2011 on animal species under protection (Law Gazette No 237, item 1419).
2. Central Statistical Office. Environment 2012, Statistical information and elaborations. Warsaw; 311.
3. Halley DJ, Rosell F (2002): The beaver's reconquest of Eurasia: status, population development and management of a conservation success. *Mammal Rev* 32: 153-178.
4. Campbell RD, Rosell F, Nolet BA, Dijkstra VA (2005): Territory and group size in Eurasian beaver (*Castor fiber*): echoes of settlement and reproduction. *Behav Ecol Sociobiol* 58: 597-607.
5. Zalewski K, Falandysz J, Jadacka M, et al. (2012): Concentrations of heavy metals and PCBs in the tissues of European beavers (*Castor fiber*) captured in northeastern Poland. *Eur J Wildl Res* 58: 6550660.
6. Giżewska A, Spodniewska A, Barski D (2014): Concentration of lead, cadmium, and mercury in tissues of European beaver (*Castor fiber*) from the north-eastern Poland. *Bull Vet Inst Pulawy* 58: 77-80.
7. Rosell F, Zedrosser A, Parker H (2010): Correlates of body measurements and age in Eurasian beaver from Norway. *Eur J Wildl Res* 56: 43-48.
8. Siwicki AK, Skopińska-Różewska E, Hartwich M, et al. (2007): The influence of *Rhodiola rosea* extracts on non-specific and specific cellular immunity in pigs, rats and mice. *Centr Eur J Immunol* 32: 84-91.
9. Wagner U, Burkhardt E, Failing K (1999): Evaluation of canine lymphocyte proliferation: comparison of three different colorimetric methods with the H-thymidine incorporation assay. *Vet Immunol Immunopathol* 70: 151-159.
10. Siwicki AK, Anderson DP (1993): Nonspecific defence mechanisms assay in fish. *Fish Disease Diagnosis and Prevention's Methods*. FAO-Project GCP/INT/526/JPN, IFI Olsztyn: 105-109.
11. Rice EW, Wagman E, Takenaka Y (1986): Ceruloplasmin assay in serum: standarization of ceruloplasmin activity in terms of international enzyme units. *Diagnostic Laboratory* 12: 39-53.