

Pathogenesis of herpesviruses in lower vertebrates: influence of ictalurid herpesvirus 1 (IcHV-1) on macrophage and lymphocyte activities in fish

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Abstract

Interest of lower vertebrates viruses has recently increased for a variety of reasons despite the fact that none of them infect warm-blooded animals and man. Several DNA viruses infecting fish have been studied for analysis the pathogenesis and for developing the effective methods of prevention and therapy. In this paper, we present the *in vitro* influence of ictalurid herpesvirus-1 (IcHV-1) on the phagocytic activity of splenic macrophages and proliferative response of pronephric lymphocytes stimulated by mitogens ConA and LPS isolated from wels catfish (*Silurus glanis*) and tench (*Tinca tinca*). The results showed that IcHV-1 induced suppressive influence on the phagocytic ability of splenic macrophages in wels catfish, compared to the tench. Also the results showed that IcHV-1 decreased the proliferative response of pronephros lymphocytes stimulated by mitogens ConA in wels catfish, compared to the tench where the immunostimulating effect was observed. The analyses of this study suggested that IcHV-1 inhibited of cell-mediated immunity only in wels catfish.

Key words: Fish, IcHV-1, macrophage activity, lymphocyte proliferation.

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Introduction

Fish, amphibians and reptiles diverged from mammals about 400 million years ago and the viruses most probably co-evolved with their hosts. Interest of lower vertebrates viruses has recently increased for a variety of reasons despite the fact that none of them infect warm-blooded animals and man. Some of lower vertebrates viruses have been used as models for studying disease mechanisms. The family *Herpesviridae* contains the three subfamilies: *Alphaherpesvirinae*, *Betaherpesvirinae*, *Gammaherpesvirinae* and the unassigned genus "Ictalurid herpes-like viruses" [1, 2]. The *Herpesviridae* are the most extensively studied fish viruses. First of all because they are ubiquitous in aquatic organisms, since they have been isolated all over the world from both marine and freshwater fishes of different species,

and is responsible for severe losses in aquaculture. Several viruses isolated from different species of fish have been classified as herpesviruses [2-5]. The icosahedral capsid is made of 162 capsomeres that are embedded in a protein matrix and are surrounded by an envelope, which includes virus-encoded glycoproteins. The capsid contains a single linear double-stranded DNA molecule, which is replicated in the nuclei of the infected cells. In fish 25 types of herpes-like viruses have been detected but only few are well characterised. Ictalurid herpesvirus-1 (IcHV-1) and salmonid herpesvirus 1 (SaHV-1) are the most pathogenic viruses for fish and induce significant diseases. Experimental infection of catfish fingerlings with IcHV-1 induces haemorrhagic, oedematous and anaemic disease and strong mortality [2, 6].

The aim of the present study was to determine the *in vitro* influence of ictalurid herpesvirus-1 (IcHV-1) on the

phagocytic ability of splenic macrophages and proliferative response of pronephric lymphocytes stimulated by mitogen in wels catfish (*Silurus glanis*) and tench (*Tinca tinca*).

Materials and methods

Animals

Two healthy freshwater species of fish were used for this experimental study: european wels catfish (*Silurus glanis*) and tench (*Tinca tinca*), purchased from the Experimental Station of Inland Fisheries Institute in Żabieniec. Before dissection, the fish were anaesthetized in Propiscin (IFI, Poland) and bled from the caudal vein to reduce the blood volume in the spleen and pronephros. The spleen and pronephros (head kidney) were isolated from 10 healthy wels catfish and tench with mean weight about 150 g. The leucocytes were separated and cell culture prepared. Single cell suspensions were obtained by placing the spleen and pronephros in medium RPMI-1640 and teasing it through a steel mesh. They were isolated on Histopaque-1077 (Sigma) or Gradisol (Polfa) gradients.

The *Epithelioma papulosum cyprini* (EPC) cell line in minimum essential medium (BHK21 Medium, Glasgow MEM, Life Technologies) with 10% tryptose phosphate broth (DIFCO) and 10% foetal calf serum (FCS, Life technologies) was used to grow the virus and for the assays [7].

A modification of the Secombes [8] method was used to study the respiratory burst activity of splenic macrophages stimulated by phorbol myristate acetate (PMA, Sigma). Cells suspension (1×10^6 /ml of medium) were incubated with IcHV-1 (1×10^5 p.f.u./ml of medium)

for 2 h at 22°C. The plates were read on a micro-reader at 620 nm.

The proliferative response of the pronephric lymphocytes was determined by the MTT colorimetric assay method according to Carmichael *et al.* [9] as modified for fish by Siwicki *et al.* [10]. Concanavalin A (ConA, Sigma) at a concentration of 64 µg/ml was used for stimulation of lymphocyte proliferation. The 100 µl of lymphocytes suspension (5×10^6 /ml of medium) were incubated with IcHV-1, with mitogen + IcHV-1 and only with mitogen. The virus was added at doses of 1×10^5 p.f.u./ml of medium (20 µl per well). The plates were read on a microreader (OD 620 nm). In all experimental protocol the control group was non-stimulated cells by mitogen or virus. The level of optical density (OD) at 620 nm in control group was less near 0.1 nm.

The results were statistically analyzed with STATISTICA PL (StatSoft, Cracow, Poland). Single-factor analysis of variance (ANOVA) and the Tukey test (when statistically significant differences were determined among groups $p \leq 0.05$) were applied.

Results and discussion

Herpesviruses are very pathogenic for lower vertebrates. Systemic infections of fish farming caused by herpesviruses have recently been recognized in Europe, Asia, USA and Australia. A unifying theme among the herpesviruses is the intimate interrelationship of virus infection with host cellular immunocompetence. Actually, we have a few informations about effect of herpesvirus on the cell-

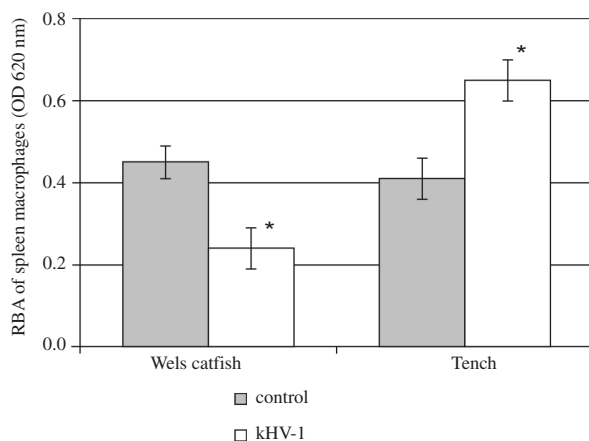


Fig. 1. The influence of Ictalurid herpesvirus 1 (IcHV-1) on the respiratory burst activity (RBA) of spleen macrophages in wels catfish and tench (OD 620 nm, $n = 10$; mean \pm SD; * statistically significant $p \leq 0.05$)

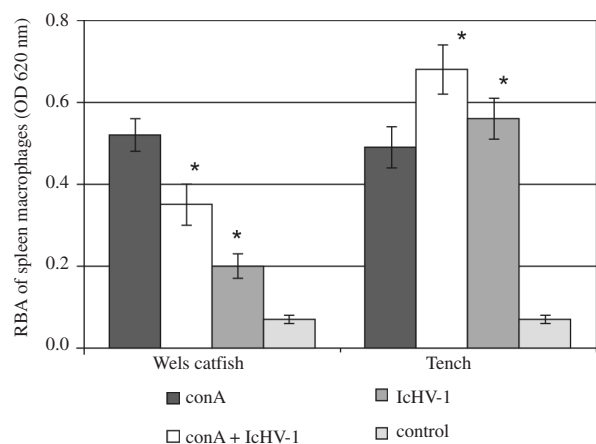


Fig. 2. The influence of Ictalurid herpesvirus 1 (IcHV-1) on the proliferative responses (LP) of pronephric lymphocytes stimulated by mitogen ConA in wels catfish and tench (OD 620 nm; $n = 10$; mean \pm SD; * statistically significant $p \leq 0.05$)

mediated immunity in fish. In the present study the influence of the Ictalurid herpesvirus 1 (IcHV-1) on spleen macrophage metabolism was assessed by examining their respiratory burst activity. The effects of IcHV-1 on the respiratory burst activity in wels catfish and tench are presented on Figure 1. The results showed that Ictalurid herpesvirus-1 significantly ($p < 0.05$) decreased the macrophage activity level in wels catfish, compared to the control and tench. Compared to the wels catfish, in tench the stimulating influence of IcHV-1 on the spleen macrophage respiratory burst activity was observed.

The proliferative response of pronephric lymphocytes showed a similar pattern. The influence of IcHV-1 on the proliferative response of pronephric lymphocytes stimulated by mitogen ConA is presented on Figure 2. In wels catfish the IcHV-1 statistically significant ($p < 0.05$) decreased the proliferative response of lymphocytes, compared to the tench and control. In tench the stimulating influence of IcHV-1 on lymphocyte proliferation induced by ConA was observed.

The IcHV-1 virus was tested *in vitro* on cell-mediated immunity in two species of fish: tench and european wels catfish. In our study, a strong suppressive influence of the IcHV-1 on macrophage respiratory burst activity and lymphocyte proliferation was observed in wels catfish, compared to the tench where IcHV-1 increased the macrophage and lymphocyte activities. The effect of DNA viruses on the wels catfish and carp leucocytes has been previously reported by Siwicki *et al.* [10, 11]. The authors clearly demonstrated a suppressive effect of iridovirus on the phagocyte and lymphocyte activity and hypothesised that this suppression led to a reduction in the number of cells or to an impairment of phagocytic function. This preliminary *in vitro* study also demonstrated a strong inhibitory influence of the IcHV-1 on cell-mediated immunity, but on selected species. In tench the immunostimulatory influence was observed, compared to

the wels catfish where the strong suppression was observed. These results suggested that the IcHV-1 suppressed the intracellular metabolism of the european wels catfish leucocytes, and has immunomodulatory effect on the cell-mediated immunity dependent to the species of fish.

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