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Review Article

Vaccinal Control of Marek's Disease: The Present and Future - A Review

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ABSTRACT

Marek's disease is an economically relevant lymphoid neoplasm of poultry, caused by oncogenic strains of Marek's disease herpesvirus. The disease has been controlled effectively by vaccination with attenuated or non-pathogenic MDV strains. Different vaccines have been tried out and the underlying principle for immunity is the action of antibodies targeted against membrane specific antigens and cytotoxic effect against tumor cells. Marek's disease virus is a particularly unwieldy herpesvirus to manipulate molecularly and many of the techniques performed routinely for other herpes viruses are not yet available for the MDV machinery. The postulated mechanisms of immunity against Marek's disease have been discussed here in detail. Vaccine breaks do occur as field strains continue to evolve towards pathotypes of increased virulence, and this evolution is of course vaccine driven. Experimental solutions to improve protection against the disease, like recombinant vaccines, have been discussed in this paper.

Key words: Herpesvirus, Immunity, Marek's, Pathogenic, Poultry, Vaccines.

INTRODUCTION

Marek's disease virus-1 (MDV1) causes the lymphoproliferative condition called Marek's disease (MD) in chickens (Churchill & Biggs, 1967; Payne, 1985). Herpesvirus of turkeys (HVT/MDV3) and MDV2 are apathogenic strains of the same genus and are serologically related to MDV1.They are extensively used as vaccines against Marek's disease singly or in combinations (Witter et al., 1970). HVT antiserum neutralizes MDV1 and vice versa but the real mechanism of defense contributed by HVT vaccines has not been defined till date.

Even through MDV is highly cell-associated, it is a readily transmissible and constantly evolving virus (Spencer and Calnek, 1967; Calnek and Hitchner, 1969). Although vaccination is effective in safeguarding the poultry population, the persistent evolution of MDV1 towards pathotypes of increased virulence (Witter, 1997; Witter et al., 2005) is ascribed to the selection pressure imposed on these viruses in vaccinated birds. In the mission to develop more efficient vaccines to control Marek's disease, genetically engineered vaccines may be the ultimate solution.

The detection of MDV DNA in human sera has raised doubts about interspecies transmission of the virus between poultry and human beings (Laurent et al., 2001). The re-emergence of epidemics like avian influenza strongly suggests the probability of such a doubt and it is the need of the hour that effective vaccination strategies against poultry viral diseases like Marek's have to be formulated.

How MDV/HVT Vaccines work?

A two-step hypothesis of immunity accorded by HVT vaccine was postulated by Payne et al (1976).The first step is against MDV1, by lowering the viral load in the bird, and the second against neoplastically transformed cells, leading to riddance of the tumour. When infected chickens were immunised with noninfectious viral antigens (Kaaden and Dietzschold, 1974; Lesnik and Ross, 1975) and with glutaraldehyde-inactivated cells of a MD lymphoma derived lymphoblastoid cell line protection was conferred against the disease (Powell, 1975).

The induction of suppressor T-cells which curbs the proliferation of neoplastic cells (Rouse and Warner, 1974), T-cell response to MD tumour-associated antigens (Schierman et al, 1976), T cell mediated cytotoxicity against MDV-infected cells (Ross, 1977) and the generation of antibody to viral envelope and virus specific membrane antigens (Kaaden and Dietzschold, 1974) or antibody to the tumour cell or virus-infected cell (Purchase and Sharma, 1974) etc., may be the reasons for this protection.

HVT-associated antibody or immune lymphocytes elicited by HVT vaccination confer a protective effect by interacting with MDV-infected cells and MD tumour cells. Purified HVT stocks,

inactivated HVT preparations, membrane fractions of HVT-infected chick embryo fibroblasts, all were found to be equally effectual in immunization against Marek's disease through years of analyses. Several studies have proved the efficiency of envelope specific glycoproteins of HVT to induce neutralization of pathogenic MDV1, MDV2 and HVT strains.

Kaaden and Dietzschold (1974) showed that antisera prepared against plasma membranes isolated from MDV- or HVT-infected cells neutralized extracellular infectious MDV. It was also found that after incubation of plasma membranes isolated from MDV-infected cells with Marek's disease antibody, the buoyant density of the membranes increased due to the binding of immunoglobulin to the virus-induced membrane proteins. Since it was known that the envelope of most herpes viruses are derived from a fraction of the membrane of the infected cells (Darlington and Moss, 1969; Roizman et al., 1969), anecdotal evidence suggested that virus-induced membrane antigen becomes part of the mature virus particle (Pearson et al., 1970).

Several virus-induced antigens have been detected in MDV- or HVT-infected cells; they have been demonstrated to be of two types (Intracellular antigen and Membrane antigen) by immunofluorescence techniques in cell cultures infected with MDV or HVT (Mikami et al., 1980). Intracellular antigen (IA) has been detected in both the nucleus and the cytoplasm of acetone-fixed cells (Purchase, 1969; Purchase et al., 1971) and was found only in cells that produced MDV particles (Nazerian and Purchase, 1970). Membrane antigen (MA) has been found on the surface of live MDV-or HVT-infected cells (Chen and Purchase, 1970; Ishikawa et al., 1972).

Membrane antigens are subdivided into two subclasses and designated as early membrane antigen (EMA) and late membrane antigen (LMA) which differ with respect to their sensitivity to inhibitors of DNA synthesis, their appearance in arginine-deficient Japanese quail embryo fibroblast (QKF) cultures and antigenic specificity (Ishikawa et al., 1972: Mikami et al., 1973; Onuma et al., 1976; Inage et al., 1979).

Supplementary studies on virus-induced proteins in HVT-infected chick embryo fibroblast cells revealed that glycoproteins isolated from membrane rich fractions of infected cells neutralize and precipitate antibody raised against HVT in rabbits and chickens (Wyn-Jones and Kaaden, 1979). After analytical electrophoresis, such isolates were found to contain three polypeptide bands which were not present in glycoprotein extracts of uninfected cells. It was also established that inoculation of chickens with purified material results in the production of precipitating and neutralizing antibody, indicating that these high-molecular-weight polypeptides contribute to immunity against Marek's disease.

Challenge of these chickens with virulent Marek's disease virus proved that a partial protection was afforded by the inoculated glycoproteins (Kaaden Dietzschold, 1974: Lesnik and Ross, 1975). Moreover, the virus-associated antigens on membranes of infected cells appear to be common in both HVT and MDV infected cells since an appreciable degree of protection was obtained after challenge of the chickens inoculated with the purified material isolated from membrane or infected cells by HVT and MDV (Wyn Jones and Kaaden, 1979).

Role of HVT Glycoproteins

Herpesvirus glycoproteins as virion surface components represent potent immunogens and hence, many of them have acquired immunoevasive functions (Lubinski et al., 1998), in addition to their basic function in occurrence of infection such as initial attachment, membrane fusion, virion penetration, trafficking of virion components, virion assembly, egress and cell-to-cell spread (Rajcani and Vojvodova, 1998). HVT and MDV have conserved homologues of 10 of the 12 glycoproteins found in Herpes Simplex Virus-I (gB ,gC ,gD ,gE, gH, gl ,gK ,gL, gM and gN).

Both gl and gE glycoproteins have been shown to form heterodimeric complexes and function in virus particle fusion with the host cell (Roop et al., 1993; Milne et al., 1998). The interaction of gI and gE is required for maturation and subcellular translocation of these two molecules, and is functionally essential for virus entry and cell-lo-cell spread (Wu et al., 2000).

The HVT gN has been shown to form a disulphide cross-link to gM (Wu et al., 1998). Several studies have revealed that the gE-gI and the gM-gN complexes serve overlapping but different functions in alpha-herpes virus egress and cell-to-cell spread. Deletion of either gE or gl, in MDV-I infected cells, resulted in the production of virus progeny that were unable to spread from cell to cell in either chick embryo fibroblasts or quail muscle cells (Schumacher et al., 2001). MDV is unable to replicate in the absence of two major membrane protein complexes, the gE-gl and the gM-gN complex (Schumacher et al., 2001; Tischer, 2002).

Additional putative glycoprotein genes include gB, gC,gD and gK.. One of these, gD does not appear to participate in infection processes or induction of immune responses, since it has been shown to be poorly expressed during MDV infection (Tan et al., 2001).The minor relevance of gD for cell-mediated immunity and the fact that it is a non-essential gene for invivo infectivity (Parcells et al., 1994) suggests that this gene is dispensable in MDV (Anderson et al., 1998) and can be a candidate locus for the development of recombinant MDV vaccines expressing genes for other poultry pathogens such as Newcastle disease virus, infectious bursal disease virus, and others (Hirai and Sakaguchi, 2001).

Examination of gC envelope glycoprotein of HVT, suggest that they have multiple functions in vitro and in vivo. gC plays an important role in binding heparan sulphate, an initial step in virus infection (Shieh et al., 1992; Spear *et al.*, 1992). Apart from this gC binds and inhibits complement C3k which may be important for immune evasion (Lubinski et al., 1998). It can be generalized that gC orthologues have a pivotal role in attachment of free virus to heparin and

chondroitin-like glycosaminoglycans on the surface of the plasma membrane, thereby conferring the primary contact between the virion and host cell (Roizrnan and Knipe, 2001).

It has been demonstrated that MDV mutants lacking gB were nonviable (Tischer et al., 2002). Comparison of HVT glycoproteins with those from both MDV and HSV-I reveals that gB exhibits the greatest level of conservation among the glycoproteins. In particular, domains involved in HSV-I gB oligomerization (Sarmiento et al., 1979; Claesson-Welsh and Spear, 1986), which is important for fusion with host cells (Laquerre et al., 1996), may be conserved in HVT gB.

Recombinant Vaccines Based on MDV Glycoproteins

Almost 40 years ago, when it was established that virus-induced proteins prepared from cells productively infected with MDV or HVT are protective in chickens vaccinated against tumor development (Kaaden et al., 1974; Lesnik and Ross, 1975), arguments for the development of a vaccine against Marek's disease from virus-induced antigens in infected cells began.

The first strategy was to use live virus vectors based on HVT (Morgan et al., 1992: Ross et al., 1993; Cronenberg et al., 1999) and MDV-I (Nakarnura et al., 1992) which express inserted genes obtained from other MDV serotypes or from other avian viruses. Some of the approaches were: (a) construction of recombinant HVT virus in which Newcastle disease virus (NDV) genes were inserted into a non-essential gene in the Unique Short region of HVT to convey dual protection against MDV and NDV (Sondermeijer et al., 1992: Morgan et al., 1993) and (b) construction of a recombinant fowl pox viruses (rFPVs) that expressed a variety of MDV genes including gC, gD, gB and tegument proteins from all three serotypes.

Numerous studies that described the construction and testing of a range of recombinant fowlpox virus vaccines expressing MDV gK, gl, gH genes were also initiated. A remarkable level of protection has been reported against MD with such vaccines (Nazerian et al., 1992 and 1996; Reddy et al., 1996). Certain studies showed that recombinant vaccines expressing gC or gD are not as effective as a gB-expressing vaccine (Heine et al., 1997).

Another research proved that recombinant fowlpox virus vaccines expressing the gB gene of MDV-I was found to elicit a more efficacious response when compared to the recombinant vaccine that encoded gB of other serotypes (Lee et al., 2004). The studies also clearly showed that combined vaccines of gB, gC and gD are more effective than individual vaccines (Lee et al., 2003).

In another attempt construction of an infectious Marek's disease virus bacterial artificial chromosome (MDV-BAC) and HVT bacterial artificial chromosome (HVT-BAC) have been tested as vaccine candidates. BAC clones containing MDV genome could elicit partial protection ranging from 42-56% and BAC clones of HVT could induce protection which was comparable to the efficacy of HVT (Baigent et al., 2006).

The next strategy used was gene deletion within serotype I MDVs (Zelnik et al., 1995; Lupiani et al., 2004). The very virulent strain Md5 lacking the oncogene Meq appears to be the most promising candidate at the moment (Lupiani et al., 2004; Lee et al., 2007). The most recent technique has been modification of domains within the oncogene Meq in the very virulent strain RBI B (Brown et al., 2006). Any of the recombinant DNA vaccines have yet been licensed for authorized use, as none of them exceed the efficacy of other commercial vaccine strains.

Vaccines Based on MDV Glycoproteins-Future of Marek's Disease Prophylaxes

MD is a significant concern in commercial poultry production due to its highly contagious nature and prevalence in the field. It is possible that MDV will continue to increase in virulence and overcome the protection conferred by CVI9R8 strain. This being the case, since there are currently no new vaccine strains available for commercial use, it would be extremely difficult to find a better alternative to fight a further evolved MDV strain on short notice (Gimeno, 2008)

It is obvious that recombinant vaccines will be the basis for control of MDV in the years ahead; however there exist number of limitations. Since there are 10 homologous glycoproteins conserved between HVT and MDV, the question arises that which one or a combination of them acts as common antigenic protein and are involved in immunity conferred by HVT against MDV. Future beckons for more research to find out which viral genes are involved in immunity or virulence and what combination of genes must be expressed or deleted to produce an effective vaccine.

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