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Marek's disease vaccines: Current status, and strategies for improvement and development of vector vaccines

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ABSTRACT

Marek's disease (MD) is a lymphoproliferative viral disease of chickens, which has been controlled through vaccination since 1969. MD vaccines protect against tumors but do not provide sterilizing immunity, and thus it is generally believed that their use has contributed to increase virulence of field strains with the ability to cause MD in vaccinated chickens. Traditional methods of developing vaccines, like cell culture attenuation, have proved unsuccessful for the development of improved vaccines to protect against highly virulent MD virus (MDV) field strains. With the advent of recombinant DNA technology, it is now possible to study MDV gene function and develop rational vaccines that protect against highly pathogenic strains. In addition, the long term protection conferred by MD vaccines, their excellent safety profile, their efficacy when administered early (at hatch or *in ovo*), and their ability to overcome maternal antibodies, has made MDV an excellent candidate vector to protect not only against MD but also against other important viral poultry diseases. In this review we will discuss the current status of MD vaccines and their use as vector vaccines to control important viral poultry diseases.

1. Introduction

Marek's disease (MD) is a common, highly contagious, lymphoproliferative disease of chickens characterized by lymphoid infiltrations in peripheral nerves, visceral organs, eye, muscle and skin, and immunosuppression. The causative agent of MD is Marek's disease virus (MDV), a member of the genus *Mardivirus*, sub-family *Alphaherpesvirinae* in the family *Herpesviridae*. MDVs are classified into 3 different species which correspond to previously described serotypes: *Gallid herpesvirus 2* (GaHV-2, MDV serotype 1 or MDV-1), *Gallid herpesvirus 3* (GaHV-3, MDV serotype 2 or MDV-2), and *Meleagrid herpesvirus 1* (MeHV-1, MDV serotype 3, MDV-3 or HVT) (Davison et al., 2009). MDV-1 includes oncogenic viruses of variable virulence, MDV-2 include non-oncogenic viruses from chickens, and MDV-3 includes non-oncogenic viruses from turkeys.

During the first half of the 20th century management changes in poultry production, including the move to intensive production methods and increase flock size, resulted in significant increase of MD incidence. Just prior to the introduction of MD vaccines, tumor diseases, like MD and avian leukosis, caused significant losses to the poultry industry. The introduction of vaccines to control MD in the early 1970s was a first significant step to reduce mortality and condemnation rates at slaughter and was essential for the sustainability of the modern poultry industry. However, due to the cost of vaccination and occasional outbreaks, MD continues to have a significant economic impact on the poultry industry, with the most recent losses estimated around US $1–2 billion annually (Morrow and Fehler, 2004).

Although MD vaccines have been very successful at protecting chickens against tumors and mortality, they do not provide sterilizing immunity and vaccinated chickens still support replication and shedding of virulent field viruses. The widespread use of MD vaccines is thought to have contributed to the evolution of MDV field viruses towards greater virulence (Davison and Nair, 2005; Gandon et al., 2001; Gimeno, 2008; Witter, 1997), and there is a need for the development of sterilizing MD vaccines. As we increase our knowledge of MDV gene function and host pathogen interactions, it is expected that recombinant technology will play a major role in the development of next generation vaccines. In addition, an improvement in our currently limited understanding of the mechanisms by which vaccines confer immunity (Haq et al., 2013) will positively affect our ability to produce more efficacious vaccines. In this review, we will discuss the current status of MD

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vaccines and their use as vector vaccines to control important viral poultry diseases.

2. Traditional Marek's disease vaccines

MD vaccines consist of viruses of three different serotypes: MDV-1, MDV-2 and MDV-3. Below, following the order in which they were licensed in the U.S., we will describe in more detail their identification and use (Table 1).

2.1. Serotype 3 vaccines: MDV-3

MDV-3 or HVTs are non-oncogenic viruses from turkeys that are antigenically related to oncogenic MDV-1. HVT were first isolated from turkeys by Anderson and Kawamura (Kawamura et al., 1969) and Witter (Witter et al., 1970) and soon after it was shown that, under experimental conditions, could protect chickens against MDV challenge (Okazaki et al., 1970). These initial studies followed by large-scale validation resulted in HVT being licensed in the US in 1971 (Purchase et al., 1971). HVT is the most widely used vaccine to control MD and is commonly used in broilers as a monovalent vaccine or as part of a polyvalent vaccine in breeders and layers (Dunn and Gimeno, 2013). Although several HVT strains are licensed, the most commonly used strain is FC126.

Like all other MDV serotypes, HVT is a highly cell-associated virus and, as a consequence, vaccine preparations require special handling and storage. However, unlike the MDV-2 and MDV-1 viruses, it is possible to make cell free vaccine preparation by sonication of HVT infected cell cultures (Calnek et al., 1970). Although these cell free preparations are easier to transport and handle, they are susceptible to neutralization by maternal antibodies thus are less efficacious than cell associated HVT vaccines (Prasad, 1978; Witter and Burmester, 1979), and thus their use is restricted to small backyard flocks or countries where liquid nitrogen transportation is not practical.

2.2. Serotype 2 vaccines: MDV-2

Following the isolation of MDV-1 strain CVI988 from clinically healthy non-vaccinated flocks (see below), research groups in Europe (Biggs and Milne, 1972) and the US (Schat and Calnek, 1978) isolated other naturally avirulent viruses from healthy chickens. These viruses were serologically distinct from pathogenic MDV-1 and apathogenic HVT and were classified as novel serotype 2 viruses (Bulow and Biggs, 1975a, 1975b). However, the limited protection provided by MDV-2 virus against very virulent (vv) MDV and their susceptibility to maternal antibodies (Witter, 1982) resulted in these viruses being used in combination with other serotypes and are components of bivalent or trivalent vaccine formulation (Witter and Lee, 1984).

2.3. Serotype 1 vaccines: MDV-1

MDV-1 vaccines have been generated by serial passage in cell culture, resulting in the introduction of random mutations in the viral genome and subsequent attenuation (Churchill et al., 1969a). Although several MDV-1 vaccines have been developed, only few have been commercialized or used. The first MD vaccine, HPRS-16/att, was generated by serial passage (33 passages) of a virulent MDV in chicken kidney cells and was shown to provide protective immunity against virulent MD viruses (Churchill et al., 1969b). HPRS-16/att was only used for a few years and was soon replaced by HVT which provided better protection.

CVI988 or Rispens is the most efficacious vaccine currently available. It was isolated in The Netherlands and showed low level of oncogenicity (Bulow, 1977), but was attenuated by serial cell culture passage. Compared to HPRS-16/att, CVI988 showed superior protection (Rispens et al., 1972a,b) and was extensively used in The Netherlands, although it took several years to be fully accepted in the rest of the world. The delayed acceptance of CVI988 was due to this vaccine's residual virulence in highly susceptible chickens and it ability to spread among chickens. To eliminate this residual pathogenicity, de Boer et al. developed higher passage cloned derivatives, CVI988 Clone C (de Boer et al., 1986, 1987) and CVI988 Clone C/R6 (back-passaged 6 times in chickens) (De Boer et al., 1988); however, although commercialized, their use was limited due to inferior protection compared to parental CVI988 (Witter, 1992). It is important to note, however, that studies by Witter at al. (Witter et al., 1995) showed that commercial stocks of CVI988 lack oncogenicity and transmit poorly among chickens, probably due to a higher passage of these vaccine stocks.

After the emergence of vv and very virulent plus (vv+) MDV field strains, CVI988 became the vaccine of choice worldwide because of superior protection (Witter, 1992; Witter et al., 1995) and is now considered the "gold standard" of MD vaccines. Over time, attempts have also been made to attenuate highly virulent MD virus strains to be used as vaccines. Witter found that a vv MDV (Md11) (Witter, 1982) and vv+ MDV (648A and 584A) (Witter, 2002) become fully attenuated around passages 70–100 and, at this high level of attenuation, did not provide adequate protection. On the other hand, partially attenuated viruses, obtained at lower passage, replicated better in chickens and provided significantly higher levels of protection against virulent MD challenge. However, these vaccines did not provide significantly better protection than bivalent HVT/SB-1 or CVI988, retained residual virulence, and reverted back to virulence when passaged in chickens (Witter and Kreager, 2004; Witter and Lee, 1984), making them non-viable for commercialization.

2.4. Protective synergism of MD vaccines

In the early 1980s, there were increased reports of vaccine breaks in HVT vaccinated flocks with concurrent increase in condemnation in broilers at slaughter. This was followed by

Table 1
MD licensed vaccines in the US.

Serotype	Vaccine	Description	Used	Reference
MDV-1	CVI988	Mildly pathogenic-Attenuated	Yes	Rispens et al. (1972a)
	CVI988 Clone C	Cloned CVI988	No	de Boer et al. (1986)
	CVI988 Clone C/R6	Backpassaged CVI988 Cone C	No	De Boer et al. (1988)
	Md11/75C/R2	Very virulent – Attenuated, cloned and backpassaged	No	Witter (1995)
MDV-2	SB-1	Naturally apathogenic- cloned	Yes (bivalent with HVT)	Schat and Calnek (1978)
	301B/1	Naturally apathogenic-cloned	Yes (bivalent with HVT)	Witter (1987); Witter et al. (1990)
MDV-3 (HVT)	FC126	Naturally apathogenic- cloned	Yes	Witter et al. (1970)