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Antibodies specific for channel catfish virus cross-react with Pacific oyster, *Crassostrea gigas*, herpes-like virus

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Herpes-like viruses were first described in the larval Pacific oyster, *Crassostrea gigas*, during the summer of 1991 in France (Nicolas *et al*, 1992) and New Zealand (Hine *et al*, 1992). Since these first reports, sporadic incidence with high mortality rates (90–100%) have occurred among *C gigas* larvae in several French hatcheries in 1992, 1993 and 1994 (Renault *et al*, 1994a). Moreover, a similar virus was found in association with sporadic high mortality rates (80–90%) of *C gigas* spat in France in 1993 (Renault *et al*, 1994b) and 1994 (Renault, personal communication). In addition, the pathogenicity of the herpes-like virus was demonstrated by inoculating virus suspensions into axenic cultures of *C gigas* larvae (Le Deuff *et al*, 1994).

As a result of these reports and the economic importance of *C gigas* to shellfish culture, it seems important to characterize this virus and to develop sensitive diagnostic methods in order to study the epidemiology of the disease and prevent its potential spread. Due to the lack of marine mollusc cell lines, the study of the viral cytopathogen effects in homologous cell culture is impos-

sible. Thus, in an effort to overcome the limitations of existing methodologies, the direct detection of the oyster herpesvirus was attempted using monoclonal and polyclonal antibodies specific for a fish herpes-like virus, the channel catfish virus (CCV). Such antibodies were expected to provide the basis for the development of sensitive diagnostic immunoassays for direct detection of herpesvirus in oyster tissues.

Using the immunogold method, the monoclonal antibody specific for a CCV envelope glycoprotein was found to specifically label the enveloped virus particles (fig 1a) when diluted 1/10, but this labelling disappeared when the monoclonal antibody was diluted 1/100. The same results were obtained for oyster herpes-like virus infected spat and larvae from different origins and for CCV-infected channel catfish ovary (CCO) cells. One or more colloidal gold particles was found in association with the viral particle envelope both extracellularly (fig 1b) and in the cytoplasm (fig 1c). No labelling of unenveloped nucleocapsids or empty particles was observed (fig 1d).

* Correspondence and reprints

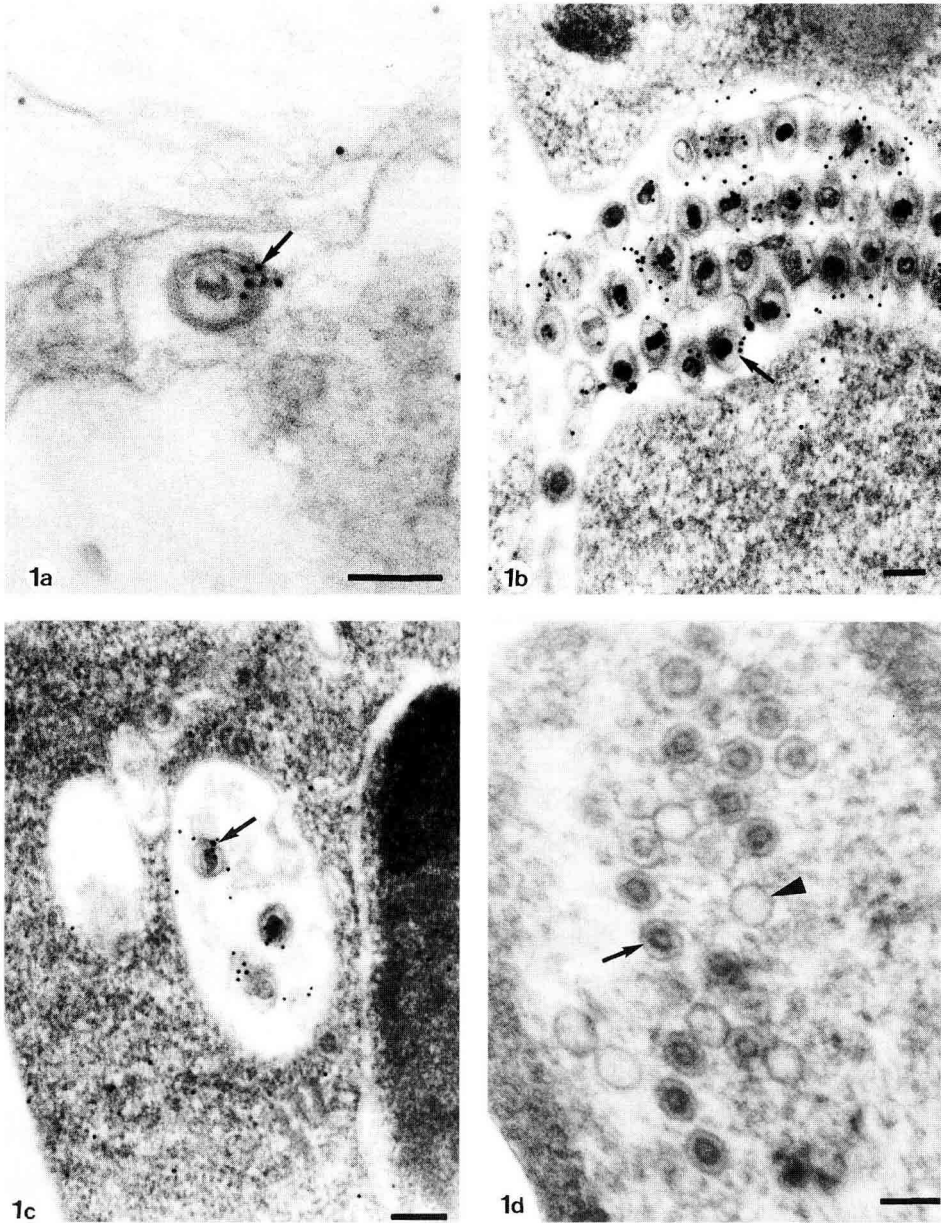


Fig 1. Transmission electron micrographs of colloidal gold labelling using a monoclonal antibody specific for a CCV envelope glycoprotein. **1a**) Colloidal gold particle binding (arrow) corresponded to the viral envelope of the herpes-like virus infecting Pacific oyster. **1b**) Extracellular, enveloped, labelled virus particles (arrow). **1c**) Cytoplasmic, enveloped, labelled virus particles (arrow). **1d**) Unlabelled, unenveloped nucleocapsids (arrow) and empty particles (arrowhead). Bar = 100 nm.

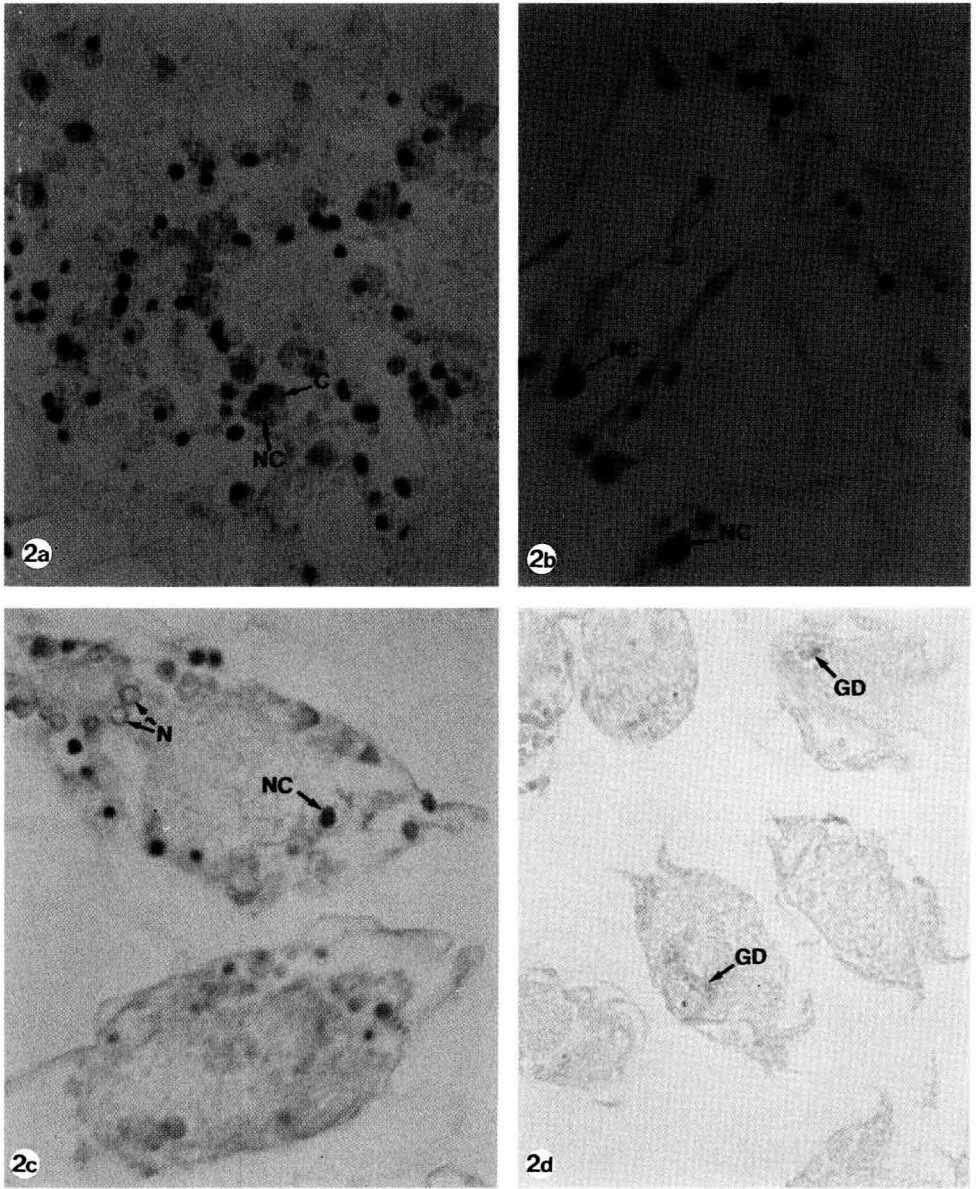


Fig 2. Immunohistochemistry on Pacific oyster spat (**2a** and **2b**) and larvae (**2c**) infected by a herpes-like virus and on healthy Pacific oyster larvae (**2d**). Nuclear (N) and cytoplasmic (C) or both (NC) labelling could be observed in infected cells. The background was observed as a diffuse labelling on the digestive gland (DG) of the healthy larvae.

The binding of the monoclonal antibody could not be observed using immunohistochemical methods when the ascitic fluids were diluted 1/50 or 1/10. As no 'positive' control was performed on the CCV-infected CCO cells, it was not possible to determine whether the antigens were too few in number to produce a visible labelling or if the fixatives used denatured the target antigens.

Using the immunogold method, the polyclonal antibody diluted 1/10 produced a high level of background labelling on the CCV-infected CCO cells and the herpes-like virus infected oyster spat and larvae. When the polyclonal antibody was diluted 1/100, little residual labelling could be observed on the capsids. These results could be improved by using better blocking of unspecific binding sites and also by testing serial dilutions of the polyclonal antibody between 1/10 and 1/100.

However, when immunohistochemical methods were used, the polyclonal antibody-diluted 1/200 specifically labelled cells in infected oyster spat, localized in the conjunctive tissues of the mantle, the digestive gland and the gills. Depending on cells, the labelling was localized on the nucleus, the cytoplasm or both (figs 2a,b). This was similar to that of the transmission electron microscope observations (Nicolas *et al*, 1992; Renault *et al*, 1994a) of virus particles localized in the cell nucleus and/or in the cytoplasm, depending on the stage of the viral replication cycle. Moreover, a few cells were labelled even in animals that had numerous lesions in histological examination. This is in agreement with the transmission electron microscope observations (Renault *et al*, 1994b) which revealed that few cells in the tissues of infected spat contained particles.

Immunohistochemistry on infected oyster larvae was difficult to interpret since it

was not possible to completely eliminate the background effect due to unspecific tissue labelling. However, the labelling observed on healthy and herpesvirus-infected larvae was different. The background in healthy larvae was diffuse and mostly localized on the digestive gland (fig 2d). The labelling in the virus-infected oyster larvae was observed on specific cells in the mantle tissue (fig 2c).

The results described here failed to provide a useful diagnostic tool for oyster herpesvirus infection of larvae and spat. However, this work could help to the taxonomic classification of this new agent and might further elucidate the relationships between viruses and their hosts (Hayashi *et al*, 1993).

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