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Characterization of structural proteins of koi herpesvirus

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Since the late 1990s a virus disease leading to mass mortality of common carp and koi has spread over major parts of the world. The causative agent was designated koi herpesvirus (KHV) or *Cyprinid herpesvirus 3*, and classified as a member of the family *Alloherpesviridae* within the order *Herpesvirales*. Up to now adequate, safe and efficacious vaccines are not available, and diagnostics are still almost limited to PCR detection of viral DNA in tissues of infected fish. Therefore we started to investigate predicted immunogenic virion proteins of KHV, and prepared monospecific rabbit antisera against ten of them. However, only the type III membrane protein pORF81, the type I membrane proteins pORF25 and pORF149, and the major capsid protein pORF92 were sufficiently abundant and immunogenic to permit unambiguous detection in western blot analyses of KHV-infected cells. Vice versa, in indirect immunofluorescence tests (IIFT) sera from KHV-infected carp and koi reacted with cells transfected with eukaryotic expression plasmids for pORF25, pORF65, pORF148, and pORF149 which represent a family of related KHV membrane proteins. Moreover, several monoclonal antibodies raised against KHV virions proved to be specific for pORF149 in IIFT of transfected cells, and in immunoelectron microscopic analyses of KHV particles. Since pORF149 obviously represents an immunorelevant envelope protein of KHV, recombinant baculoviruses permitting its overexpression in transduced vertebrate cells, as well as in infected insect cells were generated. Remarkably, pORF149 was also incorporated into pseudotyped baculovirus particles. The suitability of these tools for serological diagnostics, and for vaccination of carp will be further investigated.

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