Oncogenesis of Marek’s Disease

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Summary

Marek’s disease is a lymphoproliferative disease of chickens and is caused by a herpesvirus. Attempts to show the exclusive role of either thymus or bursa of Fabricius in pathogenesis of the disease have been unsuccessful. Infection of chickens with Marek’s disease virus usually remains latent and does not cause a clinical disease. Early infection with apathogenic strains of the virus and viral specific maternal antibody in newly hatched chicks may play important roles in the latency of the virus.

Introduction

MD is a lymphoproliferative disease of domestic fowl and is characterized by lymphoid infiltration in the peripher al nerves and development of lymphoid tumors in the visceral organs, muscle, and skin. The lymphoma appears to be multifocal and affects the ovary, testes, kidney, liver, lung, and heart. The incidence of lymphoma in the ovary seems to be higher than in the other organs. The disease is highly contagious and affects chickens of all ages with predilection for younger chickens. Existing evidence is against the vertical transmission of the disease; most chickens are infected with the virus shortly after hatching.

Among several neoplastic diseases of man and animals with possible herpesvirus etiology, MD is the only naturally occurring lymphoma with a proven etiology. The virus is ubiquitous in nature and can be isolated from chickens raised under natural conditions. However, only a low percentage of infected chickens develop the lymphoma. Chicks hatched from a previously infected hen have maternal antibodies to the virus. The level of this antibody declines and becomes undetectable by 3 weeks of age. However, shortly after infection the level of acquired antibody increases. Infection may induce the development of progressive lymphoma with death or, in some instances, regression. In most instances, the infection remains latent without the development of any signs of disease.

The nature of the natural resistance to MD is not well understood. Experimental inbred lines of chickens have been developed that are highly resistant to the lymphoma. This resistance, however, is not to the infection with the virus but rather to the development of the lymphoma.

Lymphoid cells are not the only type of cells that become infected with the virus. Other types of cells and in particular the epithelial cells of the feather follicle also become infected with the virus. The viral genome is present but usually not expressed in tumour cells and most lymphoid tissues of infected chicken. It can be detected only by in vitro cultivation. Occasionally, however, viral replication proceeds further and viral antigens and viral particles are detected in cells of the bursa of Fabricius, thymus, kidney, and some tumor cells. In the feather follicle epithelium virus replication is complete and highly infectious virus particles are produced. Such infectious virus is presumably released into the atmosphere via dust and dander and can account for the rapid transmission of the disease from infected to noninfected chickens.

Discussion

Cellular Composition of MD Tumors and Nerve Lesions. Marek (19) first described the disease and reported the infiltration of many mononuclear lymphocytes in the peripheral nerves. He described the disease as inflammatory and referred to it as polyneuritis. Later Pappenheimer et al. (25) observed grossly visible lymphoid tumors to be associated with the disease. Because knowledge about the etiology of the disease was lacking, it was not clear whether the nerve lesions and the lymphoid tumors were caused by the same agent. They found the cellular composition of the lymphoid tumors to be similar to the advanced lymphoid infiltrations in the nerves; however, the extent of the lymphoid infiltration varied considerably depending on the stage and severity of the disease. This caused a disagreement among some of the investigators as to the neoplastic nature of the disease.

Lesions observed in MD-infected chickens are either lymphoproliferative, which occur in lymphoid tissue and peripheral nerves, or degenerative. Both types of lesions can be observed in progressively infected chickens, whereas one may find only the degenerative lesions in chickens with little or no disease manifestations.

The microscopic picture of the lymphoproliferative changes has been studied by several investigators. Wight (36) examined rather advanced cases of the disease and classified the nerve lesions into 3 types: (a) a simple cellular infiltration in the nerves with no edema; (b) development of edema with little cellular infiltration; and (c) a massive infiltration of lymphocytes. It was concluded that all 3 types of microscopic lesions were manifestations of the same disease and that the neoplastic development was secondary to the inflammatory response.

Payne and Biggs (27) studied the chronological develop-
ment of lesions induced experimentally. They also described 3 types of lesions but came to a different conclusion. Type A lesion was observed in 14 to 21 days after infection and consisted of massive proliferation of lymphoid cells, demyelination, and proliferation of Schwann cells. A dark, pyknotic cell (MD cell) resembling a degenerating blast cell was also observed in this type of lesion. The type B lesion became common later in the course of the disease and was manifested by a diffuse cellular infiltration and some edema. The type C lesion was observed in clinically normal, mature chickens and consisted of a very light cellular infiltration in the nerves. On the basis of these observations the authors concluded that lesions of MD were related to a primary neoplastic process.

Recently, Okada and Fujimoto (24) examined the nerve lesions of MD with the electron microscope and found 3 types of lesions: type 1 was composed of small lymphocytes; type 2 was composed of a pleomorphic population of small, medium, and large lymphocytes; and the type R was composed of small lymphocytes, plasma cells and reticulum cells.

Lesions in the visceral organs that may have solid tumors have a similar microscopic appearance, regardless of the organ involved. In general, tumors have the same composition as the type A lesions of the nerves and are composed of a mixed population of lymphocytes. The lymphoid lesions of skin are more inflammatory in appearance.

The degenerative changes induced by MDV are usually accompanied by viral replication. These changes occur in the bursa of Fabricius, the thymus, and the feather follicle. Changes in the bursa occur in both the medullary and the cortical regions and consist of atrophy, necrosis, and cyst formation (15, 29). Atrophy also occurs in the thymus and involves the cortical and the medullary regions (29). The degenerative changes of the feather follicle are restricted to the epithelial layer (5, 23) and the lymphoid cells in the area are not usually affected. The degenerative changes are most pronounced in 3 to 4 layers of epithelial cells of the feather follicle where many intranuclear and cytoplasmic inclusions are seen and complete replication of the virus takes place (4, 23).

The Origin of MD Tumor Cells. Although the heterogeneity of cells in MD tumors is rather well established, the origin of these cells is less well understood. Theoretically, MD tumors could originate from a thymus-dependent system that is responsible for cell-mediated immunity, a bursa-dependent system that is responsible for the humoral response in chickens (11), or a combination of these 2 systems. If a single system were responsible for the development of MD tumors, its removal could prevent the development of the tumor. Examples are the failure of avian lymphoid leukosis viruses to induce any lymphoid tumors in bursectomized chickens (10) and the failure of mouse leukemia viruses to induce leukemia in thymectomized mice (14). The situation in MD is not as clear. Data on bursectomy are considerable but not completely conclusive and very little is known about the role of thymus. Both organs are affected by MD (15) and a depression in both cellular and humoral response has been observed as indicated by delayed homograft rejection and poor antibody response (26, 27).

The possibility that the target cells for MD tumors are in the bursa of Fabricius has been studied by several investigators but the data are not completely concordant. Morris et al. (20) observed some decrease in the incidence of the disease after bursectomy whereas Kenyon et al. (16) did not observe any significant effect. Other investigators (13, 28), who studied more critically the effect of bursectomy on MD tumors, found no effect on the development of the disease. Payne and Rennie (28) performed their experiments on chicks neonatally bursectomized, which then were sublethally X-irradiated. They were monitored for their ability to produce antibodies against sheep erythrocytes. They found the chickens negative for immunoglobulin G and immunoglobulin M, germinal centers, and plasma cells. The response of these chickens to infection with MDV did not differ from control chickens. On the basis of these observations, Payne and Rennie (28) and later Fernando and Calnek (13) concluded that target cells for MDV were not in the bursa of Fabricius.

Payne (26) reported that thymectomy has no effect on the development of MD in naturally susceptible chickens whereas thymectomy increases the incidence of lymphoma formation in chickens naturally resistant to the disease. It was suggested that the thymus may have 2 roles in MD: (a) it may contain MD target cells that after becoming infected with the virus are transformed and develop the tumor; and (b) the thymus may also contain immunologically active cells which react against the MD target cells. In naturally susceptible chickens the cellular immune response may not be effective against the target cells, whereas in resistant chickens cellular immune response may destroy the cells transformed by MDV. Thymectomy in such chickens would result in the removal of these groups of active cells and subsequently enhance the development of the lymphoma. However, since thymectomy does not change the development of MD in susceptible chickens it is more likely that MD target cells are not strictly thymus dependent. Thus, existing evidence does not indicate that the MD target cells are either exclusively in bursa or exclusively in thymus. Perhaps both lymphoid systems contain target cells for MDV.

The Nature of Lymphoid Proliferation in MD. The causative agent of MD is now demonstrated to be a herpesvirus and the response of susceptible chickens to the experimental inoculation of the cell-free virus is a typical lymphoma (4, 231). However, the question is still valid as to whether the lymphoid proliferation is a direct result of infection and transformation of the lymphoid cells or is merely a response to 1 or several external stimuli.

Evidence in favor of transforming capacity of MDV is not conclusive. Campbell and Wood (6) demonstrated that in vitro stimulation of MD lymphocytes with phytohemagglutinin causes a blastoid transformation. The authors found viral specific immunofluorescent antigens and viral particles in some of the cells but did not establish a cell line.

Lee (8) also found a high rate of DNA synthesis in leukocytes from infected chickens after a short term of in vitro culture. The rate of DNA synthesis in cultures derived from MDV-infected chickens was significantly higher than that in similar cultures derived from control uninfected chickens.

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or chickens inoculated with the nononcogenic herpesvirus of turkeys (38). Within the group of chickens infected with the oncogenic MDV, the rate of DNA synthesis was also higher in those with lymphoid tumors than in those without tumor. The peak of DNA synthesis was observed 3 weeks post-inoculation and corresponded well with the fulminating state of the disease when mortality due to the lymphoma was highest. From these experiments Lee (18) concluded that lymphoid cells from chickens inoculated with the oncogenic strains of MDV and particularly those bearing tumors have a higher growth rate than similar cells from control chickens.

Viral antigens and viral particles are generally absent in MD tumor cells (5, 21) and in vitro recovery of the virus from lymphoid tumors is relatively low. These findings may indicate that only a low percentage of tumor cells is infected with the virus and the massive lymphoid proliferation is due to an external stimulus. This stimulus may be viral infected cells, viral antigens, or antigens released from the host cells as a result of infection. However, these observations are not strong enough to rule out the possibility of an internal factor being responsible for the lymphoid proliferation in MD. The in vitro assay for the recovery of the virus may not be sensitive enough to detect a high rate of infection in tumor cells. Also, the fact that viral antigens and viral particles are not detected in tumor cells does not rule out the presence of viral genome in these cells. A similar situation exists in tumor cells from Burkitt lymphoma. Viral antigens and viral particles are usually absent in biopsy materials and the established cell lines derived from these biopsies. However, viral genome was demonstrated in these cells by DNA-DNA hybridization (39).

MDV-producing cells cannot be the external stimulus for the lymphoid proliferation since very few, if any, virus-producing cells are found in tumors. Also, little lymphoid proliferation is observed in the site where complete replication of the virus takes place with the exception of the cutaneous form of MD where lymphoid accumulations in the area of the feather follicle were found adjacent to the degenerative changes caused by virus replication (17). The release of normal host antigens as a result of virus replication may also act as an external stimulus for proliferation of the lymphoid cells. Such antigens if not recognized as “self” may create an autoimmune reaction in the chicken and be responsible for the lymphoid proliferation. This aspect of the argument has not been fully investigated and at the present there is no solid evidence to support this hypothesis.

Latency in MD. The fact that infection with MDV is not necessarily followed by the development of progressive lymphoma has been mentioned. In fact, the incidence of lymphoma in chickens raised under natural conditions is relatively low compared to the widespread infection. Host-related and virus-related factors can influence the outcome of infection and may be responsible for latency of the virus. Among these factors the oncogenic property of the virus and the effect of maternal antibody are discussed.

Strains of MDV may vary considerably in their capacity to produce nerve lesions and lymphoma in chickens. There are mild or “classical” strains of the virus and there are also acute strains. Certain strains such as the B14 (29) and the JM (33) produce a high incidence of nerve lesions whereas other strains such as the HPRS-16 (29), GA (12), and the RPL-39 (30) produce a high incidence of visceral tumors. In addition, there are field isolates of MDV with very little or no oncogenicity for chickens (31). It has been shown (9) that experimental inoculation of chickens with attenuated strain of the virus provides protection against subsequent challenge with a highly oncogenic virus. Also, the early exposure of chickens to naturally mild or apathogenic viruses provides (P. M. Biggs, personal communications, 1972) protection against the development of lymphoma upon subsequent natural challenge with oncogenic virus. Therefore, it seems that mild strains of the virus may play an important role in natural control of the disease and the latency of the virus.

The exact role of maternal antibody and actively acquired antibody in latency of MDV infection is not well understood but it has been shown (2, 3, 7) that maternal antibody delays the onset of the disease, reduces the incidence of mortality, reduces the titer of viremia, and indirectly raises the level of antibody in survivors. The peak of virus titer in antibody-free chicks experimentally infected (K. Nazerian, unpublished data) may be reached in 2 weeks postinoculation whereas about 4 weeks are required to reach this peak in antibody-positive chicks; therefore, it seems that maternal antibody, if not completely effective against the development of the disease, at least reduces the level of infection and delays the onset of the disease.

The presence of maternal antibody and its level and the degree of oncogenicity of the virus may have a combined effect on the latency of MDV. Chickens positive for maternal antibody, on one hand, may be capable of limiting the level of infection of the virus as a result of an as yet unknown mechanism mediated by the antibody and thereby delay the onset of the disease. The infection of the chickens with mild strains, on the other hand, may mobilize the immunological system(s) by an as yet unknown mechanism to enable the chicken to resist the lymphoid proliferation that could result from infection with an oncogenic strain of the virus. Once the onset of the disease is delayed, other host-related factors such as age resistance and genetic resistance may play further roles in latency of the virus and natural control of the disease.

References

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