Natural Mechanisms of Controlling Lymphotropic Herpesvirus Infection (Marek’s Disease) in the Chicken

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Summary

Four categories of natural resistance phenomena against Marek’s disease in chickens, i.e., maternal antibody, age resistance, genetic resistance, and natural immunization, are described. In nature, these forms of resistance probably act in concert through complex interrelationships. The mechanisms are not well understood but include humoral immunity (maternal antibody), cell-mediated immunity, and possibly other mechanisms. The possible role of newly discovered tumor-specific antigens in cell-mediated immunity against Marek’s disease lymphomas is discussed.

Introduction

MD\textsuperscript{1} is a lymphoproliferative disease of chickens caused by a herpesvirus (24, 43, 74). The pathology and pathogenesis of MD contain many analogies to that of infectious mononucleosis or Burkitt lymphoma (46, 51). Thus, on both etiological and pathological grounds, MD appears to be a valuable model for the study of herpesvirus-related lymphoproliferative disease in man.

This paper is designed to review those factors occurring naturally in chicken populations that enable the MD-infected chicken to resist the lethal consequences of the disease. The exploitation of these or other factors by man for control of MD in commercial chicken populations has been discussed elsewhere (51). For this discussion, natural resistance to MD is subdivided into: (a) that conferred by maternal antibodies; (b) that occurring in older chickens; (c) that occurring in certain genetic strains of chickens; and (d) that conferred by exposure to naturally avirulent strains of MD virus. Some background on the epidemiology of MD, as well as a general discussion of tumor immunity and the role played in such immunity by MD tumor-specific antigens (85), is also presented.

The Epidemiological Scenario

The environment of commercially reared chickens has become increasingly more conducive in recent years to the spread of infectious disease. Chickens are reared in high density not only within houses but also within farms and entire geographic areas. There is generally no practical way to prevent the carry-over of infection between succeeding flocks of chickens or the spread of infection between groups of chickens in adjacent houses. Under these conditions, MD became in the late 1960’s the most economically devastating disease of chickens, causing mortality losses of up to 30\% or higher in flocks raised for egg production and condemnation rates for gross tumors of 15\% or higher in 7- to 9-week-old broiler chickens. The isolation of the nononcogenic HVT (82) and the discovery of its immunogenic potentials against MD (45, 55) paved the way for the new widespread immunization of commercial broiler and layer chickens with HVT vaccine that has resulted in substantial reductions in MD incidence.

Despite the reduction in MD mortality and condemnations achieved through vaccination, the frequency of MD virus infection remains high. Not only are most flocks exposed to MD virus (22, 36), but virtually every chicken in exposed flocks also is infected and is a permanent carrier of the virus (84). MD virus is not spread by congenital infection of embryos (58, 72, 74, 78, 79) but is instead most commonly acquired through exposure to a contaminated environment. The environment becomes contaminated with enveloped, infectious MD virus produced in and shed from the feather follicle epithelium of the infected chicken (16, 18, 44). Infection has been associated with air (26, 63), dust (5, 38), and litter (80). The presence of such infectious material in the environment undoubtedly accounts for the extreme contagiousness of the disease. Filtration studies of contaminated air indicate that MD virus is associated with larger (\textgtrsim 2 \mu m) physical particles (13, 17) such as desquamated epithelial cells (19); however, some infectious cell-free MD virus has also been derived from dust extracts (19, 33). Virus shed into the environment is relatively stable under optimum conditions and may constitute a source of infection over a period of many weeks (5, 37, 38, 80). Vaccination with HVT has not greatly influenced this aspect of the epidemiological picture although the rate of MDV shedding in HVT-vaccinated birds is reduced to some degree (28, 54).

Several factors influence the occurrence of MD in infected chickens; some of these, including maternal antibody, age, genetic constitution, and immunization, form the principal subject matter of this report. Others not discussed here in detail include the pathogenicity and strain of virus, the exposure dose, and stress. Isolates of MD virus vary widely in their pathogenicity (7, 52), ranging from highly oncogenic to apathogenic, and viruses of all types...
Maternal Antibody

Because most adult chickens carry humoral antibodies against MD virus, virtually all progeny chicks are hatched with levels of passively acquired maternal MD antibody. Moreover, because of vaccination with HVT, many chicks now receive antibodies against both MD virus and HVT from their dams. 

Chickens that are derived from infection-free dams and that thus lack maternal MD antibodies are substantially more susceptible to MD mortality and induction of lymphoproliferative lesions than are chickens that are derived from infected dams and thus possess maternal antibodies (23). Furthermore, the severity of acute necrotizing lesions and the incidence of virus-infected cells in the blood were reduced (11, 14) in chicks with maternal antibody, and the latent period for development of lymphoproliferative lesions was extended (11). The relationship of this protective effect to the passively acquired antibodies is further supported by the disappearance of protection concomitantly with the disappearance of antibody at about the 3rd week of age (75). Some characteristics of the resistance induced by maternal antibodies are given in Table 1.

The mechanism by which maternal antibody exerts its protective effect is not fully understood. However, antibody has a neutralizing effect and appears to inhibit the dissemination of virus within the host even though the infection appears closely cell associated (11, 14). Because naturally occurring antibody is reactive against the viral membrane antigen synthesized by productively infected cells in vitro (20) and against a presumably similar membrane antigen on certain lymphoid cells in vivo (3), direct lysis or inhibition of infected cells in the chicken might occur through the coating action of cytophilic antibodies directed at viral membrane antigens. However, direct supportive evidence for this hypothesis is lacking.

Antibodies directed against HVT occurring in progeny of HVT-vaccinated dams also have significant effects on MD. Such antibodies have been found protective against MD in some (11, 27) but not all experiments (14). From the established immunological relationships between HVT and MD virus (53, 82), HVT antibodies would probably be reactive against MD virus in vivo but possibly to a lesser extent than homologous MD antibodies.

The presence of maternal MD antibody in chicks will not prevent infection and will prevent the lethal consequences of the disease at best in only a small percentage of the exposed population. However, the phenomenon is of interest as a distinct type of natural MD resistance. No parallel resistance appears to be induced through active antibody formation because the susceptibility of chickens to MD is unaltered by bursectomy (30, 47) and because passive antibody administered 7 days after infection is nonprotective (14). Thus, the influence of maternal antibody is apparently directed against the initial dissemination and replication of virus in the host and has little influence on the lymphoproliferative lesions subsequently formed.

Age Resistance

For some time, we have known that older chickens are more resistant to MD inoculation than are youngsters (6, 62). More recently, age resistance has also been demonstrated in isolation-reared chickens free of prior MD virus infection (78, 80, 83), a finding that clearly differentiated this resistance from that induced by avirulent MD virus infection. Witter et al. (83) found 20-week-old chickens refractory to 10^3 gross-tumor-inducing doses of MD virus, but they were no more resistant to virus infection than 1-day-old chicks. Variations in the degree of resistance (4, 83) and the age at which this resistance is manifested (15) have been noted.

Sharma et al. (68) studied the resistance of 12-week-old chickens to inoculation with the JM strain of MD virus. Microscopic and even gross lesions were observed at a high frequency shortly after infection, but these lesions apparently regressed because no chickens died during the 20-week observation period and the survivors had only a low frequency of microscopic lesions. Although humoral immune responses in these older birds occurred slightly earlier than in similarly exposed day-old chicks, neither frequency nor magnitude of antibody response differed over the long term. These data (Chart 1) indicated that age resistance was characterized by the ability of the chicks to regress its neoplastic lesions (68). Because the resistance of older chickens was unaffected by bursectomy (67) but was markedly reduced by surgical thymectomy and X-ray (69), this resistance and accompanying lesion regression are

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Degree (%)</th>
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<tr>
<td>&quot;Neutralization&quot; of infectivity</td>
<td>~90</td>
</tr>
<tr>
<td>Cell-associated MD virus</td>
<td>~99</td>
</tr>
<tr>
<td>Cell-free MD virus</td>
<td>~90</td>
</tr>
<tr>
<td>Depression of viremia at 3 wk</td>
<td>~90</td>
</tr>
<tr>
<td>Lengthening of latent period for lesion development</td>
<td>50–60</td>
</tr>
<tr>
<td>Reduction of tumor mortality</td>
<td>Variable</td>
</tr>
</tbody>
</table>

* From data of Burgoyne and Witter (11).

* Both lymphoproliferative (nerve) and necrobiotic (bursa) lesions.
Chart 1. Data on lesion regression in age resistance in MD from Sharma et al. (68). Four to 6 birds were killed at each interval and examined for microscopic lesions in the peripheral nerves and gonad. In addition to these microscopic lesions, gross lesions were observed in one-half of the older chicks at the 4th week postinoculation.

probably a manifestation of cell-mediated immunity. In fact, these studies (Table 2) offer the most convincing evidence to date for the participation of cell-mediated immune responses in Marek’s disease.

The practical implication of age resistance in chicken flocks is not wholly clear. Although natural exposure to MD may occur early (9, 81, 84), this is not invariably true and many chickens are likely to become exposed after age resistance has developed. Also, virulent MD viruses can be isolated from flocks that have never experienced a significant rate of disease (R. L. Witter, unpublished data); but because such flocks are also usually exposed to less virulent strains, their resistance cannot be related solely to their age at initial exposure.

Because all birds exposed even at 1 day of age may not necessarily succumb from the disease, some degree of resistance is possibly manifested at hatching or is acquired shortly thereafter during the period of tumor development. Whether such resistance differs in character from that present in older birds has not been determined.

Genetic Resistance

So-called genetic resistance of chickens to MD has been the subject of considerable study. The principal features of this resistance that characterize it as genetic are: (a) susceptibility differences between lines, sire families, etc.; (b) intermediate susceptibility of F1 hybrid chickens of resistant and susceptible parent stocks; and (c) ability to select for resistance or susceptibility in heterogeneous populations.

Resistant and susceptible lines were developed at Cornell University by Hutt and Cole (25, 34) and at the Regional Poultry Research Laboratory by Waters, Crittenden, and Stone (77). Breeding programs for the selection of resistant stocks in commercial populations have been proposed for the control of the disease (10, 25). Cole’s experiments (25) showed that random-bred stock, in which the MD susceptibility was 51%, yielded resistant and susceptible stocks with susceptibilities of 13 and 91%, respectively, after only 2 generations of selection. Susceptibility was determined by inoculation of 1-day-old chicks that usually had maternal antibodies and by measurement of mortality responses.

Genetic resistance in maternal antibody-positive chickens has been characterized by Sharma and Stone (66) who followed the response of resistant line 6 chickens at weekly intervals and at no time found evidence of lymphoproliferative lesion development. From this observation, genetic resistance appeared different from age resistance where lesions developed but later regressed. Also, viremia in genetically resistant line 6 chickens was lower than in comparable exposed susceptible chickens (66), and the resistance was not overcome by inoculation with 10⁶ times the dose of MD virus required to induce neoplastic response in the susceptible line 7 chicks (65). However, both line 6 and line 7 chickens were equally susceptible to infection.

Workers subsequently noted that genetically resistant chicks with maternal antibodies were considerably more resistant than were chicks lacking maternal antibodies (15, 65, 76). The dramatic acquisition of resistance by antibody-free, genetically resistant line N chicks within the 1st 3 to 4 weeks of life prompted Calnek (15) to propose that genetic resistance was an early expression of age resistance. Rapid
acquisition of resistance has also been observed in line 6 chickens, and resistance in different lines of chickens is clearly acquired at different rates (J. M. Sharma, unpublished data). If this age-dependent manifestation of genetic resistance is similar to age resistance, it also may be related to the ability of the host to mount cell-mediated immune responses. One could hypothesize, therefore, that this aspect of genetic resistance is simply an expression of cellular immunocompetence, the rate of acquisition of which is under genetic control.

On the other hand, maternal antibody-free chicks of genetically resistant lines are generally somewhat more resistant to inoculation at 1 day of age than are comparable chicks of genetically susceptible lines (Refs. 4, 15, and 73; J. M. Sharma and R. L. Witter, unpublished data). Thus, the susceptibility of different lines of chickens to MD virus challenge at hatching is variable even in the absence of maternal antibody. It is not clear whether this early, maternal antibody-independent resistance differs from that expressed in the presence of antibody or that acquired with increasing age. Evidence supporting a distinction in mechanisms lies principally in the presumption that cell-mediated immunity is not fully functional in the newly hatched chick and thus could not account for resistance present at this time. However, this presumption has not been proved to be true; indeed, the several weeks required for lymphoma formation may also result in the acquisition of cellular immunocompetence (61, 71). Nevertheless, it is of possible value to speculate on the existence of 2 types of genetic resistance, one expressed with increasing age through cell-mediated immunity and the other expressed independently upon hatching by other mechanisms. Data in Table 3 illustrate the characteristics of these 2 putative types of genetic resistance. Further work to test the above hypothesis should be of interest.

Some interesting associations have been drawn between MD resistance and the presence of specific alleles at the avian B blood group locus. The original association of the $B^{2}$ allele with MD resistance (32) has now been elegantly confirmed by recent experiments involving crosses and backcrosses of line N, which is homozygous $B^{1}B^{2}$, and line P, which is homozygous for a different allele. However, this $B^{2}$ allele is not present in the resistant line 6 chicken, and, interestingly, line 6 and the susceptible line 7 chickens are both homozygous for the same $B$ allele ($B^{1}$) (49). Thus, although the $B^{2}$ allele seems to be invariably associated with resistance when present, other genetic factors also appear of importance in MD resistance. Of the known $B$ alleles, both $B^{1}$ and $B^{2}$ are associated with low-immune competence, which has been interpreted as a Mendelian restraint of lymphocyte proliferation (41, 42). The possible association of the $B$ blood group locus in chickens with immune responsiveness and tumor resistance may prove to be analogous with the H-2 locus in the mouse, which is clearly associated with immune response genes (40).

### Natural Immunization

The variation in virulence of naturally occurring MD isolates (7) has already been pointed out. It now appears that infection with low virulence or avirulent viruses may constitute an important naturally occurring resistance phenomenon. Low-virulence MD viruses are widespread, coexist with virulent viruses in the same flock of birds (7), and can exist as a dual infection with virulent virus in the same chickens (21). Infection with MD viruses of low virulence offers protection against subsequent challenge with virulent viruses (7, 58-60, 73, 86). In fact, the use of such mild viruses as a control procedure for MD has received considerable attention (59, 60, 86).

There is some disagreement, however, on the extent to which natural exposure with low-virulence viruses modifies the occurrence of MD under normal field conditions. In 1 trial, lanconescu et al. (35) found chickens on commercial farms exposed for 5 weeks to viruses of unknown virulence no more resistant than comparable isolator-reared, infection-free chickens. On the other hand, Biggs et al. (6, 9) found that the ultimate incidence of MD was markedly influenced by the rearing house environment (through 8 weeks of age) and suggested that this effect was related to the pathogenicity of the virus to which the birds were initially exposed; i.e., low-virulence virus protects if it precedes infection with virulent strains. There now seems little doubt,
however, that this phenomenon is a major factor in influencing the response of field chickens to virulent MD exposure and may account for a great deal of the variation in MD incidence between groups of birds reared under comparable conditions.

The mechanism of such protection is probably similar to that induced by immunization of chickens with HVT vaccines. Although the precise mechanism is not known, it appears to have an immunological basis (56) but it is not abrogated by bursectomy (29, 71). On this basis, the protective effect may, like age and genetic resistance, have a basis in cell-mediated immunity.

The various types of MD resistance thus far described probably have in nature a complex interrelationship with each other. For example, the newly hatched chick may be protected initially by maternal antibody and subsequently by exposure to avirulent viruses or increasing age or both. Also, several of the types of resistance described separately may actually have a common mechanism. Although firm evidence is lacking, age resistance, the aspect of genetic resistance expressed through increasing age, and resistance produced by natural vaccination may all have a common basis in the host's cell-mediated immune responsiveness. Based on this assumption, a simplified categorization of the types of resistance would be as follows: (a) humoral immunity as conferred by maternal antibodies; and (b) cell-mediated immunity. The innate resistance present at hatching may constitute a 3rd type of resistance.

Tumor-specific Antigens and Immunity

In the model just presented, humoral immunity would serve to reduce the initial dose of infecting virus and limit its dissemination after infection. Cell-mediated immunity would then be stimulated and offer protection against lymphoma development. This stimulation would occur more rapidly in older, immunocompetent birds and may be influenced by genetic factors. The stimulus for such a cell-mediated immune response is not known but could consist of either viral antigens present in productively infected cells in vivo (1, 48) or of tumor-specific antigens contained on transformed cells (50, 85).

Tumor-specific antigens associated with MD have only been recently recognized (50, 85). We have found such tumor antigens on 3 classes of MD tumor cells, i.e., MD lymphoma cells, cultured cells of the MSB-1 lymphoblastoid cell line (2), and JMV lymphoblastic leukemia cells (64), by indirect membrane immunofluorescent staining with serum from chickens immunized with JMV cells or from rabbits immunized with MSB-1 cells (85). We have provisionally designated this antigen as a MATSA. Evidence for the tumor specificity of MATSA and its lack of relation to viral antigens is given in Table 4. The MATSA's on JMV, MSB-1, and MD lymphoma cells were immunologically related but not identical. Although the MATSA on MD tumor cells stimulates few, if any, humoral antitumor antibodies in infected hosts (85), MATSA may be responsible for induction of cell-mediated, antitumor immune responses. Whereas tumor antigen-stimulated, cell-mediated immunity may explain tumor immunity in older and genetically resistant chickens, it would not necessarily hold for immunity induced by avirulent, non-transforming vaccine viruses. However, the possibility that HVT and perhaps other avirulent viruses may actually induce MATSA-positive, transformed cells is favored by our recent observation of T-cell-dependent, transient lymphoproliferative lesions at 8 to 12 days in HVT-vaccinated chickens. However, MATSA has not thus far been directly associated with these HVT-induced lesions.

The possible stimulatory role of viral-induced cell surface antigens in antitumor immunity is supported by the work of Kaaden and Dietzschold (39), who have reported the immunization of chickens with purified membranes of HVT-infected chick embryo fibroblasts. Moreover, we have found that chickens immunized with HVT do not develop reticular cell proliferative lesions (R. L. Witter and J. M. Sharma, unpublished data) that normally occur 5 to 7 days after MD virus challenge in the thymus and other lymphoid organs and that appear to be a manifestation of productive (lytic) viral infection rather than neoplastic transformation (48). The question of whether tumor antigens or viral membrane antigens or both have roles in inducing MD tumor immunity is an area of considerable current interest.

Table 4

<table>
<thead>
<tr>
<th>Cells</th>
<th>Frequency of antigen-containing cells</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>MATSA⁺</td>
</tr>
<tr>
<td>MSB-1 lymphoblastoid cell line</td>
<td>++++</td>
</tr>
<tr>
<td>MD lymphoma</td>
<td>+ to +++</td>
</tr>
<tr>
<td>JMV lymphoblastic leukemia</td>
<td>++++</td>
</tr>
<tr>
<td>RPL-16 tumor transplant (RNA-virus induced)</td>
<td>-</td>
</tr>
<tr>
<td>REV-transformed spleen cells</td>
<td>-</td>
</tr>
<tr>
<td>Normal spleen lymphocytes</td>
<td>-</td>
</tr>
<tr>
<td>MD-infected fibroblasts</td>
<td>-</td>
</tr>
<tr>
<td>Normal fibroblasts</td>
<td>-</td>
</tr>
</tbody>
</table>

* From data of Witter et al. (85). Staining frequencies scored from ++++ (80 to 100%) to - (0%). Frequencies for MD lymphomas are variable, ranging from 10 to 40%.

* MSB-1 lymphoblastoid cell line was derived from an MD lymphoma by Akiyama et al. (2); MD lymphoma cells were derived from tumors in chickens inoculated with the JM or GA strains of MD virus (85); JMV lymphoblastic leukemia cells were from spleens of chickens inoculated with the JMV tumor, originally derived by rapid passage of MD lymphomas (64); RPL-16 tumor transplant was derived from chickens with lymphoid leukemia (12); REV-transformed spleen cells were derived from chickens inoculated with reticuloendotheliosis virus, strain T (57).

* Detected by indirect membrane immunofluorescent staining on unfixed cells with antisera made in rabbits or chickens against MSB-1 cells or with antisera made in chickens against JMV cells.

* Includes the cellular antigen detectable in fixed cells and the viral membrane antigen detectable in unfixed cells by indirect immunofluorescence with convalescent sera from MD-infected chickens.

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