Immunohistochemistry and Morbillivirus Infections in Sea Mammals

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INTRODUCTION

Starting from 1987, at least eight morbillivirus infection (MI) epidemics have caused mass mortality in several free-living pinnipeds and cetacean populations around the world. The responsible agents all belong to the genus Morbillivirus (Family Paramyxoviridae) and have been characterized as either canine distemper virus (CDV) strains, infecting pinnipeds, or as three new morbilliviruses, namely phocid (phocine) distemper virus (PDV) of pinnipeds, morporkise morbillivirus (PMV) and dolphin morbillivirus (DMV). The latter two viruses are currently gathered under the common denomination of cetacean morbilliviruses (CMV) [1-3].

MI diagnosis in sea mammals may be achieved by means of several laboratory techniques, including immunohistochemistry [4-9]. This article specifically deals with the use of immunohistochemistry, describing in detail and showing some laboratory results obtained using an immunoperoxidase technique on tissue samples from affected animals [5].

MATERIALS AND METHODS

The protocol, representing a slight modification of a previously published methodology [5], is a peroxidase-linked streptavidin-biotin (LSAB) technique that we commonly use in order to demonstrate morbillivirus antigen in tissue specimens from affected sea mammals. This protocol includes the following reagents: a monoclonal antibody (mab) to either the haemagglutinin (H) or the phosphoprotein (P) antigens of PDV is utilized as primary antibody. Alternatively, mabs to other closely related agents, canine distemper virus, may be successfully used for both diagnostic and research purposes. This article reports the immunohistochemical technique and its results.

KEYWORDS

sea mammals, pinnipeds, cetaceans, morbillivirus infection, pathology, immunohistochemistry, monoclonal antibodies

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RESULTS AND DISCUSSION

The main histopathological findings in affected animals consisted of a non-suppurative broncho-interstitial pneumonia, characterized by type II pneumocyte hyperplasia and intranuclear, intrabronchiolar and endoalveolar Warthin-Finkeldey type syncytia (Fig 1), along with a multifocal, non-suppurative encephalitis, associated with a severe and generalized lymphoid tissue depletion. Furthermore, eosinophilic viral inclusions were often detected, at both intracytoplasmic and intranuclear level, within bronchial and bronchiole epithelial, pulmonary syncytial, neuronal (Fig 2) and other cell types [2-9].

Immunohistochemically, morbillivirus antigen could be easily demonstrated by means of the immunoperoxidase technique described above. In more detail, intense immunolabelling could be detected in lung (Fig 3), brain (Fig 4), lymph nodes and a number of other organs and tissue districts [2-9].

Specific immunostaining was frequently characterized by a diffuse brown granular cytoplasmic reaction, which was superimposed upon a more intense labelling of both cytoplasmic and nuclear viral inclusion bodies (Figs 3 and 4). A relevant number of such inclusions could not be easily detected in haematoxylin and eosin (H&E)-stained sections [5].

As far as individual organ and tissue immunoreactivity is concerned, the lung commonly showed a widespread antigen staining within the cytoplasm and the nucleus of bronchial, bronchiolar and alveolar epithelial cells, as well as of endobronchial, endobronchiolar and endoalveolar macrophages, lymphoid cells and syncytia (Fig 3). At the brain level, specific cytoplasmic and nuclear immunolabelling was frequently observed...
within single or large groups of neurons scattered throughout the cerebral cortex (Fig 4), with positive immunostaining being mainly characterized by a laminar pattern. Neuronal cell nuclei, perikarya, axons and dendrites all reacted positive, along with cytoplasmic and nuclear viral inclusion bodies (Fig 4). Specific immunolabelling was frequently detected also in perivascular and meningeal mononuclear cells. Similar to the lung and the brain parenchyma, lymph nodes and spleen commonly harboured large amounts of morbillivirus antigen, which could be immunocytochemically demonstrated mainly in macrophages and lymphoid cells [2-9].

Immunohistochemistry is not the only option for the laboratory diagnosis of MI in sea mammals. To this aim, in fact, virus isolation on suitable cell cultures, along with serology, electron microscopy and reverse-transcriptase polymerase chain reaction (RT-PCR) techniques, may also be successfully employed [1,2]. As far as RT-PCR is specifically concerned, such methodology has the advantage of a possible use even on tissue samples affected by severe autolysis [10], a commonly occurring situation in stranded cetaceans. Immunohistochemistry warrants, similarly to RT-PCR, a great diagnostic specificity and reliability, though the former technique appears to be more adequate than RT-PCR in addressing pathogenetic studies, a need which is markedly reinforced by the almost absolute lack of scientific information concerning the pathogenesis of MI in aquatic mammals [2].

**CONCLUSIONS**

Although much high quality scientific work has been carried out during the last 15 years in the context of morbillivirus infections in aquatic mammals, there still remain a number of relevant issues requiring further research activity. Among these, hitherto unsolved questions concern the origin and the evolutionary phylogeny of such viruses, as well as their host range (including also terrestrial mammals), pathogenicity, ecology and epidemiology. It should be also underlined that, apart from the lack of detailed scientific information regarding the pathogenesis of morbillivirus infections in sea mammals, such infections may represent useful comparative pathology models in the study of similar disease conditions in man and terrestrial mammal species. In this respect, immunohistochemistry should be regarded as a highly efficient and reliable laboratory technique able to provide a number of relevant answers to the many hitherto unsolved pathogenetic questions in this investigation field.

**REFERENCES**