

Control Strategies for Marek's Disease: A Perspective for the Future

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ABSTRACT Marek's disease virus is an evolving pathogen, acquiring virulence in response to increasingly effective vaccines. Although vaccine efficacy is generally good, industry has placed a high priority on more effective products. The search for better vaccines has been conducted mainly in the arena of molecular

biology, and has been thus far disappointing. Various conditions prevail that currently limit the potential to develop suitable long-term solutions. A new paradigm based on reduction of early exposure, multiple levels of host resistance, and improved cooperation among stakeholders is proposed for consideration.

(Key words: Marek's disease, vaccine, serotype, genetic resistance, transgene)

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INTRODUCTION

Marek's disease (MD) is a common and important neoplastic disease of chickens. The causative herpesvirus (MDV) belongs to Serotype 1, and is related antigenically to nononcogenic herpesviruses of chickens (Serotype 2) and turkeys (Serotype 3); the latter is also known as turkey herpesvirus (HVT). Within Serotype 1 MDV four pathotypes are recognized; mild (m), virulent (v), very virulent (vv), and, recently, very virulent plus (vv+). All pathotypes of Serotype 1 cause disease, and can be attenuated by serial passage in cell cultures. Vaccination of chickens *in ovo* or at hatch with attenuated Serotypes 1, 2, or 3 viruses induces protection against subsequent challenge with virulent Serotype 1 MDV (Calnek and Witter, 1991). A classification of serotypes and pathotypes is given in Table 1.

Following the introduction of HVT vaccine about 1971, losses from MD in broiler and layer chickens were dramatically reduced. Based on this early success, the poultry industry has relied on vaccination as the principal means of control. However, control can also be achieved through selection for host genes associated with resistance to tumor induction. Because MDV is not transmitted vertically, partial control may also be achieved through biosecurity procedures sufficient to delay exposure, such as placement of newly hatched chicks in thoroughly cleaned and disinfected houses that are well separated from houses with older chickens. Genetic selection and management have been used more frequently as adjuncts to vaccination rather than as

primary control strategies but are critical components of an integrated control system.

PATHOTYPES AND EVOLUTION OF VIRULENCE

The increase in virulence of MDV over the past 50 yr is of major significance. The shift from mMDV to vMDV strains in the late 1950s, the shift from vMDV to vvMDV in the late 1970s and, more recently, the appearance of the putative vv+ pathotype in the early 1990s have each resulted in the potential for greater disease losses that have persisted until introduction of a more effective vaccine. The main basis for the pathotype classification is the ability to cause disease in chickens immunized with increasingly effective vaccines, as shown in Table 2 (Witter, 1997). Thus, vvMDV are poorly protected by HVT but better protected by HVT + SB-1 vaccine. The vv+MDV are protected poorly by both vaccines (above), but CVI988/Rispens vaccine appears to offer somewhat better protection. Thus far, no antigenic differences have been noted among viruses of different Serotype 1 pathotypes, suggesting that the higher virulence associated with newer pathotypes is not likely due to antigenic drift.

Some other heretofore unrecognized biological properties have been noted with vv+ strains, although it is not yet clear whether the changes are pathotype specific. We have recently noted, for example, that vv+ strains cause a particularly acute form of transient paralysis in 3-wk-old chickens, resulting in very high

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Abbreviation Key: bpr = base pair repeats; HVT = turkey herpesvirus; M = mild; MD = Marek's disease; MDV = Marek's disease virus; v = virulent; vv = very virulent; vv+ = very virulent plus.

TABLE 1. Viral serotypes

| Serotype | Pathotype | Code | Vaccine |
|----------|--------------------|------------------|---------|
| 1 | Virulent | v | no |
| | Very virulent | vv | no |
| | Very virulent plus | vv+ | no |
| | Mild | m | no |
| | Attenuated | | yes |
| 2 | Nononcogenic | | yes |
| 3 | Nononcogenic | HVT ¹ | yes |

¹Turkey herpesvirus.

mortality (Witter, 1996; Witter and Gimeno, unpublished data). Calnek and coworkers (B. W. Calnek, Cornell University, Ithaca, NY 14853, personal communication, 1997) have associated greater lymphoid organ pathology with vv+ strains. The appearance of MD as a clinical disease of turkeys in France and Israel (Davidson *et al.*, 1996; Malkinson *et al.*, 1996; Gaudry, 1997), although not directly associated with vv+ strains, seemed to occur at the same time that the pathotype was first emerging. Finally, Rosenberger and coworkers (1997) have noted instances in which recently isolated MDV strains of high virulence appeared to have increased pathogenicity for adult chickens, a finding consistent with that reported by Witter (1996).

The conditions that favor evolution of MDV towards greater virulence are not known but are probably created in part by vaccination with increasingly effective products. As vaccinal immunity is compromised by factors such as early exposure or immunosuppressive stress, mutant clones have an increased opportunity to selectively multiply and to be seeded in the environment. Because vaccines are the major barrier to the disease, single point mutations may be sufficient to result in the emergence of a mutant strain.

MD VACCINES: CURRENT TECHNOLOGY

Modified live vaccines are the cornerstone of current MD control programs. In the U.S., seven vaccine strains are currently licensed (Table 3). Additional strains are licensed in other countries. The vaccine strains may be used singly or in combinations that are of three general types or formulation in ascending order of efficacy: 1) HVT alone, 2) HVT plus a Serotype 2 strain, and 3) CVI988 (Rispen) with or without viruses of Serotypes 2 or 3. The CVI988 (Rispen) strain appears to be the most efficacious of the Serotype 1 strains and, at present, is widely used in layer and breeder chickens (Table 4).

Although much attention has been given to selection of the optimal combination of vaccine strains, a number of other considerations may (or may not) be important. Protective synergism is the property of two vaccine viruses to induce greater protection in combination than either could induce alone (Witter and Lee, 1984). This phenomenon is easily demonstrated between Serotypes

TABLE 2. Proposed Marek's disease (MD) pathotype nomenclature

| Pathotype designation | | Criteria for assigning pathotype ¹ | |
|-----------------------|-----------|---|--|
| Short form | Long form | Percentage MD is | In chickens ² vaccinated with |
| m | mMDV | < JM | none |
| v | vMDV | = JM | HVT |
| vv | vvMDV | > JM | HVT |
| | | = Md5 | HVT + SB-1 |
| vv+ | vv+MDV | > Md5 | HVT + SB-1 |

¹Percentage MD in chickens challenged at 5 to 7 d of age with the isolate to be typed is less (<), more (>), or no different (=) as determined by statistical analysis than that induced by challenge with the prototype JM or Md5 strains or equivalent.

²A genetically susceptible strain, positive for maternal antibodies.

2 and 3, but is less obvious with other combinations (Witter, 1988, 1992). The common practice of mixing serotypes in virtually all combinations has little support from controlled studies, but is not detrimental and often seems to be advantageous in the field.

In ovo vaccination has become the predominant method of administration of MD vaccines to broilers, mainly due to lower cost and improved reliability (Miles *et al.*, 1992; Sarma *et al.*, 1995; Johnston *et al.*, 1997). This technique is efficacious with HVT vaccine (Sharma and Burmester, 1982) but more studies may be needed to optimize this technique for vaccines of Serotypes 1 and 2. In the field, *in ovo* vaccination has not appeared to reduce MD condemnations compared to day-old vaccination (Miles *et al.*, 1992; C. Ricks, Embrex, Inc., Research Triangle Park, NC 27709-3989, personal communication, 1997).

Based on estimates derived from comparison of actual condemnation losses in vaccinated broiler chickens and predicted losses in the absence of vaccination, vaccine efficacy is extremely high, at least when measured as a national average (Figure 1). Anecdotal reports on the high losses that occur in flocks that, through error, did not receive viable vaccines supports this contention. However, excessive losses continue to occur sporadically in properly vaccinated flocks, sometimes in specific geographic regions, and certain vaccines protect poorly against certain challenge strains in the laboratory. Although the efficacy of MD vaccines may be viewed

TABLE 3. Licensed vaccine strains in the U.S.

| Vaccine strain | Serotype |
|-----------------|----------|
| FC126 (HVT) | 3 |
| SB-1 | 2 |
| 301B/1 | 2 |
| CVI988 clone C | 1 |
| CVI988/C/R6 | 1 |
| CVI988 (Rispen) | 1 |
| R2/23 (Md11/75) | 1 |

TABLE 4. Most common vaccine formulations for Marek's disease (MD)

| Most common vaccine formulations | Relative efficacy vs MDV pathotype | | | Current use in commercial flocks | |
|---|------------------------------------|-----|-----|----------------------------------|---------------|
| | v | vv | vv+ | Broiler | Layer/breeder |
| FC126 alone | +++ | + | + | + | + |
| FC126 plus Serotype 2 | +++ | +++ | ++ | +++ | ++ |
| CVI988 (Rispens) alone or plus Serotype 3 or plus Serotypes 2 and 3 | +++ | +++ | +++ | + | +++ |

¹Plus values indicate relative frequency (approximate).

from two perspectives, it seems fair to conclude that in general practice and use, MD vaccines are extremely effective.

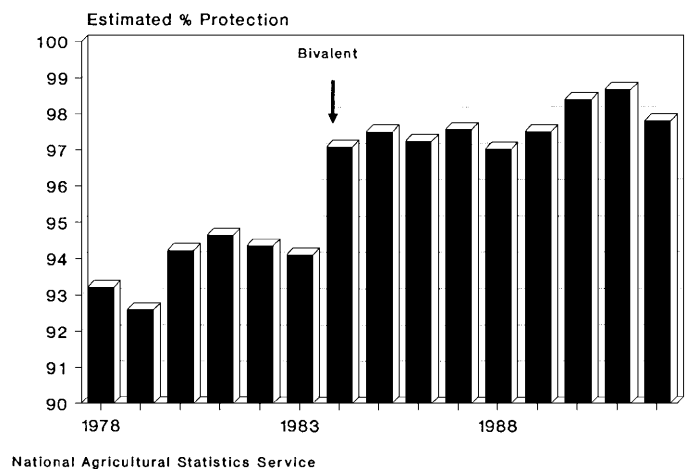
ARE IMPROVED VACCINES NEEDED?

If current vaccines are generally efficacious, safe, and cost effective, why then does the research community and the poultry industry give such high priority to development of improved vaccines? There are several reasons. The current level of control is considered fragile at best, because significant disease outbreaks not only continue to occur but also are totally unpredictable. This unpredictability factor is particularly troublesome to producers. It is usually impossible to determine, by retrospective analysis, the reasons for outbreaks. Although most serious outbreaks are resolved with time, it is usually difficult to determine the extent to which such change resulted from human intervention. Also, because of the tendency of the virus to mutate towards greater virulence with concomitant reduction in efficacy of vaccines (Witter, 1996), many fear that current vaccines will not provide adequate protection indefinitely. Worse yet, no new generation of improved vaccines is on the horizon. Other factors are the increased competitiveness within the industry and the tendency to base management on cost rather than disease control criteria. For these and other reasons, MD has continued to rank high on industry priority lists despite a relative low level of loss as measured by current industry averages (Table 5). The most common plea is for the development of another generation of better vaccines.

THE SEARCH FOR IMPROVED VACCINES

Recombinant DNA technology has stimulated a variety of sophisticated approaches towards the production of superior vaccines. Vaccines in which MDV immunogenes are expressed in live fowl pox virus or herpesvirus (usually HVT) vectors have been described (Morgan *et al.*, 1992; Nazerian *et al.*, 1992; Ross *et al.*, 1992; Sondermeijer *et al.*, 1993), are known to induce protective immunity, and seem the closest to commercial use. Interestingly, protection by a fowl pox virus vector

expressing the *gB* gene of Serotype 1 MDV has shown significant enhancement in the presence of HVT (Nazerian *et al.*, 1996). Deletion mutants of Serotype 1 MDV have been difficult to produce. Robin Morgan and colleagues (R. Morgan, University of Delaware, Newark, DE 19717-1303, personal communication, 1997) found deletion of *gC*, *gD*, or *meq* may result in varying degrees of attenuation but the mutant viruses have thus far not replicated well *in vivo* and have provided only limited protection. The potential for deletion mutant vaccines is still considered promising by some workers, but progress is hindered by technical difficulties and the lack of knowledge of which genes are critical to virulence or oncogenicity. A naked DNA vaccine has been also described (Li *et al.*, 1996). For any such novel vaccine to be commercially successful, a competitive advantage over existing products must be demonstrated—and this has proved a formidable challenge. Filling one small niche is the recombinant fowl pox virus expressing *gB* of Serotype 1 MDV which, when combined with cell-free HVT, constitutes a bivalent, totally cell-free vaccine that provides better protection than cell-free HVT alone (Nazerian *et al.*, 1996). Cell-free vaccines are advantageous as they do



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FIGURE 1. Estimated efficacy of protection by Marek's disease (MD) vaccines based on a comparison of actual condemnation losses for the U.S. with losses projected at the 1.5% rate that prevailed just prior to the introduction of vaccines in 1971.

TABLE 5. Disease priorities as determined by the Committee on Transmissible Diseases of Poultry, U.S. Animal Health Association¹

| Strain | 1992 | 1993 | 1994 | 1995 | 1996 |
|----------|---------|---------|----------------|---------|----------|
| Layers | SE | SE | SE | AI | SE |
| | Marek's | AI | Marek's | Marek's | Marek's |
| | IB | MG/MS | <i>E. coli</i> | SE | fowl pox |
| Broilers | IB | MG/MS | IB | Marek's | IB |
| | Marek's | Marek's | Marek's | AI | Marek's |
| | MG/MS | IB | MG/MS | IB | others |

¹AI = avian influenza; *E. coli* = colibacillosis; fowl pox = variant fowl pox; IB = infectious bronchitis; Marek's = Marek's disease; MG/MS = mycoplasmosis due to *Mycoplasma gallisepticum* and *Mycoplasma synoviae*; SE = *Salmonella enteritidis*.

not require storage at ultra low temperatures, but are generally less effective in the presence of maternal antibodies. Up to now, the only licensed cell-free vaccine is composed of HVT alone.

The fortuitous insertion of portions of a retrovirus proviral genome into the JM strain of MDV resulted in attenuation of virulence without the usual alterations in other biological properties (Witter *et al.*, 1997). This mutant virus, which may be considered a natural deletion mutant, was also highly protective in laboratory studies (Witter *et al.*, 1997) but is probably precluded from consideration as a vaccine because of the retroviral insert and induction of thymic atrophy.

We recently examined the possibility that detection of expanded 132 base pair repeats (bpr), normally a marker for attenuation (Becker *et al.*, 1993; Kopacek *et al.*, 1993; Silva, 1992; Zhu *et al.*, 1992), would permit selection of attenuated clones at early passage. However, several clones selected by this method were still highly oncogenic and unsuitable as vaccines (Witter, Niikura, and Silva, unpublished data).

Bacon and colleagues (Bacon and Witter, 1994a,b) recently found that vaccine efficacy was influenced by the MHC and that this influence was different for different vaccine serotypes. This finding suggested that the well recognized role of the MHC in antigen presentation was also important in vaccinal immunity and that manipulation of the vaccine-host relationship might provide an alternate approach towards better control (Witter and Hunt, 1994); however, this approach has proved a daunting task. Hunt and colleagues have sequenced several chicken class I MHC molecules (Hunt *et al.*, 1994) but the search for the specific peptide configurations presented by each haplotype is just beginning. Recent improvements in technology to detect specific haplotypes through simple serologic tests (Fulton *et al.*, 1996), however, may be very helpful in the implementation of any MHC-based control strategies.

Thus, there is no lack of novel ideas or strategies. For control of MD the problem is that many of the strategies have been unsuccessful thus far for technical reasons. Those products that have provided protection have generally failed to exceed the efficacy of existing

products. On balance, the effort has thus far been disappointing.

There are a number of explanations for this failure. Marek's disease virus is a particularly difficult herpesvirus to manipulate molecularly and many of the procedures performed routinely for other herpesviruses are not yet available for the MDV system. There is a lack of basic understanding of the process of immunity induction following vaccination. We do not know enough about which viral proteins or which host immune responses are important. Also, given that existing vaccines are able to induce high levels of protection within 7 to 10 d of hatching, we may be approaching the limit of the capacity of the immune system to respond.

CONDITIONS THAT IMPACT THE POTENTIAL FOR IMPROVED MD CONTROL

A number of circumstances exist that constitute, to varying degrees, obstacles to progress in MD control. Some of these circumstances relate to conditions of science and academia. Other conditions are controlled or influenced by one or more segments of the poultry industry.

In academia and the scientific community, a world wide shrinkage of funding and scientific expertise on MD has been evident for several years. Although international symposia on MD still generate much interest, the number of laboratories with long-term commitments to MD research have been reduced and many of the existing programs are focused on the molecular biology of the virus. Many established principal investigators have retired and heirs to this legacy are not immediately apparent. Funding of government research programs is undergoing attrition and competitive grant opportunities are increasingly competitive. The unique technical demands of the MD system, already addressed, suggest that, compared to other herpesviruses, more effort may be required for the same amount of knowledge gain.

Within the poultry industry, each major sector owns certain issues that impact MD control. The broiler and

layer producers, in an attempt to reduce costs, have implemented practices such as placing chicks on built-up litter, rearing flocks of multiple ages on the same premises, and diluting vaccines. Vaccine producers have escalated plaque-forming unit doses (probably for marketing reasons), have promoted a large and sometimes confusing menu of vaccine options, and have reacted to market pressures that have resulted in abnormally low profit margins for MD vaccines which tends to discourage research and development. Part of the responsibility for the latter point rests also with producers who are reluctant to pay more for vaccines. Suppliers of *in ovo* technology have created a new system of vaccine administration for broilers, which has resulted in a need for additional research on the effects of *in ovo* administration on vaccinal immunity, especially by the different serotype vaccines. Although some breeders have been quietly selecting for MD resistance for years, this selection is prioritized along with other economic traits and progress is both slow and costly, partly because there is only limited knowledge about relevant host genes and the methods to efficiently conduct selections. For all sectors, cost is an overriding concern and new investment for MD control is largely crisis-driven. In addition, industry efforts to influence the amount of funding for MD research have been inconspicuous. In my experience, there has been relatively little evidence of close communication between stakeholders and little effort by these stakeholders to develop an industry-wide control strategy. None of this means to detract from the large number of dedicated industry veterinarians and geneticists who are committed to MD control and are doing the best they can within existing economic constraints.

CONCLUSIONS

So what do we do? A good place to start would be to acknowledge three truths: 1) good vaccines are not enough, 2) the two conditions most likely to defeat vaccines are early exposure and viral evolution, and 3) improved control, whether short- or long-term, will not be cheap.

The potential for early exposure can easily be reduced through changes in management practices and facility design. It has been gratifying to see certain layer producers, in response to unacceptable MD losses in 1994 to 1995, convert from multiple- to single-age pullet growing units. This change, in concert with upgraded sanitation and biosecurity, and with increased use of CVI988/Rispens vaccine, was directly associated with a significant reduction in MD losses (Kreager, 1996).

Solutions for viral evolution are somewhat more problematic, but are perhaps even more important. There seem to be two strategies: eliminate (eradicate) the virus altogether or erect multiple barriers in the host chicken to reduce the impact of single mutational events.

Eradication is currently practiced by producers of specific-pathogen-free flocks, both commercial and research. This procedure is facilitated by the absence of egg-transmitted infection but requires filtered air, positive pressure housing and has not yet been considered economically feasible for the industry. Multiple barriers, however, are worthy of consideration and are of three types. Polyvalent vaccination is already practiced and is known, at least with some combinations, to provide more complete protection than single vaccines. It may also be important to use these vaccines at the recommended dose, as partial dose vaccination may create lower level immunity that may well encourage the generation of more virulent strains. By itself, however, vaccination will not be sufficient.

The presence of multiple host resistance genes that individually and collectively may restrict both viral replication and tumor susceptibility is a powerful strategy, just beginning to be developed. The MHC-related resistance genes have been known for many years. Recent evidence from Cheng and coworkers (Vallejo *et al.*, 1996) suggests the existence of additional, non-MHC-related genes associated with certain marker sequences in the host genome that will ultimately aid selection programs. In the future, it may be possible to produce chickens with multiple host resistance genes of both MHC and non-MHC types.

A third barrier may be the introduction, through gene transfer technologies, of pathogen-derived genes that may directly interfere with the replication pathway of virulent Serotype 1 MDV. This strategy has several advantages. First, there is ample precedent. This principle is currently in use for disease control in many plant species (Grumet, 1995; Baulcombe, 1996). A truncated form of the *ICP4* gene, a gene of herpes simplex virus involved in replication, when expressed in transgenic mice has been shown to limit the replication of the wild type parent virus (Smith and DeLuca, 1992). In addition, chickens carrying germ line inserts of avian leukosis virus envelope antigen were resistant to infection with wild-type virus (Salter *et al.*, 1986a,b) thus demonstrating an example of pathogen-derived-resistance in the chicken. Second, it should be both feasible and desirable to insert two or more viral resistance genes to establish redundancy. Third, it may be possible to transfer Serotype 1-specific genes that will not interfere with the ability of the chicken to be immunized with live vaccines of Serotypes 1 and 2, or with non-herpesvirus vectored MD vaccines, such as fowl pox virus expressing the glycoprotein B of MDV (Nazerian *et al.*, 1996).

For maximum effect, all three barrier levels must work in concert and be coordinated with management changes to significantly reduce early exposure (Table 6). In theory, chickens bearing multiple host resistance genes, multiple viral resistance genes, vaccinated with multiple vaccines and held under conditions so that exposure is delayed should be far less likely to spawn new pathotypes of MDV. Moreover, such chickens

TABLE 6. Multiple barriers to limit the potential of single point mutations to create virulent pathotypes¹

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- Polyvalent vaccines used at sufficient dose to provide robust immunity (selected for ability to replicate in chickens genetically resistant to Serotype 1 Marek's disease virus challenge)
plus
 - Multiple host genes (MHC and non MHC) to provide resistance to challenge and augment vaccine efficacy
plus
 - Two or more pathogen-derived transgenes expressed in germ and somatic cells to limit replication of challenge virus without precluding use of vaccines
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¹Best used in concert with management and biosecurity procedures to reduce probability of early exposure.

should also be far more resistant to challenge with current MDV pathotypes.

The disadvantage of the proposed paradigm lies in the need to overcome important and difficult issues such as technology development, cost, time, and regulatory and public policy issues. It is important not to underestimate the magnitude of these obstacles. However, it is difficult to envision any alternate strategy with better potential for success over the long term.

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