Marek's Disease as a Model for the Landry–Guillain–Barré Syndrome

Latent Viral Infection in Nonneuronal Cells Accompanied by Specific Immune Responses to Peripheral Nerve and Myelin

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In the chicken, Marek's disease virus (MDV) induces a demyelinating peripheral neuropathy that, early in the course of the disease, is histopathologically indistinguishable from that seen in the Landry–Guillain–Barré syndrome in man. A continuing role for a productive infection in the pathogenesis of this disease is unlikely, since neither MDV nor MDV antigens can be characteristically detected in nerves or spinal ganglia examined at necropsy. The authors investigated the possible role of a latent viral infection by explanting and maintaining *in vitro* the sciatic nerves and spinal ganglia from diseased birds. In these tissues, viral specific products were induced and detected by immunofluorescence and ultrastructural methods early after explanation in well-isolated Schwann cells, satellite cells, and lymphocytes. Later, virus was detected in fibroblasts, macrophages, and neoplastic lymphoblastoid cells. Neurons and myelinating Schwann cells, in contrast, did not replicate the agent. Specific cell-mediated and humoral immune responses to chicken peripheral nerve and peripheral nerve myelin were demonstrated early in the course of the disease. When considered relative to potential pathogenetic mechanisms, these results suggest that Marek's disease neuropathy is initiated by the establishment of a latent viral infection in neuronal supporting cells. A specific immune response to viral-induced antigens on these cells could, in turn, result in subsequent demyelination. (Am J Pathol 1981, 103:309–320)

DESPITE years of study, the pathogenetic mechanisms operating in postinfectious demyelinating diseases of the peripheral and central nervous systems remain speculative. Among peripheral nervous system diseases of this type, the Landry–Guillain–Barré syndrome (LGBS) is presently defined as a paralytic, demyelinating disorder frequently preceded by infection with common viruses.[1] Most prevalent amongst these agents are herpesviruses, particularly the Epstein–Barr virus (EBV).[2,3] Marek's disease virus (MDV), an avian herpesvirus which can be considered the chicken counterpart to EBV, induces a paralytic, demyelinating peripheral neuropathy in chickens. Early lesions are histopathologically indistinguishable from those seen in the LGBS of man.[3,4] Because of these similarities, MD provides an attractive, and as yet unexploited model for the study of demyelinating disease of infectious origin.

Central to our understanding of MD (and the LGBS) are the mechanisms underlying the massive lymphocytic infiltration and demyelination of peripheral nerves. Replication of MDV in myelinating Schwann cells with resultant cell destruction and demyelination is unlikely, since neither MDV nor MDV antigens can be characteristically detected in Schwann cells of affected nerves (reviewed by Payne et al[5]).

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Since herpesviruses are the classic examples of agents that induce latent infections,[6] it seemed possible that a latent infection of Schwann cells, either alone or in conjunction with an immune response, might play a role in the pathogenesis of the disease. In the simplest case, a latent infection of myelinating Schwann cells could alter cellular metabolism and result in primary demyelination. However, an immunologic component was considered more likely, since ultrastructural studies have indicated that demyelination does not occur in the absence of infiltrating mononuclear cells.[4]

We first studied the potential role of latent infections by explanting the sciatic nerves and spinal ganglia from diseased birds and maintaining them on monolayers of susceptible cells. This procedure induces active replication of latent herpesviruses, and the presence of virus is scored by the appearance of specific cytopathic effects (CPE) on the monolayers. In addition, the neural cells producing virus can be identified by immunohistochemical and ultrastructural methods. We then assessed a potential immunologic contribution by testing for humoral and cell-mediated immunity to components of peripheral nerves. We report here the results of these studies, and discuss them in the context of mechanisms underlying this demyelinating disease.

Materials and Methods

Chickens

Specific-pathogen-free Single Comb White Leghorn chickens (line 15 × 7 and s-strain) were hatched in our laboratories from eggs obtained from the Regional Poultry Research Laboratory, USDA, East Lansing, Michigan, and the Division of Avian and Aquatic Animal Medicine, New York State College of Veterinary Medicine, Ithaca, New York. To prevent accidental infections, we housed infected and uninfected chickens in separate buildings. We monitored the flocks routinely for MDV infection serologically by employing indirect immunofluorescence tests specific for MDV antigens, and virologically by testing for CPE in primary chick kidney (CK) cultures made from birds randomly selected from the flock.

Viruses: Methods of Propagation and Assay

A classic, neurovirulent strain of MDV, JM 3379, was obtained from the Regional Poultry Research Laboratory. This virus was passaged three times in primary CK cultures and was then maintained *in vivo* throughout the remainder of the study by serial blood inoculations from infected, syngeneic donor chickens. Viral titrations were performed on secondary CK cells grown in plastic flasks (Falcon Plastics, Oxnard, Calif; 25 sq cm growth area) and maintained in minimal essential medium (MEM) supplemented with 10% fetal calf serum (Sterile Systems, Inc., Logan, Utah), 5% tryptose phosphate broth, and 10% lactalbumin hydrolysate. After allowing an aliquot of the pooled, infected blood cells to cocultivate for a 24-hour period, the CK monolayer was then washed free of blood cells, and an agarose overlay was substituted for the liquid medium.[7] Focus-forming units (FFU) were counted after 10 days.

Stocks of herpes simplex virus (MacIntyre strain) were replicated in rabbit skin cells and possessed a titer of about 1×10^8 plaque-forming units (PFU)/ml.

Inoculation of Chickens

One-day-old chickens were inoculated intraperitoneally with a 2-ml aliquot of pooled, whole blood obtained from infected, syngeneic chickens inoculated in the same manner 6 weeks earlier. Each inoculum contained 340–500 FFU of MDV.

Cocultivation of Tissues

Immediately after removal from chickens, relevant tissues were finely chopped and incubated on confluent CK monolayers at 39 C. The monolayers were examined for MDV-specific CPE daily from 3 to 9 days after inoculation.

Immunofluorescence Techniques

Pooled convalescent serum from MDV-infected chickens served as the anti-MDV serum used in an indirect immunofluorescence test. Frozen sections of appropriate tissues were fixed for 20 minutes in acetone at room temperature, air-dried, reacted for 30 minutes with anti-MDV serum, washed four times in phosphate-buffered saline (PBS), pH 7.2, and stained for 45 minutes with fluorescein-isothiocyanate–conjugated rabbit antichicken IgG (Cappel Labs, Cochranville, Pa). After another PBS wash, the tissues were mounted with elvanol and examined with a Leitz fluorescence microscope.

Specificity of the method was established in the following ways: 1) uninfected tissues and CK tissue cultures infected with Newcastle disease virus (NDV) did not fluoresce; 2) infected tissues did not fluoresce when treated with normal serum or anti-NDV serum; 3) bursas of Fabricius from MDV-infected birds demonstrated positive immunofluorescence at 5 days, a

time when active viral replication occurred but not at 14 days, when replication had ceased[8] (M. L. Cook, unpublished observations); 4) absorption of anti-MDV serum with MDV-infected CK cells markedly decreased the staining intensity of infected tissues.

Spinal Ganglia Organ Culture

Chickens were sacrificed by cervical dislocation, and the spinal ganglia were aseptically removed and chopped into small pieces. Ganglia were maintained on collagen-coated tissue culture plates (Costar Corp., Cambridge, Mass) at 39 C with the use of MEM supplemented with 50% fetal calf serum and 1.2% dextrose.[9] Fresh medium was added to the cultured nervous tissue every 2 days.

Microscopic Techniques

Tissues fixed in neutral-buffered formalin were processed for light microscopy by the standard methods described earlier.[10] Since it was necessary to perform other tests and manipulations on the same tissues to be examined ultrastructurally, infected birds were not perfused with fixative. Tissue found to contain substantial amounts of viral-specific antigen following *in vitro* cultivation were selected for study by electron microscopy.

Experimental Allergic Neuritis (EAN)

EAN was induced as described by Petek and Quaglio.[11] Roosters 4 weeks of age and older were injected intraperitoneally with 0.5 ml of diphtheria, pertussis, tetanus toxoids vaccine (Wyeth Laboratories, Marietta, Pa) followed 24 hours later by an intradermal injection of 0.2 ml of a 1:1 homogenate containing 10% rabbit peripheral nerve and Freund's complete adjuvant (Difco Labs, Detroit, Mich).

Skin Testing

Normal birds or those with EAN or MD were tested for specific cell-mediated immunity to a 10% suspension of normal chicken nerve in pyrogen-free PBS (Flow Labs, Inglewood, Calif) by injecting 0.05 ml of the suspension into one wattle.[12] The same volume of PBS was injected into the other wattle as a negative control. Both wattles were biopsied at the site of inoculation 48 hours later. The tissue was fixed in neutral formalin and deep-cut cross-sections were processed and examined by light microscopy.

Purification of Myelin and Detection of Antimyelin IgG Antibodies

Myelin was isolated and purified from normal chicken peripheral nerve by an adaptation of the method described by Wolfgram and Kotorri.[13] Purity of the preparation was determined by electron-microscopic examination.

Peripheral nerve and dorsal ganglia from MDV-infected chickens and birds with EAN were tested for tissue-bound antimyelin IgG antibodies using an indirect immunofluorescence technique. To preserve myelin, cryostat-cut frozen sections were fixed in 90% ethanol at 4 C for 15 minutes. They were then air-dried, stained for 45 minutes with fluorescein-isothiocyanate–conjugated rabbit antichicken IgG, and washed four times in PBS. Peripheral nerves from uninfected chickens provided negative controls. Specificity of the fluorescein conjugate against purified chicken IgG was established with the use of an Ouchterlony technique.[14]

Serums from chickens with MD or EAN were assayed for IgG antibodies reacting with myelin sheaths by the indirect immunofluorescence test described previously. Specificity of the method was determined in the following ways: 1) the reaction of MD and EAN test serums with normal chicken peripheral nerve resulted in the specific staining of myelin sheaths; 2) control chicken serums did not stain normal chicken peripheral nerve; 3) test serum that had been preabsorbed with normal chicken peripheral nerve or with myelin purified from normal chicken peripheral nerve no longer stained the target tissue.

Results

Latent Infections

Recovery of MDV From Tissues Cocultivated on CK Monolayers

To determine which tissues contained virus, samples of blood, kidney, sciatic nerve, and dorsal ganglia from diseased birds were taken at 5, 11, 18, 25, and 35 days after infection and cocultivated on chicken kidney cell monolayers. The results summarized in Table 1 indicate that a viremia was detected at Day 5 and persisted throughout the 35-day period. Virus was recovered from the kidney tissue at all time points and from sciatic nerve and dorsal ganglia starting at 11 days after infection. MDV was cocultivated from 75% of the samples of nervous tissue taken at 18 days and from all tissues at Days 25 and 35. No virus was recovered from identical samples of homogenized tissues, a result consistent with the infection being a latent one.

Table 1—Recovery of Marek's Disease Virus From Tissues Cocultivated on CK Monolayers

Tissues Cocultivated	Days after infection				
	5	11	18	25	35
Blood	4/4*	2/4	4/4	4/4	4/4
Kidney	0/4	4/4	4/4	4/4	4/4
Sciatic nerve	0/4	1/4	3/4	4/4	4/4
Dorsal ganglia	0/4	1/4	3/4	4/4	4/4

* Tissues positive/tissues tested (4 birds/group). Foci first appeared after 5–6 days of cocultivation. Details concerning methods are given in the text.

A histologic examination of the nervous tissues revealed a lymphocytic infiltration of dorsal ganglia by 11 days after infection, followed by similar lesions in the sciatic nerves at Day 18 and later. This paralleled the virologic findings. Since it has been well established that peripheral blood lymphocytes harbor a latent infection with MDV,[15] it was not possible in this experiment to establish whether virus was induced from the invading mononuclear cells or came from parenchymal cells of nerve and ganglia. In an effort to resolve this issue, a timed immunofluorescence and ultrastructural study of dorsal ganglia from infected birds maintained in organ culture was initiated.

Demonstration of Viral Specific Products in Organ Cultures of Spinal Ganglia

Immunofluorescence Microscopy: To localize individual cells that reactivated latent MDV following transfer to organ culture, we explanted and maintained the spinal ganglia of 4–6-week-old infected chickens on collagen-coated tissue culture plates and, at various intervals, tested for viral-specific antigens by immunofluorescence methods. MDV antigens were detected in a variety of well-isolated cells beginning as early as 2 days of cultivation and were expressed in the maximum number of cells after 3–4 days in culture (Figure 1). Although most cells containing viral antigens could not be positively identified, satellite cells were an exception. Thus, as Figure 1 indicates, isolated satellite cells were found to express viral antigens. As a control experiment, ganglia from uninfected chickens were explanted and cocultivated with peripheral blood lymphocytes taken from MDV-infected birds. After 2 and 3 days of cultivation, viral antigens could be detected in the added lymphocytes, but not in any cells populating the ganglia.

Electron Microscopy: Extensive electron-microscopic examinations revealed MDV capsids and nucleocapsids in well-isolated nonmyelinating Schwann cells and in lymphocytes after the tissues had been in organ culture for 2 or more days. The Schwann cells enclosed axons and were surrounded by an intact basement lamina (Figure 2). In addition, nucleocapsids were found in satellite cells (Figure 3), confirming the results of immunofluorescence studies. After 3–5 days in culture, viral capsids were also detected in lymphoblastoid "Marek's disease cells" (considered to be neoplastic and characteristic for this disease) and in fibroblasts and macrophages. In this and the immunofluorescence studies, no viral-specific products were ever detected in neurons or in myelinating Schwann cells.

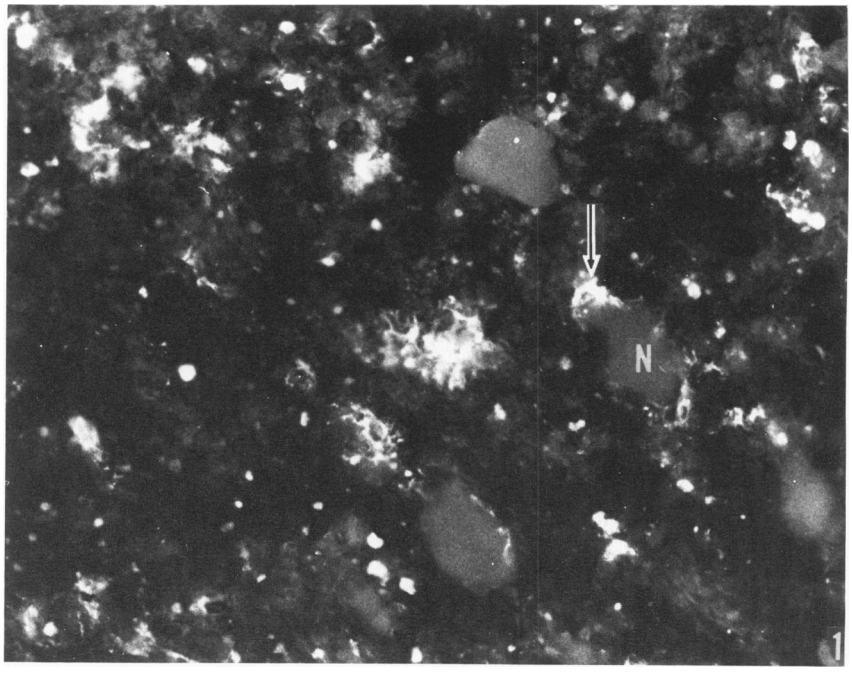

Figure 1—Photomicrograph of fluorescent antibody-labeled Marek's disease antigens in well-isolated cells of a dorsal ganglion. The tissue was explanted from an MDV-infected chicken and maintained in organ culture for 3 days. MDV antigens are present in various cell types, including satellite cells (*arrow*). Neurons (*N*) are not involved. (×440)

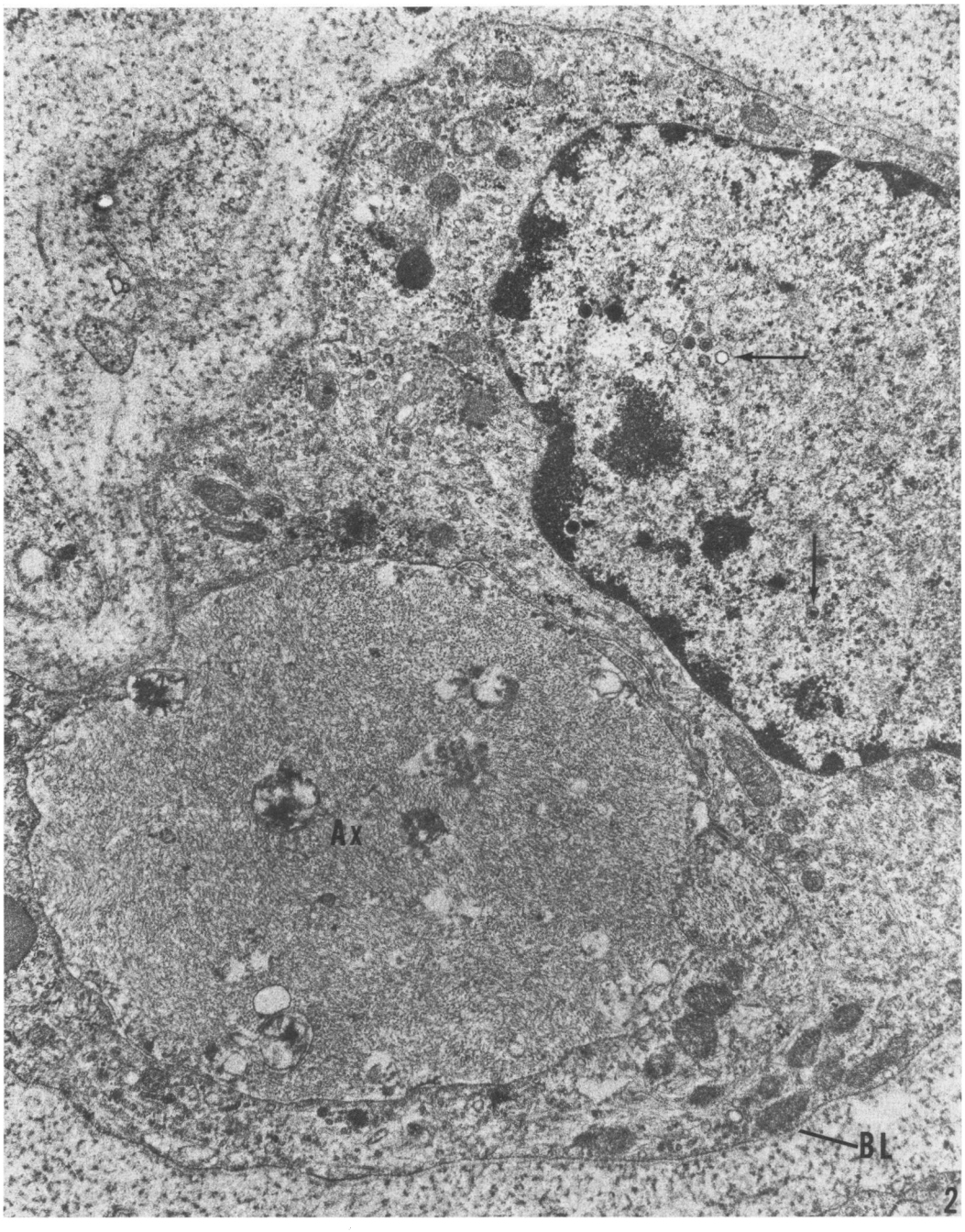

Figure 2—Electron micrograph of a nonmyelinating Schwann cell in a dorsal root ganglion from a MDV-infected chicken. The ganglion was explanted and maintained for 4 days in organ culture. Viral capsids and nucleocapsids are present in the nucleus (*arrows*). The Schwann cell encloses an axon (*Ax*) and is surrounded by an intact basement lamina (*BL*). (×20,000)

Viability of Neurons and Myelinating Schwann Cells

To establish whether our failure to observe signs of viral infection in neurons and myelinating Schwann cells was due either to poor viability or the inability of these cells to support productive viral infection under these conditions, we explanted age-matched control ganglia and infected them *in vitro* with herpes simplex virus. As shown in Figure 4, herpes simplex virions and capsids were readily demonstrated in the nuclei of neurons after 3–5 days in organ culture. Thus, the neurons were still viable and capable of supporting viral replication. An extensive ultrastructural study of myelinating Schwann cells in the herpes-simplex-infected ganglia revealed viral capsids in the cytoplasm of only a single cell. Productive infection in these cells

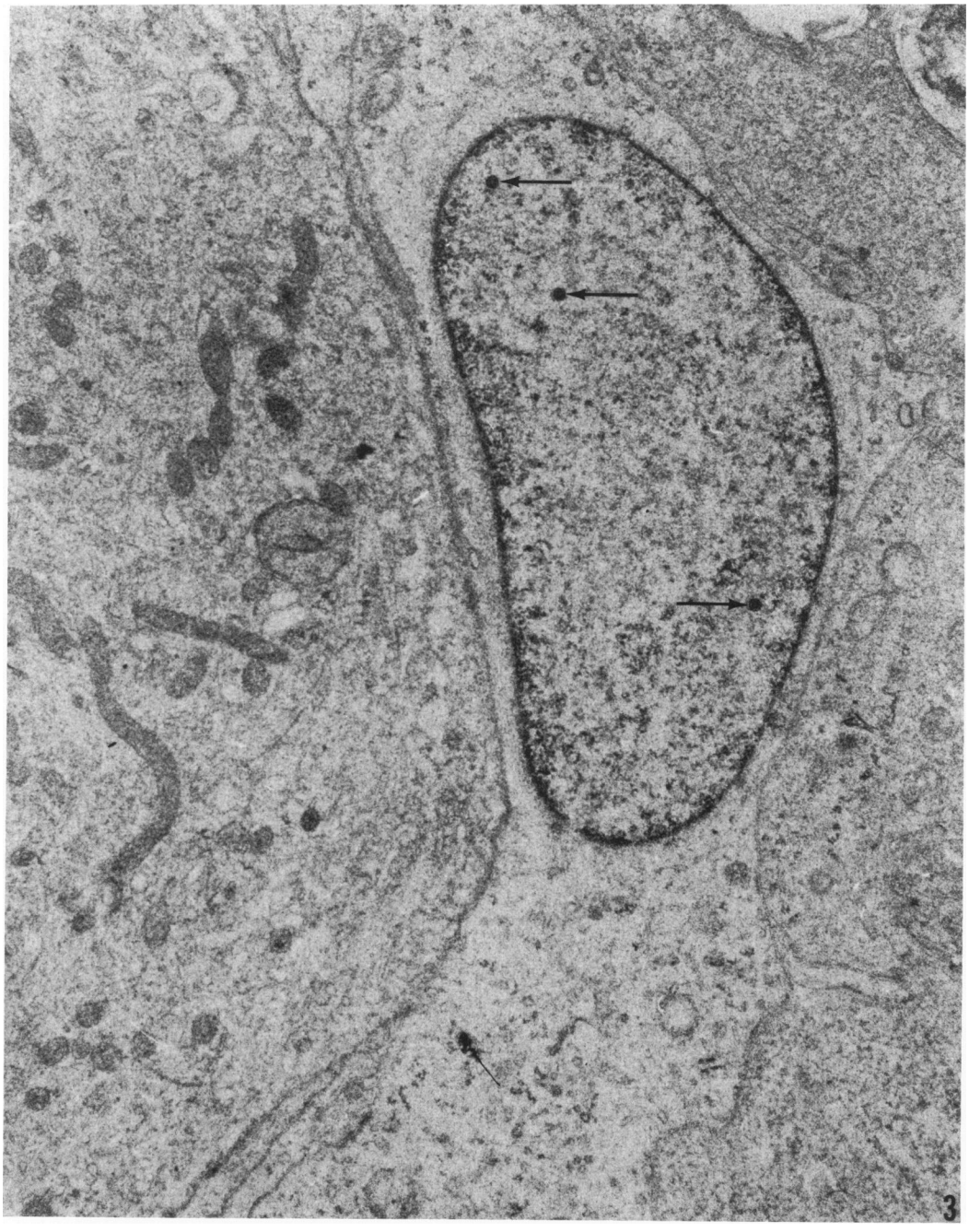

Figure 3—Electron micrograph of a satellite cell in a dorsal root ganglion explanted from an MDV-infected chicken and maintained for 5 days in organ culture. Viral capsids are present in the nucleus (*arrows*). The cytoplasm of the contiguous neuron is also seen. (× 18,000)

is, therefore, rare. In contrast, many nonmyelinating Schwann cells in these ganglia contained virions and nucleocapsids. Although this experiment does not permit us to conclude unequivocally that the myelinating Schwann cells were viable in explants, their morphologic integrity remained intact, strongly suggesting that they were alive, but refractory to infection.

Immunology

Demonstration of Cell-Mediated Immunity to Normal Chicken Peripheral Nerve in MD and EAN

Initially, experiments were designed to determine whether diseased birds mounted a specific cellular immune response to normal chicken peripheral nerve.

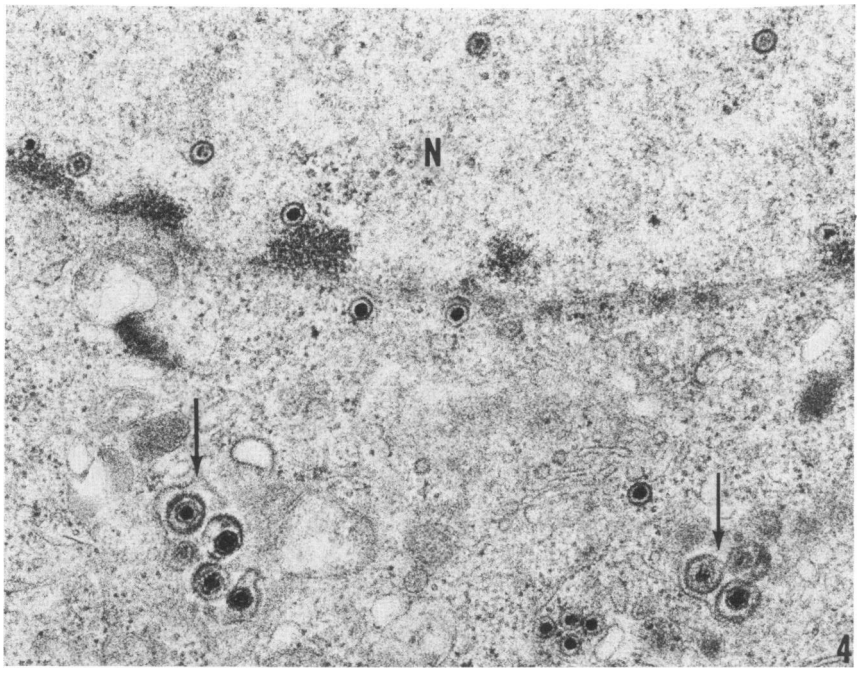

Figure 4—Electron micrograph of a herpes-simplex-virus-infected neuron in a dorsal root ganglion from a chicken. The ganglion had been removed and infected with virus 3 days previously and then maintained in organ culture. Note the capsids and nucleocapsids in the nucleus (*N*) and numerous virions in the cytoplasm (*arrows*). (×32,000)

To first establish the validity of the *in vivo* "wattle test" to be employed, we induced EAN in two age groups of chickens and applied the test to these birds as a positive control. Chickens were tested for delayed-type hypersensitivity to 10% normal chicken peripheral nerve homogenate in PBS by intradermal inoculation of the wattle. The injection of an equal volume of PBS in the opposite wattle failed to produce a histologic reaction at 48 hours in any of the birds tested.

Histologic EAN was confirmed by the detection of microscopic sciatic nerve lesions in 6 of 8 birds 23 days after the injection of homogenized rabbit peripheral nerve in Freund's complete adjuvant. A positive delayed-type hypersensitivity reaction consisting of a lymphocytic perivascular inflammation (Figure 5a) was elicited 48 hours after inoculation in all 8 of the 7-week-old birds with EAN tested and in 7 of 8 of the 12–16-week-old chickens tested 21 days after the induction of EAN.

In studies of MD, birds were tested at 7 and 12–16 weeks after infection. As is shown in Table 2, 11 of 13 birds at 7 weeks after infection demonstrated a typical delayed type hypersensitivity reaction 48 hours after inoculation, again consisting of a pronounced lymphocytic perivascular inflammation (Figure 5b). There was no correlation between the extent of inflammation seen in the wattle test and the number or intensity of microscopic nerve lesions in each diseased bird. A light and electron-microscopic study revealed active demyelination in these nerves examined at 7 weeks after infection. In contrast to the results at 7 weeks, only one of 8 chickens at 12–16 weeks after infection demonstrated delayed type hypersensitivity to chicken peripheral nerve. The intensity and number of sciatic nerve lesions in this group of birds was moderate in comparison to those at 7 weeks after infection, suggesting that some regression of nerve lesions had occurred. Finally, delayed-type hypersensitivity to chicken peripheral nerve was not seen in any age-matched control bird given an identical injection of peripheral nerve homogenate. As shown in Figure 5c, the only histologic reaction observed in the wattles of control birds was that of edema and occasional collections of histiocytes.

Detection of Serum- and Tissue-Bound Antimyelin IgG Antibodies in Birds With MD or EAN

We next initiated studies of humoral immunity in MD by testing the serums and sciatic nerves of 5–7-week-old birds with MD for the presence of antimyelin IgG antibodies. EAN was again induced in age-matched control chickens to serve as a positive control. The test took two forms, both utilizing the indirect immunofluorescence method. First, we tested sections of sciatic nerves from birds with EAN or MD *in situ* for tissue-bound antimyelin IgG antibodies by allowing them to react with fluorescein-isothiocyanate-conjugated rabbit antichicken IgG and then examining the sections for the staining of myelin sheaths. Secondly, we tested serums from birds with MD or EAN for antimyelin IgG antibodies by permit-

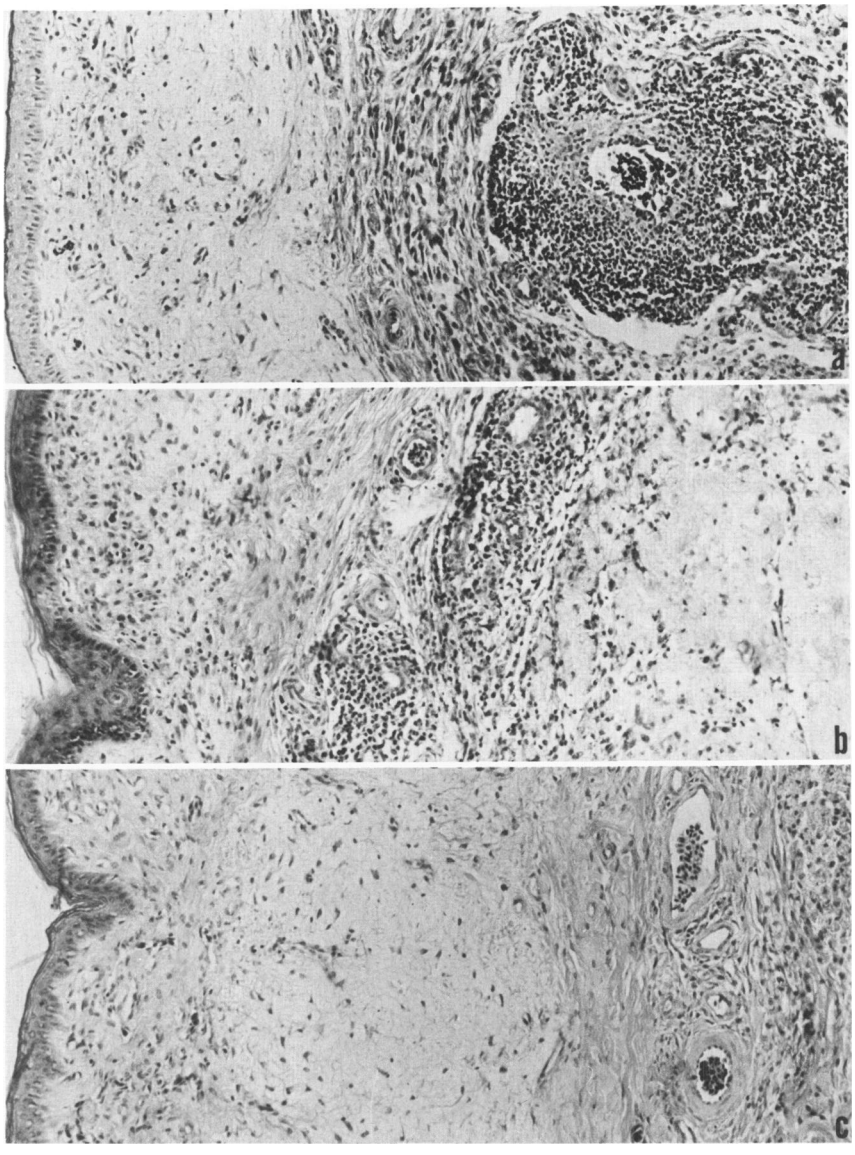

Figure 5—Photomicrographs of wattle tests for delayed-type hypersensitivity to peripheral nerve in birds with EAN and Marek's disease. In all cases, biopsies on wattles were performed 48 hours after intradermal injection of a 10% suspension of peripheral nerve. **A**—Positive reaction in a bird tested 21 days after induction of EAN. An infiltration of lymphocytes is present in the dermis. (H&E, ×150) **B**—Positive reaction in a bird tested 7 weeks after infection with MDV. As in Figure 5a, an infiltration of lymphocytes can be seen in the dermis. (H&E, ×150) **C**—Negative reaction in a hatch-mate to the bird described in Figure 5b. Edema is present in the area of injection. (H&E, ×150)

ting serial dilutions of the test serums to react with sections of normal chicken peripheral nerve.

The results of this study are summarized in Table 3, and specificity controls were as described in the Materials and Methods section. Antimyelin antibodies were detected *in situ* in 3 of 9 MD derived nerves and in 5 of 6 EAN-derived nerves. When antibodies in serums were studied (1:10 dilution or greater considered positive), 7 of 9 serums from MD birds (Figure 6) and 6 of 6 serums from EAN birds were positive. In addition, a direct correlation was found between the antimyelin IgG serum titer and the amount of tissue-bound antimyelin antibodies detected *in situ* in nerves. Thus, only the 3 of 9 MDV-infected birds tested whose serum antimyelin IgG was detectable at a 1:40 dilution or greater demonstrated antimyelin IgG in the sciatic nerves. Antimyelin IgG antibodies were found in all of the EAN serums tested at a dilution of 1:40 or greater. It should also be noted that there was no clear relationship between the level of serum or tissue bound antimyelin IgG and the intensity of the nerve lesions in either MD or EAN. Finally, the antimyelin IgG antibodies detected in MD and EAN were specific for peripheral nerve myelin and did not stain central nervous system myelin or various other control organs in an indirect immunofluorescence test.

Discussion

We have shown here that inoculation of chickens with MDV results in the establishment of a latent viral infection in nonneuronal cells populating peripheral

Table 2—Delayed-Type Hypersensitivity to Chicken Peripheral Nerve in Birds With MD

Age of birds at testing	No. positive/ MDV-infected birds	No. birds tested/ control birds
7 weeks	11/13	0/8
12-16 weeks	1/8	0/8

Biopsies of the wattles were performed 48 hours after the injection of 10% chicken peripheral nerve in PBS and examined by light microscopy for a delayed-type hypersensitivity reaction. Further details concerning the methods are given in the text.

nerves and ganglia. In addition, specific-cell-mediated and humoral immune responses to peripheral nerve and myelin were demonstrated early in the course of the disease. These latter results are consistent with the suggestion that hypersensitivity to components of peripheral nerve (eg, myelin) plays an important role in the pathogenesis of this disease.

As general evidence for the latent infection, virus and viral-specific products were detected in spinal ganglia and sciatic nerves from diseased birds that were explanted and maintained *in vitro*, but not in tissues examined at the time of explant. The ultrastructural observations further indicated that the latent infection was selective with respect to neural cell type. Thus virus could be detected in satellite and nonmyelinating Schwann cells, but not in neurons and myelinating Schwann cells. The isolated location and paucity of supporting cells containing MDV capsids and antigens after 2 days of "induction" in culture, the failure to detect viral products in nonlymphoid cells 2 to 3 days after *in vitro* infection of control ganglia, and the extended replication cycle of this virus give substantial support to our suggestion that satellite and nonmyelinating Schwann cells harbor a latent viral genome. Presently, the only technique which might give more definitive information is *in situ* molecular hybridization. However, our experiments in this area have not been rewarding, since we have not been able to prepare a nucleocapsid preparation sufficiently free of cellular DNA sequences, and a sufficient level of sensitivity has not been reached (J. S. Pepose, unpublished observations). In this regard, it should be noted that the method also lacks sufficient sensitivity for detection of herpes simplex virus genomes in latently infected neurons[6] (D. Galloway and J. McDougall, personal communication) and in lymphocytes latently infected with EB virus.[16]

Clearly, viral infection of the nerve is followed by a specific cellular immune response to peripheral nerve, and a humoral immune response to peripheral nerve myelin. The wattle tests employing syngeneic antigens are consistent with an earlier report indicating that myelin from horse sciatic nerve evokes a similar response.[17] Parallel findings have been reported in EAN[18,19,20] and the LGBS,[21] where protein component P_2 of peripheral nerve myelin was used as the test antigen. A central role for cell mediated immunity (CMI) in the pathogenesis of MD is strongly implicated by two additional findings. First, thymectomy has been reported to reduce the incidence of disease,[5] and secondly, nerve lesions are populated predominantly by T lymphocytes.[22] Mechanisms underlying the anergic reaction and nerve lesion regression observed late in the course of the disease may be interrelated and involve the generation of suppressor T cells.[23] These cells could theoretically suppress specific CMI to nerve antigens such as myelin, as reported in studies of experimental allergic encephalomyelitis (EAE),[24] or induce a nonspecific suppression of CMI. Generalized impairment of CMI in MD has been illustrated by delays in the rejection of skin allografts,[25] transplantable MD lymphomas,[26] and Rous sarcomas,[27] particularly in tumor-bearing chickens. An *in vitro* correlate of CMI, the mitogenic responsiveness of splenic lymphocytes, is also markedly depressed in MD[28,29] and has been recently attributed to a population of suppressor macrophages.[30]

IgG antibodies directed against peripheral nerve myelin reported here have also been found in the LGBS[31,32] and EAN.[33] However, the role played by antibody in the disease is unclear. In our experiments, no correlation existed between antimyelin antibodies in the nerves of MDV-infected birds and the intensity of the nerve lesions. In addition, others have shown that bursectomy produced either no effect[34] or actually enhanced MD,[35,36] and passive transfer of MD serum into naive birds both delayed and lessened the intensity of peripheral nerve lesions when the chickens were subsequently exposed to MDV.[37] Of course, these latter results could be explained by neutralization of infectivity by antiviral antibody present. It is

Table 3—Detection of Serum and Tissue-Bound IgG Antibodies Directed Against Peripheral Nerve Myelin in MD and EAN

Test for antimyelin IgG in	No. positive/No. tested
MD nerves	3/9
EAN nerves	5/6
Normal chicken nerves	0/9
MD serums	7/9
EAN serums	6/6
Normal chicken serums	0/9

Specific staining of the myelin sheaths of normal chicken nerve in an indirect immunofluorescence test using test serum at dilutions of 1:10 or greater was considered positive. Specificity of the reaction was determined by absorption with myelin derived from normal chicken peripheral nerve. Further details concerning the methods are given in the text.

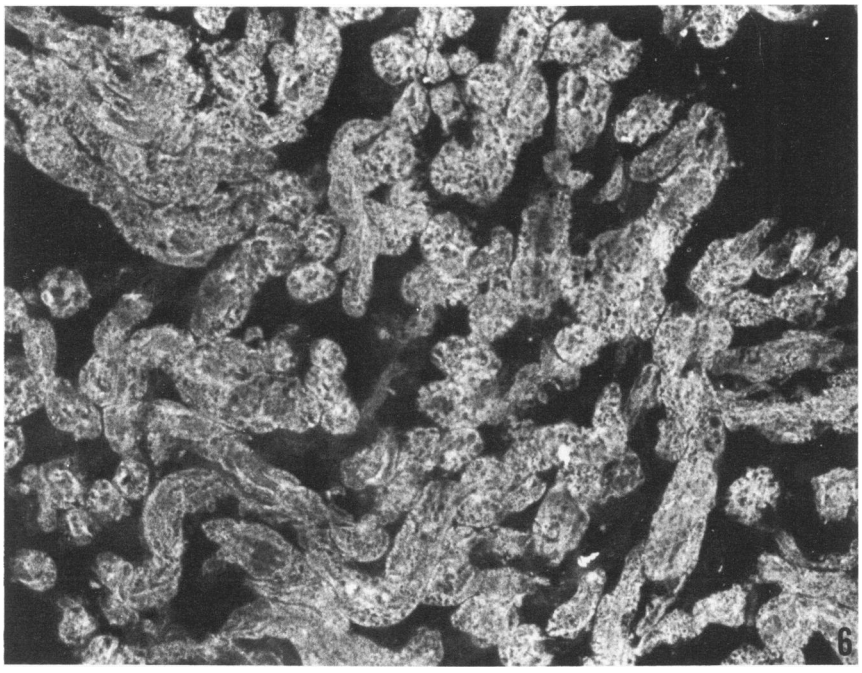

Figure 6—Photomicrograph of sciatic nerve from a normal chicken stained by indirect immunofluorescent methods involving serum from a bird with Marek's disease (primary reagent) and fluorescene-labeled rabbit antichicken IgG (secondary reagent). Myelin sheaths are selectively stained in this micrograph. Controls establishing specificity are described in the text. (× 700)

also possible that the antimyelin antibodies have a protective effect. Such a phenomenon has been demonstrated in passively transferred EAE[38] and in EAN.[1]

When the cell types harboring latent virus and the specific immune responses to nerve components are considered, the following working hypothesis for the pathogenesis of MD seems most reasonable. A persistent cell-associated viremia disseminates to peripheral nerve by means of peripheral blood lymphocytes, resulting in a latent infection of nonmyelinating Schwann cells and satellite cells. As a consequence of the latent infections, a viral-induced antigen is expressed on the cell membrane, and it can be detected by reactive lymphocytes. A cellular immune response directed against viral antigens on neuronal supporting cells may release factors, such as lymphotoxins and proteolytic enzymes, that disrupt the integrity of a few myelin sheaths. Such nonspecific "bystander" demyelination has been reported following the release of myelinolytic proteases from activated macrophages.[39,40] Myelin derived from such disintegrating sheaths, together with viral antigens (which may act as adjuvants), could then sensitize the host against myelin and set the stage for an autoimmune demyelinating process characterized by the selective destruction of myelin lamellas without primary damage to Schwann cells.[41] A mediator in this specific-cell-mediated immune reaction to myelin may be similar to the soluble EAE-producing factor demonstrated in the supernatants of cultured lymphocytes derived from animals with EAE.[42,43]

Obviously, the phenomenon central to these considerations is the presence of the viral-induced antigen in the Schwann cell or satellite cell membrane. Although not yet described in these cells, Ross has presented convincing evidence for a viral-induced membrane antigen in lymphocytes latently infected with MDV,[44] and an antigen with similar properties is known to occur on lymphocytes that are latently infected with Epstein–Barr virus.[45] Neither of these antigens can be detected by standard immunofluorescence methods, but they are demonstrated by their reaction with sensitized lymphocytes from infected hosts.

Further incisive studies of MD are likely to be immunologic in nature and will involve the passive transfer of cells and serums. While histopathologic effects from the passive transfer of serums and an evaluation of the cellular membrane components against which the antibodies are directed at various times during the course of the disease are straightforward, studies involving cellular immunity are not. Thus, while it has been possible for us to induce EAN in naive animals by the transfer of lymphocytes from symptomatic EAN donors,[46,47] a similar experiment in MD[48] is difficult to interpret, because, although MD was produced, the transfused lymphocytes harbor latent MDV and thereby simultaneously transfer the infection. As an alternative approach, the development of an *in vitro* test of CMI to peripheral nerve myelin and myelin components in an avian system would be useful.

References

1. Arnason BGW: Inflammatory polyradiculoneuropathies, Peripheral Neuropathy. Vol 2. Edited by PJ Dyck, RK Thomas, EH Lambert. Philadelphia, W. B. Saunders, 1975, pp 1110-1148
2. Grose C, Henle W, Henle G, Feorino PM: Primary Epstein-Barr virus infections in acute neurologic diseases. N Engl J Med 1975, 292:392-395
3. Borit A, Altrocchi PH: Recurrent polyneuropathy and neurolymphomatosis. Arch Neurol 1971, 24:40-49
4. Lampert P, Garett R, Powell H: Demyelination in allergic and Marek's disease virus-induced neuritis: Comparative electron microscopic studies. Acta Neuropathol 1977, 40:103-110
5. Payne LN, Frazier JA, Powell PC: Pathogenesis of Marek's disease. Int Rev Exp Pathol 1976, 16:59-154
6. Stevens JG: Latent characteristics of selected herpesviruses. Adv Cancer Res 1977, 26:227-256
7. Ubertini T, Calnek BW: Marek's disease herpesvirus in peripheral nerve lesions. J Natl Cancer Inst 1970, 45: 507-514
8. Payne LN, Rennie M: Pathogenesis of Marek's disease in chicks with and without maternal antibody. J Natl Cancer Inst 1973, 51:1559-1573
9. Winkler GF, Wolf MK: The development and maintenance of myelinating tissue cultures of rat trigeminal ganglion. Am J Anat 1966, 119:179-197
10. Cook ML, Stevens JG: Replication of varicella-zoster virus in cell culture: An ultrastructural study. J Ultrastruct Res 1970, 32:334-350
11. Petek M, Quaglio GL: Experimental allergic neuritis in the chicken. Pathol Vet 1967, 4:464-476
12. Warner NL, Ovary Z, Kantor FS: Delayed hypersensitivity reactions in normal and bursectomized chickens. Intl Arch Allergy App Immunol 1971, 40:719-728
13. Wolfgram F, Kotorri K: The composition of the myelin proteins of the central nervous system. J Neurochem 1968, 15:1281-1290
14. Ouchterlony O: Antigen-antibody reaction in gels. Acta Pathol Microbiol 1949, 26:507-515
15. Witter RL, Offenbecker L: Duration of vaccinal immunity against Marek's disease. Avian Dis 1978, 22: 396-408
16. Moar MH, Klein G: Detection of Epstein-Barr virus (EBV) DNA sequences using in situ hybridization. Biochem Biophys Acta 1978, 519:49-52
17. Schmahl W, Hoffman-Fezer G, Hoffman R: Zur Pathogenese der Nervenläsionen bei Marekscher Krankheit des Huhnes: I. Allergisch Hautreaktion gegen Myelin peripherer Nerven. Z Immun Forsch 1975, 150:175-183
18. Brostoff S, Burnett P, Lampert P, Eylar EH: Isolation and characterization of a protein from sciatic nerve myelin responsible for experimental allergic neuritis. Nature New Biol 1972, 235:210-212
19. Abramsky O, Teitelbaum D, Arnon R: Experimental allergic neuritis induced by basic neuritogenic protein (P_1L) of human peripheral nerve origin. Eur J Immunol 1977, 7:213-217
20. Brostoff SW, Levit S, Powers JM: Induction of experimental allergic neuritis with a peptide from myelin P_2 basic protein. Nature 1977, 268:752-753
21. Abramsky O, Webb C, Teitelbaum D, Arnon R: Cellular hypersensitivity to peripheral nervous antigens in the Guillain-Barre syndrome. Neurology 1975, 25:1154-1159
22. Rouse BT, Wells RJH, Warner NC: Proportion of T and B lymphocytes in lesions of Marek's disease: Theoretical implications for pathogenesis. J Immunol 1973, 110:534-539
23. Sharma JM, Witter RL, Burmester BR: Pathogenesis of Marek's disease in old chickens: Lesion regression as the basis for age-related resistance. Infect Immun 1973, 8: 715-724
24. Swierkiosz JE, Swanborg RH: Suppressor cell control of unresponsiveness to experimental allergic encephalomyelitis. J Immunol 1975, 115:631-633
25. Purchase HG, Chubb RC, Biggs PM: Effect of lymphoid leukosis and Marek's disease on the immunological responsiveness of the chicken. J Natl Cancer Inst 1968, 40:583-592
26. Calnek BW, Fabricant J, Schat KA, Murphy KK: Rejection of a transplantable Marek's disease lymphoma in normal versus immunologically deficient chickens. J Natl Cancer Inst 1978, 60:623-631
27. Calnek BW, Higgins DA, Fabricant J: Rous sarcoma regression in chickens resistant or susceptible to Marek's disease. Avian Dis 1975, 19:473-482
28. Lu Y, Lapen RF: Splenic cell mitogenic response in Marek's disease: Comparison between non-infected tumor-bearing and non-tumor bearing infected chickens. Am J Vet Res 1974, 35:977-980
29. Theis GA, McBride RA, Schierman LW: Depression of in vitro responsiveness to phytohemagglutinin in spleen cells cultured from chickens with Marek's disease. J Immunol 1975, 115:848-853
30. Lee LF, Sharma JM, Nazerian K, Witter RL: Suppression of mitogen-induced proliferation of normal spleen cells by macrophages from chickens inoculated with Marek's disease virus. J Immunol 1978, 120:1554-1559
31. Cook SD, Dowling PC, Murray MR, Whitaker JN: Circulating demyelinating factors in acute idiopathic polyneuropathy. Arch Neurol 1971, 24:136-144
32. Tse KS, Arebesman CE, Tomasi TB, Tourville D: Demonstration of anti-myelin antibodies by immunofluorescence in Guillain-Barre syndrome. J Exp Immunol 1971, 8:881-887
33. Yonezawa T, Ishihara Y, Matsuyama H: Studies on experimental allergic peripheral neuritis. II. Demyelinating patterns studied *in vitro*. J Neuropathol Exp Neurol 1968, 27:453-463
34. Payne LN, Rennie M: Lack of effect of bursectomy on Marek's disease. J Natl Cancer Inst 1970, 85:387-397
35. Carte IF, Weston CF, Smith JH: The effect of bursectomy and testosterone propionate treatment on Marek's disease produced by artificial exposure. Poultry Sci 1969, 48:1793-1794
36. Morris JR, Jerome FN, Reinhart BS: Surgical bursectomy and the incidence of Marek's disease in domestic chickens. Poultry Sci 1969, 48:1513-1515
37. Calnek BW: Effects of passive antibody on early pathogenesis of Marek's disease. Infect Immun 1972, 6:193-198
38. Paterson PY, Harwin SM: Suppression of allergic encephalomyelitis in rats by means of antibrain serum. J Exp Med 1963, 117:755-774
39. Wisniewski HM, Bloom BR: Primary demyelination as a non-specific consequence of a cell-mediated immune reaction. J Exp Med 1975, 141:346-359
40. Norton WT, Cammer W, Bloom BR, Gordon S: Neutral proteinases secreted by macrophages degrade basic protein: A possible mechanism of inflammatory demyelination. Adv Exp Med Biol 1978, 100:365-381
41. Lampert PW: Autoimmune and virus-induced demyelinating diseases. Am J Pathol 1978, 91:176-208
42. Whitacre CC, Paterson PY: Transfer of experimental allergic encephalomyelitis in Lewis rats using supernatants of incubated sensitized lymph node cells. J Exp Med 1977, 145:1405-1410
43. Whitacre CC, Paterson PY: Experimental allergic encephalomyelitis transfer activity in Lewis rats: Immunobiologic and initial biochemical properties. J Immunol 1980, 124:1784-1788

44. Ross LJN: Anti-viral T cell-mediated immunity in Marek's disease. Nature 1977, 268:644–646
45. Svedmyr E, Jondal M: Cytotoxic effector cells specific for B cell lines transformed by Epstein-Barr virus are present in patients and infectious mononucleosis. Proc Natl Acad Sci USA 1975, 72:1622–1626
46. Aström KE, Waksman BH: The passive transfer of experimental allergic encephalomyelitis and neuritis with living lymphoid cells. J Pathol Bacteriol 1962, 83:89–106
47. Arnason BGW, Chelmicka-Szorc E: Passive transfer of experimental allergic neuritis in Lewis rats by direct injection of sensitized lymphocytes into sciatic nerve. Acta Neuropathol 1972, 22:1–6
48. Hoffman-Fezer G, Schmahl W, Hoffman R: Zur Pathogenese der Nervenläsionen bei Marekscher Krankheit des Huhnes: II. Übertragbarkeit von Nervenveränderungen mit Milzzellen Marek-Kranker Tiere. Z Immun-Forsch 1975, 150:300–308