Prevention of Marek's Disease: A Review¹

H. Graham Purchase
National Program Staff, Agricultural Research Service, Department of Agriculture, Beltsville, Maryland 20705

Summary

Marek's disease (MD) is a highly infectious neoplastic condition of chickens caused by a herpesvirus. The virus is cell associated in tumors and in all organs except in the feather follicle where enveloped infectious virions egress from the body. From this source, infection is spread horizontally by the airborne route to the environment and to other chickens. Vertical transmission from dam to offspring does not occur or at best is very rare.

The nonpathogenic herpesvirus of turkeys (HTV) is ubiquitous in turkeys and is probably spread horizontally by the airborne route. When chickens are inoculated with this virus, they do not subsequently develop MD even after infection with virulent Marek's disease virus. The Marek's disease virus, not the HTV, will spread horizontally from dually infected birds. The HTV vaccine is safe and highly effective in preventing MD under field conditions, and most chickens throughout the world are vaccinated with this vaccine.

Other vaccines that have been used but have disadvantages over HTV include the following: (a) the highly pathogenic HPRS 16 strain of Marek's disease virus was attenuated by passage in cell culture. The attenuated virus protects against MD and does not spread, but "over-attenuated" virus does not protect; (b) naturally apathogenic strains virologically, immunologically, and epizootiologically similar to pathogenic strains will protect when administered before infection with the virulent strains; (c) virus preparations that have been chemically treated to inactivate infectivity protect only slightly.

When a candidate vaccine virus for the prevention of herpesvirus-induced cancer in humans is developed, the purity of the vaccine preparations will be easily determined by modern techniques. However, measurements of safety and effectiveness are a significant problem. If, analogous to the MD model, the vaccine will have to be administered shortly after birth and the incubation period to development of neoplasms is long, then pathogenicity tests in nonhuman primates and other animals may be of limited valued. However, biochemical demonstration that the segment of the nucleic acid responsible for oncogenesis is absent from the vaccine virus may be the major indication that the vaccine is nononcogenic and therefore safe. Because of the low incidence of neoplasia and long incubation period, the effectiveness of the vaccine will be difficult to test. The vaccine possibly will protect against an acute manifestation of viral infection.

Future research on MD will be directed to determining the mechanism of protection against disease, i.e., whether immunity is mediated by thymus- or bursa-dependent systems, and to identifying the protective antigen, i.e., which cell surface or an interior antigen induces the protective immunity. The prevention of MD by vaccination may become a very fruitful area for model studies on prevention of human cancer by vaccination.

Introduction

MD² is a preventable, highly infectious, neoplastic condition of chickens and is caused by a herpesvirus. After an incubation period of 1 to 4 months, susceptible chickens infected with a virulent strain of virus develop lymphoid tumors of the nerves; many visceral organs, including liver, gonad, and kidneys; and other organs, including the muscle, eye, and skin. The virus is ubiquitous; but depending on conditions, mortality may vary from less than 1% to over 50% during the life of the chicken population. The virus is cell associated in tumors and in all organs except in the feather follicle where enveloped infectious virions egress from the body. Infection is spread horizontally by the airborne route to other chickens. Infection is not spread vertically from dam to offspring or, at best, only rarely. For more details on the nature of MD and its etiological agent, the reader is referred to the paper of Witter (51) and to other reviews (4, 5, 7, 19, 20, 24-28, 48, 49).

Natural mechanisms of controlling MD have been reported elsewhere (51). The review will concern itself with ways in which man may prevent MD and in which the prevention of MD may be a model for the prevention of human cancer. The 4 methods currently in use will be described: genetic resistance, eradication, chemoprophylaxis, and vaccination.

Genetic Resistance

Chickens vary in their innate susceptibility to MD. The variation in susceptibility has been exploited in laboratory situations to develop resistant and susceptible lines and in commercial situations to render commercial chickens more resistant to disease (14). The rapid success obtained in only

¹ Presented at the symposium "Immunological Control of Virus-associated Tumors in Man: Prospects and Problems," April 7 to 9, 1975, Bethesda, Md.

² The abbreviations used are: MD, Marek's disease; MDV, Marek's disease virus; HVT, herpesvirus of turkeys; EBV, Epstein-Barr virus.
3 or 4 generations in the laboratory (13) has not been attainable under commercial conditions, because the complexity of measuring susceptibility to disease and of breeding for it have resulted in only minimal selection pressure.

Genetic resistance may be a model whereby the variable behavior of putative human tumor viruses in different families or people could be explained. However, it is not amenable to manipulation in man by man. Exceptions to this rule may be developed in the distant future when “genetic engineering” becomes possible.

Eradication

The MDV is highly contagious and, even under the best laboratory conditions, occasional breaks in isolation have occurred. Breaks in isolation are believed by this author to be the source of contamination in some experiments where egg transmission was thought to have occurred (38). Whether or not the virus is transmitted vertically, a large volume of convincing evidence indicates that, at most, egg transmission is extremely rare and is unimportant under commercial conditions (15, 44, 46, 54). Thus, large groups of commercial chickens from infected dams can be reared in isolation, free of infection. Even chicks from eggs containing ovules with maternal hemorrhage or pieces of maternal tissue hatch free of infection (43).

Because MDV is not spread from parent to offspring through the egg, all progeny are hatched free of infection. In poultry houses supplied with biologically filtered air and maintained under positive pressure relevant to the outside, chicks can be reared free of infection (15). However, because of the expense of construction and maintenance and great contagiousness of the virus, this procedure has had limited application and limited success in the field. The possibilities are too great for the virus to enter the isolated flock on personnel, introduced equipment, or feed that under commercial conditions is not adequately decontaminated. Nevertheless, chickens brooded in filtered air positive pressure houses, even for only the first few weeks of life, had a lower incidence of disease than those reared under conventional conditions (3).

Human virus infections can be contained only with difficulty, particularly when the virus is shed well in advance of the signs of the disease. However, isolation of newborn children might delay infection with putative oncogenic viruses until such a time that age resistance (51) develops; i.e., older children may still be susceptible to infection but not susceptible to the development of neoplasms.

Chemoprophylaxis

Although much effort has been expended in the search for chemoprophylactic drugs against MD, there has been little success. A substituted benzimidazole (AUS) appears to partly prevent tumor development but not replication of the virus (Refs. 9 and 42; D. P. Jacobus, personal communication). Also, there has been some success with statolon and exogenous interferon (45) administered around the time of infection with MDV.

MD in chickens is probably an excellent, inexpensive, safe, and rapid system in which to test chemoprophylactic drugs. When a drug is found to be effective against MD, very possibly it will be effective against similar virus-induced neoplasms in humans.

Vaccination

The HVT Vaccine against MD. Herpesviruses similar or identical to HVT (52) have been isolated from all flocks of turkeys thus far tested. They may be present in most turkeys within the flock and may persist in some turkeys throughout their lives. Like MD in chickens, HVT in turkeys is most readily spread horizontally through the air (53). However, under usual conditions, HVT does not spread horizontally in chickens (31, 52, 53). HVT is closely related to MDV serologically (22, 29, 52). Thus, these viruses share at least one A-antigen and at least one B-antigen in common when tested by double diffusion in agar. Antiserum to both viruses will stain antigens in cells infected with either virus in the indirect fluorescent antibody test although homologous staining is much brighter than heterologous staining and the pattern of staining with the 2 antisera differs. The viruses produce morphologically distinct plaques in cell culture. Hence, HVT can be distinguished from MDV, and antibody to HVT can be distinguished from antibody to MDV.

By a variety of tests, HVT is nonpathogenic, although recently this virus has been reported to cause minor lesions in the nerves (R. L. Witter, personal communication). In short- and long-term trials, when chickens are inoculated with high doses, HVT does not produce any untoward effects (30, 32). The virus is also nonpathogenic for turkeys (50) and primates (40, 41). Even when blind passed 10 times consecutively in chickens, it did not gain pathogenicity (30). Thus, the HVT vaccine can be considered to be nonpathogenic or nononcogenic, or both, for all species thus far tested, and no evidence indicates that it contains genetic information for the induction of tumors.

Both in laboratory and in field trials, the HVT is highly effective in preventing MD (22, 28, 31, 32). As expected, vaccination with HVT must precede challenge with virulent MDV in order to obtain protection (23). As few as 5 cell culture plaque-forming units will protect 50% of the chickens when their immunity is challenged 3 weeks after vaccination (33). Some protection is obtained with massive doses of HVT given at the same time as the MDV challenge (W. Okazaki and H. G. Purchase, unpublished observations). Under these circumstances, the race between the induction of immunity by HVT and production of tumors by MDV is won by HVT because of the large dose of the virus inoculated. Thus, HVT is safe and highly effective in preventing MD under field conditions, and most chickens throughout the world are now vaccinated with this vaccine.

In the United States, the vaccine is the cell-association virus and consists of HVT-infected live tissue culture cells preserved with dimethyl sulfoxide in liquid nitrogen until just before application of the vaccine. In other countries and recently also in the United States, virus extracted from disrupted cultured cells (there is no cell-free virus released
into cell culture supernatants) is lyophilized and used as a vaccine. As might be expected, maternal, passively acquired antibody increases the amount of cell-free virus required to produce a satisfactory immunity but has little or no effect on the amount of cell-associated virus required to produce a satisfactory immunity (28). The widespread use of the HVT vaccine against MD in the United States since 1970 has resulted in more than an 80% reduction in the number of chickens condemned from MD in poultry processing plants. A similar reduction in mortality during the growing period has been observed in vaccinated egg-laying chickens, and the vaccinated chickens lay approximately 4% more eggs than do unvaccinated chickens. Indirectly, from this information, one must conclude that "subclinical" infection with MDV has resulted in a suboptimal performance of the chickens. Many of the chickens may have had a disease resembling infectious mononucleosis. Because of the ubiquitous nature of the MDV, its full effect was appreciated only when the improved performance after vaccination was noticed.

In spite of the effectiveness of the vaccine against MD, HVT does not appear to interfere with the replicative cycle of MDV. Thus, MDV will infect HVT-vaccinated birds, replicate, persist, and be shed from their feather follicles in the usual manner although at a slightly reduced rate. Recent observations that immunosuppression with large doses of cyclophosphamide will compromise the protection offered by HVT indicate that the immune system of the host plays a major role in protection (34). However, humoral neutralizing antibody, cellular antiviral immunity, and interferon, although present and partly effective, do not fully account for the protection observed. Thus, the major mediators of protection are considered to be immunological reactions against the cells that form the tumor. The most likely appear to be cell-cell interactions involving "killer" or "suppressor" thymus-derived cells and virus-infected cells with unique surface antigens. Antigens have been detected on the surface of infected cells in culture (10, 17). Recently, a common antigen has been detected on the surface of MD tumor cells, a transplantable MD tumor, and a MD tumor cell line (R. L. Witter, personal communication). This antigen is an excellent candidate for the mediator of immunity and is presently being studied with this in mind. The antigen does not appear to be analogous to the membrane antigen in EBV-infected cells (18), because the MD antigen is also present on cells not producing virus.

Alternate Vaccines against MD. The 1st vaccine found to be effective in preventing MD was derived from a highly virulent strain by attenuation in cell culture (11, 12). The attenuated virus was also highly effective in the field (6) and was sold commercially before it was superseded by the HVT. Like the HVT, the attenuated MDV does not spread from chicken to chicken. Also, it is distinguishable from the virulent virus by plaque morphology and by loss of the A-antigen that is normally present in the supernatant of infected cell cultures. However, the attenuated virus does not replicate as rapidly as does HVT, and the yield of cell-free virus on extraction is very much lower than that from HVT.

Mild strains of MDV have been modified in cell culture (7, 35–37) or in embryonated eggs (47), so that they no longer cause disease and yet retain the ability to spread from chicken to chicken. Apart from their loss of pathogenicity, these viruses have properties similar to those of field strains of MDV. When these viruses are used, protection against MD should be obtainable if a few vaccinated "seeder" chicks are placed in the flock and the virus is allowed to spread by natural means. If this technique were successful, the amount of vaccine and labor required for administration of the vaccine would be greatly reduced. In practice, however, the virulent virus enters most commercial flocks before immunity can be established by use of the seeder chick technique; thus, in commercial situations all chicks must be vaccinated (7). An additional disadvantage of these viruses over the HVT is that they replicate more slowly than HVT does and little or no cell-free virus can be extracted from cultures infected with them.

An apathogenic virus isolated from chickens in the field (55) has been used successfully as a vaccine. This virus does not replicate well in cell culture and must be distributed and used as infected whole chicken blood. This form of vaccine is expensive to produce, and each batch is impossible to control sufficiently for safety and effectiveness; therefore, it has only limited use.

The limited attempts to prepare "killed" virus vaccines against MD have until recently been unsuccessful. Preliminary results (17) with crude membrane extracts have shown promise, but the work needs confirmation. Viral envelope or cell membrane antigens from material known to be onco- genic must be proved free of all traces of nucleic acids before they can be used in humans and the proof may be hard to obtain. Not until the antigen responsible for protection is identified and purified will a subunit vaccine be a possibility.

Relevance to the Prevention of Human Cancer. When a candidate live virus for the prevention of herpesvirus-induced cancer in humans is identified, its purity will be easily determined by modern techniques. However, measurements of safety and effectiveness are a significant problem. Pathogenicity or safety tests in nonhuman primates and other animals may be the only in vivo tests permissible and may be of limited value. Biochemical demonstration that the segment of the genetic information responsible for oncogenesis is absent from the clone purified vaccine virus may be the major indication that the vaccine is nononcogenic and therefore safe. The difference between the genetic information in MDV and HVT, when elucidated, will undoubtedly be a model for differentiating between oncogenic and nononcogenic viruses of mammalian origin.

Because of the low incidence of neoplasia among humans, the long incubation period, and the difficulties in challenging vaccinated people with oncogenic viruses, the effectiveness of the candidate human vaccine will be difficult to test. With present techniques, testing would require extremely large numbers of, possibly, newborn babies, and the time required to obtain results could run into decades. The problem may be facilitated if the oncogenic virus produces an acute manifestation against which the vaccine will protect. New chemical and immunological techniques that indicate the effectiveness of the vaccine are needed.

Some, although not all, of the above problems would be
eliminated if a killed virus vaccine was developed. The MD model has not been exploited to any extent, probably because the live virus vaccines are so safe and effective. However, there is great potential for killed MD vaccines as models for vaccines against human cancer.

**Nonspecific Immune Alteration.** Results of attempts to influence the immune system nonspecifically, e.g., with mycobacterial products, have been variable (J. M. Sharma, personal communication). Because of the clear separation of the central organs for immunity in the fowl, namely, the bursa of Fabricius and thymus, the immune system can be manipulated in ways that would not be possible in mammals. The potential for exploring the mechanism of protection (and of pathogenesis) are enormous, and MD in chickens is an exciting model for human cancer.

**Conclusions**

The EBV and MDV are in many ways similar. Both are herpesviruses that on infection immortalize lymphoid cells (2, 21). Infections *in vitro* may be productive or nonproductive; and a variety of antigens, some of which are analogous in the 2 systems, are produced. Whereas, *in vivo* infection with MDV may be nonproductive, productive of viral proteins, or productive of complete virus, only the 1st host cell-virus relationship has been found *in vivo* with EBV. The site of productive replication is still being sought. MDV infection may be silent (55), as EBV infection may be (16). MDV may produce a transient disability (1, 39) with complete or partial recovery and decrease the performance of the chicken. EBV induces infectious mononucleosis that has many similar properties (8). Under certain conditions, MDV may be highly oncogenic, and EBV may also be highly oncogenic and the cause of Burkitt’s lymphoma. If the analogy between EBV and MDV carries further, exciting possibilities emerge. Nonpathogenic human herpesviruses related to EBV, attenuated strains of EBV, and nonpathogenic viruses of animals closely related to humans (nonhuman primates) may already be in existence and could be candidates for the prevention of herpesvirus-induced human cancer. The challenge for virologists, immunologists, and biochemists is to develop techniques that will allow us to infer the safety and effectiveness of these viruses as vaccines for humans.

Future research on MD will be directed to determining the mechanism of protection of HVT against disease, i.e., whether immunity is mediated by thymus- or bursa-dependent systems, and to identifying the protective antigen, i.e., which cell-surface or interior antigen produces the protective immunity. The prevention of MD by vaccination may become a very fruitful area for model studies on prevention of human cancer by vaccination.

**References**

H. G. Purchase

Prevention of Marek's Disease: A Review

H. Graham Purchase