Short communication

Immunosuppressive influence of Anguillid herpesvirus-1 (AngHV-1) infection on cellular defense mechanisms in European eel (*Anguilla anguilla*)

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Abstract

The European eel (*Anguilla anguilla*) is a catadromous fish with a complicated life cycle. The long-term impact of anthropopressure, environmental pollution and diseases have led to a risk of extinction. The aim of the present study was to determine the influence of Anguillid herpesvirus-1 infection on the innate immunity of European eel from natural conditions. Spleen phagocyte respiratory burst activity and potential killing activity, as well as pronephros lymphocyte proliferation stimulated by concanavalin A or lipopolysaccharide were measured. The analyses of the results showed that all studied parameters were significantly higher ($P<0.05$) in AngHV-1-negative fish compared to the ones where the presence of viral DNA was confirmed.

Key words: alloherpesviruses, cellular immunity, immunosuppression, innate immunity

Introduction

Eel is facultatively catadromous fish. The number of European eels in the environment decreased significantly worldwide during the last several decades. In result, this species is considered critically endangered. Many factors are mentioned as an explanation of this phenomenon. Swimbladder nematode, some pathogenic bacteria as well as viruses (van Beurden et al. 2012) have been suggested as the most important reasons for the declining population of the wild European eel.

Anguillid herpesvirus-1 (AngHV-1) is one of the biggest viral threats to eel along with the eel virus European (EVE) and the eel virus European X (EVEX) (van Beurden et al. 2012). The virus is present in most eel farms nowadays (EFSA 2008). It is able to persist and establish latency in clinically healthy fish

Table 1. The effect of AngHV-1 on the cell-mediated innate immunity in European eel (*Anguilla anguilla*) (mean ± SD).

Immunological parameters	Group of fish	
	AngHV-1 negative	AngHV-1 positive
Metabolic activity of spleen macrophages (OD 620 nm)	0.48 ± 0.05	0.21 ± 0.04 *
Potential killing activity of spleen macrophages (OD 620 nm)	0.35 ± 0.04	0.13 ± 0.03 *
Proliferative response of pronephros lymphocytes stimulated by ConA (OD 620 nm)	0.52 ± 0.05	0.21 ± 0.04 *
Proliferative response of pronephros lymphocytes stimulated by LPS (OD 620 nm)	0.40 ± 0.04	0.28 ± 0.04 *

* statistically significant $p < 0.05$

(van Nieuwstadt et al. 2001). No vaccines or antivirals against AngHV-1 are commercially available. The aim of this study was to evaluate the effect of AngHV-1 on the cell-mediated innate immunity in European eel (*Anguilla anguilla*). So far, not much research has been done on AngHV-1, and according to the authors' knowledge, none of them is related to the virus's influence on the immune system of the European eel.

Materials and Methods

In our study 50 European eel individuals at 50-200 g body weight were examined. The fish were caught in the basin of the Vistula and Odra rivers.

Material for the test were kidney, gill, spleen, and liver. Organs were collected aseptically from fish to detect AngHV-1. The DNA was isolated using the Qiagen DNA Mini Kit according to the manufacturer's instructions. PCR was performed with the total volume of the reaction of 25 μl, containing 1xHotStarTaq Plus Master Mix (Qiagen), 1 μL eel tissue DNA, 0.4 μM of each primers AngHV_F 5'GTGTCGGGCCTTTGTGGTGA3' and AngHV_R 'CATGCCGGGAGTCTTTTTGAT3'. The amplification was done using the following program: 5 min at 95°C; 40 cycles of 30 s at 94°C; 45 s at 65°C and 60 s at 72°C; followed by 7 min at 72°C. The samples were analyzed electrophoretically in agarose gel. A positive result was found for band of 394 bp.

Among the studied fish 20 AngHV-positive and 20 AngHV-negative were selected for further study. The spleen and pronephros of each fish were pressed through a 60 μm nylon mesh. Single cell suspensions were obtained using Gradisol L (Aqua-Medica, Łódź, Poland) gradients, as described by Siwicki and Cossarini-Dunier (1990). Cells were suspended in RPMI-1640 medium containing 10% fetal calf serum (FCS, Sigma-Aldrich) and 1% antibiotic-antimycotic solution (Sigma-Aldrich), then dispensed into 96-well plates and cultured/incubated at 24°C and used for the following assays.

The respiratory burst activity (RBA) and potential killing activity of splenic, as well as the pronephros lymphocyte proliferation were measured using a method described by Terech-Majewska et al. (2016).

Data are reported as means ± SD. Student's t-test was used to determine the significant difference in immunological parameters between the groups. All calculations were determined to be significant at $p < 0.05$.

Results and Discussion

The European eel is threatened with extinction, therefore the state of its non-specific immunity and susceptibility to diseases is an important factor affecting the reproductive capacity of the species. Herpesviruses are very pathogenic for lower vertebrates. A number of mass mortalities in fish and oysters caused by herpesvirus infections has occurred in recent decades. The main feature of herpesviruses is their latency capability in the infected host (van Nieuwstadt et al. 2001). An additional risk is the possibility of transmission of the virus by other fish species to eel (Nguyen et al. 2016). AngHV-1 is considered by some authors to be the most dangerous for European eel due to its high pathogenicity (Davidse et al. 1999). The potential risk of AngHV-1 in open waters of Poland is indicated by the results of the Kempter et al. (2014), which confirmed the presence of AngHV-1 genetic material in 50% of studied fish from Lake Dąbie and 28.6% from the Szczecin Lagoon.

According to the information provided by the International Committee on Taxonomy of Viruses AngHV-1 belongs to the *Alloherpesviridae* family as a fourth known member of cypriniviruses together with Cyprinid herpesvirus-1 (CyHV-1), CyHV-2 and CyHV-3. They represent an important group of pathogens affecting fish. Much less work has been done on AgHV-1 compared with the other three cypriniviruses. According to the authors' knowledge, no studies have been conducted on the effects of AngHV-1 on the immune system of the European eel. Because all these viruses belong to one group, a similar effect on the host's immune system can be expected. A unifying theme among the herpesviruses is the intimate interrelation-

ship of virus infection with host cellular immunocompetence. CyHV-3 is the best-studied virus belonging to this family. It is the etiological agent of koi herpesvirus disease (KHVD) which causes significant morbidity and mortality in koi and common carp.

Comparisons of the innate cellular defense mechanism in European eel (Table 1) showed that AngHV-1 induced suppression of the phagocytic ability and potential killing activity of splenic phagocytes, compared to the eel AngHV-1-negative. Also, the AngHV-1 decreased the proliferation of lymphocytes T and lymphocytes B isolated from pronephros of AngHV-1-positive eel, compared to AngHV-1-negative eel. Earlier research by Siwicki et al. (2012) showed that CyHV-3 significantly decreases the spleen phagocyte and pronephros lymphocyte activities in common carp compared with the control. These results harmonize with our results on AngHV-1, which may be related to the close relationship of these viruses. According to the authors' knowledge, this is the first data regarding AngHV-1 influence on cell-mediated immunity in European eel.

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