INTERNATIONAL SYMPOSIUM on MAREK'S DISEASE

Abstracts - Program

JULY 23-26, 1984
CORNELL UNIVERSITY
ITHACA, NEW YORK, U.S.A.

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INTERNATIONAL SYMPOSIUM ON MAREK'S DISEASE
JULY 23-26, 1984
CORNELL UNIVERSITY, ITHACA, N.Y. 14853

Organizing Committee

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SPECIAL CONTRIBUTION: The Wednesday evening barbecue is generously provided by ISA/Babcock, Ithaca, New York.

The Organizing Committee expresses sincere appreciation to all of the many organizations who, through their generous support, have made this Symposium possible.

PROGRAM

Sunday, July 22

6:00 pm - 8:00 pm Registration and informal wine and cheese party. First floor lounge of the Robert Purcell Union

Monday, July 23 - Morning Session

8:00 Registration--Morrison Hall

8:30 Opening--B.W. Calnek
Official Welcome--E.C. Melby Jr., Dean, New York State College of Veterinary Medicine

8:45 Overview Paper--Comparative molecular characteristics of herpesviruses. Elliott Kieff, (1)

9:45 Coffee Break

SESSION I. MOLECULAR CHARACTERISTICS OF MDV (L.F. Lee, Chairperson)

10:00 Review Paper--Molecular characteristics of Marek's disease virus. K. Nazerian. (2)

10:30 Molecular cloning and hybridization studies with viral DNAs of the Marek's disease herpesvirus system. C.P. Gibbs, K. Nazerian, L.F. Velicer*, H.-J. Kung. (3)

10:50 Possible functions of MDV DNA fragments that might be related to the virulency and to the cross-reactivity with HVT. K. Hirai*, K. Ikuta, K. Nakajima, S. Kato. (4)

11:10 Restriction enzyme analysis of MDV DNA and homology between strains. N. Ross*, B.S. Milne, K.A. Schat. (5)

11:30 The structure of Marek's disease virus (MDV) genome: expansion of a specific region of DNA in non-pathogenic strains of MDV. K. Fukuchi, A. Tanaka, M. Suto, J. Donovan, L. Eklund, J. Jessip, M. Nonoyama*. (6)

11:50 Discussion

12:30 Lunch - Big Red Barn

Monday, July 23 - Afternoon Session

SESSION II. BIOLOGICAL CHARACTERISTICS OF MDV. (T. Mikami, Chairperson)

2:00 Review paper--Biological characteristics of Marek's disease virus. V. von Bulow. (7)

2:30 Isolation and partial characterization of viral polypeptides common to Marek's disease virus and herpesvirus of turkeys. R.F. Silva*, L.F. Lee. (8)

2:50 Analysis of virus proteins specific to and cross-reactive with Marek's disease virus and herpesvirus of turkeys using monoclonal antibodies. S. Kato*, K. Ikuta, S. Ueda, K. Hirai. (9)

3:10 Coffee Break

3:30 Analysis of the precursor polypeptide of Marek's disease herpesvirus A antigen. R.J. Isfort*, R.A. Vrable, K. Nazerian, L.F. Velicer. (10)

3:50 Enzyme-linked immunoabsorbent assay (ELISA) for serotype-specific monoclonal antibodies to Marek's disease virus. L.F. Lee. (11)

4:10 Discussion

5:10 Adjourn

Monday, July 23--Evening Workshops-New York State College of Veterinary Medicine

8:00 pm - 9:00 pm Workshop I - Public Health Concerns
Hagan Room
H.G. Purchase, Moderator

Objectives:

1. Identify how best to respond to allegations of hazard from MD or vaccines.
2. Determine what additional work needs to be done and who should do it.

Workshop II - Monoclonal Antibodies and Cell Lines
Conference Room, Diagnostic Laboratory
K.A. Schat, Moderator

Objectives:

1. Update on availability and characteristics of new materials.
2. Do we need to consider nomenclature or establishment of a registry for MCAs?

9:00 pm - 10:00 pm Workshop III - Recombinant DNA Vaccines
Hagan Room
P.M. Biggs, Moderator

Objectives:

1. What proteins currently identified are most likely to be immunogenic?
2. How best to test candidate proteins for immunogenicity?
3. Is there a need for recombinant DNA vaccines?
4. If so, what is the best strategy?

Tuesday, July 24 - Morning Session

8:30 Overview Paper--Comparative oncology of herpesviruses: Virus-host interactions influencing tumor development. J. Menezes. (12)

SESSION III. VIRUS-HOST CELL INTERACTIONS (E.F. Kaleta, Chairperson)

9:30 Review Paper--Interactions between Marek's disease virus and the host cell. K.A. Schat. (13)

10:00 In vitro infection of lymphocytes with Marek's disease virus. B.W. Calnek, K.A. Schat, L.J.N. Ross, C-H.L. Chen (14)

10:20 Coffee Break

10:50 Use of monoclonal antibodies to identify lymphoid cells in Marek's disease tumors. L. Cauchy, O. Mazzella, F. Coudert*, J. Richard, E. Esnault, F. Kumbaniduwa, G. Dambrine. (15)

11:10 Differential diagnosis of Marek's disease and lymphoid leukosis on the basis of cell surface antigens. C.N. Dren*, I. Nemeth. (16)

11:30 Detection of cell surface antigens on MDCC-MSB-1 cells using monoclonal antibodies. T. Mikami*, T. Higashihara, K. Ohashi, H. Kodama, H. Izawa. (17)

11:50 Discussion

12:30 Lunch - Big Red Barn

Tuesday, July 24 - Afternoon Session

SESSION IV. HOST RESISTANCE FACTORS: IMMUNE RESPONSES (J.M. Sharma, Chairperson)

2:00 Review Paper--Host resistance factors:immune responses. P.C. Powell. (18)

2:30 Pathogenesis of Marek's disease in turkeys. K. Nazerian*, J.M. Sharma. (19)

2:50 Studies on prostaglandin E_2 production in Marek's disease. J.M. Bumstead, I. Flack, L.N. Payne*. (20)

3:10 Coffee Break

3:30 Inhibition of natural killer activity in chickens by Marek's disease virus-transformed cell lines. E.D. Heller*, K.A. Schat. (21)

3:50 Studies on tumor immunity induced by Marek's disease lymphoblastoid cells. L.W. Schierman. (22)

4:10 Potential use of immunomodulators to enhance immune response of turkey herpesvirus (HVT) vaccinated chickens. V. Sivanandan*, D.A. Ogbogu, D.A. Halvorson, J.A. Newman. (23)

4:30 A chromium release assay for the study of cell-mediated immune responses to Marek's disease antigens. K.A. Schat*, E.D. Heller. (24)

4:50 Discussion

5:30 Adjourn

Tuesday, July 24 - Evening

7:00-8:00 Cocktail Hour--Moakley House

8:00 Banquet--Moakley House

Wednesday, July 25 - Morning Session

SESSION V. HOST RESISTANCE FACTORS: NATURAL RESISTANCE (L.W. Schierman, Chairperson)

8:30 Review Paper--Natural resistance to Marek's disease, R. Cole. (25)

9:00 Mortality, virus shedding and immunity to Marek's disease in relationship to chicken-line-specific properties, D. von dem Hagen, H-C. Loliger*. (26)

9:20 Genetic resistance to Marek's disease in congenic strains of chickens. H. Abplanalp*, K.A. Schat, B.W. Calnek. (27)

9:40 Factors influencing resistance and distribution of lesions in chickens exposed to BC-1 and RB-1B isolates of Marek's disease virus. J.L. Spencer*, J.S. Gavora, S.S. Chen. (28)

10:00 Discussion

10:30 Coffee Break

SESSION VI PATHOGENESIS (L.N. Payne, Chairperson)

10:50 Review Paper--Pathogenesis of Marek's disease. B.W. Calnek. (29)

11:20 MDV-induced atherosclerosis and evidence for a herpesvirus role in the human vascular disease. C.G. Fabricant*, J. Fabricant. (30)

11:40 Immunological studies on Marek's disease virus-induced atherosclerosis. J. Fabricant*, C.G. Fabricant. (31)

12:00 The organ distribution of cells expressing tumor antigens in Marek's disease: evidence for spontaneous remission. P.C. Powell*, M. Rennie. (32)

12:20 Extrinsic factors and host resistance to Marek's disease: effects of dietary selenium. B.R. Cho. (33)

12:40 Early mortality syndrome in chickens dually infected with Marek's disease virus (MDV) and chicken anaemia agent (CAA). V. v. Bulow, R. Rudolph, B. Fuchs, H. Landgraf, E. Vielitz. (34)

1:00 Lunch - Big Red Barn

Wednesday, July 25 - Afternoon Session

2:00 Pathogenesis of the turkey herpesvirus (HVT) in chickens inoculated as embryos. J.M. Sharma. (35)

2:20 Studies on the oncogenic properties of various Marek's disease virus strains. J.M.A. Pol*, G.L. Kok, G.F. de Boer. (36)

2:40 Discussion

4:00 Adjourn

Wednesday, July 25 - Evening

5:15 Buses leave for Taughannock Falls State Park for Barbeque (hosted by ISA-Babcock)

9:00 Return

Thursday, July 26 - Morning Session

SESSION VII. VACCINES, VACCINATION AND FIELD PROBLEMS (J.L. Spencer, Chairperson)

8:10 Review Paper--Strategies for the control of Marek's disease through vaccination, R.L. Witter. (37)

8:40 Ten years of experience in the prevention of Marek's disease in the Socialist Republic of Romania. I.V. Patrascu*, P. Stiube, I. Muntean, T. Coman, D. Mihailescu, D. Mihailescu. (38)

9:00 Marek's disease in Egypt. H.H. Tantawi. (39)

9:20 Field studies on the incidence and epizootiology of Marek's disease in broiler breeders in Germany. E.F. Kaleta*, U. Lohren, E. Vielitz. (40)

9:40 Application of partial flock inoculation with an apathogenic strain (HN-1) of chicken herpesvirus (CHV) of Marek's disease to immunize chicken flocks against pathogenic field strains of MD. D.V. Zander*, R.G. Raymond. (41)

10:00 Coffee Break

10:20 Protective efficacy of clones of Marek's disease virus strain. G.F. de Boer*, J.E. Groenendal, H.L. Oei, J.M.A. Pol. (42)

10:40 Lack of association between natural infection with serotype-2 Marek's disease virus and Marek's disease condemnations in broiler chickens. R.L. Witter. (43)

11:00 Polyvalent vaccines for improved control of Marek's disease. A. Zanella*, R. Marchi. (44)

11:20 Some experiences with mono- and bivalent Marek's disease vaccines. E. Vielitz*, H. Landgraf. (45)

11:40 Discussion

12:30 Lunch - Big Red Barn

Thursday, July 26 - Afternoon Session

2:00-3:30 Workshop IV - Control of Marek's disease. W. Baxendale, Moderator

Objectives

1. Define the magnitude of the current problem by identifying the extent of protection and the frequency of vaccine breaks worldwide.
2. How much additional improvement can be realistically expected?
3. What are the most promising strategies for further improvement in control?

3:30 Summing-up. P.M. Biggs

4:00 Closing remarks. B.W. Calnek

4:05 Adjourn

SOCIAL PROGRAM FOR ACCOMPANYING PERSONS

*All tours leave from the front of the North Campus Union

Monday, July 23:

9:00 am-11:00 am Bus tour of Cornell Campus (fee: $5.00/person)

A guided tour by bus of the Cornell University Campus which includes the Johnson Art Museum and the Laboratory of Ornithology at Sapsucker Woods.

12:00-4:30 pm Lunch and Downtown Ithaca

Transportation will be provided into Ithaca for lunch at Oldport Harbour Restaurant - courtesy of the Symposium - followed by an afternoon of shopping, etc. in downtown Ithaca.

Tuesday, July 24:

9:00 am-5:00 pm Bus trip to Corning, N.Y. (fee: $10.00/person includes bus, and admission fees)

To visit the Rockwell-Corning Museum, the Corning Glass Center, and the historic Market Street in downtown Corning.

Lunch available (own expense) on premises at Glass Center or on Market Street.

7:00 pm Cocktails and Banquet--Moakley House (included in registration)

Wednesday, July 25:

8:45 am-4:30 pm Bus trip to Rose Hill Mansion, Geneva, New York and to Sonnenberg Gardens, Canandaigua, New York.(fee: $10.00/person includes bus, entrance fees)

Lunch available (own expense) on premises at Sonnenberg Gardens.

Rose Hill Mansion is a restored early 19th century American mansion. There will be a guided tour. Coffee provided.

Sonnenberg Gardens is a 59-acre estate and botanical garden. Wine tasting.

5:15 pm Buses leave for Taughannock State Park for Barbecue (hosted by ISA-Babcock)

Thursday, July 26: Free

Please sign up for all activities Sunday, July 22 at the registration table.

COMPARATIVE MOLECULAR CHARACTERISTICS OF HERPESVIRUSES (OVERVIEW PAPER)(1)

E. Kieff

Div. of Biological Sciences
The University of Chickago
910 East 58th Street
Chicago, IL 60637

Abstract not submitted

MOLECULAR CHARACTERISTICS OF MAREK'S DISEASE VIRUS (REVIEW PAPER) (2)

K. Nazerian

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Agricultural Research Service
Regional Poultry Research Laboratory
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East Lansing, MI 48823

Abstract not submitted

MOLECULAR CLONING AND HYBRIDIZATION STUDIES WITH VIRAL DNAs OF THE MAREK'S DISEASE HERPESVIRUS SYSTEM (3)

C.P. Gibbs, K. Nazerian, L.F. Velicer, J. Kung

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Michigan State University
East Lansing, MI 48824-1101

Marek's disease (MD) is a lymphomatous disease of chickens, caused by infection with a herpesvirus, Marek's disease virus (MDV). MD is the only neoplastic disease for which a successful vaccine has been developed. The vaccine virus, herpesvirus of turkeys (HVT), is non-oncogenic in chickens. Despite the extensive immunologic relationship between these viruses, previous studies by others showed that the two viral DNAs share little or no homology. Using less stringent hybridization conditions and new methods that greatly improve the reassociation kinetics, we have re-examined the sequence homology between MDV and HVT DNA. The data obtained indicate that HVT and MDV are far more closely related than previously reported. The homology between these two viral DNAs ranges between 70 and 80% at the nucleotide level and extends over 90-95% of the respective genomes.

POSSIBLE FUNCTIONS OF MDV DNA THAT MIGHT BE FRAGMENTS RELATED TO THE VIRULENCY AND TO THE CROSS-REACTIVITY MDV WITH HVT (4)

Kanji Hirai[1], Kazuyoshi Ikuta[2], Kazuhiro Nakajima[2], and Shiro Kato[2]

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Tokai University School of Medicine
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[2]Department of Pathology
Research Institute for Microbial Diseases
Osaka University
Suita, Osaka 565

We have previously shown that the avirulent strains of MDV were probably generated through DNA arrangement that occured in the limited region of the virulent MDV genome. The region was mapped to a 1.22 kilobase (kb) DNA fragment, which was located within the inverted repeated sequences of the MDV genome. By Northern blot hybridization of the polyadenylated RNA from MDV-infected cells with ^{32}P-1.22 kb fragment, the majority of the transcripts were found at the position of approximately 2.5 kb. We also attempted to identify the polypeptides encoded by the 1.22 kb fragment by hybrid selection and *in vitro* translation. The translated polypeptides were immunoprecipitated with MB2 monoclonal antibody reactive with MDV-specific phosphorylated proteins.

Southern blot hybridization under stringent conditions showed that the homology between MDV and HVT is located in the restricted portion within the unique sequences of these viral genomes. The homologous sequences were estimated to consist of 400 to 500 base pairs. By Northern blot hybridization with ^{32}P-labeled HVT DNA fragment containing the homologous sequences, one predominant polyadenylated transcript of, at most, 2.4 kb could be detected in both MDV and HVT infected cells.

RESTRICTION ENZYME ANALYSIS OF MDV DNA AND HOMOLOGY BETWEEN STRAINS (5)

N. Ross[1], B.S. Milne[1], K.A. Schat[2]

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[2]Dept. Avian & Aquatic Animal Medicine
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The results of restriction enzyme analysis have shown that the patterns obtained with SB-1 and RB-1B were similar to those of HPRS-24 and HPRS-16 respectively. Minor differences, which were probably strain-specific, were noted among the strains of each serotype.

A study of the pathogenicity and of the restriction enzyme patterns of several plaque-purified clones of HPRS-16/att indicated that the changes in genetic structure which occur during attenuation were not due to the accumulation of defective molecules during attenuation but probably reflected the selection of variants with mutations in regions of viral DNA that are not essential for infectivity but which could be important for pathogenicity.

Libraries of cloned DNAs derived from type-1 and type-2 virus strains were used in hybridization experiments to identify homologous sequences between the serotypes. The results showed that there is extensive homology among the strains of each serotype but that there is little inter-typic homology (<10%). By decreasing the stringency of hybridization, it has been possible to identify homologous fragments among the serotypes.

THE STRUCTURE OF MAREK'S DISEASE VIRUS (MDV) GENOME: EXPANSION OF A SPECIFIC REGION OF DNA IN NON-PATHOGENIC STRAINS OF MDV (6)

K. Fukuchi, A. Tanaka, M. Suto, J. Donovan, L. Eklund, J. Jessip, and M. Nonoyama

Showa University Research Institute for Biomedicine in Florida
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St. Petersburg, FL 33702

Twenty-seven BamHI fragments of MDV DNA were cloned into bacterial plasmids and restriction maps for BamHI, BglI and SmaI endonucleases were constructed. On the map, as found in HSV-1 and -2, the inverted repeat regions were identified. SmaI digestion gave evidence of the presence of direct repeats SmaI-M (0.9 Kb) in BamHI-F. A majority of the population had two copies of SmaI-M, some containing up to 16 copies.

We have examined restriction patterns of viral DNAs of pathogenic and non-pathogenic strains and observed the disappearance of BamHI-D and -H fragments in DNA of nonpathogenic strains, confirming previous results reported by Hirai, et al. (Microbiol. Immunol. 25 671-681, 1981). BamHI-D and -H are located at the joint regions of U_L and TR_L, or U_L and IR_L. Southern blot hybridization with BamHI-D as a probe revealed that the disappearance of BamHI-D and -H is due to the heterogeneous expansion in TR_L and IR_L.

BIOLOGICAL CHARACTERISTICS OF MAREK'S DISEASE VIRUS (REVIEW) (7)

V. v. Bulow

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In infected chickens, Marek's disease virus (MDV) is present in a cell-associated form in most organs and tissues, and a persisting cell-associated viremia is one of the characteristics of MDV infection. Whole blood cells, purified blood leukocytes, or tumor cells are the most commonly used sources of MDV for virus isolation and infectivity assays. A productive MDV infection, yielding cell-free infectious virus, is consistently present only in the feather follicle epithelium.

Genetically susceptible chickens provide the most sensitive host system for the assay of cell-associated MDV derived from chickens. MDV infection which does not cause visible lesions may be detected by the presence of cell-associated viremia or humoral antibodies.

Chick embryo inoculation is a convenient and sensitive method for qualitative and quantitative assays of cell-associated MDV. Inoculation via the yolk sac or the chorioallantoic membrane (CAM) results in the formation of pocks on the CAM and a splenomegaly. Cell-associated MDV can be recovered from lymphoid tissues of the infected embryo.

A wide range of avian cells can be infected in culture by either cocultivation with infected cells or inoculation of cell-free MDV. Chicken kidney cells (CKC) and duck embryo fibroblasts (DEF) are most commonly used for primary MDV isolation and for virological studies because MDV causes a readily recognizable cytopathic change in these types of

cells. The size and morphology of plaques varies for different strains of MDV, and for each cell type. Infection in culture is rarely fully productive but usually abortive. Virus-associated antigens can be detected by immunofluorescence in the nucleus, cytoplasm, and on the cell memebrane. Several soluble antigens in the culture medium (A antigen) and in extracts of infected cells (B and C antigens) can be detected by immunodiffusion tests. The A antigen is also present at high concentrations in feather follicles and feather tips of infected chickens. Biochemical and immunological properties of membrane antigen and the soluble A antigen suggest that they are closely related.

Multiple passage of virulent MDV in cell culture usually results in attenuation of the virus. Loss of pathogenicity is mostly accompanied by loss of the A antigen and membrane antigen, loss of the ability of the virus to spread horizontally in chickens, and better growth in cell culture, resulting in larger plaque formation.

Three different serotypes of MDV and the closely related herpesvirus of turkeys (HVT) have been identified on the basis of immunofluorescence, agar-gel precipitation and serum neutralization. Serotype-1 comprises pathogenic strains of MDV and their attenuated variants. Naturally apathogenic MDV, which is also characterized by forming small plaques in CKC culture, has been classified as serotype-2. Serotype-3 is represented by HVT.

Different pathotypes of serotype-1 MD viruses, including classical, acute and very virulent strains, could not be distinguished serologically. The very virulent strains of MDV differ from other serotype-1 viruses by their increased pathogenicity, and in that they are not, or only poorly, protected against by HVT or serotype-2 MDV.

ISOLATION AND PARTIAL CHARACTERIZATION OF VIRAL POLYPEPTIDES COMMON TO MAREK'S DISEASE VIRUS AND HERPESVIRUS OF TURKEYS (8)

R.F. Silva and L.F. Lee

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Agricultural Research Service
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Monoclonal antibodies were used to identify Marek's disease virus (MDV) and turkey herpesvirus (HVT) proteins. The monoclonal antibodies immunoprecipitated a total of four proteins of 79,000, 41,000, 38,000, and 24,000 daltons (p79, p41, p38, p24) and three glycoproteins of 100,000, 60,000 and 49,000 daltons (gp100, gp60, and gp49) from MDV or HVT infected cells. Competition immunoprecipitations and limited proteolytic digestion confirmed that p79, gp100, gp60, and gp49 express MDV-HVT-common epitopes as well as serotype-specific epitopes. We could only detect MDV-specific epitopes on the p41, p38, and p24 proteins. We believe the three glycoproteins may be involved in the vaccinal immunity provided by HVT because: (1) The three glycoproteins co-migrate in polyacrylamide gels with three of the five major protein bands immunoprecipitated by convalescent chicken plasma, (2) The glycoproteins possess serotype-common antigenic determinants, expressed on both pathogenic MDV and HVT vaccine strains, and (3) An antigenic determinant responsible for antibody-mediated virus neutralization is present on the three glycoproteins.

Further studies on the three glycoproteins were initiated since they appeared to be involved in the cross protection afforded by HVT against Marek's disease. Pulse-chase experiments and the use of inhibitors of glycosylation suggested that a 44,000 dalton protein is a precursor which is rapidly glycosylated. gp60 and gp49 apparently result from the cleavage of gp100.

ANALYSIS OF VIRUS PROTEINS SPECIFIC TO AND CROSS-REACTIVE WITH MAREK'S DISEASE VIRUS AND HERPESVIRUS OF TURKEYS USING MONOCLONAL ANTIBODIES (9)

S. Kato[1], K. Ikuta[1], S. Ueda[1], and K. Hirai[2]

[1]Department of Pathology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565
[2]Department of Molecular Biology, Tokai University School of Medicine, Bohseidai, Isehara 259-11, Japan

We prepared a series of monoclonal antibodies specific to and cross-reactive with Marek's disease virus (MDV) and herpesvirus of turkeys (HVT). These antibodies formed two groups which reacted with different antigens on the surface of cells infected with MDV or HVT. One group consisted of monoclonal antibodies against glycoproteins (gA) secreted into the medium of cultures infected with MDV or HVT. These glycoproteins may correspond to the "A" antigens of these two viruses. Differences between MDV- and HVT-gA and between virulent and avirulent MDV-gA were observed in the glycosylated or unglycosylated precursor forms. The other group of monoclonal antibodies reacted with three glycoproteins (gB) of MDV and HVT related to viral neutralization. Immunization of chickens with the HVT-gB, purified by affinity chromatography and coupled with monoclonal antibodies, resulted in partial protection against Marek's disease (MD).

Monoclonal antibodies against the MDV antigen purified from extracts of MDCC-MSB-1 cells were found to react with four phosphorylated polypeptides in the MW ranges of 40K to 20K in the MDV-infected cells. The antigen was detected by immunofluorescence tests on several MD lymphoblastoid cell lines cultured at 33°C or treated with 5-iodo-2-deoxyuridine.

ANALYSIS OF THE PRECURSOR POLYPEPTIDE OF MAREK'S DISEASE HERPESVIRUS A ANTIGEN (10)

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Michigan State University
East Lansing, MI 48824-1101

Marek's disease, a lymphomatous disease of chickens caused by Marek's disease herpesvirus (MDHV), can be prevented by the vaccine virus, herpesvirus of turkeys (HVT). These two viruses share several common antigens, including A antigen (MDHV-A, HVT-A), a 61-65 dalton glycoprotein shed in the culture medium of infected cells and immunoprecipitable with rabbit anti-A antisera. As a first step toward determining that portion of the viral genome coding for MDHV-A, experiments were conducted to identify its unglycosylated polypeptide. We have previously reported the immunoprecipitation of a 47K ^{35}S-methionine-labeled molecule formed in the presence of tunicamycin, an inhibitor of glycosylation. Pulse labeling for very short periods of time also revealed an immunoprecipitable 47K polypeptide that was rapidly processed to the fully glycosylated antigen. Finding the same sized polypeptide immunoprecipitatable by rabbit anti-A using two independent methods strongly suggests the 47K molecule is the unglycosylated precursor polypeptide. This was further supported by immunoprecipitating the same size molecule as a translation product after cell-free translation of poly A + RNA isolated from MDHV-infected cells. These data have made it possible to attempt comparison of the MDHV-A antigen and HVT-A antigen precursor polypeptides and to begin hybrid selection and hybrid arrest of translations studies designed to identify the region of the MDHV genome encoding the polypeptide.

ENZYME-LINKED IMMUNOABSORBANT ASSAY (ELISA) FOR SEROTYPE-SPECIFIC MONOCLONAL ANTIBODIES TO MAREK'S DISEASE VIRUS

(11)

Lucy F. Lee

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An ELISA was developed for detection of serotype-specific monoclonal antibodies to Marek's disease virus (MDV). Antigens of all three MDV serotypes (Md11/75c, SB-1, HVT) were propagated in chicken embryo fibroblasts (CEF). Sonicated cell extracts and purified virus were unsatisfactory as the source of antigen for ELISA as they gave low specific and high non-specific reactivities. The virus-infected cells, on the other hand, were found to be a good antigen source with high specific and low non-specific reactivities. The optimum number of MDV-infected cells was determined, by a checkerboard titration of MDV-infected cells and monoclonal antibodies, to be about 5×10^4 cells/well. ELISA and immunofluorescence (IF) were closely comparable in specificity when convalescent chicken sera were used, but ELISA was found significantly more sensitive than IF. ELISA was used to screen over 3,000 hybridomas for monoclonal antibodies against HVT and SB-1, and serotype-specific antibodies with titers up to 10^7 were obtained. Parallel studies using ELISA and IF identified 3 groups of monoclonal antibodies: 1) antibodies which reacted with MDV in ELISA and IF; 2) antibodies which reacted only in IF; and 3) antibodies which reacted only in ELISA. A concordance of 53-80% was found between ELISA and IF. The results showed that the assay is efficient, sensitive, simple and suitable for large-scale screening of monoclonal and chicken antibodies.

COMPARATIVE ONCOLOGY OF HERPESVIRUSES: VIRUS-HOST INTERACTIONS INFLUENCING TUMOR DEVELOPMENT (12)

Jose Menezes

Laboratory of Immunovirology
Pediatric Research Center and
Department of Microbiology
Faculty of Medicine
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A number of herpesviruses show oncogenicity experimentally and are associated with certain tumors in their host, including man. In subjects with immunodeficiencies or immunosuppression, some herpesviruses may, in addition to producing serious or fatal primary or reactivated infections, generate tumors. Several herpesviruses interact intimately with the lymphoid system: for example, Epstein-Barr virus (EBV), besides being a B-cell mitogen, infects and immortalizes B-lymphocytes and produces lymphoproliferative disorders and malignancies under certain conditions. Marek's disease virus and herpesviruses ateles and saimiri produce lymphoid T-cell tumors. Moreover, immunosuppression appears to be a commonly observed, but poorly understood, phenomenon in herpesviruses-associated infections and malignancies. Relevant observations from our and other laboratories bearing on virus-host interactions influencing tumor development will be reviewed, and an attempt will be made to synthesize an up-to-date appreciation of the problem, particularly using the EBV-system as a model.

INTERACTIONS BETWEEN MAREK'S DISEASE VIRUS AND THE HOST CELL (REVIEW) (13)

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Infection with Marek's disease virus (MDV) can result in a variety of interactions with the host cell. The final outcome of the infection of the cell depends on a complex set of factors. These factors are at least partly defined by the infecting virus, the genetic resistance of the host, and the cell type (B-cells, T-cells, epithelial cells, smooth muscle cells, etc.). Cells in the spleen and thymus can adsorb MDV and HVT, suggesting the presence of virus receptors which may differ among genotypes. B-lymphocytes are the major target cells for the early cytolytic, productive-restrictive infection. Epithelial cells undergo a productive or productive-restrictive infection.

Latent infection and transformation are two important features of infection of T-cells with MDV. Both latently infected and transformed lymphocytes express Ia-antigen. It is unknown if the expression of Ia is a prerequisite for or a consequence of infection. The mechanism(s) responsible for the induction and maintenance of latency are not yet elucidated. The transformation of the T-cell to a lymphoblastoid tumor cell most likely requires the continuous activity of a part of the viral genome. Thus far, the transforming sequences and their products have not been identified. Apparently, MDV can maintain the transformed state without the need for virus-produced thymidine-kinase and polymerase. Transformed cells express a number of antigens normally not detected on T-cells (MATSA, fetal antigen, etc).

The interaction of MDV with smooth muscle cells may result in alterations of the lipid metabolism. Presently, it is not known if this is an abortive or a transforming infection.

IN VITRO INFECTION OF LYMPHOCYTES WITH MAREK'S DISEASE VIRUS (14)

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Marek's disease virus (MDV) was serially passaged in lymphocytes and infection was monitored by: 1) virus isolation after co-cultivation on monolayer chicken kidney cell cultures, 2) immunofluorescence tests for viral internal antigen (VIA) and 3) viral DNA hybridization. Dual fluorescence tests detected surface markers (T-cell, B-cell, Ia-antigen) on VIA-positive cells. Most infected cells in cultures at 48 hours postinoculation (PI) were B-cells, but after 96 to 120 hours PI, many Ia-bearing (i.e. activated) T-cells were found to be infected. The proportion of infected T-cells was higher with cells from genetically susceptible donors than from resistant donors.

Only low levels of infection could be serially passaged in spleen cultures free of B-cells, and the level appeared to be virus strain-dependent. The presence of Ia antigen suggested that activation may enhance T-cell susceptibility. Attempts to increase T-cell susceptibility by mitogen (Con-A) stimulation gave confusing results, since the number of VIA-positive cells was less than in nonstimulated cultures in some cases. Possible explanations include: 1) interference with cell-to-cell virus transfer by surface-bound Con-A, and 2) influence of activation on viral genome expression. Activated T-cells resulting from mixed lymphocyte reactions permitted serial passage of MDV.

USE OF MONOCLONAL ANTIBODIES TO IDENTIFY LYMPHOID CELLS IN MAREK'S DISEASE TUMORS (15)

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T- and B-cells were harvested from thymus and bursa of Fabricius of SPF 10-week-old chickens. The cells were treated in 4% tween-40-supplemented Tris HCl bufferred saline (TBS) to obtain crude membrane extracts: Nuclei were discarded. Supernatant was centrifuged and the pellet purified by banding in discontinuous gradients of 10 to 48% sucrose. The lightest major band was collected. The purified membrane extracts constituted the antigens for immunization of mice and antibody testing. ELISA was established using purified membrane extracts. To obtain monoclonal antibodies (MA), Balb C mice were injected 3 times with purified membrane extracts from T- or B-cells. Positive clones were tested for specificity against various lymphoid cells. Immunoconjugate assays of MA on T-cells extracted from different sources were performed. One MA labeled only thymocytes and another labeled thymocytes, peripheral blood lymphocytes and Marek's disease lymphoblastoid cell lines (MDCC-MSB1, MDCC-PAT9, MDCC-PAT5). Assays are in progress to determine the specificity of other MA reacting with T-cells and to identify the specific lymphoid populations present in the tumors.

DIFFERENTIAL DIAGNOSIS OF MAREK'S DISEASE AND LYMPHOID LEUKOSIS ON THE BASIS OF CELL SURFACE ANTIGENS (16)

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A highly specific new diagnostic method described by Neumann and Witter (1979) is based on the detection of characteristic cell surface antigens of Marek's disease (MD) and lymphoid leukosis (LL) tumor cells using the indirect immunofluorescence (IF) test. This technique, however, requires viable tumor cells. We attempted to further improve their method by applying the peroxidase-anti-peroxidase (PAP) technique of Sternberger et al. (1970), according to Manson et al. (1975), on cell smears fixed by buffered formol-acetone.

Antisera against chicken thymus (T), bursa (B), two lymphoblastoid cell lines (MDCC-HP2 and MDCC-RP1), and the heavy chain of chicken IgM were raised in rabbits. To remove irrelevant antibodies, the antisera were extensively absorbed with various normal chicken tissues and glutaraldehyde-insolubilized chicken immunoglobulins and bovine serum. The specificity of antisera was tested by the standard IF method on living cells and the PAP method on fixed cell smears.

Cell surface markers of normal T and B cells, as well as MD virus- and LL virus-transformed cells were detected by IF and PAP methods. The advantage of the PAP method over the IF method is that permanent slides can be prepared which can be counterstained by conventional histological dyes. We found the PAP method to be as sensitive as IF procedure.

DETECTION OF CELL SURFACE ANTIGENS ON MDCC-MSB-1 CELLS USING MONOCLONAL ANTIBODIES (17)

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Monoclonal antibodies against MDCC-MSB-1 cells were established by somatic cell hybridization between mouse myeloma cells and spleen cells from mice immunized with MDCC-MSB-1 cells to analyze the surface antigens expressed on MDCC-MSB-1 cells. The reactivities of the antibodies against various kinds of normal chicken cells and lymphoblastoid line cells derived from chicken tumors of Marek's disease (MD), lymphoid leukosis and reticuloendotheliosis were examined by the membrane immunofluorescence test.

The following monoclonal antibodies were established: three (2B9, 2D8 and 2D5) against MD tumor-associated antigen, two (2E2 and 2G1) against T-cell antigen, one (2H3) against embryonic antigen and one (2C12) against chicken thrombocytes. The three monoclonal antibodies against tumor-associated antigen could be divided into two types: one (2B9) which associated only with the MDCC-MSB-1 cell line and the other (2D8, 2D5) with all of the MD lymphoblastoid cell lines tested. 2E2 and 2G1 reacted with both the MD line cells and thymus cells. 2H3 reacted not only with the various lymphoid cells and erythrocytes from one-day-old chicks but also with three different types of lymphoblastoid cell lines. However, it did not react with erythrocytes from adult chickens. 2C12 reacted with MD line cells, normal chicken thrombocytes, bone marrow cells, spleen cells and 12-day-old embryo liver cells. None of these antibodies reacted with chick kidney cells inoculated with MD virus <u>in vitro</u>.

HOST RESISTANCE FACTORS: IMMUNE RESPONSES (REVIEW) (18)

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Immunity to Marek's disease is complicated by antagonism between virus-induced immunosuppression on the one hand and protective immune responses on the other. Immunosuppression appears to have an important role in the pathogenesis of the disease. Early immunosuppression, following soon after virus infection, probably contributes to lymphoma formation; later immunosuppression, coinciding with the presence of lymphomas, is a consequence of it. Immunosuppression may result from virus-induced cell damage or from the suppressor activities of lymphocytes and macrophages. Nevertheless, protective immunity can develop, and this feature is utilized regularly when chickens are vaccinated against Marek's disease. Protective immunity has both antiviral and antitumor components, and the effector mechanisms involved include B-cells, T-cells, macrophages, natural killer cells, antibody-dependent cell-mediated cytotoxicity and interferon. Chickens resistant to Marek's disease as a result of vaccination, age and some forms of genetic constitution are primarily dependent on the immune system for their privileged status. Although all branches of the immune system are involved in resistance, indications are that cell-mediated immunity is the most important single mechanism. Autoimmunity is also a feature of the disease, and sensitivity to myelin has been detected in birds with neural lesions; it is, however, believed that cell-mediated primary demyelination is a late event occurring after lymphoid infiltration, and the stimulus for the infiltration remains unknown.

PATHOGENESIS OF MAREK'S DISEASE IN TURKEYS (19)

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Turkeys are naturally resistant to Marek's disease (MD) and there has been no confirmed report of isolation of MD virus (MDV) from this species. Nevertheless, several investigators have reported on successful laboratory transmission of MD in turkeys when high doses of virulent MDV were used for inoculation. Even these experimental cases appeared to be different from MD in chickens in their lesion spectrum and pattern of virus replication. Furthermore, highly effective MD vaccines such as the herpesvirus of turkeys (HVT) failed to protect turkeys against the disease. In this study, we have attempted to repeat vaccination trials in progeny poults from a commercial flock and those of a small white Beltsville laboratory flock. Results indicated that although marginal protection was offered by HVT in some experiments, turkeys from both strains remained refractory to vaccination against MD. Surgical bursectomy caused an increase in the level of lymphoma formation by MDV in turkeys. This observation along with previously reported results that all lymphoblastoid cell lines established in our laboratory from MD tumors in turkeys were of B-cell type further sharpens the difference between pathogenesis of MD in chickens and turkeys. A significant difference in susceptiblity to MD between commercial turkeys and the small white Beltsville turkeys was also noticed.

STUDIES ON PROSTAGLANDIN E_2 PRODUCTION IN MAREK'S DISEASE (20)

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Marek's disease (MD) lymphoblastoid cell lines, derived from MD lymphomas, produce a soluble suppressor factor (SF) which inhibits mitogen responses of normal spleen lymphocytes. Results show that MD cell line SF stimulates normal spleen cell cultures to produce prostaglandin E_2 (PGE_2), which could be at least partially responsible for the observed suppression. MD cell lines themselves produce only low levels of PGE_2.

Further experiments studied PGE_2 production by cultures of lymphoid organs during the course of MDV infection. Preliminary results showed that there were significantly higher concentrations of PGE_2 in cultures of bursal and thymus lymphocytes from infected birds than control birds. PGE_2 concentrations were either lowered or unchanged in cultures of spleen cells from infected birds. Lymphoma cultures also produced high levels of PGE_2. The possible role of PGE_2 in immunosuppression in MD will be discussed.

INHIBITION OF NATURAL KILLER ACTIVITY IN CHICKENS BY MAREK'S DISEASE VIRUS-TRANSFORMED CELL LINES (21)

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Very little is known about natural killer (NK) cells in chickens. It was first reported in 1979 by Sharma and Coulson that spleen cells of normal SPF chickens, with no detectable exposure to pathogenic agents, were cytotoxic for lymphoma cell lines. We have studied NK cytotoxicity using a chromium release assay. Several cell lines were examined for their sensitivity to NK cytotoxicity of spleen lymphocytes and peripheral blood lymphocytes (PBL). All Marek's disease (MD) cell lines were refractory to NK cell activity; only RP-9, an avian leukosis virus-transformed cell line, was sensitive to NK cytotoxicity. NK activity could be demonstrated in spleen cells and PBL in P-2, N-2 and UCD-003 chickens. Differences in NK activity could not be detected between these chicken lines. Nylon wool treatment of spleen cells or PBL did not affect cytotoxicity, suggesting that the responsible cells are non-adherent. NK activity was found to develop with age. Activity could not be detected in chicks of one week of age, but from this age on, NK cytotoxicity developed gradually reaching a plateau at about 4 weeks of age. NK activity against RP-9 was inhibited by the addition of unlabeled MD cell lines. All MD cell lines tested were able to cause inhibition of NK activity to RP-9 independent of the genetic background of the cell line and the characteristics of the virus.

STUDIES ON TUMOR IMMUNITY INDUCED BY MAREK'S DISEASE LYMPHOBLASTOID CELLS (22)

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Chickens from inbred lines G-B1 were immunized with cells from *in vitro* cultured Marek's disease (MD) lymphoblastoid cell lines (MDCC). They were subsequently challenged with highly virulent syngeneic transplantable MD lymphomas (MDCT) or with MD virus (MDV). The greatest degree of protection against the early lethal effects associated with the transplantable lymphomas was obtained when the lymphoblastoid cells were syngeneic with the recipient. Immunization with cells from syngeneic MDCC were also the most effective in preventing *in situ* growth and metastases of transplantable lymphomas. Similarly, inoculation of lymphoblastoid cells protected G-B1, G-B2 and F_1 (G-B1 x G-B2) chickens against primary MDV-induced lymphomas and, in addition, caused a marked decrease in virus shedding among syngeneic and semi-syngeneic (F_1) recipients. The latter finding is of particular interest because the MD lymphoblastoid cells used for immunization are virus non-producers *in vivo*. While the MDCC are not considered to be a practical vaccine for MD, the findings may lead to new insight regarding specificity of host immune responses against MD tumor and viral antigens.

POTENTIAL USE OF IMMUNOMODULATORS TO ENHANCE IMMUNE RESPONSE OF TURKEY HERPESVIRUS (HVT) VACCINATED CHICKENS (23)

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Two immunomodulators (Levamisole and Inosiplex) were studied to evaluate the immunopotentiating effects on turkey herpesvirus (HVT)-vaccinated chickens. They were administered orally on 1 day post-vaccination. Initially, a dose-response study was made to determine the optimal concentration of the agents that would enhance the immune response of the vaccinated chickens using the whole blood lymphocyte stimulation assay as a measure of cell-mediated immune response. Within the limits of the conditions employed in this experiment, 2.5 and 10.0 mg/kg body weight gave the optimal immunopotentiation effect.

A challenge study, using a virulent pathogenic field isolate of Marek's disease (MD) virus to assess the degree of protection, also was performed. The results of this study showed that the vaccinated Levamisole-treated group had a significantly lower percentage of Marek's disease mortality and/or lesions compared to other groups (non-vaccinated treated, vaccinated non-treated and non-vaccinated non-treated groups). The enhancement of protection was found to be better when Inosiplex was given early at 1 day post-vaccination, than when given late at 21 days post-vaccination. The results of this study support the possible use of these immunomodulators to potentiate HVT vaccination.

A CHROMIUM RELEASE ASSAY FOR THE STUDY OF CELL-MEDIATED IMMUNE RESPONSES TO MAREK'S DISEASE ANTIGENS (24)

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Few studies have been reported on the cell-mediated immune responses against the virus infection associated with Marek's disease (MD). It has been demonstrated that vaccination against MD will protect against the early lytic phase of infection with MDV. The protection is most likely an immune response, but little is known about the effector mechanism(s). A chromium release assay was developed using chick kidney cells (CKC) infected with HVT. Cells were labeled for 3 hours with 0.10 uCi/75 cm^2 tissue culture flask. The monolayers were washed twice, trypsinized, washed and counted. Allogeneic and syngeneic cells were mixed with spleen effector cells in U-shaped 96-well microtiter plates. After an incubation of 4 or 8 hours, the supernatant fluids and cell pellets were harvested separately and counted. Nonspecific release was generally between 7 and 15%. Low levels of cell-associated cytotoxicity could be detected at 4 and 5 days post inoculation with HVT. The specific release was found against allogeneic HVT-infected and control CKC. Syngeneic HVT-infected CKC were lysed very infrequently and only by effector cells that also lysed the allogeneic target cells. These results suggest that the lysis was caused by natural killer cells.

NATURAL RESISTANCE TO MAREK'S DISEASE (REVIEW) (25)

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Resistance to Marek's disease (MD) can be markedly influenced by appropriate genetic selection. The factors involved, such as exposure, pathogenicity of virus (MDV), the testing and breeding procedures to use, and how best to develop stock with a high degree of resistance are known. Evaluation of potential breeders can be done without exposing or infecting them with MDV. Selection for increased susceptibility can provide excellent controls for all types of basic and applied research on MD and MDV. The ranking of various stocks for genetic resistance tends to be maintained following exposure to different isolates of MDV as well as following vaccination.

Resistance associated with a marker gene (Ea-B^{21}), in the major histocompatibility complex, is expressed as a dominant trait. Other genes are involved because resistance can exist in the absence of B^{21}.

The objective of a breeding program should be to increase the frequency of both major and modifying genes that affect resistance. The commercial industry can and should utilize this approach for there is no evidence that resistance is negatively associated with any other trait of economic importance. Field control of MD should utilize all factors known to be effective: - genetic resistance, vaccination, and minimum exposure to MDV.

MORTALITY, VIRUS SHEDDING AND IMMUNITY TO MAREK'S DISEASE IN RELATIONSHIP TO CHICKEN LINE-SPECIFIC PROPERTIES (26)

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Marek's disease (MD) mortality rates were compared in chicken lines and sublines which differed in resistance to avian leukosis viruses, and which had different B-alleles (line differences only). Newly hatched chickens were experimentally infected with Marek's disease virus (MDV) by contact exposure for 14 days to MDV-infected "seeder" chicks. MD mortality was recorded for at least 30 weeks.

Subline R11 (homozygous B^{15}) and Line UM (homozygous B^{19}) were relatively resistant with MD rates below 12%. Lines which were susceptible to MD were homozygous for B^{2}, B^{15} or B^{21}, or had various combinations of those or other alleles (B^{13} or B^{14}). Susceptible lines had MD rates which varied from 25 to 55% or more. The rate of MDV production in the feather follicles of MD-resistant chickens was reduced in comparison to that in MD susceptible chickens. Relationships between MDV or HVT antibodies and MD mortality rates were not evident.

It is concluded that resistance or susceptibility to MD depended on the MDV multiplication rate in the infected chickens and was influenced by interactions between the infecting virus and involved cell systems. The influence of B-allele types on MD-development seemed to be of secondary importance.

GENETIC RESISTANCE TO MAREK'S DISEASE IN CONGENIC STRAINS OF CHICKENS (27)

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Chickens representing 10 major histocompatibility complex (MHC) antigens maintained congenically in the highly inbred White Leghorn line UCD-003 ($\underline{B}^{17}\underline{B}^{17}$) were exposed to different strains of Marek's disease (MD) virus. Mortality from MD was determined to compare the relative influence of the various B-alleles associated with the MHC. The birds with $\underline{B}^{2}$, $\underline{B}^{Q}$ and $\underline{B}^{21}$ alleles were more resistant to challenge with MDV strains than the other groups. The effect of the MHC-alleles on the early pathogenesis was assessed by the detection of viral antigen in the lymphoid organs and virus isolation from spleen lymphocytes. Differences in the early pathogenesis could not be detected regardless of the resistance to MD.

Adult hens of the various MHC types also were assessed for mortality from natural causes, laying performance, and fertility and hatchability of eggs.

FACTORS INFLUENCING RESISTANCE AND DISTRIBUTION OF LESIONS IN CHICKENS EXPOSED TO BC-1 and RB-1B ISOLATES OF MAREK'S DISEASE VIRUS (28)

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Chickens of 23 strains were inoculated at 3 weeks post hatching with BC-1 (Exp. 1) or RB-1B (Exp. 2) isolates of Marek's disease (MD) virus. Another experiment included 8 strains of chickens vaccinated with turkey herpesvirus (HVT) at one-day of age and non-vaccinated chickens all of which were exposed to RB-1B by contact at 2 weeks of age. While strains of chickens differed in resistance to MD, the emphasis herein is on other factors that influence resistance to the disease.

Females were more susceptible than males to the BC-1 isolate. The predominant lesion in females was in the ovary and in males it was in nerves; the incidence of tumors at these sites was significantly higher ($p < .01$) than in counterpart organs of the other sex. There was no sex difference in the combined incidence of tumors in visceral organs, excluding gonads. In RB-1B inoculated chickens, the predominant lesions were in viscera and the only significant sex differences ($p < .01$) was a higher incidence of neural lesions in males than females.

Vaccination not only reduced the incidence of MD but in birds diagnosed as positive it reduced the overall prevalence of lesions. Surprisingly, there was a trend for progeny of dams vaccinated with HVT and exposed to MD virus to be more susceptible to RB-1B than were those from comparable dams free of these viruses.

PATHOGENESIS OF MAREK'S DISEASE (REVIEW) (29)

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The sequential events in genetically susceptible chickens infected with oncogenic Marek's disease virus (MDV) can be divided into 4 phases: (1) cytolytic infection in lymphoid organs (bursa, spleen, thymus), involving mostly B-cells plus some T-cells, which occurs at 3-6 days post infection (DPI) and ultimately causes atrophy of the bursa and thymus, (2) partial recovery at about 7 DPI associated with splenomegaly, immune responses, appearance of MATSA-bearing cells, a switch to latent infection involving mostly T-cells plus some B-cells, and a transient loss of mitogen responsiveness, (3) later (2nd to 3rd week PI onward) cytolytic infection in epithelial tissues, permanent immunosuppression, reappearance of cytolytic infection in lymphoid tissues, mononuclear cell infiltrations and primary demyelination in nerves, and (4) cellular alterations (after 2-3 weeks PI) including lymphoma development and atherosclerosis.

Infections with mononcogenic chicken or turkey herpesviruses share some of the above features but generally fail to cause cytolytic infections of lymphocytes and do not induce any known cellular alterations. Also, the lymphocyte populations or subpopulations involved appear to be different from those in oncogenic MDV infections.

A hypothesis is advanced in which B-cells but not resting T-cells are susceptible to oncogenic MDV. Cytolytic B-cell infection would cause a reticulitis with infiltration of inflammatory cells. T-cells become "activated" and are then susceptible, but do not undergo cytolytic infections because of inherent differences or imposed (host response) controls.

Activated, infected T-cells replicate and undergo neoplastic transformation for unknown reasons, but perhaps following integration of MDV-DNA sequences. Permanent immunosuppresion resulting from suppressor activities of tumor cells, or loss of effector cells through cytolytic infection, or both, permits transformed cells to develop into lymphomas.

MDV-INDUCED ATHEROSCLEROSIS AND EVIDENCE FOR A HERPESVIRUS ROLE IN THE HUMAN VASCULAR DISEASE (30)

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Atherosclerosis has been induced in repeated experiments in SPF chickens infected with MDV without dietary cholesterol supplementation. The virus-induced arterial lesions are remarkably like those of chronic human atherosclerosis, both in character and distribution. The virus has been shown to be directly linked to the atherosclerotic lesions because: (1) Uninfected hypercholesterolemic or normocholesterolemic chickens did not develop atherosclerosis, (2) Viral-specific internal antigens were found in a few arterial smooth muscle cells (SMC) throughout the time-span of lesion development, and (3) Immunization of chickens with HVT prevented not only MDV-induced tumors, but MDV-induced atherosclerosis as well. *In vitro and preliminary in vivo studies have shown that* infection with MDV enhances the accumulation of cholesterol and cholesteryl esters in SMC - the two principal lipids which accumulate in human arterial lesions. These findings suggest that a principal mechanism of MDV-induced atherosclerosis may be the viral-induced alteration of SMC lipid metabolism. Furthermore, they have introduced two new important concepts in the pathogenesis of the human arterial disease: (1) Virus infection may be a primary etiologic agent, and (2) Viral alterations of SMC lipid metabolism may lead to atherosclerosis. Recent reports from other laboratories and findings in our own laboratory support a herpesvirus role in human atherosclerosis. The present evidence suggests that CMV may be a likely etiologic agent.

IMMUNOLOGICAL STUDIES ON MAREK'S DISEASE VIRUS-INDUCED ATHEROSCLEROSIS (31)

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We have reported previously that infection of SPF chickens with Marek's disease virus will induce gross and microscopic atherosclerotic lesions. This paper describes research on some immunologic facets of this infection process.

Immunofluorescent studies were carried out in correlation with investigations on the pathogenesis. Viral internal antigens (VIA) could be detected in the arterial media of virus-infected chickens as early as 4 weeks after infection and were found throughout the course of the disease. However, VIA occurred only in a few cells and at the periphery of the atherosclerotic plaques. Antigen-antibody complexes were not found in the arteries at any time during the course of the disease when the tissues were stained with fluorescein isothiocyanate-labelled antibodies to virus, immunoglobulin G or the C_3 component of complement.

Either embryonal bursectomy or vaccination with turkey herpesvirus markedly reduced the development of atherosclerotic lesions as well as tumors in virus-infected chickens.

These findings serve to further relate Marek's disease virus to the development of atherosclerosis in chickens and also to offer some suggestions as to the nature of the mechanisms of pathogenesis of these lesions.

THE ORGAN DISTRIBUTION OF CELLS EXPRESSING TUMOR ANTIGENS IN MAREK'S DISEASE: EVIDENCE FOR SPONTANEOUS REMISSION (32)

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The organ distribution of cells expressing Marek's disease tumor-associated surface antigen (MATSA) has been studied in chickens following infection with the HPRS-16 or B-14 strains of MDV or with HVT. No significant differences were found in the percentages of MATSA-expressing cells in the spleens, bursas, thymuses or gonads of chickens infected with the HPRS-16 or B-14 strains. However, the peripheral nerves of chickens infected with HPRS-16 contained a higher proportion of MATSA-positive cells than those from chickens infected with B-14. In a second experiment, chickens vaccinated with HVT, or infected with MDV (HPRS-16), or both vaccinated and infected were compared. No differences were found between treatments in the numbers of MATSA-positive cells in spleens, but differences were noted in the numbers of such cells in the peripheral blood at six weeks after infection and thereafter, and large differences were found in the numbers present in the gonads. In a third experiment, repeated sampling of the same individual birds allowed comparisons to be made between the numbers of circulating MATSA-positive cells in the blood of chickens that had been either vaccinated, or challenged, or vaccinated and challenged. These studies showed that: (1) it was not possible to predict mortality on the basis of the numbers of MATSA-bearing cells, (2) some birds showed progressively increasing numbers of MATSA-bearing cells from the time of infection until death, and (3) some individual birds that survived showed firstly an increase in the numbers of MATSA-bearing cells, to levels equal to or in excess of those seen in birds destined to die, and secondly a rapid fall in the numbers of these cells. It is suggested that in these birds, immunological rejection of transformed cells was occurring.

EXTRINSIC FACTORS AND HOST RESISTANCE TO MAREK'S DISEASE: EFFECTS OF DIETARY SELENIUM (33)

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Carcinostatic effects of increased dietary selenium (Se) on various forms of tumors have been repeatedly shown in mice and rats.

The incidence of Marek's disease (MD), as determined by the development of gross MD lesions, was often reduced in chickens given supplemental Se. Chickens were given ad libitum 1, 2, 4 or 8 ppm of sodium selenite in the drinking water from the first day of life through the 8-week experimental period, and challenged at 4 days of age with virulent MD virus. Though statistically insignificant, the reduction in the incidence of MD was consistently observed in a commercial strain of White Leghorn (WL) chickens, whereas it was not as consistent or apparent in a more susceptible WL strain of commercial specific-pathogen-free chickens. Plasma Se levels were significantly elevated by Se supplementation at levels of 1 ppm or more, and MD incidence was numerically higher in the birds with lower plasma Se levels (< 250 ng/ml) than in those with higher Se levels.

EARLY MORTALITY SYNDROME IN CHICKENS DUALLY INFECTED WITH MAREK'S DISEASE VIRUS (MDV) AND CHICKEN ANEMIA AGENT (CAA) (34)

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Inoculation of one-day-old SPF chickens with a strain of Marek's disease virus (MDV) isolated in 1970 in Germany consistently resulted in an early mortality syndrome associated with bone marrow aplasia, anemia, almost complete atrophy of the thymus and high mortality between 10 and 16 days postinfection (p.i.). It has been shown that this syndrome was due to dual infection with virulent MDV and an agent, designated as Cux-1 isolate, with the same properties as the Gifu-1 strain of chicken anemia agent (CAA).

Single infection with CAA caused an aplastic anemia in up to 100% of experimental chickens and a mortality of 0% to 25% until 14 - 16 days p.i. The pathogenicity of CAA was considerably increased if chickens were dually infected with CAA and virulent strains of MDV. Dually infected chickens died as early as 10 - 14 days p.i., and the mortality ranged from 42% to 92%. Single infection with acute or classical MDV never caused anemia or bone marrow aplasia.

Histological lesions in anemic chickens were similar, regardless of whether they were infected singly or dually. There was an almost complete aplasia and atrophy of the bone marrow, virtually complete atrophy of the thymus, and lymphoid atrophy in the bursa of Fabricius, spleen and other tissues. In several instances, focal necrosis was present in lymphoid tissues and in the bone marrow.

It has been suggested that dual inoculation of chickens with virulent MDV may improve detection of CAA in test samples.

PATHOGENESIS OF THE TURKEY HERPESVIRUS (HVT) IN CHICKENS INOCULATED AS EMBRYOS (35)

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Deposition of cell-associated HVT in the amniotic sac of 18-day-old chicken embryos results in the infection of the embryo and in rapid replication of the virus in various tissues of the embryo. We have noted that embryonic lung was the first site of HVT-replication. The virus reached peak levels in the lungs within 2 to 3 days of inoculation; subsequently, the virus spread to other parts of the body. This result indicated that the embryo acquired infection with HVT via the respiratory tract. Presumably, the virus deposited in the extra embryonic fluid came in contact with the respiratory epithelium of the embryo. Within the lung, the virus was associated with a morphologically diverse population of cells that were adherent to plastic substrate and were retained by rayon fiber columns. These adherent cells were not removed by carbonyl iron treatment and resisted treatment with antisera to B- and T-cells.

STUDIES ON THE ONCOGENIC PROPERTIES OF VARIOUS MAREK'S DISEASE VIRUS STRAINS (36)

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The oncogenicity of four Marek's disease virus strains was investigated by in ovo inoculation on day 8 of incubation and observation of the chickens during 16 weeks after hatching. Macroscopic and microscopic examination of the chickens revealed lymphomas in 85% of the chickens after inoculation of virulent GA-5 virus.

The 36th chick embryo fibroblast passage of MDV CVI-988, obtained by further attenuation of a serotype-1 MDV strain of low virulence, caused an endoneural lymphoma in one chicken (2%).

MDV HPRS-24, belonging to serotype-2, caused an endoneural lymphoma in one chicken and a visceral lymphoma in another chicken (4%). MDV SB-1 of serotype-2 was nononcogenic in this test model. The pathogenic properties of the virus strains are discussed and related to the experimental method. Quantitative differences in histological lesions caused by the three vaccine strains are discussed.

STRATEGIES FOR THE CONTROL OF MAREK'S DISEASE THROUGH VACCINATION (REVIEW) (37)

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Control of Marek's disease (MD) can now be accomplished by the use of vaccines of 4 basic types: attenuated serotype-1 MD virus, nononcogenic serotype-2 MD virus, nononcogenic serotype-3 virus (turkey herpesvirus), and polyvalent vaccines. Several factors have an important influence on vaccine efficacy and may from time to time be responsible for excessive losses in vaccinated chickens. Maternal antibodies and challenge virus strain are among the most important of these factors. Maternal antibodies are especially detrimental when directed against the same serotype as the vaccine or when a cell-free vaccine is used. Certain strains of serotype-1 MD virus, identified as "very virulent", have unusually high pathogenicity for chickens vaccinated with serotype-3 virus. Many vaccine failures can be traced to improper administration of vaccine. Stress, genetic strain of chicken, and environmental exposure to less virulent MD field strains are also important. Although a minimum dose is required, further increase in vaccine dose or multiple inoculations have little benefit. Three vaccination strategies may be considered. Monovalent vaccination involves the selection and use of a single vaccine virus. This is the historic procedure and provides satisfactory protection with any of the commercial vaccine strains in many situations. Alternate generation vaccination is a strategy that requires the use of vaccines of different serotypes in parents and progeny. Most

commonly, parents are vaccinated with a serotype 1 or 2 vaccine and progeny are vaccinated with serotype 3, so as to avoid interference with homologous maternal antibody. Polyvalent vaccination is a third strategy that utilizes the principle of synergism between vaccine viruses to achieve improved protection, and may be particularly useful where chickens are exposed to very virulent serotype 1 challenge strains. Although vaccination will be the basis of MD control for years to come, further improvements in vaccines will be difficult because of the extremely high efficacy and low cost of existing products. Even with improved vaccines, further gains in MD control may be dependent on the development of more immunologically competent and genetically resistant chicken strains which will respond better to vaccines, and the use of better husbandry and sanitation procedures which will lower MD exposure and reduce other immunodepressive stress.

TEN YEARS OF EXPERIENCE IN THE PREVENTION OF MAREK'S DISEASE IN THE SOCIALIST REPUBLIC OF ROMANIA (38)

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More than 250,000,000 chickens representing both egg laying and meat type breeds were vaccinated against Marek's disease in the period between 1972 and 1983. Chickens with or without MDV and HVT maternal antibodies were vaccinated at one day of age with turkey herpesvirus strain FC-126, clone 1, in either the cell-associated or cell-free form. All vaccinations were done in the hatcheries and the chickens were placed in ordinary poultry houses. Minimum HVT doses were 1,000 FFU during the period of 1972 to 1980, 1,500 FFU for 1980 to 1983, and 2,000 FFU since 1983. No significant differences in protective effects had been detected when HVT doses of 140-300 FFU per chick were compared with doses of 1,000 FFU or more per chick.

A decrease in Marek's disease mortality during the period of 10-40 weeks of age was observed in vaccinated birds and it appeared that vaccination at one day of age provided long lasting protection. A total of 32 outbreaks of Marek's disease were recorded from 1972 to 1983, and the protective effect of HVT vaccine was determined to be 81.3-99.2%. The Romanian turkey virus vaccine, strain FC-126 (ROMVAC) offered satisfactory protection against Marek's disease when used as either cell-associated or cell-free virus.

MAREK'S DISEASE IN EGYPT (39)

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Abstract not submitted

FIELD STUDIES ON THE INCIDENCE AND EPIZOOTIOLOGY OF MAREK'S DISEASE IN BROILER BREEDERS IN GERMANY (40)

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A total of 24 broiler breeder flocks on different geographic locations were vaccinated against Marek's disease (MD) at day-old with lyophilized or cell-associated HVT vaccines, respectively. Additional vaccinations against Newcastle disease, infectious bursal disease and infectious bronchitis were standard procedures in the growing period. After a normal growing period general production records were acceptable for all flocks.

Tumor mortality, probably associated with MD, started between 19 and 35 weeks of age in 7 of 24 flocks. Total tumor-associated losses in a 35-40 week period were in the range of 3 to 25%. Pathological lesions consisted of tumors predominantly in visceral organs: proventriculus, ovary, kidney, spleen and also quite frequently in the serosa of the body cavity. Mortality was approximately two-fold higher in males than in females.

HVT viremia tests were performed at approximately 12 days of age on randomly selected chicks (10 birds per flock). The viremia rate varied between 20 and 100%, but no direct association between viremia rate and tumor incidence could be established. Precipitating and neutralizing antibodies against HVT were detectable in affected and non affected birds in similar proportions. The exact reason(s) for the scattered but partly significant incidence of the tumor-associated losses remain obscure at present.

APPLICATION OF PARTIAL FLOCK INOCULATION WITH AN APATHOGENIC STRAIN (HN-1) OF CHICKEN HERPESVIRUS (CHV) OF MAREK'S DISEASE TO IMMUNIZE CHICKEN FLOCKS AGAINST PATHOGENIC FIELD STRAINS OF MD (41)

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In 1969 a flock of SPF chickens held in extreme isolation for many generations without evidence of tumors was found endemically infected (viremic) with an apathogenic strain (HN-1) of chicken herpesvirus (CHV) of MD. Laboratory and field flocks inoculated subcutaneously at 1 day of age with 0.2 ml of a 50% suspension of whole blood from SPF donors prepared with a solution containing sodium citrate and antibiotics showed excellent protection against virulent MD. Flocks were also protected when only 10% (called "seeders") were inoculated, and the apathogenic virus was allowed to spread.

Broiler flocks reared on built-up litter were protected when a number of seeder chicks equal to 2 or 3 percent of the final flock placement were inoculated and held in isolation for 4, 7, or 10 days prior to introduction into flocks of day-old penmates - "head start infection." "Concurrent inoculation" of 2 to 3 percent of the flock also provided protection.

When commercial turkey herpesvirus (THV) vaccines became available, 100% of the chicks in breeder and layer flocks were vaccinated, then 3 to 5 percent were additionally inoculated with CHV to serve as seeders for added MD protection. In 1976 all seeding of commercial laying flocks was discontinued and some local broiler producers began vaccinating with THV only.

Soon after discontinuing CHV seeders, disastrous MD losses began to occur in many layer flocks, usually late in the

grow period or early lay period, and mortality and leukosis condemnations began to rise in broilers reared on certain farms despite the use of THV vaccine. Extensive investigation of vaccine brands, types, titer, handling, mixing, and administration, failed to reveal a cause for THV vaccine failure.

Resumption of the seeding program to supplement vaccination with THV once again eliminated the MD breaks, sometimes dramatically. On some farms repeated flock seeding was necessary before the MD problem was completely eliminated. On several problem broiler farms, the owners re-instituted the supplemental seeding procedure with dramatic reduction in mortality and leukosis condemnations. In laboratory experiments, cell-cultured HN-1 CHV proved less satisfactory than blood from SPF donors.

PROTECTIVE EFFICACY OF CLONES OF MAREK'S DISEASE VIRUS STRAIN CVI-988 (42)

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Marek's disease virus strain CVI-988 vaccines, belonging to serotype-1, provide satisfactory protection and are safe for chickens in the field. However, in the laboratory some pathogenicity was demonstrated for RIR chickens, which are extremely MD susceptible. By serial cell culture passages and plaque purification, MDV CVI-988 clones were obtained which were innocuous for RIR chickens, but which demonstrated vaccine characteristics similar to commercial vaccine batches. Comparative 50% protective dose (PD_{50}) assays were performed with three clones at various stages during adaptation to chick embryo fibroblasts. One MDV CVI-988 clone, at about the 60th passage level, was selected, showing an improved protective efficacy to against GA-5 and MDV K challenge.

In addition, this virus clone provided good protection against challenge with MDV RB-1B and MDV Tun, two representatives of so-called very virulent MDV strains. The latter strain was recovered from a poultry farm in Tunesia after severe MD losses were observed in HVT-vaccinated chickens.

LACK OF ASSOCIATION BETWEEN NATURAL INFECTION WITH SEROTYPE-2 MAREK'S DISEASE VIRUS AND MAREK'S DISEASE CONDEMNATIONS IN BROILER CHICKENS (43)

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In the United States, the frequency with which broiler chickens are condemned for lesions of Marek's disease (MD) varies widely among different geographical regions. One environmental factor presumed to influence MD frequency is natural infection with nononcogenic serotype-2 MD virus strains. In the laboratory, such strains are protective themselves, and they also augment the protection afforded by turkey herpesvirus (HVT) vaccine. It seemed possible, therefore, that the low frequency of MD condemnations typically experienced in certain areas was due to high natural infection with serotype-2 virus. To test this hypothesis, 58 HVT-vaccinated broiler flocks from 7 selected states were tested for MD viremia between 7 and 8 weeks of age. Viruses isolated were typed by fluorescent staining with monoclonal antibodies. Overall, serotype-2 virus was isolated from 34% of flocks and from 7% of individual birds. Although the isolation frequency from individual birds in Virginia was 35%, the frequencies in 3 other low condemnation states (Georgia, Mississippi, California) and in 3 high condemnation states (Delaware, Maryland, Maine) were equally low, ranging from 0 to 6.6%. Thus, low levels of MD condemnations in certain states are probably not due to high serotype-2 virus exposure. However, the frequency of infection with virulent serotype-1 MD was significantly greater in high condemnation states than in low condemnation states.

POLYVALENT VACCINES FOR IMPROVED CONTROL OF MAREK'S DISEASE (44)

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In an effort to develop strategies for providing improved protection of chicken flocks exposed to Marek's disease virus (MDHV), particularly to the highly virulent strains, the protective efficacy of polyvalent vaccines was evaluated experimentally and in large scale field trials. The polyvalent vaccine was prepared by combining turkey herpesvirus (HVT) with naturally or artificially attenuated MDHV serotype-1.

The vaccines used in experimental trials were: 1) HVT strain FC-126; 2) HVT + MDHV strain CVI-988; 3) HVT + MDHV strain LCBS-216/68. The vaccines used in field studies were the same ones already mentioned, plus a bivalent vaccine prepared with HVT + MDHV strain HPRS-16 and a trivalent vaccine combining HVT with MDHV strains CVI-988 and LCBS-216/68.

The results confirmed those previously reported by us and by other authors, on the protective synergism of polyvalent vaccines and the enhancement of the efficacy of heterologous virus (HVT), by combining it with homologous attenuated virus (MDHV). Their use is particularly recommended for use against the highly virulent "pathotypes" of MDHV, first isolated in USA, but apparently present also in Europe. Polyvalent vaccines could be useful in areas or on farms where vaccinal immunity, using HVT alone, is suboptimal for a variety of causes (overcrowding, multi-age breeding, high and long-lasting risk of exposure, etc.). Obviously, the application of hygienic measures remains a factor of primary importance.

SOME EXPERIENCES WITH MONO- AND BIVALENT MAREK'S DISEASE VACCINES (45)

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Apathogenic serotype 1, 2 and 3 Marek's disease herpesviruses and bivalent preparations combining these viruses were used in laboratory- and field-trials in Germany as vaccines against Marek's disease. All experimental vaccines were safe and protective against field virus challenge, but there were differences in the rate of protection in favor of the bivalent serotype 3/serotype 1 combination.

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