AVIAN ONCOGENIC VIRUSES RECENT ADVANCES ON DIAGNOSIS, PREVENTION AND CONTROL

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Cancer remains one of the major challenges facing modern Human Medicine. It is also a major challenge in Veterinary Medicine, particularly in poultry where it is a major risk to the 55 000 million chickens produced commercially every year. Unlike in humans, where more than 80% of the cancers are non-infectious, the vast majority of avian neoplastic diseases are caused by viruses. Two major groups of avian oncogenic viruses that threaten poultry health are the *retroviruses* and *herpesviruses*. In addition to their role in inducing diseases in chickens, avian oncogenic viruses have been instrumental in laying the foundations for much of the basic understanding on the molecular mechanisms of cancer. Thus the oncologist's debts to avian tumour viruses are enormous. For example, a number of major discoveries including those by Peyton Rous (Rous, 1911), Howard Temin (Temin, 1976), David Baltimore (Baltimore, 2006), Michael Bishop (Bishop, 1990) and Harold Varmus (Varmus, 1990), all of whom were subsequently awarded Nobel prizes, have come from studies avian viruses.

RETROVIRUS DISEASES

Avian retroviruses are lipid-enveloped particles belonging to avian leukosis/sarcoma virus (ALV) and Reticuloendotheliosis virus (REV) groups. Each particle contains a homodimer of linear, positive-sense, single-stranded 7-11 kb genomes that encode the viral genes *gag* (expressing the viral capsid, matrix and other nucleocapsid proteins), *pol* (expressing the protease, reverse transcriptase and integrase) and *env* (that express the bipartite membrane-anchored envelope glycoprotein) as well as a number of *cis*-acting regulatory sequences. These viruses produce tumours affecting multiple cell types resulting in multiple tumours including lymphoid/myeloid/ erythroid leukosis or multiple sarcomas. Historically, avian retroviruses have played major roles in contributing to the fundamental understanding of several molecular mechanisms of cancer. One of the major triumphs that have come from the investigations into the molecular mechanisms of retroviral oncogenicity is the discovery of oncogenes. Since the first discovery of the *src* oncogene in Rous sarcoma virus in mid 1980s, hundreds of oncogenes, many of which were directly involved in the induction of tumours, have been identified (Moore and Chang, 2010). As simple viruses with only a limited set of viral genes, ALV relies on modulating the host gene expression to bring about neoplastic transformation. Although this can be achieved by the downregulation of tumour suppressor genes such as retinoblastoma or p53, in most cases this is achieved by the activation of cellular oncogenes that occur either by retroviral insertional activation or by transduction of viral oncogenes. More recently, insertional activation of small non-coding RNAs such as microRNAs have been demonstrated in neoplastic transformation by avian retroviruses (Nair, 2008; Thompson et al., 2011).

Avian retroviruses are almost ubiquitous in commercial chickens worldwide, although many primary egg-type and meat-type breeding companies have instituted ALV eradication schemes to eliminate these pathogens from their flocks. Apart from losses from tumours, the presence of exogenous ALV infection can have an adverse effect on egg production, egg size, fertility, hatchability, growth rate, and non-specific mortality. Transmission of viruses between birds occurs by either (a) Vertical (congenital) transmission through the egg or (b) by horizontal transmission by bird to bird contact. The egg transmission is considered the

most important as congenitally infected birds remain the most important source of infection for the flocks. The infection by the congenital route is strongly associated with the presence of virus in vaginal swabs, egg albumen, and embryos, and the detection and elimination of these infected birds form the basis for diagnosis of infection and eradication programmes. The infective status of chickens in an ALV-infected flock can be categorised as to whether they are viraemic (V+ or V-), have antibodies (A+ or A-), and whether they shed ALV in droppings and egg albumen (S+ or S-). Congenitally-infected birds are usually viraemic, antibody-negative, shedders (V+A-S+) and form the most significant group in relation to the danger to other birds.

Diagnosis of retroviral infections is done at different levels. *Pathological diagnosis* can be made from the gross and microscopic lesions. Gross lesions of lymphoid, myeloid and erythroid leukosis are not easily distinguishable to the untrained pathologists. However, histopathological examination can usually help in making a conclusive diagnosis. The identity of the tumour cell lineage can also be confirmed by identification of specific markers by immunocytological methods. *Virological diagnosis* include the isolation of the causative retrovirus in tissue culture using infected materials such as serum, buffy coat cells, tumour tissue, cloacal or vaginal swabs, egg albumen, embryos and meconium. Virus isolation is considered the 'gold standard' for diagnosis and is usually the starting point for further detailed studies. Avian retroviruses usually do not induce extensive cytopathic effects in cultured cells and the viral replication is detected indirectly by detecting the group-specific p27 antigen in cultured cells. Once isolated, further typing can be done by additional tests such as interference assays, host range analysis and neutralisation assays. More recently, Polymerase chain reaction (PCR) tests specific for various subgroups are also used for typing of viral isolates. These tests are sensitive, rapid, and can be used to detect proviral sequences in tumour material or cultured cells. By using a reverse transcription step, the test can also be used to detect and quantify viral RNA. Detection of antibodies against retroviruses can be used in flock surveillance and to identify particular classes of birds in epidemiological studies and in eradication programmes. This can be done using tests such as virus neutralisation and ELISA.

Prevention and control of avian retroviral diseases: As no specific treatments or vaccines are available for the control of retroviral infections, the main method of control is the eradication of the virus from the infected flocks. This is done at the commercial primary breeding level, by continuous process of flock testing and removal of infected birds. Many of the primary breeding companies have made much progress in eradicating retroviruses from their elite lines. Additionally, genetic selection for disease resistance can also be attempted to create flocks resistant to retroviral infections.

MAREK'S DISEASE

Named after the Hungarian pathologist Jozsef Marek, Marek's disease (MD) is a neoplastic and neuropathic disease of poultry caused by a highly contagious, cell-associated herpesvirus. MD virus (MDV) is one of the first and by far the most oncogenic herpesviruses known and remains the only major neoplastic disease for which an effective vaccine has been widely used successfully. As a naturally occurring neoplastic disease, it still serves as an elegant model for understanding the molecular mechanisms of herpesvirus-induced latency and oncogenesis, as well as for dissecting the mechanisms of genetic resistance to tumours. With increasing reports of vaccination breaks and emergence of more virulent pathotypes, MD continues to pose severe threats to the poultry industry around the world. Developing more effective control strategies against MD remains a major challenge today.

MDV genome has a size of 160-180 kb encoding more than 100 genes (Osterrieder et al., 2006). Advance in technologies for genetic manipulation of the viral genome has enabled the examination of the functions of a number of MDV genes. For example, development of the bacterial artificial chromosome (BAC)-based infectious clones of a number of MDV strains (Petherbridge et al., 2004; Petherbridge et al., 2003) has identified the functions of important genes such as Meq (Nair and Kung, 2004) and vTR (Jarosinski et al., 2010; Jarosinski and Osterrieder, 2010) in the induction of

disease. More recently, we have demonstrated the role of virus-encoded microRNAs in the induction of lymphomas (Zhao et al., 2011).

MD usually occurs in birds from 4 weeks of age. In some of the virulent pathotypes that produce severe cytolytic disease, the incubation period can be shorter. Different clinical forms of the disease can be observed depending on the virulence of the virus and the genetic resistance of the host. These include the *classical form* with predominant neural involvement, a*cute form* with multiple lymphomas in visceral organs, *acute cytolytic form* with severe atrophy of the lymphoid organs, and *transient paralysis form* with birds suddenly developing ataxia and paralysis. As in the case of retrovirus diseases, diagnosis of MD is not extremely difficult, although differential diagnosis for the identification of the etiological agents can pose problems. However, gross pathological lesions assisted by histological studies can be valuable in confirmation of MD. Virological diagnosis can be made by isolating the virus in cell culture as well as by detecting viral proteins or genome in the samples. Materials commonly used for the isolation of the virus are the buffy coat cells from heparinised blood samples, or suspensions of lymphoma and spleen cells. As MDV is highly cell-associated, it is essential that the suspensions contain viable cells. These cell suspensions are inoculated into monolayer cultures of chick kidney cells or duck and chicken embryo fibroblasts. Evidence of MDV replication in the culture can be seen as plaques that appear in 3-4 days. Less commonly feather tips, from which cell-free MDV can be extracted, are also used for virus isolation. More recently, molecular biological techniques such as PCR tests have been used widely to differentiate between the oncogenic and vaccine strains, as well as for the quantitation of MDV genome copy numbers in various tissues and for assessing the efficiency of vaccination in flocks (Baigent et al., 2006).

Vaccination with live attenuated vaccines forms the cornerstone of the control of MD. It is estimated that more than 22,000 million doses of MD vaccines are used annually for the control of the disease worldwide. The types of vaccines used are derived from the different serotypes of MDV and include the strains such as CVI988 (Rispen's strain), SB-1 and herpesvirus of turkey (HVT). These vaccine strains are used either alone or in combinations as multiple vaccines to benefit from their synergestic effects and improved the efficiency. Although vaccination has been widely used successfully in controlling the disease since the 1970s, evolution of virus towards increasing virulence and emergence of strains with the ability to break through the vaccine-induced immune responses does occur at alarming rates in the poultry houses regardless of the vaccination status. As a result, MDV pathotypes referred to as *very virulent* (vvMDV) or *very virulent plus* (vv+MDV) were isolated from flocks vaccinated with different vaccination regimes (Witter, 1997). Although the fundamental mechanisms of this evolution are not fully known, the role of vaccines themselves in assisting the drive towards increasing virulence has not been ruled out (Nair, 2005). If the viral evolution is allowed to continue at the present rate with the current vaccines and the vaccination strategies, MD could again emerge as a major economic problem for the industry. Continued introduction of newer vaccines that may succeed on short-term is unlikely to be a sustainable long-term strategy. The failure to prevent the infection, replication and shedding of virulent virus strains is a serious limitation of the current vaccines. Future research should aim at developing vaccines capable of inducing vaccines capable of inducing 'sterile immunity' that would prevent virus replication in the vaccinated hosts.

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He then moved to the Institute for Animal Health (IAH) to join to work on avian oncogenic viruses, and became the Head of the Viral Oncogenesis group. He has since been leading the research on the pathogenesis of avian oncogenic viruses such as Marek's Disease (MD). Dr. Nair is the OIE (Office International des Epizooties) Expert on MD and heads the International Reference Centre on MD at the IAH. He is also a Visiting Professor at Imperial College London and a Jenner Investigator at the Jenner Institute in Oxford. Currently, he is the Head of the Avian Viral Diseases (AVD) Programme at IAH, overseeing the entire programme dedicated to the viral diseases in poultry.

His research group investigates the molecular mechanisms involved inthe induction of lymphomas and continuing increase in virulence of MDV strains. He has published more than 100 science publications in number journals and has contributed to a number of book chapters. He is also one of the Editors of the *Diseases of Poultry.*