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ABSTRACT

FUNCTIONAL ANALYSIS OF GLYCOPROTEIN H AND L COMPLEX OF MAREK'S DISEASE VIRUSES

By

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Serotype 1 Marek's disease virus (MDV) is oncogenic, which rapidly induces T cell lymphoma and mononuclear cell infiltration of peripheral nerves in susceptible chickens. The mechanism of cell to cell spread has not been characterized for MDV, but is thought to occur by intracellular bridge formation which would require the expression of several MDV glycoproteins on the surface of the infected cell. Glycoproteins H (gH) and L (gL) form a hetero-oligomeric function unit, which plays an important role in virus infection and cell to cell spread, in most herpesviruses described to date. The objective of this research project is to identify the gH gene in MDV-1 GA strain, and analyze its potential biological functions. A 2439 bps open reading frame (ORF) was identified from the DNA sequence of BamHI F and K2 fragments of MDV GA strain, which predicts a 813 amino acid polypeptide. This peptide is homologous to herpes simplex virus (HSV-1) gH, and has typical glycoprotein features: 1. a single signal sequence, 2. a large extracellular domain, 3. transmembrane domains, and 4. a small cytoplasmic domain. There are 9 potential N-linked glycosylation sites within the extracellular domain. A fragment of the gH ORF was cloned into a vector in frame with GST to produce a GSTgH fusion protein in an E. coli expression system. The GSTgH fusion protein was

used to develop gH monoclonal antibodies (Mab) and antiserum. The gH expression was detected in MDV GA strain infected DEF by immunofluorescence assay (IFA) with gH Mabs and serum. To investigate the interaction of MDV gH and gL, both gH and gL were expressed with baculovirus expression system, fowlpox virus (FPV) expression system, and vaccinia virus MVA/T_7 pol enhanced transient expression system. IFA was performed with gH and gL antibodies. The results suggested that co-expression of gH and gL in the same cell is required and is sufficient for both gH and gL subcellular transportation and cell surface expression in Sf9 cells. The gH requires gL for cell surface expression, the reverse is also true in Sf9 cells, whereas gL alone can be detected on the cell surface in DF1 cells with small patch appearance. Co-expression of gH and gL in DF1 cells results in gH-L patches in the cell surface, suggesting the fusogenic function of gH-L complex. Evidence from the FPV expression system indicated that gH is required for protecting gL secretion by providing a membrane anchor for the gL molecule. The results from in vitro translation and transient expression in DF1 cells indicated that the amino acids 451-659 (the SacI-HindIII fragment) of the gH polypeptide are essential for gH-L complex formation. By co-immunoprecipitation from pulse-chase labeling MDV-infected DEF samples with gL serum, specific and unique bands ranged from 100 to 110 kDa were precipitated, which reflect the immature and mature from of gH molecules. There was not enough neutralization and plaque-forming inhibition activity of the rabbit serum against GSTgH fusion protein. Further evaluation of the biologic functions of MDV gH is necessary.

This dissertation is dedicated to
My mother, Caizheng Huang
My father, Weixie Wu
My wife, Caiyun
My sons, Nan and Peter

Their patience and love have supported me through these studies

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LIST OF ABBREVIATIONS

2YT	two yeast-tryptone
AGP	agar gel precipitation
BHV	bovine herpesvirus
ALV	avian leukosis virus
bps	base pairs
°C	centigrade
CEF	chick embryo fibroblast
CMV	cytomegalovirus
CsCl	cesium chloride
DEF	duck embryo fibroblast
DF1	line 0 chicken embryo fibroblast cell line
DMSO	dimethyl sulfoxide
DNA	dioxyribonucleic acid
DR	direct repeats
ds	double stranded
EBV	Epstein-Barr virus
E. coli	Escherichia coli
EDTA	ethylenediaminetetraacetic acid
E gene	early gene
EHV	equine herpesvirus
ELISA	enzyme linked immunosorbant assay
FFE	feather follicle epithelium

FITC	flourescein-5' isothiocyanate
FPV	fowlpox virus
GA	Marek's disease virus serotype 1 GA strain
gB	glycoprotein B
gC	glycoprotein C
gD	glycoprotein D
gE	glycoprotein E
gH	glycoprotein H
gHe	EcoRI truncated glycoprotein H of Marek's disease virus
gHh	HindIII truncated glycoprotein H of Marek's disease virus
gHs	ScaI truncated glycoprotein H of Marek's disease virus
gI	glycoprotein I
gK	glycoprotein K
gL	glycoprotein L
GST	glutathione-S-transferase
GSTgH	glutathione-S-transferase and MDV gH fusion protein
HCMV	human cytomegalovirus
hsp	(HSV) host-shot-off protein
HSV-1	herpes simplex virus type 1
HSV-2	herpes simplex virus type 2
HVT	turkey herpesvirus
IBDV	infectious bursal disease virus
ICP0	(HSV) infected cell protein number 0

ICP4	(HSV) infected cell protein number 4
ICP22	(HSV) infected cell protein number 22
ICP27	(HSV) infected cell protein number 27
ICP47	(HSV) infected cell protein number 47
IE gene	immediate early gene
IFA	indirect immunofluorescence assay
IgG	immunoglobulin G
IgM	immunoglobulin M
IPTG	isopropyl β-D-thiogalactopyranoside
IR_L	internal repeat long
IR_S	internal repeat short
kDa	kilo-Dalton
L gene	late gene
LL	lymphoid leukosis
LM	Leibovitz-McCoy medium
LPDV	lymphoproliferative disease virus of turkey
Mab	monoclonal antibody
MD	Marek's disease
MDV-1	Marek's disease virus serotype 1
MDV-2	Marek's disease virus serotype 2
MDV-3	Marek's disease virus serotype 3
Meq	Marek's disease virus genome EcoRI Q fragment
MHC	major histocompatibility complex

ml	milliliter
mMDV	mild Marek's disease virus
mM	millimolar
moi	multiplicity of infection
mRNA	message RNA
ng	nanogram
NLS	nuclear localization signal
NS-1	mice myoloma cell line
NuLS	nucleolar localization signal
OBP	origin binding protein
ORF	open reading frame
Ori	replication origin
PAA	phosphonoacetic acid
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
pCNDAgH	plasmid pCDNA3.1zeo inserted with Marek's disease virus gH gene
pCDNAgL	plasmid pCDNA3.1zeo inserted with Marek's disease virus gL gene
PCR	polymerase chain reaction
pBacgH	Marek's disease virus gH gene in baculovirus transfer vector pBlueBac4
pBacgHL	Marek's disease virus gH&L genes in baculovirus transfer vector pBlueBac4
pBacgL	Marek's disease virus gL gene in baculovirus transfer vector pBlueBac4
pfu	plaque forming units

pGEMgHe	truncated gHe in pGEM-7Zf+ vector
pGEMgHh	truncated gHh in pGEM-7Zf+ vector
pGEMgHs	truncated gHs in pGEM-7Zf+ vector
pM	picomole
pp38	Marek's disease virus phosphate protein 38
PRV	pseudorabies virus
rBacgH	Marek's disease virus gH recombinant baculovirus
rBacgHL	Marek's disease virus gH and gL recombinant baculovirus
rBacgL	Marek's disease virus gL recombinant baculovirus
reFPV	recombinant fowlpox virus
reFPVgHL	Marek's disease virus gH and gL recombinant fowlpox virus
reFPVgL	Marek's disease virus gL recombinant fowpox virus
REV	reticuloendotheliosis virus
RNA	ribonucleic acid
rpm	revolutions per minute
SDS	sodium dodecyl sulfate
TBS	Tris buffered saline
TE	Tris-EDTA
TK	thymidine kinase
TR_L	terminal repeat long
TR_S	terminal repeat short
U	international unit
µl	microliter

U_L	unique long
µm	micrometer
U_S	unique short
vMDV	virulent Marek's disease virus
VP16	(HSV) virion protein No.16
vvMDV	very virulent Marek's disease virus
vv+MDV	very virulent plus Marek's disease virus
VZV	varicela-zoster virus

INTRODUCTION

Marek's disease is one of the most common of the lymphoproliferative neoplastic diseases of chickens, characterized by a mononuclear infiltration of multiple tissues and organs (Calnek and Witter, 1997). The first account of the disease was probably Josef Marek's report of paresis in six roosters with mononuclear infiltration of peripheral nerves and spinal nerve roots (Marek, 1907). As the disease gained importance to the poultry industry, it was clear that the lesions of the disease were not restricted to the spinal cord and peripheral nerves. The wide variety of clinical signs and the location of lesions led to promulgation of an equally wide variety of terms to identify the conditions. For instance, the related pathologic conditions, associated to peripheral nerve or spinal cord lesions, was variously described as range paralysis for the clinical signs, or polyneuritis, neuritis, or neurolymphomatosis gallinarum for the lesions of the disease. The ocular lesions, characterized by mononuclear infiltration of the iris, were commonly referred to as gray eye for its appearance, or iritis, uveitis and ocular lymphomatosis for the lesions. Lesions in various visceral organs and muscles were often referred to as visceral lymphomatosis, and those in skin as skin leukosis (Calnek and Witter, 1997). To distinguish the condition clearly from etiologically different lymphoproliferative diseases, Biggs (Biggs, 1961) proposed the use of the term Marek's disease (MD) in 1961. This term is in common use today.

Prior to use of vaccines, poultry industries worldwide experienced heavy economic losses due to the death and condemnation resulting from MD between 1950s and 1960s. The disease has been effectively controlled through the use of live virus vaccines since the 1970s. However, both clinical, field, and laboratory data have provided

compelling evidence that the virus has undergone a series of mutations to greater virulence, with an increase in the severity of the disease (Witter, 1997a). The increase in virulence appears associated with the vaccine application, and may partially result from the natural selection and adaptation against the changes of host (MDV) resistance due to the vaccination.

To effectively control MD, it is necessary to study new strategies for vaccine development. Viral glycoproteins represent the first line of virus-host interaction. Attachment of virus to host cell surface activates a cellular process mediated by viral glycoproteins that lead to the fusion of the viral envelope with the cellular plasma membrane. Multiple viral glycoproteins are required for this process, including glycoproteins B (gB), C (gC), D (gD), H (gH), and L (gL). The gB, gD, and gH-L complex can act individually or in combination to trigger pH-independent fusion (Spear, 1993). The focus of this research and dissertation is on gH identification and gH-L interaction. gH is conserved structurally and functionally in most herpesviruses described to date and plays an important role in virus infection and cell to cell spread,. However, available information for MDV gH, gL, and gH-L complex is limited. To further understand MDV infection and the cell fusion processes, it is essential to identify gH expression by infected cells, to investigate gH and gL post-translational modification, subcellular translocation, as well as gH-L interaction, and to study the relationship between gH-L complex and viral infection. These studies will expand our knowledge on the mechanisms of MDV infection for scientific purposes, as well as vaccine development.

CHAPTER I. LITERATURE REVIEW

I. Marek's disease and Marek's disease virus (MDV)

1. Etiology of Marek's disease

A. Identification and classification

Attempts to transmit the disease were not successful until the early 1960s. The successful and regular experimental transmission of the disease was the first major breakthrough in modern MDV research (Biggs and Payne, 1963; Sevoian and Chamberlain, 1962a; Sevoian et al., 1962b). Many efforts were made, but it was not until 1967 that a herpesvirus was identified as the etiologic agent of MD. The virus was later designated as Marek's disease virus (MDV). In the same publications, MDV was successfully propagated in tissue culture for the first time (Churchill and Biggs, 1967; Nazerian et al., 1968; Solomon et al., 1968). The successful growth of MDV in vitro in cell culture facilitated the identification of many biological, pathological, and virological characteristics in various experimental studies. MDV, a strictly cell-associated herpesvirus (Churchill and Biggs, 1967; Nazerian et al., 1968; Solomon et al., 1968; Witter et al., 1969), has some properties similar to α-herpesviruses, and some similar to γ-herpesviruses. Originally, MDV was classified as a γ-herpesvirus based on its lymphotropic properties, which was similar to Epstein Bar virus (EBV), a member of the γ-herpesvirus family. However, the genomic structure and gene organization of MDV more closely resembles α-herpesvirus (Buckmaster et al., 1988). MDV is the prototype virus of the MDV group, and is designated as serotype 1 (Calnek and Witter, 1997).

On the basis of agar gel precipitation (AGP) and indirect immunofluorescence assay (IFA), the MDV group was divided into three serotypes (Bulow and Biggs, 1975).

Type specific monoclonal antibodies (Ikuta et al., 1982; Lee et al., 1983b) are normally used to determine viral serotype today. MDV serotype 1 viruses (MDV-1) include oncogenic strains and their attenuated derivatives. Two additional groups of non-oncogenic herpesviruses isolated from turkeys (Kawamura et al., 1969; Witter et al., 1970) and chickens (Biggs et al., 1972; Cho and Kenzy, 1972) are also considered part of MDV group. The non-oncogenic chicken isolates are designated as MDV serotype 2 (MDV-2), such as SB-1 strain (Schat and Calnek, 1978a; Calnek et al., 1979). On the basis of the antigenic relationship to MDV-1, the turkey isolate, turkey herpesvirus (HVT) is designated as MDV serotype 3 (MDV-3). Also due to the closer antigenecity between MDV-2 and MDV-3 to MDV-1, MDV-2 and -3 are normally used as vaccines to protect chickens from virulent MDV-1 challenge.

There is a wide variation in pathologic potential within MDV-1. Based on pathogenicity or oncogenicity, MDV-1 strains are further subdivided into three classes, which are designated as mild (mMDV), virulent (vMDV), very virulent (vvMDV) (Witter, 1983; Witter, 1985). The prototype strains for each class are the CU2 strain (Smith and Calnek, 1973) for the mMDV class, the JM (Purchase and Biggs, 1967), and GA (Eidson and Schmittle, 1968) strains for the vMDV class, and the MD5 (Witter et al., 1980) and RB1B (Schat et al., 1982) strains for the vvMDV class. Pathotypes with virulence exceeding vvMDV (vv+MDV) have been suspected, and at least one such strain, 584A (Witter, 1992), has been reported. The pathotyping of MDV-1 isolates involves pathogenicity or oncogenicity tests in vaccinated or unvaccinated chickens (Witter, 1989). The vv+MDV pathotypes are assigned to those isolates that induce MD lesions in chickens vaccinated with MDV-2 & MDV-3 bivalent vaccine (Witter, 1997b).

The vvMDV strains induce visceral and neural tumors, and result in excessive MD losses in HVT vaccinated flocks. The vMDV strains only induce tumors in susceptible chickens, but not in resistant lines of chickens or HVT vaccinated birds. The mMDV strains rarely cause tumors even in susceptible birds. Repeated passage of oncogenic MDV-1 virus in cell culture results in attenuation of the viruses (Nazerian, 1971).

B. Isolation and cultivation

MDV can be isolated as early as 1 or 2 days post-inoculation (Phillips and Biggs, 1972), or 5 days after contact exposure (Adldinger and Calnek, 1973), and throughout the life of the chicken. Intact viable cells are the preferred source, due to the highly cell-associated properties of MDV. Samples for virus isolation may consist of blood lymphocytes, splenocytes, or isolated tumor cells. Probably the most widely used method for primary isolation of MDV is inoculation of susceptible tissue cultures with blood lymphocytes, or single cell suspension from lymphoid tissues of infected chickens. Cultured duck embryo fibroblasts (DEF) and chicken kidney cells are suitable for isolation of MDV-1 viruses (Churchill and Biggs, 1967; Solomon et al., 1968), whereas chicken embryo fibroblasts (CEF) are normally used to isolate MDV-2 and MDV-3 viruses (Biggs et al., 1972; Schat and Calnek, 1978a). Infected cell cultures usually develop discrete focal lesions, consisting of clusters of degenerate rounded cells. These lesions are called plaques, which are normally less than 1mm in diameter. Low passage MDV-1 viruses grow better in DEF and chicken kidney cell culture, but grow slowly and produce only small plaques. Usually, the plaques develop in 5-14 days on primary isolation and in 3-7 days after adaptation to culture. MDV-2 viruses grow better in CEFs, grow slowly and produce medium sized plaques with some large syncytia. MDV-3

viruses (HVT) grow better in CEFs, grow rapidly and produce large plaques. High titers of infectious virus can be produced in HVT-infected cell cultures than from cultures infected with MDV-1 and MDV-2

C. Morphology and Ultrastructure of MDV

Most studies on morphology have been conducted with MDV-1 and HVT. Qualitative differences have not been detected between these two serotypes with electron microscopy (Nazerian et al., 1971; Okada et al., 1972). The structures of MDV-1 and HVT are similar to other herpesvirus. The mature virus particles consist of three layers: nucleocapsid, tegument and envelope membrane. The tegument is an amorphic space between nucleocapsid and envelope membrane. It consists of globular material, which is frequently asymmetrically distributed and variable in amount. The physical structure of nucleocapsid is described as cubic icosahedral symmetry, which measures between 85-100nm in diameter and has 162 hollow central capsomers (Nazerian, 1968). The nucleocapsid appears to be assembled in the infected nucleus via the fusion of 6 small nuclear protein particles (35nm in diameter) into a cylindrical mass (Hamdy et al., 1974; Nazerian, 1968; Nazerian and Purchase, 1970; Okada et al., 1974; Okada et al., 1972). The double-stranded DNA (dsDNA) genome is added into this cylindrical structure (Okada et al., 1980). The mature nucleoid measures 45-60nm in diameter and has a toroidal structure (Nazerian, 1974). The envelope membrane is the outer layer of the mature virus particle. Spikes, the small surface projections of the envelope, are dispersed evenly over the virion surface. Most glycoproteins are located in the spikes.

The first mature nucleocapsid can be observed 10 hours post-infection, but the first enveloped virion particles appear at 18 hours post-infection (Hamdy et al., 1974).

The 150-160nm in diameter enveloped particle can be found free in the host cell nucleoplasm or in membrane bond nuclear vesicles. Sometimes, naked and enveloped virions can also be detected in the cytoplasm and rarely in the extracellular space. Large numbers of cytoplasmic enveloped virus particles can be found in the feather follicle epithelium (FFE) (Calnek et al., 1970). In negative-stained preparations, the particles have large envelopes measuring 270-400nm in diameter, and often appear as an irregular amorphous structure (Calnek et al., 1970).

In general, the morphology of HVT resembles that of MDV-1. In thin sections, however, nucleocapsid of HVT has a unique crossed appearance (Nazerian et al., 1971). The morphology of MDV-2 has not been studied in detail, but virion particles, morphologically similar to MDV-1 and HVT, have been visualized (Schat and Calnek, 1978a).

2. Pathology of MD

A. Virus infection

Three general virus-cell interactions are recognized: productive infection (also known as cytolytic infection), latent infection, and transformation. There are two types of productive infection, fully productive and productive-restrictive. Fully productive infection has only been observed in FFE, which results in development of a large number of enveloped and fully infectious virions (Calnek et al., 1970). Whereas, productive-restrictive (or semi-productive) infection occurs in the other tissues and in cell cultures, where most of the virions produced are not enveloped, and not released in an infectious form. Therefore, cell to cell fusion becomes the major mechanism of virus spreading in

productive-restrictive infection (Calnek et al., 1970). However, a variable number of enveloped virions may be produced in cell cultures. When disrupting these cells in distilled water, infectious cell free virions are recovered (Cook and Sears, 1970). In vivo productive infection normally leads to formation of intranuclear inclusion bodies, cell destruction, and necrosis. Polykaryocytosis is a major component of viral plaques, and frequently used as a marker in virus assays in cultured fibroblasts.

Latent infection is not productive. There are very few copies (about 5) of the virus genome in latently infected cells, and viral gene expression is also highly limited. Mostly translation does not occur, and normally no virus or tumor associated antigens can be detected (Calnek et al., 1981; Sharma, 1981), although some genes may be transcribed (Sugaya et al., 1990). Latent infection may be released to productive, or selectively activated in vitro with biochemical treatments (Buscaglia et al., 1988b; Buscaglia and Calnek, 1988a).

The third form of MDV infection is transforming, which is non-productive, and occurs only in cells transformed by MDV-1 virus. Transformed cells usually contain more copies of MDV genome than latently infected cells (Ross, 1985), and there is more extensive viral gene expression, occasionally resulting in antigen production (Nakajima et al., 1987; Nakajima et al., 1989). The viral DNA in transformed cells is highly methylated, whereas methylation has not been detected in viral DNA from productively infected cells (Kanamori et al., 1987).

B. Host range

Chickens are the natural host for MDV. Quail may become infected. However, quail-origin MDV appears to be more pathogenic than chicken-origin MDV for quail.

The pathogenesis for quail MD was thought different from that for chicken MD. Some genera of the order Galliformes have virological or serologic evidence of infection with MDV. Pheasants can be infected with MDV experimentally. Ducks become infected but without developing disease after MDV inoculation. There is evidence that the natural host range of MDVs is expanding to include turkeys. Close contact with chickens has been associated with outbreaks of MD in turkeys (Davidson et al., 1996; Witter, 1997a). The recent field isolates from chickens in the United States also have high oncogenic potential for turkeys (Davidson et al., 1996; Witter, 1997a). Mammalian species are refractory to infection with virulent MDV (Calnek and Witter, 1997).

C. Clinical signs of MD

MD is horizontally transmitted by direct or indirect contact between birds. Many apparently normal chickens may be carriers. Viruses in FFE replicate into a fully infectious form (Calnek et al., 1970). Feather dander serves as a source of contamination of the environment, where the virus can remain infectious for a prolonged period at low temperatures (Beasley et al., 1970; Calnek et al., 1970). Chicks inoculated at a day of age shed virus about 2 week post-inoculation, whereas clinical signs and gross lesions appear about 3 to 4 weeks post-infection. In general, the clinical signs of MDV infection are associated with asymmetric progressive paresis and later, complete paralysis of one or more of the extremities, which is common in both classic and acute MDV infections. A particular characteristic sign in infected birds is one leg stretched forward and the other backward (range paralysis). A transient paralysis syndrome associated with MD may develop 8-12 days post-inoculation and last 1-2 days. Ocular lesions are another common

signs in MDV infection. Blindness may result from infection of the iris (neoplastic mononuclear infiltration) (Calnek and Witter, 1997).

D. Morbidity and Mortality

Incidence of MD is quite variable. In general, the mortality is nearly equal to morbidity. Prior to use of vaccines, losses in affected flocks were estimated to range from a few birds to 25-30% and occasionally up to 60%. Since vaccination, the losses in egg-type chickens were reduced to less than 5%, and broilers may experience losses of 0.1-0.5% and condemnation of 0.2% or more (Purchase, 1985). After the disease appears, mortality builds gradually and generally persists for 4-10 weeks. Both infectious agent and host factors can influence the losses in affected flocks. Host factors, virulence of the virus, dosage and route of exposure are usually correlated with losses. Host factors which affect pathogenesis and immune response, such as sex, age, genotype, and maternal antibody may also influence the losses in affected flocks.

E. Gross and microscope lesions of MD

The lesions of MD were systemically reviewed by Calnek and Witter (1997). The most common gross lesions are associated with peripheral nerves and lymphoid tumor formation in various organs. The peripheral nerves, especially the celiac, brachial, and sciatic nerves are grossly enlarged, and appear gray to yellow, occasionally there is obvious edema, and a loss of cross striations. The celiac plexus is more frequently involved than other peripheral nerves (Goodchild, 1969). Lymphoid tumors may occur in one or more organs, including gonads, lungs, heart, mesentery, kidneys, liver, spleen, bursa, thymus, adrenal glands, pancreas, proventriculus, intestine, iris, skeletal muscles, and skin. Visceral tumors are common in acute MDV infections.

Mocroscopically, lesions are found in peripheral nerve, central nerve system, lymphometous lesions on various organs, skin, bursa of Febricous and thymus. There are two main types of lesions in peripheral nerves. The type A lesion is neoplastic in character, consisting of masses of lymphoblastic cells, along with demyelination and Schwann cell proliferation. The type B lesion is inflammatory in character, consisting of diffuse mild to moderate infiltration by small lymphocytes, and plasma cells, along with edema, demyelination and Schwann cell proliferation. A mild version of the type B lesion was referred as type C lesion (Payne and Biggs, 1967), but this terminology is not commonly used today. The MD lesions in the central nervous system are usually inflammatory in character. Lymphomatous lesions in visceral organs are uniformly proliferative in nature. Their cellular composition consists of diffusely proliferating lymphoblasts, and lymphocytes, along with activated and primitive reticular cells (Payne and Biggs, 1967). Plasma cells are rarely present (Purchase, 1985). Skin lesions are mainly inflammatory in nature. Occasionally, lymphomatous lesions are present. Skin lesions are usually located around infected feather follicles. The bursa of Fabricius and thymus are commonly smaller than normal in chickens infected with MDV. The lesions are characterized by degeneration, atrophy, necrosis, and lymphoid infiltration.

3. Pathogenesis of MDV infection

The pathogenesis of MDV-1 infection is well established. Virus gains entrance via the respiratory tract where it is most likely internalized by phagocytosis. Shortly thereafter, the lymphoid organs, such as spleen, bursa of Fabricius, and thymus, are infected. The cytolytic infection is primarily confined to B cells, although some T cells may also involved (Calnek and Spencer, 1985; Shek et al., 1983). This first phase

infection is called the productive-restrictive infection, and cause primarily degenerative changes. The local necrosis associated with this phase can provoke an acute inflammatory reaction, attracting macrophages, granulocytes, and lymphocytes (Payne and Roszkowski, 1973). The severity of this early cytolytic phase may be related to the virulence of virus strain.

About 6-7 days post-infection, the infection switches to the latent stage, due to the development of host immune response. T- cell mediated immunity (CMI) plays a central role in this switch (Calnek and Spencer, 1985; Payne, 1985). The activation of T cells in response to the necrotizing infection of B cells acts as a key event in pathogenesis by providing an abundant supply of cells that are the usual target cells for transformation (Calnek, 1986; Schat et al., 1982).

The concept of latency is central to our understanding of herpesviruses. Latency is the reversibly nonproductive infection of a cell by a replication-competent virus. Almost all herpesviruses are capable of becoming latent, which can generally be considered to be a lifelong state. Latency involves several important properties of herpesviruses. First, they must successfully evade the host immune response, and second, they must be able to insert their genome into cells of the body, and have that genome persist in the latently infected cell. This is relatively easy for neurotropic herpesviruses (α-herpesviruses), which infect non-dividing cells such as neurons. It is more difficult for lymphotropic herpesviruses (γ-herpesviruses), which infect dividing or mitotic cells such as B cells. In the later case, the virus requires a specialized origin of DNA replication to ensure that its genome is replicated and retained in daughter cells, upon cell division. An example of such an element is the Epstein-Bar virus (EBV) oriP element, which is required for

latent-phase EBV DNA replication. In MDV latent infection, the CD4+ T-helper cells are the principal targets of the virus, although a few B cells may be involved (Schat et al., 1991). The latent infection is persistent and can last for the lifetime of the host (Witter et al., 1971). The mechanism of MDV genome persisting in T-cells is unknown.

Only susceptible birds will progress past the latent stage and develop a second wave of cytolytic infection, coincident with permanent immunosuppression. This second productive infection is much more severe and extensive than the early productive-restrictive infection. In this phase, affected tissues are not limited to the lymphoid organs, but also include epithelial cells in various visceral organs. Lymphoproliferation and the development of T-cell tumors are commonly observed in this stage (Buscaglia and Calnek, 1988a; Calnek and Witter, 1997). The composition of lymphomas is complex, consisting of a mixture of neoplastic, inflammatory and immune cells. Both B and T cells are present, but T cells are predominant (Hudson and Payne, 1973; Rouse et al., 1973). The usual target cells for transformation are CD4+ active T-helper cells. But various subsets of T-cells, such as CD4+, CD8+, and CD4-/CD8- cells, are transformable experimentally (Schat et al., 1991), and both T and B cell tumors have been reported in turkeys infected with MDV-1 (Nazerian et al., 1985; Powell et al., 1984). The infections of transformed cells are mostly non-productive in vivo and in vitro.

MDV transformed cell lines can be established from the tumor tissues. Lymphoblastoid cell lines developed from MD lymphomas grow continuously in cell culture. All cell lines have T cell markers, usually CD4+/CD8- from lymphomas. The cell line also can be established from spleen cells of early MDV lesion, which may be CD4+/CD8-, CD4-/CD8+, or CD4-/CD8-. The cell lines can be distinguished as either

producer or non-producer cell lines. Producer cell lines are those cells MDV can be rescued from after in vitro co-cultivation, or following inoculation into susceptible chickens. In non-producer cell lines, viral antigens are not detectable and virus can not be rescued by co-cultivation (Schat et al., 1989).

Inoculation with attenuated MDV-1 viruses, or challenge of vaccinated chickens with virulent virus, will alter the pathogenesis by reducing or eliminating the early productive infections (Calnek et al., 1980; Payne et al., 1976; Schat et al., 1985; Smith and Calnek, 1974). Consequently, the level of latent infection is markedly reduced, and neither late cytolytic infection, nor immunosuppression, nor transformation occurs.

Several host factors, such as age, sex, maternal antibody, immunological impairment, and vaccination may affect the susceptibility of chickens to MDV infection in various degrees. One of the potential important factors is genetic resistance. Genetic effects are not obvious during the initial phase of productive infection. Early cytolytic lesions are equivalent in susceptible and resistant strains of chickens. However, the infection level in resistant bird will drop rapidly and markedly 8-10 days post-infection, whereas infection in susceptible birds remains at high levels or after a brief drop, rises to constitute the second cytolytic infection which then continues at a high level until the death of the birds (Calnek, 1985). The genetic resistance to MDV infection is associated with the B-F region of the major histocompatibility complex (MHC), B complex (B). The MHC type resistance can markedly reduce the infection level during latent phase, and eliminate the second wave of productive infection, which occurs in susceptible chickens. The chickens with B^{21} allele are highly protective against MD (Briles et al., 1983; Briles et al., 1977; Calnek, 1985; Hansen et al., 1967; Longenecker et al., 1976). Other B alleles have ranged

from susceptible to various degree of resistance (Bacon, 1987; Hepkema et al., 1993). Recently a MHC-like, Rfp-Y haplotype (Rfp-Y is a second region in the genome of the chicken containing MHC class I and II genes) was reported to significantly influence the outcome of infection with MDV, too (Wakenell et al., 1996). Non-MHC genes may also be involved in resistance. Lines 6 and 7 type (Crittenden et al., 1972) exhibit resistance associated with the Ly-4 locus. This non-MHC resistance may be more importantas demonstracted in studies involving several commercial chicken lines (Groot and Albers, 1992).

4. Molecular biology of MDV

A. Physical map and genomic structure

The DNA of MDV is a linear double-stranded molecule that has a density of 1.705g/cm^3 in CsCl, and a base composition of 46% guanine and cytosine. The molecular weight of MDV DNA is about 108-120 x 10^6 Dalton (Da), equivalent to 166-184 kilo base pairs (kbps) (Cebrian et al., 1982; Fukuchi et al., 1984; Hirai et al., 1979; Lee et al., 1971). The composition and density of HVT DNA is similar to MDV-1 DNA (Lee et al., 1972). The genomic structure and gene arrangement of MDV DNA are similar to that of α-herpesviruses, including HSV and varicela-zoster virus (VZV) (Buckmaster et al., 1988; Davison and Scott, 1986b; Karlin et al., 1994; Roizman et al., 1992; Wu et al., 1988), although biologically MDV is close to γ-herpesviruses, like EBV.

The gross organization of the MDV genome consists of unique long and unique short regions (U_L and U_S), each bounded by inverted repeats, named terminal repeat long (TR_L), internal repeat long (IR_L), internal repeat short (IR_S) and terminal repeat short

(TR_S), respectively (Fig. 1). The TR_L/U_L, U_L/IR_L, IR_S/U_S and U_S/TR_S junctions have been determined (Makimura et al., 1994; Brunovskis and Velicer, 1995). The TR_L/U_L junction is located 192 bps downstream of the last EcoRI site in the TR_L region, while the U_L/IR_L junction is located 192 bps upstream of the first EcoRI restriction enzyme site in the IR_L region. The IR_S/U_S junction is located 914 bps downstream of the last EcoRI site in the IR_S region, while the U_S/TR_S junction is located 914 bps upstream of the first EcoRI restriction enzyme site in the TR_S region.

In addition to the inverted repeats, several direct repeats have been identified in the MDV genome (Hirai et al., 1988). These direct repeat sequences are mostly located within the internal and terminal repeat regions. A heterogeneous expansion region containing multiple, tandem 132 bps repeats has been identified in attenuated MDV and mapped to the inverted regions flanking the U_L region of the genome (Fukuchi et al., 1985a; Maotani et al., 1986; Silva and Witter, 1985), adjacent to the MDV origins of replication (Ori), and designated as DR1. DR1 can be expanded by serial in vitro passages of virulent MDV-1 strain in primary CEF cell culture (Fukuchi et al., 1985c; Maotani et al., 1986), with a few copies in virulent MDV-1, and multiple copies in their attenuated derivatives. The function of this DR1 repeat needs further investigation, but Kawamura's work suggested that the DR1 may be associated with viral oncogenicity (Kawamura et al., 1991).

The physical maps of BamHI restriction endonuclease (RE) fragments have been constructed from MDV-1 (Fukuchi et al., 1985b), MDV-2 (Ono et al., 1992), and HVT (Igarashi et al., 1987). All three serotypes differ in their RE digestion patterns (Gibbs et al., 1983; Hirai et al., 1979; Ross et al., 1983; Silva and Barnett, 1991), but share

significant homology at the DNA level, based on cross hybridization and certain individual gene comparison results (Coussens and Velicer, 1988; Igarashi et al., 1987; Ono et al., 1992; Zelnik et al., 1992; Scott et al., 1993; Yoshida et al., 1994c). The RE maps have become a basis for most gene identification and localization, and also are useful for comparative studies.

B. MDV replication and gene expression

For initial infection of cultures or chickens with cell free virus, enveloped virions enter susceptible cells by conventional absorption and penetration. By contact with cell associated virus, infection is initiated by cell-to-cell fusion or direct contact with infected cells (Hlozanek, 1970). Due to the highly cell-associated features of MDV, cell-to-cell transfer is normally accomplished through formation of intracellular bridges (Kaleta and Neumann, 1977), and is presumed to be the principal mode of virus spread both in vitro and in vivo. Several envelope glycoproteins are involved in these initial events. After penetrating into the cytoplasm, the naked virions are transported to the nuclear pores, where viral DNA is released from the capsid into the nucleus. Transcription, replication of viral DNA, and assembly of new capsid take place in the nucleus. The α-herpesviruses (or Standard group E genomic herpesviruses) have similar replication and transcription patterns, and presumably MDV replication may also follows this pattern.

In HSV-1, several tegument proteins have been shown to have important functions for the initiation of viral replication. Among these are the virion host shut-off (vhs) protein (U_L41 gene product), involving in the early shut-off of host macromolecule synthesis, and turning the infected cell into a viral replication machine. Another protein of importance is U_L48 gene product, named VP16 (αTIF), acting as trans-activator to

induce transcription of immediate early genes (IE, or α gene in HSV-1) which are the first set of genes to be expressed. The αTIF and vhs are structural proteins located in the tegument. They enter the cell along with the virion and perform their functions in different compartments of the cell. The U_L46 and U_L47 gene products appear to modulate the VP16 function (Roizman and Sears, 1995). Homologs to all these genes are present in MDV genome (Yanagida et al., 1992a), but their functions are not yet defined.

HSV genes are grouped by their timing of expression. Each group is sequentially ordered in a cascade fashion (Roizman and Sears, 1995). MDV gene expression is also believed to follow the same pattern (Maray et al., 1988; Schat et al., 1989). Generally, herpesvirus genes have been grouped into three kinetic families: IE genes, early genes (E or β in HSV-1), and late genes (L or γ in HSV-1), based on the requirement for viral protein synthesis and/or viral DNA replication (Honess and Roizman, 1974).

(1). MDV IE gene expression

IE genes are expressed immediately upon infection, in absence of viral protein synthesis. There are five α genes in HSV, referred to as infected cell peptides, or ICP, with numbers designation: 0, 4, 22, 27, and 47. All these proteins, except of ICP47, have been shown to have regulatory functions, and functional proteins are required for the synthesis of subsequent polypeptide groups. Early attempts to identify the IE genes of MDV have had little success due to the highly cell-associated properties of the virus. With protein synthesis inhibitor, cycloheximide, numerous IE transcripts have been detected in MDV infected cells and MDV transformed lymphoblastoid cell lines (Maray et al., 1988; Schat et al., 1989). Only limited information was achieved due to the fact that cycloheximide also inhibits the translation of these IE transcripts. Therefore no IE

proteins were detected. Recently, at least three IE genes of MDV were identified, with homologues to HSV-1 ICP4 (Anderson et al., 1992), ICP27 (Ren et al., 1994), and ICP22 (Brunovskis and Velicer, 1995). The MDV ICP4 homologue is located in the BamHI-A fragment within the short inverted repeats, and there are two copies per MDV genome. The open reading frame (ORF) of MDV ICP4 consists of 6972 bps, and predicts a polypeptide with 2323 amino acids. (This data is derived from MDV DNA sequence on Genbank with access number U17705) (McKie et al., 1995). MDV ICP27 homologue has been mapped to the EcoRI-B fragment of MDV DNA. MDV ICP27 is a 473 amino acid polypeptide and shows 26% amino acid identity with HSV ICP27. A cysteine rich domain and a potential zinc-binding motif within the C-terminus of the ICP27 proteins appear to be highly conserved between α-herpesviruses. Two slightly different MDV ICP27 species, 52 and 55 kDa, have been detected in MDV infected cells with ICP27 specific antibodies (Ren et al., 1994). The biological functions of the MDV IE gene products, including ICP4 and ICP27, are poorly understood.

(2). MDV E gene expression and viral DNA synthesis

E genes are a second group of genes expressed following IE gene expression, and are regulated by IE gene products. Most E gene products are involved in viral DNA replication, which are essential for viral origin dependent DNA synthesis. Any inhibitors of viral DNA synthesis, such as phosphonoacetic acid (PAA), can lead to E gene product accumulation.

In HSV, available evidence suggests that the viral DNA is circularized immediately after the DNA is released into the nucleus. The DNA replicates by a rolling circle mechanism, and the synthesized DNA is a large circular or head-to-tail

concatemeric molecule. Seven genes mapped in U_L region are required for viral origin dependent DNA synthesis, including a DNA polymerase (U_L30), a single strand specific DNA binding protein, ICP8 (U_L29), an origin binding protein, OBP (U_L9), a subunit of DNA polymerase (U_L42), and a primase and helicase complex of U_L5, U_L8 and U_L52. Most of these homologues have not yet been identified in MDV genome, except the OBP (Wu et al., 1996), the DNA polymerase (Sui et al., 1995), and the partial UL29 (Wu et al., 1996).

Thymidine kinase (TK) homologue is presumably the first E gene identified in the MDV genome (Scott et al., 1989). DNA polymerase (U_L30) is also an E gene (Sui et al., 1995). A MDV unique phosphoprotein, named pp38, was identified as an E gene (Chen et al., 1992; Cui et al., 1991; Cui et al., 1990). The pp38 homologues are also present in MDV-2 and HVT (Ono et al., 1994; Smith et al., 1995). But neither gene appears closely related to MDV-1 pp38. The pp38 gene is located within BamHI-H fragment of MDV-1 genome, and spans the U_L/IR_L junction. The pp38 protein is virus-specific phosphorylated protein complex containing polypeptides of 36-39 kDa and 24 kDa, which are abundantly expressed in the cytoplasm of MDV infected cells, including FFE, and MDV transformed lymphoid cell line (MSB-1), latently infected cells, and MD tumor cells (Ikuta et al., 1985a; Naito et al., 1986; Nakajima et al., 1987; Cui et al., 1990; Ikuta et al., 1985a). The biological function of pp38 is potentially related to MDV oncogenicity (Calnek and Witter, 1997). However, some evidence (Cui, 1995. personal communication) suggest that pp38 may suppress host immune reaction, therefore promoting the virulence of MDV.

(3). MDV L gene expression

L genes comprise the large class of genes in herpesvirus. The key feature of late gene transcription is the requirement for viral DNA replication, therefore DNA replication inhibitor will affect L gene expression. Most L gene products are structural proteins, including capsid proteins, tegument proteins, and envelope glycoproteins. Based on dependence for viral DNA replication, the γ genes (L genes of HSV) can be grouped into γ1 and γ2. γ1 transcriptions occur prior to initiation of viral DNA synthesis, and is only minimally affected by inhibitors of DNA synthesis. γ2 proteins are expressed late in infection and are not detectable in the presence of effective concentrations of inhibitors of viral DNA synthesis (Roizman and Sears, 1995). The hallmark of infected cells late in infection is the appearance of reduplicated membranes and thick, concave, or convex patches, particularly in nuclear membranes. It is likely that the patches represent aggregation of viral membrane proteins, presumably including the viral glycoproteins on the outside surface of the envelope membrane and anchorage and tegument proteins on the inside surface (reviewed by Roizman and Sears, 1995).

(4). MDV oncogenicity-related proteins

Three DNA fragments or genes may be associated with oncogenecity of MDV-1, including the genes flanking the DR1, pp38, and meq. All three potential oncogenicity-related genes are mapped closely together in the repeat regions of the MDV genome. These regions are also associated with the transcriptions detected in MD lymphoma (Schat et al., 1989; Tillotson et al., 1988). DR1 and pp38 have been discussed in a previous section. meq is one of the few genes that is highly expressed in MDV-induced T-cell tumors (Jones et al., 1992; Peng et al., 1995), and is also present in the MDV transformed CEF cells (Buranathai et al., 1997). meq is homologous to the leucine-zipper

class of nuclear oncogenes, and contains a proline-rich domain characteristic of another class of transcription factors. meq contains two stretches of basic residues, designated basic region 1 (BR1) and basic region 2 (BR2). BR2 contains a primary nuclear localization signal (NLS) and a sole nucleolar localization signal (NoLS) (Liu et al., 1997). meq may function as a transcription factor in regulating viral latency or oncogenesis (Qian et al., 1995a). Overexpression of meq results in transformation of a rodent fibroblast cell line, Rat-2 (Liu et al., 1998).

5. Diagnosis

A. Virus Isolation, gross and microscope lesions

Virus isolation, gross and microscope lesions have been discussed in the previous sections. For virus isolation, intact viable cells, such as blood lymphocytes, heparinized whole blood, splenocytes, and isolated tumor cells are preferred samples for virus isolation, although cell-free preparations from skin, dander, or feather tips of infected chickens may be used (Calnek et al., 1970). A suspected isolate must be examined carefully, because all three serotypes may coexist in the same sample and are frequently isolated simultaneously. Although cell culture features, such as plaque forming time, and plaque morphology may provide information about the serotype, IFA with serotype specific monoclonal antibodies (Mab) is a convenient and more definitive method to identify the serotype of the isolate (Lee et al., 1983a).

B. Antigen detection

Although as many as 46 virus-specific polypeptides have been identified by immunoprecipitation assay (IP) from extracts of cells infected with MDV-1 or HVT

(Ikuta et al., 1981; Van Zaane et al., 1982a; Van Zaane et al., 1982b), only a few have important antigenic properties. Specific Mabs have greatly facilitated the detection of MDV antigen in tissues. Mabs have been developed against type-common and type-specific epitopes of all three MDV serotypes (Lee et al., 1983a). Mab H19 for pp38 is MDV-1 specific, except for Rispens strain (a vaccine strain), Mab 2BN90 for pp40 reacts with CEFs infected with MDV-1 (Lee, 1993; Lee et al., 1983a). These 2 Mabs may be used to distinguish Rispens from other MDV-1 strains. Mab IAN86 for glycoprotein B (gB) is common for MDV-1 and HVT, Mab Y5 is specific for MDV-2, and L72 is specific for HVT (Lee et al., 1983a). Feather tips, cytolytically infected lymphoid tissue or infected cell cultures are preferred samples for antigen detection by IFA (Spencer and Calnek, 1970), immunoperoxidase test (Cauchy, 1974), GAP (Haider et al., 1970; Lesnik et al., 1978), and ELISA (Davidson et al., 1988; Scholten et al., 1990).

C. Nucleotide acid detection

Detection of MDV DNA in infected samples is useful for laboratory research, but is less useful for clinical diagnosis. Polymerase chain reaction assay (PCR) has been used to detect MDV DNA from tissue samples and cell cultures (Rong-Fu et al., 1993; Becker et al., 1992; Silva, 1992; Smith et al., 1995). A quantitative PCR assay has been developed to determine the number of viral genomes present in samples in a restricted number of PCR cycles, which was reported to correlate significantly with subsequent development of disease (Bumstead et al., 1997). The southern hybridization and in situ hybridization have been used to detect MDV DNA from feather tips, and localize the virus infected cells in tissue samples (Holland et al., 1994; Ross et al., 1981).

D. Antibody detection

On the opposite side of antigen detection, tests for specific antibodies present in chicken sera are useful in studies of viral pathogenesis and for monitoring specific-pathogen-free flocks. A number of methods used for antigen detection are also applied for antibody detection. However, none of these tests are capable of determining antibodies to a specific viral serotype in chickens exposed to multiple viral serotypes. The biological significance of antibody detection by different methods may vary (Calnek and Witter, 1997).

E. Differential diagnosis

Most neoplasm of lymphoid and other hematopoietic cells in commercial poultry are caused by viruses, which belong to one of four distinct groups. MDV is an oncogenic herpesvirus. All the other three virus groups are oncogenic retroviruses, including avian leukosis virus (ALV), reticuloendotheliosis virus (REV), and lymphoproliferative disease virus (LPDV). The clinical diagnosis of MD has been considered difficult in practice, because there is no truly pathognomonic gross lesion and because MD lesions may resemble those of lymphoid leukosis (LL), or reticuloendotheliosis (RE). Especially, REV can induce experimental lesions (nerve enlargement and lymphoma) that closely resemble MD lesions. Indicators of virus infection are normally considered to have limited value in diagnosis of the disease, because only a small percentage of the infected chickens develop clinical MD. Therefore, differential diagnoses are traditionally based on disease-specific criteria, including epidemiology, pathology, and tumor-specific markers. Historically, chickens are diagnosed positive for MD on the basis of gross lesions and age if at least one of the following conditions are met: 1. Enlargement of peripheral nerves; 2.

Lymphoid tumors in various tissues (liver, heart, gonad, skin, muscle, and proventiculus) in birds under 16 weeks of age; 3. Visceral lymphoid tumors in birds 16 weeks or older that lack neoplastic involvement of bursa of Fabricius; or 4. Iris discoloration and pupil irregularity (Calnek and Witter, 1997). These criteria are based on following assumptions: 1. The absence of bursal tumors in cases of MD in birds older than 16 weeks, the consistent presence of gross bursal tumors in cases of LL; 2. REV infection only rarely induces nerve enlargement or lymphomas in the absence of bursal involvement in commercial chickens. However, a diagnosis based only on gross pathologic criteria can no longer be considered definitive (Calnek and Witter, 1997). The following table (Table 1) adapted from Fadly (Fadly, 1997) summarizes the combined criteria for lymphoma differentiation.

6. Immunity against MD

Both humoral and cell-mediated immunity develop in competent birds after infection with MDV. MDV immunity can be directed either against the virus infection or later against transformed and proliferating lymphoid cells (Payne and de-The, 1972).

A. Humoral immunity

Both precipitating and virus neutralizing (VN) antibodies can be detected in 1 to 2 weeks post-infection. These antibodies generally persist throughout the life of the bird. Only the VN antibodies correlate with survival of infected birds (Calnek et al., 1972; Sharma and Stone, 1972), which may be directed against gB (Davidson et al., 1991; Nazerian et al., 1992; Ono et al., 1985; Ross et al., 1993). The gC can induce the development of gC specific antibodies (Niikura et al., 1991), but the antibodies do not protect against MDV infection (Jang et al., 1996a). The pp38 also does not produce

protective antibodies (Nazerian et al., 1992). Because bursectomized birds survive MDV infection (Sharma and Witter, 1975a), it is presumed that the humoral antibody response may not be required for resistance to MD. Maternal antibodies also reduce the level of MDV infection(Ball et al., 1971; Chubb and Churchill, 1968). Bursectomy related resistance might result from the depletion of the B cells, which are the primary targets of early MDV productive infection, .

B. Cell-mediated immunity

Since antibodies may be not a required component of immune resistance to MD, it can be presumed that CMI is important. The first direct evidence for CMI response against MDV antigen came from delayed hypersensitivity reactions to various MD-associated antigens in chickens with naturally occurring infection (Byerly and Dawe, 1972). Sensitized T cells from convalescent chickens or from those vaccinated with attenuated MDV were found to be cytotoxic not only to chicken kidney cells infected with MDV in vitro but also to latently infected lymphocytes isolated from infected birds (Payne et al., 1978a; Ross, 1977). Cell mediated cytotoxicity against MD lymphoblastoid cells has been demonstrated in vitro (Confer and Adldinger, 1980; Powell, 1976; Sharma and Cooper, 1978; Sharma and Coulson, 1977). Functional T cells are required for resistance (Sharma et al., 1975b), as well as vaccinal immunity (Payne et al., 1978b). Pratt et al. (Pratt et al., 1992) found lymphoblastoid cell lines expressing pp38 to be targets for MHC-restricted lysis by cytotoxic T lymphocytes (CTL) induced by all three serotypes of MDV. The approach used by Pratt et al. was the transformation of MDV gene into REV-transformed cell lines serve as syngeneic targets for CTL in a chronium (^{51}Cr) release assay. Similar assay was used by Omar and Schat to demonstrate that REV

transformed cell lines expressing MDV gene gB, pp38, meq, or ICP4 were lysed by syngeneic MDV specific splenocytes. This syngeneic cell-mediated immune response is induced by virus specific CD8+ CTL, since depletion of CD4+ effect cells did not influence the specific release of chromium significantly (Omar and Schat, 1997).

C. Vaccinal immunity

Vaccination is one of the most important factor affecting MD morbidity and mortality. Vaccinal immunity protects chicks against early replication of virus in the lymphoid organs of challenged birds and reduces the level of latent infection (Calnek et al., 1980; Powell and Rowell, 1977; Purchase et al., 1972; Schat et al., 1982; Smith and Calnek, 1974). Immunity from live virus vaccines including HVT, attenuated MDV-1, and MDV-2 appear to be directed largely against viral antigens, and possibly tumor antigens. $CD8^+$ T cell responses are essential for anti-virus but not anti-tumor effects. CMI appears to be much more important than humoral immunity, since deletion of humoral immunity by bursectomy and X-irradiation has no effect on protection conferred by attenuated MDV (Else, 1974), although a similar treatment partially impairs vaccinal immunity from HVT (Calnek and Witter, 1997; Rennie et al., 1980).

D. Types of vaccines and vaccination strategies

Vaccination based on three serotypes of MDV, mixtures of serotypes, and recombinant DNA vaccines are all capable of protecting chickens against MD (Calnek and Witter, 1997). Attenuated MDV-1 vaccines (Churchill et al., 1969b; Rispens et al., 1972b) are derived from MDV-1 parent strains by serial passages of in vitro cell cultures, and are normally used in the cell-associated form since little cell-free virus can be extracted from infected cells (Witter, 1985). Md11.75C/R2/23 (Witter, 1991) and

CVI988 (Rispens et al., 1972a; Rispens et al., 1972b) are 2 attenuated vaccines which are currently licensed for use in the United States.

HVT vaccine (Okazaki et al., 1970) has been widely used since the 1970s. HVT cell-free virus extracted from infected cells may be lyophilized (Calnek et al., 1970) for convenient storage and handling, especially in developing countries, but cell associated form of HVT is more effective than cell-free virus in the presence of maternal antibodies (Witter and Burmester, 1979). Both attenuated MDV-1 and HVT can be used as a monovalent vaccine or mixture with others to become bivalent or trivalent vaccines.

The naturally avirulent isolates of MDV-2 (Schat and Calnek, 1978a) are usually combined with HVT to take advantage of the synergistic activity. HVT+SB-1 (Schat et al., 1982; Witter, 1982) bivalent vaccine was the first commercial vaccine based on synergism between MDV-2 and MDV-3 viruses. HVT+301B/1 (Witter, 1987) is another MDV serotype 2 & 3 bivalent vaccine.

Recombinant fowlpox virus or HVT expressing MDV-1 gB gene vaccines have been developed, and have shown some protection (Nazerian et al., 1992; Ross et al., 1993). These recombinant MDV DNA vaccines are not currently available for commercial use.

Vaccines are usually administered at hatching by subcutaneous or intramuscular inoculation with about 1,000 plaque forming units (pfu)/chick (Oei and de Boer, 1986). In ovo embryo vaccination (Sharma and Burmester, 1982) has been automated and is used in over 80% of commercial broilers in the United States, due to decreased labor costs and greater precision of vaccine administration (Calnek and Witter, 1997). In general, a solid immunity requires up to 7 days to be established. The shorter the interval

between vaccination and exposure to the virulent field virus, the poorer the level of protection (Okazaki et al., 1970).

Several factors may affect the vaccination efficacy, including genetic makeup of the bird or age at challenge with virulent virus. Immunosuppression due to stress (Powell and Davison, 1986) or infection by immunosuppressive viruses, such as infectious bursal disease (Sharma, 1984), REV (Witter, 1979), reovirus (Rosenberger, 1983), and chicken anemia virus (Otaki et al., 1988; Yuasa et al., 1988), have been reported to interfere with the induction of vaccinal immunity. "Vaccine failures", however, may mainly result from the early exposure and emergence of new higher virulent MDV strains. MDV 2+3 bivalent and serotype 1+2+3 trivalent vaccination are used to deal with the emergence of virulent strains of MDV. Strict biosecurity practices to reduce early exposure, the use of genetically resistant birds, and reasonable vaccine combination are essential adjuncts to successful vaccination program (Calnek and Witter, 1997).

E. Non-specific immunities

Macrophages. The primary function of macrophages is to phagocytose and degrade the ingested materials, including viruses, bacteria, cells debris, and whole dead or altered cells. The macrophage also plays a central role in the regulation of the immune response. It may be involved in resistance by restricting virus replication directly (Haffer et al., 1979; Higgins and Calnek, 1976) or in concert with antibody (Kodama et al., 1979; Lee, 1979). Immune B cells and macrophages can interact to inactivate cell-free virus (Schat and Calnek, 1978b). Activation of macrophages markedly reduces the incidence of MD in challenged birds in vivo (Gupta et al., 1989), and inhibits virus replication and proliferation of MD lymphoblastoid cell lines in vitro (Lee, 1979).

Interferon may be produced as a response to infection of MDV (Kaleta and Bankowski, 1972), and the level varies due to differences in genetic resistant lines of chickens (Hong and Sevoian, 1971), as well as differences in serotype and strains of infecting MDV (Sharma, 1989). Interferon protects against the transplantable JMV tumors (Vengris and Mare, 1973), and it appears to be one of the cytokines important in the development and maintenance of latency with MDV (Buscaglia et al., 1988b; Volpini et al., 1995).

Natural killer (NK) cells may be involved in age, genetic and vaccinal resistance to MD. There are increased NK cell activity after vaccination with HVT or SB-1 (AMDV-2 strain), and the NK cell activity is cytotoxic for lymphoblastoid cell lines (Quere and Dambrine, 1988). NK cells may also play a role in intratumor immunity in tumor regression (Calnek and Witter, 1997).

7. Immunosuppression

Impairment of the immune response might result directly from MDV infection through cytolytic infection of lymphocytes or indirectly through the activity of suppresser cells. Permanent immunosuppression tends to correlate with eventual tumor development (Schat et al., 1978), and coincide with the second phase of cytolytic infection. A possible association between immunosuppression, reactivation of cytolytic infection, and MD breaks during the laying cycle should be considered in egg-laying chickens (Calnek and Witter, 1997). Both humoral immunity and CMI can be depleted by MD. This is reflected by reduced antibody response to a variety of antigens and by alterations of T-cell functions (Calnek and Witter, 1997). pp38 polypeptide plays a role in antibody and CMI

depletion, which may result from the apoptosis of $CD4^+$ cells and the downregulation of CD8 expression due to the infection of MDV (Morimura et al., 1995). The pp38 might be involved in the downregulation of the MHC class 1 molecule, or interference of the antigen processes in CD4+ T cells, therefore resulting in failure of the host immune system, especially CTL response, to recognize the virus infected cells.

II. Herpesvirus glycoproteins and their roles in virus infection and spread

One of the unusual features of MDV is its strict cell-association in vitro and in vivo. MDV spreading from infected cell to neighboring normal cell is thought to occur by intracellular bridge formation (Kaleta and Neumann, 1977), which may require expression of several viral envelope glycoproteins on the infected cell surface. Recent analyses of the nucleotide sequence of MDV-1 revealed at least eight glycoproteins which are homologues to HSV-1 gB (Ross et al., 1989; Yanagida et al., 1992b), gC (Coussens and Velicer, 1988), gD (Ross et al., 1991b), gI and gE (Brunovskis and Velicer, 1995), gK (Ren et al., 1994), gH (Scott et al., 1993), and gL (Yoshida et al., 1994a).

The principal events of virus infection are attachment (binding of virus to cell surface), and penetration (the virion envelope fuses with the cellular plasma membrane, releasing the viral nucleocapsid into the host cell cytoplasm). Attachment of the virus to a cell surface activates a cellular process mediated by viral surface proteins that lead to the fusion of the viral envelope with the cellular plasma membrane. Multiple viral glycoproteins are required for HSV-1 infection and cell fusion, including gB, gC, gD, and, gH and gL complex, which can act individually or in combination to trigger pH-independent fusion of the viral envelope with the host cell plasma membrane (Spear, 1993). Transient expression of gB, gD, and gH and gL is sufficient to induce membrane fusion in the Cos cells transfection system (Turner et al., 1998).

1. Glycoprotein B homologues

A. HSV-1 gB

The gB is most important glycoprotein in herpesvirus family. HSV-1 gB is essential for viral infection (Little and Schaffer, 1981; Sarmiento et al., 1979), which is involved in virus entry and cell-to-cell fusion (Cai et al., 1988; Little and Schaffer, 1981; Manservigi et al., 1977; Sarmiento et al., 1979). The HSV-1 gB gene is located in the middle of U_L region (U_L27) (McGeoch et al., 1988). A 904 amino acid polypeptide was predicted, consisting of a co-translationally cleaved signal sequence, a large N-terminal extracellular domain, a 69 amino acid hydrophobic transmembrane domain postulated to include three membrane-spanning α-helices, and a 109 amino acid highly charged cytoplasmic C-terminal domain (Bzik et al., 1986; Bzik et al., 1984; Pellett et al., 1985).

HSV-1 gB bears several neutralization epitopes, and can induce neutralizing antibodies (Navarro et al., 1992). Immunization of mice with HSV-1 gB emulsified in Freund's complete adjuvant or with HSV-1 gB adsorbed to aluminum gel induced full protection against subsequent challenge with HSV-1 or HSV type 2 (HSV-2). Latent infection in the trigeminal ganglion was also prevented by immunization with gB (Kino et al., 1986). This protection resulted from the antibodies that block penetration of virions into host cells, and also prevents spread of virus from the infected cells to neighboring uninfected cells (Navarro et al., 1992).

B. VZV gB homologue, gpII

The VZV gB homology is also located in the center of U_L region (Davison and Scott, 1986b). A 2.6 kbps ORF potentially encodes a 98-kDa polypeptide with glycoprotein features, which is designated as gpII. The primary amino acid sequence of

gpII shows higher homology to HSV-1 gB. Unlike the mature gene products of HSV-1 gB, the primary 125-140 kDa translational product of gpII is cleaved approximately into halves, forming a pair of glycoproteins with approximate molecular weights of 66 and 68 kDa. The mature gpII (140 kDa) is a disulfide-linked heterodimer (Keller et al., 1986; Montalvo and Grose, 1987). However, VZV gpII displayed many biological and biochemical properties similar to its homologue HSV-1 gB. The anti-Mabs also exhibit both neutralization activity and inhibition of virus-induced cell-to-cell fusion. A rabbit infected with a gpII recombinant vaccinia virus produced antibodies which recognized VZV antigens and neutralized VZV infection in vitro (Massaer et al., 1993).

C. MDV gB homologues

MDV homologue of HSV gB has been identified and sequenced within BamHI fragments I_3 and K_3 of MDV-1 RB1B and GA strains (Chen and Velicer, 1992; Ross et al., 1989; Yanagida et al., 1992b). The amino acid sequence of MDV-1 gB shares similar structural features with gB of HSV-1, VZV and other mammalian herpesviruses. The percentage of amino acid identity between gBs of α-herpesviruses has a mean of 50% which was almost twice that between cytomegalovirus (CMV) and EBV (Ross et al., 1989). MDV-1 gB is composed of the B antigen complex: gp100, gp60, and gp49 as detected by immunoprecipitation with Mab1AN86 (Chen and Velicer, 1992; Ross et al., 1989; Yanagida et al., 1992b). The primary 100 kDa translational product of gB is cleaved approximately into gp60 and gp49. The mature form of 100 kDa gB is also a heterodimer (Yoshida et al., 1994b). A MDV-1 gB recombinant fowlpox virus can elicit neutralizing antibodies and protect chickens against challenge with vvMDV strains, RB1B and MD5 (Yanagida et al., 1992b). A MDV-1 gB recombinant HVT virus has

been constructed, too, but the biological significance has not been determined (Ross et al., 1993). The purified HVT gB also induced partial protection in chickens against MDV-1 challenge (Ono et al., 1985). Recently, the gB homologue in MDV-2 and HVT have been identified and sequenced (Yoshida et al., 1994c). The MDV-2 gB is structurally different from MDV-1 and –3 gBs. At least an important epitope recognized by Mab1AN86 is present in MDV-1 and -3, but not in MDV-2 (Yanagida et al., 1992b; Yoshida et al., 1994c).

D. CMV gB homologue

Human CMV (HCMV) gB is a large membrane-anchored glycoprotein. The 906 amino acid polypeptide contains a 24 amino acid signal sequence, a 690 amino acid extracellular domain with 15 N-link glycosylation sites, a 58 amino acid hydrophobic sequence that anchors the glycoprotein in the plasma membrane, and a long, charged 134 amino acid carboxyl-terminal intracellular domain (Cranage et al., 1986). HCMV gB undergoes post-translational glycosylation (Pereira et al., 1984), and is also proteolytically cleaved between amino acid 460-461 (Spaete et al., 1990; Spaete et al., 1988). The cleaved fragments are assembled into heterodimers (Britt and Vugler, 1992). Three electrophoretically distinct proteins of 170 kDa, 116 kDa, and 55kDa are identified from HCMV infected cells (Britt et al., 1990). HCMV gB is an abundant glycoprotein in the virion envelope, which elicits neutralizing antibodies in human infection and in immunized animals. Rabbit serum against HCMV gB can immunoprecipitate gB from HCMV-infected cells and neutralize HCMV infectivity in vitro (Cranage et al., 1986). Two unique neutralizing epitopes were shown to be present on the cell surface gp55-116 (gB) (Britt et al., 1990). The N-terminal 513 amino acids of HCMV gB stimulate both B-

and T-cell immune responses in humans (Liu et al., 1991). Antibodies to recombinant-derived gB after natural human cytomegalovirus infection correlate with neutralizing activity (Marshall et al., 1992), which were found to be against the functional region of gB molecule (Navarro et al., 1997). HCMV gB is a multifunctional glycoprotein which plays a central role in infectivity by promoting virion entry into cells, cell to cell spread of infection, and fusion of infected cells (Navarro et al., 1993; Tugizov et al., 1994). The HCMV gB is a ligand for the virus that mediates an interaction with a cellular receptor(s) during HCMV infection (Boyle and Compton, 1998).

E. EBV gB homologue

EBV gB homologue, designated as gp110, was identified through cross-reactivity with anti-gpII and anti-HSV-1-gB sera (Emini et al., 1987). Humans with serologic evidence of EBV infection show gp110 antibodies and gp110 activated T-cells (Roudier et al., 1989). The gp110 is one of the most abundant proteins found during the late phase of viral replication, and serves as a target for antibody-dependent cell-mediated cytotoxicity (ADCC) (Jilg et al., 1994). Although gp110 has substantial amino acid homology to HSV-1 gB, its localization within infected cells is different. The gp110 is located in the endoplasmic reticulum (ER) and nuclear membrane, and is absent from virions. gp110 contain independent signals sufficient to direct the protein to the ER/nuclear membrane. Specific transport of γ-herpesvirus gB to the nuclear membrane suggests that it may be involved in the egress of virus from the nucleus (Papworth et al., 1997). Functionally, the gp110 is also essential for EBV replication in vivo (Herrold et al., 1996; Lee and Longnecker, 1997).

2. Glycoprotein C

The initial interaction of HSV-1 virus with the host cells is binding to heparan sulphate receptor (HS). gC is principally responsible for this binding, although gC is dispensable for replication of HSV-1 in cell culture (Herold et al., 1991). Three approaches have been used to show that gC mediates the initial interaction between virus and cells. First, antibodies directed against gC inhibit HSV absorption to cells (Fuller and Spear, 1985); second, isolated HSV-1 gC can bind to cells (Herold and Spear, 1994; Svennerholm et al., 1991); and third, HSV mutants lacking gC absorb to cells inefficiently (Herold et al., 1991; Sears et al., 1991). There are several lines of evidence indicating that HS moieties of cell surface proteoglycans are serviced as receptors for gC binding (Herold et al., 1991; Shieh et al., 1992; Tal-Singer et al., 1995; Wudunn and Spear, 1989). HS is present not only as a constituent of cell surface proteoglycans but also as a component of the extracellular matrix and basement membranes in organized tissues. In addition, body fluids contain both heparin and heparin-binding proteins, either of which can prevent the binding of HSV to cells (Wudunn and Spear, 1989). As a consequence, the spread of HSV infection is probably influenced, not only by immune responses to the virus, but also by the probability that virus will be entrapped or inhibited from binding to cells by extracellular forms of heparin or HS (Spear et al., 1992). In addition to its function on attachment in viral infection, gC also is one of the viral molecules which modulate the immune response by interacting with components of the humoral immune system. gC binds the C3b and iC3b fragments of the third component of human complement (Eisenberg et al., 1987; Friedman et al., 1984; Ghosh-Choudhury et

al., 1990; Tal-Singer et al., 1991). This activity protects the virus from antibody independent complement-mediated neutralization, suggesting the role for gC early in infection, before antibodies develop (Fries et al., 1986; Harris et al., 1990).

Although HSV-1 and HSV-2 gC can bind to cell surfaces via HS, the interactions of HSV-1 gC and HSV-2 gC with cell surface HS are different, which may influence cell tropism of the viruses (Herold et al., 1996). VZV gC, which is also dispensable for replication in tissue culture, plays a critical role in the virulence of VZV for human skin (Moffat et al., 1998). MDV gC homologues (gp57-65) (Coussens and Velicer, 1988; Ikuta et al., 1985b; Isfort et al., 1987; Isfort et al., 1986), originally named A antigen, is identified in the supernatant fluids of MDV infected cell cultures by AGP test (Churchill et al., 1969a). MDV gC is also present in the cell surface and in the cytoplasm of productively infected cells, but the authentic gC is mainly secreted into the culture supernatant of MDV-infected CEF (Isfort et al., 1986). gC expression decreases with serial passage of MDV in cell culture (Churchill et al., 1969a), probably due to reduced transcription of gC gene (Wilson et al., 1994). gC appears not related to oncogenecity. MDV gC is non-essential for virus replication in vitro in cell culture. The recombinant gC expressed by the recombinant baculovirus retains the antigenic and immunogenic properties of wild type gC (Niikura et al., 1991), but it does not induce protective immune response against vvMDV challenge in chickens (Jang et al., 1996a).

3. Glycoprotein D

The gD is one of the structural components of HSV-1 envelope which is essential for virus entry into host cells. HSV-1 gD acts alone or in combination with gB, or gH-L

complex to trigger pH-independent fusion of viral envelope with the host cell plasma membrane (Spear, 1993). The biological function of gD is mainly dependent on its native conformation. gD protein and its antibodies inhibit HSV-1 infection in cultured cells in several ways.

A. HSV-1 gD specific antibodies can neutralize virus infection. HSV-1 gD or its synthetic peptide (amino acid residues 1 through 23) inoculation in mice can elicit antibodies which protect mice from a lethal challenge by either HSV-1 or HSV-2. The sera from the inoculated mice showed neutralizing activities against both HSV-1 and HSV-2 (Eisenberg et al., 1985). Several points have been made about HSV-1 gD: (i) gD is a major target antigen for neutralizing antibody, (ii) the mechanism of neutralization can involve inhibition of virus penetration of the cell surface membrane, and (iii) gD plays a direct role in the virus entry process (Highlander et al., 1987)

B. Cells that express HSV gD are resistant to infection with wild-type virus as a result of gD-mediated interference. A BJ cell line (baby hamster kidney clonal cell line) which consistently expresses HSV-1 gD is resistant to infection with HSV-1. Analysis of clonal lines of the BJ cells indicated that resistance to superinfecting virus correlates with the expression of gD. Resistance is not due to a failure of attachment to cells, but interference with fusion of the virion envelope with the plasma membrane (Campadelli-Fiume et al., 1988). This interference has been noted with several cell lines expressing gD (Campadelli-Fiume et al., 1990; Johnson and Spear, 1989), and also noted with other herpesviruses, including pseudorabies virus (PRV) and bovine herpesvirus type 1 (BHV-1) (Chase et al., 1990; Petrovskis et al., 1988). The gD-mediated interference is due to the competition between cell-associated gD and virion-associated gD for a cellular co-

receptor for entry (Campadelli-Fiume et al., 1988; Dean et al., 1994; Johnson and Spear, 1989). One of the cell surface co-receptor was identified in vitro in cell culture, which is a member of the tumor necrosis factor-nerve growth factor receptor family, and named herpesvirus entry mediator (HVEM) (Whitbeck et al., 1997).

C. Cells pretreated with soluble, truncated forms of gD are resistant to virus infection. HSV-1 and HSV-2 plaque production is inhibited by treating cells with soluble forms of HSV-1 gD (gD-1t) and HSV-2 gD (gD-2t). Both truncated gDs inhibit entry of HSV-1 and HSV-2 into the cells without affecting virus adsorption. Specific binding of gD-1t and gD-2t to a limited set of cell surface receptors is essential for subsequent virus entry into cells. This binding is not required for the initial adsorption of virus to the cell surface, which involves more numerous sites (probably including heparan sulfate) than those which mediate gD binding (Johnson et al., 1990; Nicola et al., 1996).

D. The inhibition of HSV infectivity by soluble gD is influenced by the antigenic conformation of the blocking gD mutant, as well as the form of gD in the target virus (Nicola et al., 1997). HSV-1 mutants that are resistant to gD-mediated interference have been isolated. Some of the mutants have been selected for their ability to propagate in cells expressing gD (Campadelli-Fiume et al., 1990; Dean et al., 1994). Some mutants contain altered forms of gD that can partially or fully account for any noted resistance. Others are resistant to neutralization by specific anti-gD Mabs (Mannini-Palenzona et al., 1995; Minson et al., 1986).

However, the gD is divergent functionally in α-herpesviruses. In VZV genome, there is not gD homologous gene (Davison and McGeoch, 1986a). In MDV, gD homologue is identified (Brunovskis and Velicer, 1995; Jang et al., 1996b; Ross and

Binns, 1991a; Ross et al., 1991c; Zelnik et al., 1993), which are encoded by U_S6 gene. The MDV gD contains several residues that are conserved in mammalian herpesviruses, HSV-1, PRV, and equine herpesvirus type 1 (EHV-1). In particular, six cysteines are perfectly aligned in all the gDs and there are numerous conservative substitutions (Ross and Binns, 1991a). However, the expression of the MDV-1 gD homologue in cell culture, where only cell-associated virus is produced, has not been demonstrated. The gD gene appears to be nonessential for cell culture propagation of MDV (Isfort et al., 1994; Parcells et al., 1994). The MDV-1 gD gene has been cloned into fowl pox virus (FPV). The results of an in vivo experiment suggests the gD recombinant FPV does not induce protective immunity (Nazerian et al., 1996). In fact, the gD gene is the common target for retrovirus integration, which results in disruption of the gD coding region(Isfort et al., 1994). Moreover, a MDV mutant, having a disrupted gD ORF, can establish infection and induce tumors in chickens exposed to it by inoculation and by contact, suggesting that the gD gene is also not essential for oncogenicity and horizontal transmission of MDV (Anderson et al., 1998).

4. Glycoprotein E, I, and E-I complex

The ORFs of gE and gI genes are located adjacent to one another in the U_S region. The gE and gI are conserved among the α-herpesviruses that have been sequenced, and commonly regarded as "dispensable," as their genes can be deleted from the viral genome with little or no effect on replication in vitro in cell culture (Balan et al., 1994; Neidhardt et al., 1987). However, the expressions of these gene products are required for full pathogenicity in animals. gE and gI form a noncovalently associated hetero-oligomeric

complex (Johnson and Feenstra, 1987; Whealy et al., 1993; Whitbeck et al., 1996; Yao et al., 1993; Zuckermann et al., 1988), which is considered to be a function unit. Biological functions of gE and gI complex include cell-to-cell spread, binding to antibody immunoglobulin G (IgG) (Fc receptor), and virulence, whereas the gE and gI may have separate functions in virulence because a gI null mutant is more virulent than a gE null mutant (Kaashoek et al., 1994).

The gE and gI function in cell-to-cell spread of HSV-1 and PRV in tissue culture and in animal infections (Jacobs, 1994). PRV gE mutant produces small plaques relative to wild-type virus under conditions in which extracellular progeny virus is neutralized (Zsak et al., 1992). Cell-to-cell transmission of wild-type HSV-1 occurs by at least two mechanisms: (i) release of virus from cells and entry of extracellular virus into a neighboring cell, and (ii) transfer of virus across cell junctions in a manner resistant to neutralizing antibodies. Replication of gE- and gI- mutant viruses in human fibroblasts was normal, and the rates of entry of mutant and wide type viruses into fibroblasts were similar. However, due to the defect in transfer of virus across cell junctions, spread of gE- and gI- mutant viruses from cell to cell was significantly slower than that of wild-type HSV-1, which results in small plaques on monolayers of normal human fibroblasts and epithelial cells (Dingwell et al., 1994). In VZV, deletion of both gI and gE prevents virus replication. Although VZV gI was dispensable for virus replication in vitro (whereas gE appeared to be required), its deletion or mutation resulted in a significant decrease in infectious virus yields, disrupted syncytium formation, and altered the conformation and distribution of gE in infected cells. Normal cell-to-cell spread and replication kinetics

were restored when gI was expressed from a nonnative locus in the VZV genome (Mallory et al., 1997).

The gE and gI mutants of HSV and PRV also exhibit significantly reduced virulence and a diminished or altered ability to spread to and within some, but not all, aspects of the central nervous system in animals (Jacobs, 1994; Mulder et al., 1994). Mice vaccinated with a secreted form of HSV-1 gE produced in human cells developed high serum tilters of HSV-1-neutralizing antibodies and were significantly protected from intraperitoneal lethal HSV-1 challenge (Miriagou et al., 1995). HSV-1 gE and gI form a hetero-oligomer which acts as an Fc receptor and also facilitates cell-to-cell spread of virus in epithelial tissues and certain cultured cells. Although gE-I hetero-oligomer is not required for infection of cells by extracellular virus, it is essential for efficient neuron-to-neuron transmission through synaptically linked neuronal pathways (Dingwell et al., 1995). PRV gE and gI are required for anterograde spread to a restricted set of retinorecipient neurons in the brain after infection of the rat retina. Bovine herpesvirus 1.1 (BHV-1.1), gE and gI proteins are capable of complementing the virulence functions of PRV gE and gI null mutations in a rodent model (Knapp and Enquist, 1997a; Knapp et al., 1997b).

Endocytosis is an important internalization process by which cells obtain extracellular materials. Receptor-mediated endocytosis enables selective uptake of macromolecules by the cell. Some virus-encoded proteins also undergo endocytosis from cell membrane. Herpesvirus gE is one of these proteins. Multifunctional HSV-1 gE-I complex is capable of binding the Fc portion of IgG. The domain on gE involved in IgG binding is distinct from the domain involved in mediating cell-to-cell spread (Weeks et

al., 1997). The region of gI between amino acids 128 and 145 is required for formation of the HSV-1 Fc receptor for monomeric IgG (Basu et al., 1997). Like HSV-1 gE-gI complex, VZV gE-I complex can also serve as a Fc receptor, and bind the Fc region of IgG (Johnson and Feenstra, 1987; Litwin et al., 1992). However, VZV gE is the most abundant glycoprotein (Montalvo et al., 1985). VZV gE is endocytosed from the cell surface through a tyrosine localization motif in its cytoplasmic tail, when it is expressed alone in cells (Olson et al., 1997a). Although VZV gI is normally found in association with gE in the infected cells, the gI also undergoes endocytosis and recycling when it is expressed alone in cells, which may be mediated by the methionine-leucine internalization motif in its cytoplasmic tail. The gI is co-localized with gE during endocytosis and recycling, and this endocytosis with gE-I complex is more efficient than with either gE or gI alone. The VZV gI exerts a more pronounced effect than gE on internalization of the complex, and behaves as an accessory component by facilitating the endocytosis of the major constituent gE and thereby modulating the trafficking of the entire cell surface gE-I Fc receptor complex (Olson and Grose, 1997b; Olson and Grose, 1998).

5. Glycoprotein H, L, and H-L complex

The appropriate interaction of gH and gL is required for the maturation and subcellular translocation of these two molecules, and is functionally essential for virus entry (penetration) and cell-to-cell spread. Although gH is conserved structurally and functionally throughout the Herpesviridae, the gL was thought to share homologues among herpesviruses only based on the genomic location and biologic function. The

mode of interaction and function of the gH-L complex partners appears to be quite variable. While gH and gL are covalently linked by disulfide bonds in HCMV (Kaye et al., 1992), a noncovalent interaction is present in HSV-1 (Hutchinson et al., 1992), and VZV (Duus et al., 1995). In EBV and HCMV, a third complex partner was detected, represented by a 42 kDa protein and a 125/145 kDa protein, respectively (Li et al., 1997b; Li et al., 1995; Yaswen et al., 1993). The similarity and the variation of gH-L interaction are discussed in following sections.

A. HSV-1 gH, gL and gH-L complex

A HSV-1 type-specific Mab LP11 was used to immunoprecipitate a 115 kDa glycoprotein from HSV-1 infected cells, this glycoprotein was designated as gH-1. The biological features of LP11 are able to efficiently neutralize virus infectivity, block cell fusion by syncytial virus strains, and inhibit the formation of plaques when added to cell monolayers after infection (Buckmaster et al., 1984).

The gH coding sequence was mapped to the BglII "m" fragment of HSV-1 DNA (map coordinates 0.27- 0.312). The complete nucleotide sequence of the BglII "m" fragment revealed two large ORF in addition to the thymidine kinase gene. The ORF lying immediately 3' of the thymidine kinase gene has a predicted translation product about 90 kDa with a signal peptide, a membrane anchor sequence, a large external domain containing potential N-glycosylation sites, and a charged C-terminal cytoplasmic domain (Gompels and Minson, 1986).

In in vitro transient expression systems, HSV-1 gH could be synthesized in greater amounts than those produced by a high-multiplicity virus infection, but this gH was partially processed, and accumulated intracellularly in rough endocytoplasmic

reticulum (RER) with a precursor-like form of the glycoprotein. It was not translocated to the cell surface, unless the cells were superinfected with HSV-1 or HSV-2 (Foa-Tomasi et al., 1991; Forrester et al., 1991; Gompels and Minson, 1989). The immature gH was unrecognized by LP11 (Roberts et al., 1991). Immunization with the recombinant gH did not protect mice from HSV-1 infection (Forrester et al., 1991), although gH may induce neutralizing antibodies (Ghiasi et al., 1992). The results indicated that HSV-1 gH needs to interact with another protein encoded by the virus. This interaction is required for gH maturation, cell surface localization, and formation of an antigenic structure important for its function in mediating infectivity (Gompels and Minson, 1989; Roberts et al., 1991).

The U_L1 gene has previously been implicated in virus-induced cell fusion (Little and Schaffer, 1981). With anti-U_L1 peptide sera, two protein species, a 30-kDa-precursor form and a 40-kDa mature form of the glycoprotein were identified in HSV-1 infected cells, both of which were modified with N-linked oligosaccharides. This novel glycoprotein is the 10th HSV-1 glycoprotein to be described, and was named glycoprotein L (gL). A large fraction of the gL found in infected cells was discovered to be tightly associated with gH. The gH co-expressed with gL by using vaccinia virus recombinants was antigenically normal, processed normally, and transported to the cell surface. Similarly, gL was also dependent on gH for proper post-translational processing and cell surface expression. The gH and gL form hetero-oligomer is incorporated into virions, transported to the cell surface, and play a role during entry of virus into cells (Hutchinson et al., 1992). In the absence of gL, virus particles were produced, and these particles reached the cell surface; however, the gL-negative particles purified from infected cells were also deficient in gH. The mutants lacking gH and gL were able to

adsorb onto cells, but unable to enter cells and initiate an infection (Browne et al., 1993). When attaching a ER retention motif, KKXX to the C-terminal cytoplasmic domain of gH peptide, this targeting signal conferred the predicted ER localization properties on gH in recombinant virus-infected cells, resulting in gH and gL failure to become processed to their mature forms. Cells infected with the recombinant virus released particles, which contain normal amounts of gD and VP16 but do not contain detectable amounts of gH. These particles are 100-fold less infective than those released by cells infected with the wild-type parent virus, although the number of enveloped virus particles released into the medium was unaltered. (Browne et al., 1996). A recombinant vaccinia virus expressing both gH and gL of HSV-1 was able to induce HSV-1-specific neutralizing antibody in mice. The virus clearance from the site of challenge was marginally enhanced compared to that observed following immunization with gH alone, and gH-L was found to protect mice against acute infection in the ganglia (Browne et al., 1993).

A set of linker insertion mutants in HSV-1 gH was generated and tested in transient assays for their ability to complement a gH-negative virus. The results suggested that the C-terminal third of the external domain affects the ability of gH to function in cell-cell fusion and virus entry, while the N-terminal half of the external domain induces conformational changes in gH, such that it was not recognized by LP11, although expression of the mutants on the cell surface was unchanged (Galdiero et al., 1997).

A soluble truncated gH and gL complex (gHt-L) was produced by a recombinant mammalian cell line (HL-7). Purified gHt-L reacted with gH- and gL-specific Mabs, including LP11. Polyclonal antibodies to the complex exhibited high titers of

complement-independent neutralizing activity against HSV-1, and block virus entry even when added after virus attachment. These sera also cross-neutralized HSV-2. When BALB/c mice were immunized with the gHt-L complex, the sera also exhibited high titers of virus neutralizing activity, and the mice showed reduced primary lesions, exhibited no secondary zosteriform lesions, and survived from virus challenge (Peng et al., 1998).

B. VZV gH, gL, and gH-L complex

The gH homologue of VZV was first described in 1980 as an infected cell-specific glycoprotein (Grose, 1980), designated as gp118 (VZV gH homologue). An important property of Mab to gp118 was its ability to neutralize infectious virus in the absence of complement (Buckmaster et al., 1984; Rodriguez et al., 1993).

After the complete sequence of VZV genome was published in 1986 (Davison and Scott, 1986b), VZV ORF 37 and 60 was identified to encode the gH (gpIII) and gL (gpVI) homologues, respectively. The maturation and transport of VZV gH are also dependent on co-expression of its chaperone gL. When the gH and gL transfected cells were examined by laser scanning confocal microscopy, expression of the wild-type gH-L complex was clearly visualized by uniform distribution of gH molecules across the cell surface, and causes extensive cell-to-cell fusion with polykaryocytosis (Duus et al., 1995).

In recombinant vaccinia virus expression system, a 94 kDa gH intermediate glycoprotein is synthesized in cell cultures infected with gH recombinant alone, but co-infection with both gH and gL recombinants results in the synthesis of the fully processed 118 kDa gH molecule. Simultaneous intraperitoneal inoculation of mice with high doses

of both gH and gL recombinant vaccinia viruses resulted in the development of VZV-neutralizing, complement-independent antibodies; these antibodies were not detected in mice infected solely with either the gH or the gL recombinant (Nemeckova et al., 1996).

Site-directed mutagenesis of gL cysteine residues led to a marked change in the trafficking pattern. The gH was not processed in the Golgi, and the immature gH was transported to and aggregated (patch) in the cell surface. There was also interference of fusogenic properties of the gH-L complex (Duus and Grose, 1996). Immature gH may exit the ER, when co-expressed with either gE or gI and appear on the cell surface in a patch pattern. The property of cell-to-cell fusion was also absent (Duus et al., 1995).

Analysis of VZV gL primary data suggested that VZV gL diverges greatly from other herpesvirus gL. VZV gL by itself can be processed to a mature product within the Golgi. The maturation of gH may not require transport beyond the medial-Golgi, since the gL bears an endoplasmic reticulum targeting sequence like many chaperone proteins. This property of gL results in retaining gL protein in the ER and Golgi system when expressed alone (Duus et al., 1995). VZV gL is a simpler form of the gL chaperone protein, which can be interchangeable functionally with EBV gL, although the gH and gL complexes are very different between VZV and EBV (Li et al., 1997a).

Taken together, VZV gL maturies in ER, where it escorts immature gH from the ER to the Golgi. Thereafter, mature gH is transported from the trans-Golgi to the outer cell membrane, where it acts as a major fusogen (Duus and Grose, 1996), whereas gL returns to the ER.

C. Pseudorabies Virus (PRV) gH, gL, and the gH-L interaction

PRV is an α-herpesvirus. The PRV gH and gL interaction may represent a third mode of interaction in α-herpesviruses, which is different from the interaction of the gH and gL of HSV and VZV. The primarily translated product of PRV gH protein is predicted to comprise 686 amino acids with a calculated molecular weight of 71.9 kDa. It also possess several characteristics typical for membrane glycoproteins, including a N-terminal hydrophobic signal sequence, C-terminal transmembrane and cytoplasmic domains, and domains with higher surface-localizing probability containing three potential N-linked glycosylation sites (Klupp and Mettenleiter, 1991). The PRV gL, a 20-kDa protein, was identified from purified PRV virions by Western blot assay with anti-gL synthetic oligo-peptide sera (Klupp et al., 1994; Dean and Cheung, 1993). The PRV gH is a structural component of the virion and forms a complex with another glycoprotein, gL. The absence of gH did not affect attachment of PRV to the cells, but the mutant was not infectious. The defect in infectivity could be partially overcome by experimentally induced membrane fusion using polythylene glycol (PEG), which suggests that gH is essential for entry and cell-to-cell spread in cell culture, as well as for propagation in the nervous system of mice (Babic et al., 1996). A premature translation termination codon was introduced in the gH gene by linker insertion mutagenesis. The mutant virus isolated from complementing cells, which expresses native gH, was unable to form plaques on noncomplementing cells. Immunological staining and electron microscopy showed that this mutant virus produced noninfectious progeny and was unable to spread from infected to uninfected cells by cell-cell fusion (Peeters et al., 1992).

There are several points being made about the gH and gL interaction of PRV (Klupp et al., 1997), which are different from HSV-1 gH and gL interaction. (i) PRV gL is required for penetration of virions and cell-to-cell spread. (ii) Unlike HSV-1, PRV gH is incorporated into the virion in the absence of gL. (iii) Virion localization of gH in the absence of gL is not sufficient for infectivity of PRV virions. (iv) In the absence of gL, N-glycans on PRV gH are processed to a greater extent than in the presence of gL, indicating masking of N-glycans by association with gL. (v) An anti-gL polyclonal antiserum is able to neutralize PRV viral infectivity but did not inhibit cell-to-cell spread, an important function of PRV gL in the viral entry process, which is not explained by a chaperone-type mechanism in gH maturation and processing.

D. HCMV gH, gL, and their complex.

HCMV U_L75 (gH homologue) was reconstructed into vaccinia viruses. A glycoprotein of approximately 86 kDa was immunoprecipitated from cells infected with the recombinant viruses, as well as from HCMV-infected cells with a Mab that efficiently neutralized HCMV infectivity. In HCMV-infected MRC5 cells, the gH was present on both nuclear and cytoplasmic membranes, but in recombinant vaccinia virus-infected cells it accumulated predominantly on the nuclear membrane (Borysiewicz et al., 1988).

None of ORF within the HCMV genome encodes a product with discernible sequence homology to HSV-1 gL, but the arrangement of conserved genes in HCMV suggested that the U_L115 gene is a "positional homologue" of HSV-1 U_L1, which encodes a small secreted glycoprotein. Co-expression of HCMV gH and the U_L115 gene product revealed that these proteins form a disulfide-linked complex and that the formation of this complex results in cell surface expression of gH. This complex is

analogous to the gH-L complex of HSV-1 and the HCMV U_L115 gene product is, therefore, the functional homologue of HSV-1 gL (Forrester et al., 1992).

There are three disulfide-bonded glycoprotein complexes within the envelope of HCMV, gCI, gCII, and gCIII. The gH protein is known to be a component of a 240-kDa envelope complex, gCIII (Gretch et al., 1988a; Gretch et al., 1988b; Gretch et al., 1988c). The U_L115 is a second component of gCIII. By immunoprecipitation analysis of gH and gL from HCMV-infected fibroblasts and purified HCMV virions, a 125/145 kDa protein was shown to be the third integral part of gCIII, along with gH and gL. Within the envelope of infectious extracellular virions, the mature gH exists as both a covalently complexed and noncovalently associated component of the gCIII complex (Huber and Compton, 1997; Li et al., 1997).

Functionally, the HCMV gH appears to be one of the dominant immunogens, and induces neutralizing antibodies during natural infection (Urban et al., 1996). HCMV gH also activates T helper cells, and the multiple T cell-reactive domains of gH were mapped within 15-510 amino acids of the gH polypeptide (Beninga et al., 1996).

E. EBV gH, gL, and gH-L-gp42 complex

In EBV, a viral envelope glycoprotein, gp85, is involved in the fusion process in virus infection (Haddad and Hutt, 1989; Miller and Hutt-Fletcher, 1988), and induces virus neutralizing antibodies (Strnad et al., 1982). The gene for gp85 was mapped to the BXLF2 ORF, which is predicted to code for a 706 amino acid protein (Heineman et al., 1988), and is homologous to HSV-1 gH (Cranage et al., 1988). Expression of gp85 in vitro in cell culture also resulted in an immature gH, which was absent from the cell surface (Heineman et al., 1988; Pulford et al., 1994b; Yaswen et al., 1993). The

translocation and cell surface expression of gp85 appears to require an accessory protein gp25, gL homologue, to form a heterodimeric complex (Pulford et al., 1995; Yaswen et al., 1993).

However, gp85 also complexes with one additional glycoprotein of gp42/38. Co-expression of EBV gH and gL facilitated transport of gH to the cell surface and resulted in formation of a stable gH-L complex. It also restored expression of an epitope recognized by Mab E1D1, which immunoprecipitates the native gH complex but not recombinant gH expressed alone. Co-expression of gH, gL, and gp42/38 restored expression of an epitope recognized by Mab F-2-1, which immunoprecipitates the native gH-gL-gp42/38 complex but not the complex of recombinant gH and gL. The epitope recognized by F-2-1 was mapped to the gp42 itself. The F-2-1 inhibits the ability of EBV to infect B-lymphocytes, but had no effect on the ability of the virus to infect the epithelial cell line SVK-CR2. In contrast, E1D1 has no effect on infection of the B-cell line but inhibited infection of the epithelial cell line. The gp42/38 in gH-L-gp42/38 acts as a unique adaptation to infection of B lymphocytes by EBV (Li et al., 1995). It is essential for penetration of the B-cell membrane (Wang and Hutt-Fletcher, 1998), via interacting with HLA-DR (MHC-II molecule) on the B cell surface (Li et al., 1997a).

F. MDV gH, gL, and gH-L complex

The coding regions of gH genes of MDV-1 Rb1B strain and HVT are found to contain 2439 and 2424 nucleotides, respectively. The predicted primary polypeptide products contain 813 and 808 amino acids with of a calculated molecular weight of 90.8 and 91.1 kDa, respectively. Both amino acid sequences of Rb1b and HVT gHs exhibit characteristic glycoprotein features such as hydrophobic signal, anchor sequences, and 9

potential sites for N-linked glycosylation. Polypeptide sequence comparison to the other available gH sequences revealed that MDV and HVT gH have more similarity to the α-herpesviruses than to either β- or γ-herpesviruses (Scott et al., 1993).

MDV-2 gH gene contained 2436 nucleotide and the primary translation product contained 812 amino acids with a molecular weight of 89.4 kDa. The protein encoded by MDV2 gH gene has a number of features characteristic of a membrane-associated glycoprotein. There are 9 potential N-linked glycosylation sites, too. Alignment of the amino acid sequences of the gHs among the three MDV serotypes showed 57.5% (RB1B and MDV-2), 56.2% (RB1B and HVT), and 50.1% (MDV-2 and HVT) identities (Shimojima et al., 1997).

The MDV-1 GA strain gL shares 18% identity with the HSV-1 counterpart. An antiserum to a hydrophilic region of gL expressed in E. coli can immunoprecipitate a 25 kDa polypeptide in MDV-infected cells. The gL in MDV-infected cells is resistant to Endo H, but gL expressed by gL recombinant FPV (reFPV-gL) is highly sensitive to Endo H, suggesting MDV gL alone may not be processed to its mature from (Yoshida et al., 1994a).

The biological function of gH and gH-L complex appears to be conserved in all three groups of herpesviruses, although the process of maturation and subcellular translocation of gH and/or gL products vary. However, available information for MDV gH, gL, and gH-L complex is limited. To further understand MDV infection and the cell fusion processes, it is essential to identify gH expression in infected cells, to investigate gH and gL post-translational modification, subcellular translocation, as well as gH-L interaction, and to study the relationship between gH-L complex and viral infection.

These studies will expand our knowledge on the mechanisms of MDV infection for scientific purposes, as well as vaccine development.

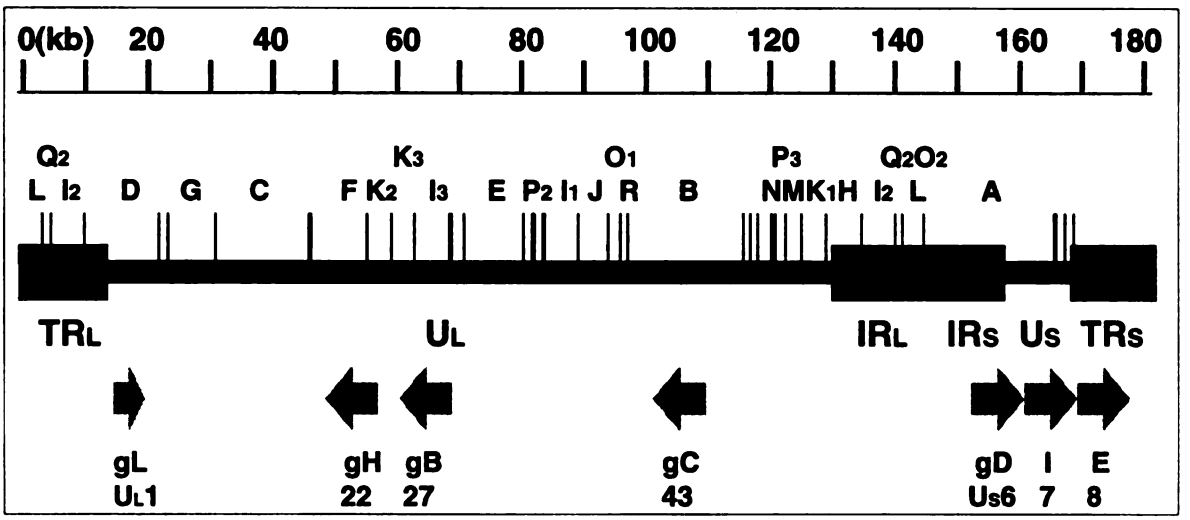

Figure 1. The physical map of MDV genome.

Two unique regions, unique long (UL) and unique short (US), are flanked by repeat fragments, which are terminal repeat long (TRL), internal repeat long (IRL), internal repeat short (IRS), and terminal repeat short (TRS). Above the genome, BamHI restriction sites are indiceted with vertical lines, the major BamHI digestion fregments are indicated with the designated letters. Under the genome, the related locations, the protein names, and the gene names of several major glycoproteins are indicated with solid arrows and letter designations.

Table 1. Diagnosis of avian lymphomas using a combined criteria

Criterion	LL	RE Bursal	RE Nonbursal	MD
Bursa lesions	+ (-)	+ (-)	-	-
Morphology of tumor cells	Homogeneity	Homogeneity	Pleomorphic	Pleomorphic
Nerve lesions	-	- (+)	- (+)	+
Age at onset (> 14 weeks)	+	+	- (+)	- (+)
Virus isolation	+/-	+/-	+/-	+/-
Antibody	+/-	+/-	+/-	+/-
B cell markers	+	+	-	-
T cell markers	-	-	+	+
Ia (MHC-II)	n/a	n/a	-	+
CD4	n/a	n/a	-	+
CD8	n/a	n/a	+	-
MATSA	-	-	-	+
H19 for pp38	-	-	-	+

LL. lymphoid leukosis by ALV; RE bursa. Bursa lymphoma by REV; RE nonbursa. Nonbursa lymphoma by REV; MD. lymphoma by Marek's disease; +. Present; -. Absent; ( ). Occasionally present; +/-. Present or absent; n/a. not applicable. MATSA. Marek's disease tumor-associated surface antigen.

CHAPTER II. IDENTIFICATION AND CHARACTERIZATION OF GLYCOPROTEIN H OF MDV GA STRAIN

Abstract

One of the unusual features of MDV-1 is its strict cell-association in vitro and in vivo. The mechanism of cell to cell spread has not been characterized for MDV infection but is thought to occur by intracellular bridge formation which may require the expression of several MDV glycoproteins on the surface of the infected cell. Glycoproteins gB, gC, gD, gH and gL have been shown to directly or indirectly mediate membrane fusion events required both for entry of virus into host cells and cell fusion in herpes simplex virus type I (HSV-1) infection. A 2439 bps open reading frame (ORF) was identified from the DNA sequence of BamHI F and K2 fragments of MDV GA strain, which predicts a 813 amino acid polypeptide. This peptide is homologous to HSV-1 gH, and has typical glycoprotein features: 1) a single signal sequence, 2) a large extracellular domain, 3) transmembrane domains, and 4) a small cytoplasmic domain. There are nine potential N-linked glycosylation sites within the extracellular domain. A fragment of the gH ORF was cloned into pGEX vector in frame with GST to produce a GSTgH fusion protein in E. coli. The GSTgH fusion protein was used to develop gH monoclonal antibodies and antiserum. The gH expression was detected in DEF infected with MDV-1 GA strain by immunofluorescence assay (IFA) with monoclonal and polyclonal antibodies. The virus neutralization and plaque-forming inhibition analyses were conducted with the gH antiserum. There was no evidence indicating neutralization or plaque-forming inhibition activities of the antiserum. Thus, it is necessary to further evaluate the biologic functions of MDV gH.

Introduction

Marek's disease (MD) is one of the most common lymphoproliferative neoplastic diseases of chickens, characterized by a mononuclear infiltration of multiple tissues or organs (Calnek and Witter, 1997). The etiology of MD is Marek's disease virus (MDV), an α-herpesvirus. For the initial infection of either cell cultures or chickens by cell free virus, enveloped virions enter susceptible cells by conventional absorption and penetration, whereas cell associated virus infection is initiated by cell-to-cell fusion, or direct contact with infected cells (Hlozanek, 1970). Due to the high cell-associated nature of MDV, the cell-to-cell transfer is normally accomplished through formation of intracellular bridges (Kaleta and Neumann, 1977), and is presumed to be the principal mode of virus spread both in vitro and in vivo. These intracellular bridges may require expression of several envelope glycoproteins from the virus on the infected cell surface. Recent analyses of the nucleotide sequence of MDV-1 revealed at least eight glycoproteins, which are homologous to HSV-1 gB (Ross et al., 1989; Yanagida et al., 1992b), gC (Coussens and Velicer, 1988), gD (Ross et al., 1991b), gI and gE (Brunovskis and Velicer, 1995), gK (Ren et al., 1994), gH (Scott et al., 1993), and gL (Yoshida et al., 1994a).

The principal events in the interaction of a virion with cellular membrane are attachment and penetration. Attachment of virus to a cell surface activates a cellular process mediated by viral surface glycoproteins that lead to the fusion of the viral envelope with the cellular plasma membrane. Multiple viral glycoproteins are required. A subset of these glycoproteins, including gB, gC, gD, gH, and gL, may directly mediate the membrane fusion events required both for HSV-1 entry of host cells and for HSV-1

induced cell fusion. The gB, gD, and gH-L complex act individually or in combination to trigger pH-independent fusion of the viral envelope with the host cell plasma membrane (Spear, 1993). Transient expression of gB, gD, and gH and gL shows sufficiency for inducing membrane fusion in the Cos cell expression system (Turner et al., 1998).

The gH is conserved structurally and functionally throughout the Herpesviridae. One common feature of gH homologues in herpesviruses is that gH can induce neutralizing antibodies, such as monoclonal antibody (Mab) LP11 of HSV-1. LP11 can efficiently neutralize virus infectivity, block cell fusion by syncytial virus strains, and inhibit the formation of plaques when added to cell monolayers after infection (Buckmaster et al., 1984). An important property of the Mab to VZV gp118 (gH homologue) was its ability to neutralize infectious virus in the absence of complement (Buckmaster et al., 1984; Rodriguez et al., 1993). In EBV, the gH homologue, gp85 , is involved in the fusion process in virus infection (Haddad and Hutt, 1989; Miller and Hutt-Fletcher, 1988), and also induces virus neutralizing antibodies (Strnad et al., 1982). The mature form of the gH molecule is always expressed on the cell surface (Duus et al., 1995; Gompels and Minson, 1989; Roberts et al., 1991), and the surface expression is required for the cell fusion function of gH. This is another feature of gH molecules.

In MDV, the DNA sequences of gH homologues of RB1B strain (serotype 1) and HVT (serotype 3) have been reported since 1993 (Scott et al., 1993), and MDV-2 gH sequence data was also introduced recently (Shimojima et al., 1997). No other information about gH is available, except the sequence data and the computer analysis results. The major problem in characterizing the function of gH is lack of gH antibodies, therefore the characteristics of MDV gH have not been defined. In the present report, the

DNA sequence of MDV GA strain gH was determined. The antibodies against gH peptide were successfully developed. Some serologic features of these antibodies are investigated, and the potential biologic functions of MDV gH are also discussed.

Materials and Methods

Cells and Virus. Duck embryo fibroblasts (DEF) and chicken embryo fibroblasts (CEF) were grown in Leibowitz-McCoy medium (GIBCO Laboratories, Grand Island, NY), supplemented with 4% calf serum (growth medium) or 1% calf serum (maintenance medium). MDV-1 GA strain (Eidson and Schmittle, 1968) was propagated in DEF or CEF. Cell free virus of GA strain made from feather follicles was a kind gift from Dr R. L. Witter (USDA-ARS Avian Disease and Oncology Laboratory, East Lansing, MI).

DNA sequence determination. DNA sequencing was performed on a double stranded plasmid by the dideoxy chain termination method using [α-^{35}S]dATP (New England Nuclear, Life Science Products, Boston, MA) and the TAQuence version 2.0 DNA Sequencing Kits (United States Biochemical Corporation, Cleveland, Ohio) as suggested by the manufacturer. Both strands of the DNA of BamHI F and K2 fragments of MDV GA strain (Fukuchi et al., 1985b) were partially sequenced. The sequence data were analyzed with various computer programs (see computer analysis in this section). The junction of BamHI F and K2 fragment was confirmed by PCR amplification of the particular area. Several regions in question were sequenced using an automated sequencer (373A DNA Sequencer, Applied Biosystems, Foster City, CA) and dideoxy sequencing methods (Prism, Applied Biosystems, Foster City, CA).

Construction of expression plasmid for GSTgH fusion protein. In order to overexpress gH antigen, the BglII-EcoRV fragment of gH gene was cloned into plasmid pGEX-5X-3 (Pharmacia Biotech AB, Uppsala, Sweden) at BamHI and SmaI sites. An adapter (5'-**GGATCCGAGCTCGAGATCT**-3') was obtained from plasmid pBlueBac4

(Invitrogen corporation, Carlsbad, CA), which allows the gH fragment to fill in Glutathione S-transferase (GST) ORF to generate GSTgH fusion protein expression vector pGSTgH. The construction was confirmed by restriction endonuclease digesting patterns and sequence analysis.

Expression and purification of GSTgH fusion protein in E. coli. The expression and purification of GSTgH fusion protein were according to the manufacture procedure (Pharmacia Biotech AB, Uppsala, Sweden). Briefly, a single colony of pGSTgH transformed E. coli (TG1 strain) cells were seeded into 50ml 2xYT-G medium (2xYT with 2% glucose, and 100μg/ml ampicillin) in a 200ml flask, and incubated at 37°C overnight with vigorous shaking. The culture was further diluted into 450ml pre-warmed 2xYT-G medium in a 2,800ml flask, and incubated for about 5 hours at 37°C with shaking until A_{260} = 1-2. The GSTgH fusion protein expression was induced with 0.1mM Isopropyl-b-D-thiogalactopyranoside (IPTG, Boehringer Mannhem corporation, Indianapolis, IN) for 3 hours. For crude GSTgH fusion protein, the 500ml E. coli culture was centrifuged at 7,700xg for 10 minutes at 4°C. The pellet was washed once with 40ml phosphate buffered saline (PBS), and resuspended in 20ml PBS. The cell suspension was sonicated, and the cell debris was removed by centrifuge, the supernatant was stored at -20°C as crude GSTgH fusion protein. For purification of GSTgH fusion protein, Triton X-100 was added into the 20ml sonicated E. coli cell suspension (final concentration is 1%), and gently mixed for 30 minutes. The cell debris was removed by centrifugation at 12,000xg for 10 minutes at 4°C. One ml PBS equilibrated 50% slurry of Glutathione Sepharose 4B (Pharmacia Biotech AB, Uppsala, Sweden) was added into the supernatant, and incubated for 30 minutes with gentle agitation at room temperature. The pellet was

retained and washed 3 times with PBS by centrifuge. The GSTgH fusion protein (or GST protein, as a negative control antigen) was eluted with Glutathione elution buffer (10mM reduced glutathione in 50mM Tris-HCl, pH8.0) (Figure 2).

Development of anti-gH monoclonal antibodies (Mab) and polyclonal antiserum. Antibodies were developed against the purified GSTgH fusion protein. For Mabs, a mouse was subcutaneously inoculated with 100μg (in 50μl PBS) purified GSTgH fusion protein emulsified in equal volume of Titlemax research adjuvant #R-1 (CytRx Corporation, Norcross, GA) at 2 sites in the base of the tail. The mouse was boosted with 100μg same protein intraperitoneally 3 weeks post-inoculation. Four days later, the spleen cells were isolated and fused with myeloma cells line, NS-1 cells. The hybridomas were screened by ELISA against crude GSTgH protein. The positive hybridomas were cloned by the limited dilution method and rescreened by ELISA with purified GSTgH fusion protein, and purified GST protein as negative control. The rabbit antiserum was produced by inoculating a New Zealand white rabbit with about 200μg (in 100μl PBS) of GSTgH fusion protein emulsified in Titlemax research adjuvant #R-1. The rabbit was given two boosters at 4 and 8 weeks with 200μg GSTgH emulsified in #R-1 at first booster subcutaneously, and 200μg GSTgH in second booster intravenously. The rabbit was bled 10 days after the last booster.

ELISA. Flat bottomed Immulon I 96-well plates (Dynatech Laboratories, Inc., Chantilly, VA) were coated with crude GSTgH protein diluted 1:10 (1:200 for purified GSTgH fusion protein, and GST protein) in carbonate coating buffer (22mM Na_2CO_3, 22mM $NaHCO_3$, pH 9.6) for 24 hours at 4°C. Plates were washed 2 times with ELISA wash buffer (PBS-T, 0.1% Tween 80 in PBS). The wells were blocked with blocking

buffer (3% bovine serum albumin in PBS) and incubated at 37°C for one hour in a humidified incubator. Plates were washed 3 times with PBS-T. Hybridoma supernatants (100µl/well) were applied without dilution, but rabbit serum was diluted to 1:1000 in blocking buffer, 100µl/well, following by 1 hour incubation at 37°C in a humidified incubator. Plates were washed 3 times with PBS-T. Goat anti-mouse (or anti-rabbit) IgG labeled with horseradish peroxidase (Kirkegaard & Perry Laboratories, Gaithersburg, MD) was diluted 1:1000 in blocking buffer and added to each well, 100µl/well. The plates were incubated 1 hour at 37°C in a humidified incubator and then washed 3 times PBS-T. 100µl subtract [Phosphate buffer, 0.2M, pH6.0, 0.8mg/ml 5-amino salicylic acid (Sigma Chemical Co., St. Louis, MO), and 0.006% hydrogen peroxide] was added to each well and the plates were placed in the dark at room temperature for 2 to 6 hours, until color was fully developed. Plates were read on an automatic ELISA reader at λ_{595nm}.

Indirect Immunofluorescence assays (IFA). CEF cells were grown into a monolayer on glass coverslips. The monolayer was infected with MDV GA strain at about 200 plaque forming unit (pfu) per 60mm tissue culture dish. When lytic plaques were observed, the coverslips were harvested by washing once in PBS, fixing 5 minutes in ice cold acetone, and air-drying. Fixed coverslips were stored at -20°C for later use. IFA was performed as previously published (Wu et al., 1997). Briefly, The fixed samples were incubated with ether anti-gH Mab32 or rabbit serum diluted 1:100 in PBS for 30 minutes at 37°C in a humidified incubator. Coverslips were rinsed 15 minutes in PBS then incubated for 30 minutes with either goat anti-mouse or goat anti-rabbit fluorescein isothiocyanate (FITC)-conjugated IgG (Kirkegaard & Perry Laboratories, Gaithersburg,

MD). The coverslips were rinsed again, and sealed with 50% glycerol in PBS. The samples were observed with a confocal microscope.

Confocal microscopy. The IFA samples were observed with a laser scanning confocal microscope (Carl Zeiss, Inc, New York, NY) with 40x oil lane, 488 argon laser line with green (BP521-650) barrier filter, fluorescence operation and confocal modes. The photographs were taken under the same conditions, and processed with Adobe Photoshop program (Adobe Systems Inc., Mountain View, Goleta, CA).

Virus neutralization and plaque-forming inhibition assays. Secondary DEF monolayer on 35mm tissue culture dish was used for virus neutralization and the plaque-forming inhibition assays. The rabbit antiserum and the pre-bleeding negative control serum were inactivated at 56°C for 30 minutes. For neutralization, 10-fold diluted sera (10^{-1}, $^{-2}$, and $^{-3}$) in SPGA (sucrose 218mM, KH_2PO_4 3.8mM, K_2HPO_4 7.2mM, monosodium glutamate 4.9mM, BSA 1% in water)-0.2% EDTA dilute solution was incubated with a certain amount of cell free virus (50pfu/dish) of GA strain for 30 minutes on ice. The serum-virus mixture (100μl per plate) was inoculated into secondary DEF monolayer. The virus was allowed to absorb for 30 minutes at 37°C, and 2ml maintenance medium was added. The plaques were counted 7 days post-inoculation. For plaque-forming inhibition assay, about 50pfu cell free virus of GA strain was inoculated into secondary DEF monolayer, and allowed to absorb for 30 minutes at 37°C, then the maintenance medium with 10-fold diluted sera (10^{-2} and 10^{-3}) was added. The plaques were counted 7 days post-inoculation.

Computer analysis. Several computer programs were used for different purposes. DNASTAR package (DNASTAR Inc. Madison, WI) was used for sequence

data input, multiple sequence analysis, and phylogenic tree generation. MacVector program (Scientific Image systems, New Haven, CT) was used to find the ORF from the DNA data, to translate ORF to polypeptide, and to generate protein profiles. Gene Construct Kit (Textco Inc., Western Lebanon, New Hampshire) was used for drawing the DNA construction map, and predicting the ORF. GCG package (Genetics Computer Group, Madison, WI) was used for pairwise comparison (GAP program), and homologous searches (FASTA program). Oligo program (National biosciences, Inc., Plymouth, MN) was used to design the oligonucleotide primers for PCR and sequencing. Blast program at National Center of Biotechnology information (http://ncbi.nlm.nih.gov/cgi-bin/blast/) was used for searching homologues, and retrieving the sequence data from Genbank and Swiss-Port databases.

Results

Analysis of the nucleotide sequence of the gH gene of MDV GA strain

The nucleotide sequence of BamHI-F and K2 fragments (Fukuchi et al., 1985b) of MDV-1 GA strain was partially determined in order to obtain complete ORF of gH gene. An overall map of the corresponding genomic region is shown in Figure 3. Computer analysis of the partial sequence revealed 3 ORFs corresponding to HSV-1 U_L21, U_L22 (gH gene) and TK gene (partial) (McGeoch et al., 1988), respectively. The ORF of U_L22 has a size of 2,439 bps with an average base composition of 30.4 % A, 19.1 % G, 19.7 % C and 30.8 % T. The U_L22 ORF is leftward, and located downstream of TK ORF.

The DNA sequence upstream and downstream of U_L22 was analyzed for putative transcription control elements. Two potential "TATA" boxes, characteristic of many eukaroytic and also herpesviral promoters (Breathnach and Chambon, 1981; Corden et al., 1980), are located -113 and -157 nucleotides upstream from the proposed initiation codon. The sequence 5'-GGCCAATAT-3' at -127, and 5'-TCACAATGA-3' at -138 exhibit similarities to the "CAT" box consensus sequence (5'-GGC/TCAATCT-3') (Figure 4). Regarding 3' elements of U_L22, there is a potential poly A sequence located at 2449 (AATAAAATTAAA) downstream from the stop codon.

The DNA sequence of U_L22 was compared with that of gH gene of MDV RB1B strain. The results indicated that only three variations of nucleotides residues are present within the ORFs at position 1414 (T to C), 2377 (T to G), and 2378 (T to G), which result in two amino acid substitutions between GA and RB1B gH. The major difference of the DNA sequence of the gH genes between GA and RB1B strains is located in the upstream

at position –55 to –129 (Figure 5). At least, a potential "TATA" box at –113 and a "CAT" box at –127 in GA gH are absent from RB1B gH.

Analysis of the deduced amino acid sequence of gH peptide

The predicted initiation codon, ATG at position 1, exhibits features of a strong translational starting signal according to Kozak's rules (Kozak, 1986). The optimal consensus sequence for initiation has been shown to be 5'-A/GCCATGG-3'. This sequence is found in the MDV U_L22 with a small difference, and reads 5'-AACATGG-3'. The proposed termination codon, TAA is found at position 2440. The polypeptide predicted from the nucleotide sequence comprises 813 amino acids with a calculated molecular weight 90.9 kDa, which is the precursor of gH peptide (pgH). The amino acid sequence is shown in Figure 6. There are four hydrophobic helices in the gH precursor, located at amino acid 1-18, 619-641, 660-682, and 770-792 (Table 2), according to the results predicted by using SOSUI system (Mitaku Laboratory, 1996). The N-terminal helix (amino acid 1-18) appears to be the signal sequence. The C-terminal helix (amino acid 770-792) might be transmembrane domain. The additional two helices may interact with or span the membrane. There is a very short cytoplasmic domain located in the C-terminus from amino acid 793 to 813. There are 9 potential N-linked glycosylation sites, N-X-T/S, with X being any amino acid except proline or aspartic acid. The locations of the 9 N-linked glycosylation sites were indicated at Figure 6.

gH peptides are highly conserved in MDV

Pairwise-comparison of pgH sequence of GA strain with the gH homologues of RB1B (Scott et al., 1993) and HVT (Shimojima et al., 1997) was conducted with GAP program in GCG package (Version 9.1). Both the sequences of RB1B and HVT are available in SWISS-PORT protein database, the accessed numbers are P36336 and P36337, respectively. The results indicated that the pgH from MDV-1 share higher percentage of identity, 99.8% between GA and RB1B strains. In fact, there are only two amino acids substituted between these two gHs, which are located at position 472 (valine to alanine) and 793 (isoleucine to arginine). There is about 50-70% identity between the gHs of MDV-1, MDV-2, and HVT (Table 3). MDV-2 sequence is not yet available in Genbank. The comparison data related to MDV-2 was directly cited from Shimojima's report (Shimojima et al., 1997).

Comparison of gH peptide to the homologues of other herpesviruses

To compare gH homologues, a total of 16 gH sequences were retrieved from Genbank, and Swiss-Port databases. The resource for each gH and the database accession number are listed in Table 5. The multiple alignment analysis was done by the cluster method of Multiple Sequence Alignment program in DNASTAR package (DNASTAR Inc. Madison, WI). The rooted phylogenic tree was generated within same program (Figure 7). This tree shows three clusters of the gH proteins which agree basically with the classification of α-, β-, and γ-herpesviruses. The gHs from β- and γ- herpesviruses are closer to each other. MDV gHs are closer to, but not absolutely located within the cluster of α-herpesviruses.

Characterization of anti-gH antibodies

The antibodies were developed according to the procedure described in the Materials and Methods section. A total of 9 Mabs and 1 rabbit serum were obtained. Three methods, ELISA, IFA and immunoprecipitation (IP) assay, were used to study the serological characteristics of these Mabs and the rabbit antiserum. Various resources of gH antigen were used in these assays. GSTgH fusion protein in bacterial lysate was used for ELISA and all the antibodies were positive in ELISA. Sf9 cells infected with gH recombinant baculovirus were used for IFA (see Chapter III). IP was done with transiently expressed gH in DF1 cells (see Chapter III). A summary of the results is listed in Table 4.

gH product is expressed in MDV GA strain infected DEF cells

To study the gH expression in MDV infected DEF cells, the coverslips of DEF was infected with MDV GA strain, and fixed with cold acetone for 5 minutes at room temperature. IFA was performed with Mab32. One positive plaque (with some single cells and some multinuclear syncytial cells) is illustrated in Figure 8. The fluorescence was mainly present in the cytoplasm, and absent from the nucleus.

The product of BglII-EcoRV fragment of gH gene does not induce neutralization, nor plaque-forming inhibition antibodies

These assays were conducted with anti-gH rabbit serum, which was developed by using GSTgH fusion protein as an immunogen. The BglII-EcoRV fragment of gH is the main antigenic region of gH molecule, according to the antigenecity profile generated by

the MacVector program (Figure 9). The results of virus neutralization test with 8 repeats are shown in Figure 10. The plaque-forming inhibition assay was composed of two trials, one trial with 4 repeats, and the other with 8 repeats. The results are summarized in Figure 11. In both assays, there were no significant differences found between the normal serum and the gH serum.

Discussion

The MDV DNA is a linear double-stranded molecule, composed of 166-184 kbps (Lee et al., 1971). The physical map of BamHI restriction endonuclease has been constructed from MDV-1 GA strain (Fukuchi et al., 1985b), which became a basis for most MDV gene identification and localization. The gH gene of MDV-1 RB1B strain has been mapped to BamHI K2 (one quarter) and F (three quarters) fragments (Scott et al., 1993). About the same time, the BamHI K2 and F fragments of the GA strain (Fukuchi et al., 1985b) were also sequenced to determine the gH location (Lee's group at USDA-ARS avian disease and Oncology Laboratory). Pairwise comparison of GA and RB1B gH DNA and deduced amino acid sequences revealed that only 3 nucleotide residues are instituted at position 1414, 2377 and 2378, which result in 2 amino acid substitutions in the deduced protein sequence at position 472 and 793. The main difference between GA and RB1B gH gene is not within the coding region, but in the upstream control elements of the gH ORFs. These differences have resulted in some changes of the potential transcription elements, such as a potential "TATA" box at −113 and a potential "CAT" box at −127 in GA strain which are absent from the RB1B sequence (Figure 5). The

biological effects of these changes on the gH expression or virus infectivity remains to be studied.

The deduced MDV gH amino acid sequence was also compared to the homologues of other herpesviruses, including α-, β-, and γ- herpesviruses. A phylogenic tree was generated from the multiple sequence alignment. All 16 entries (including 3 MDV gH homologues) are grouped into 3 main clusters, which basically agrees with the classification of α-, β-, and γ-herpesviruses. Although closely related to α-herpesvirus, the MDV subgroup is not completely located within the α-herpesvirus cluster (Figure 7). This separation may reflect the features of MDV in both biological properties (closer to γ-herpesviruses) and genomic structure and gene organization (closer to α-herpesviruses).

Fukuchi et al. reported the presence of simple repeat units (2-16 units of SmaI-M fragment repeat) in BamHI F fragment (Fukuchi et al., 1985b). After inspecting the sequence data of F fragment (data not shown), at least 4 ORF were found. They are homologous to U_L22 (gH), 21, 20, and 19 of HSV-1. However, only 1 unit of SmaI fragment was identified in the left side of the F fragment. In some herpesviruses, such as EHV-1 (Robertson et al., 1991), EHV-4 (Nicolson et al., 1990), PRV (Klupp and Mettenleiter, 1991), and infectious laryngotracheitis virus (ILTV, another avian herpesvirus) (Ziemann et al., 1998), the downstream area of gH ORF appears very active. Some direct repeat elements and putative replication origin (Ori) sequences have been found in this area. In PRV and ILTV, this region even becomes the junction for the internal reversion within the U_L region. Whereas in MDV, just like HSV-1 and VZV, the homologous or equivalent region of the direct repeat elements and Ori is not present in the downstream area of gH ORF. In fact, the MDV U_L21 ORF is located at 294 bps

downstream to the gH stop codon (Figure 3); The gH and U_L21 ORFs are arranged tail to tail.

A number of distinct hydrophobic regions were identified from the gH precursor (Table 2). Two primary hydrophobic stretches were noted at both the N and C termini, which are likely to represent signal and anchor (transmembrane domain) sequences involved in the transport to and subsequent insertion into cell membranes. The homologues of these two stretches can be found in the HSV-1 gH molecule. However, compared with HSV-1 gH protein, there is a third primary hydrophobic stretch located at amino acid 619-641, which does not have a homolog to HSV-1 gH. This fragment may also function to anchor the protein to the cell membranes. A secondary hydrophobic stretch is closer to the third primary hydrophobic stretch, and is unlikely to be membrane spanning, but may be associated to cell membrane. Therefore, from amino acid 619-792, the gH possesses a large hydrophobic area in the C-terminus which may tightly interact with cell membranes. This feature may reflect the highly cell-association properties of MDV.

Some difficulty was encountered in making gH antibodies. Several type antigens of gH protein were used to develop the antibodies, including TrpE-gH fusion protein, gH recombinant fowlpox virus (reFPV) infected CEF cell lysate (containing gH protein), as well as gH recombinant baculovirus (rBacgH) infected Sf9 cell lysate (also containing gH protein). We were unsuccessful in producing antibodies against gH protein. Finally, we tried using the GSTgH fusion protein. The GSTgH fusion protein proved to be a good immunogen for the production of monoclonal antibodies and rabbit polyclonal antibodies. The other antigens were poor immunogens most likely because of the

following: 1. The expression of gH in reFPV and rBacgH is poor, and the majority of antigens in both cell lysates are FPV or baculovirus structural proteins. 2. The TrpE-gH fusion protein was highly denatured when the fusion protein was processed [Procedure for purification of TrpE fusion protein was described previously (Wu et al., 1997)]. Antibodies against TrpE-gH fusion protein might only react with the denatured form of gH, but not the gH within MDV infected CEF (or DEF) cells. The processing of GSTgH fusion protein was under very natural conditions (see materials and Methods). This procedure offers not only more purity, but also more natural form of GSTgH fusion protein. Therefore, it was the most antigenic immunogen in this investigation. Several Mabs and rabbit serum were developed with this GSTgH fusion protein. This is the first time that the gH expression in MDV infected DEF was detected with gH antibodies (Figure 8).

The biological role of gH appears to be in virus entry into host cells and its subsequent cell to cell spread. Strong neutralization antibodies have been raised against the gH proteins from HSV-1 (Showalter et al., 1981), VZV (Keller et al., 1984), HCMV (Rasmussen et al., 1984), and EBV (Strnad et al., 1982). Plaque-forming inhibition activities were also demonstrated with these neutralizing antibodies (Desai et al., 1988; Keller et al., 1987; Miller and Hutt-Fletcher, 1988). Similar activities were not found in the present studies with rabbit serum against the MDV GSTgH fusion protein. However, the GSTgH fusion protein only contains a small portion of the gH gene (BglII-EcoRV fragment), although this portion includes the most antigenic fragment of gH protein. The results of the neutralization and plaque-forming inhibition experiments with the antibody

developed from the GSTgH fusion protein in this study may not be a good representation of the real biological activity of the entire gH molecule.

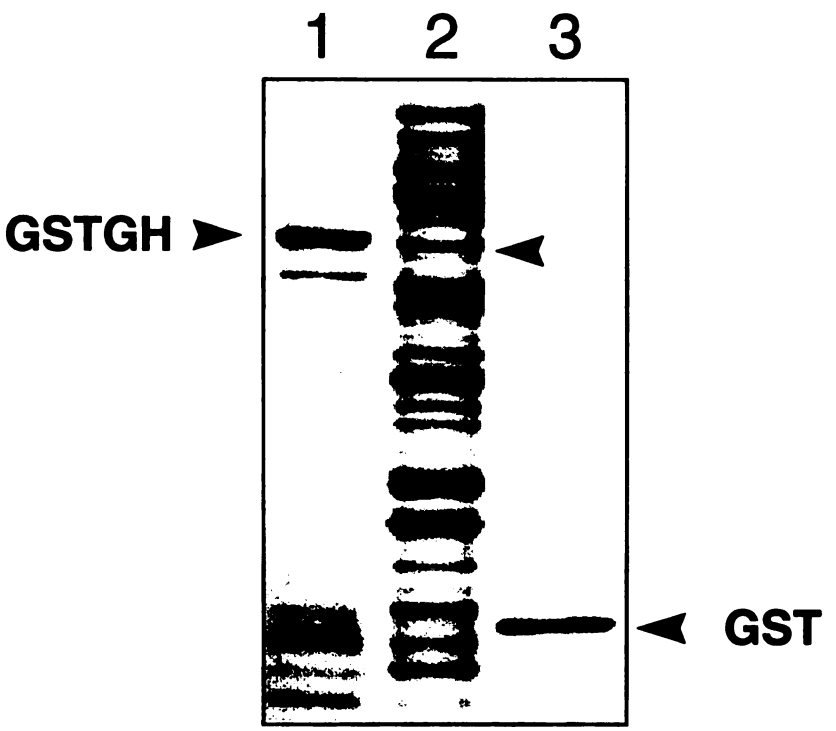

Figure 2. SDS PAGE analysis of GSTgH fusion protein.

Lane 1 is purified GSTgH fusion protein, which was used to inoculate mice and rabbit. Lane 2 is crude preparation of GSTgH fusion protein, which is used to screen the hybridoma cells. Lane 3 is the purified GST protein. The location of GSTgH fusion protein and the GST protein are indicated with arrowheads.

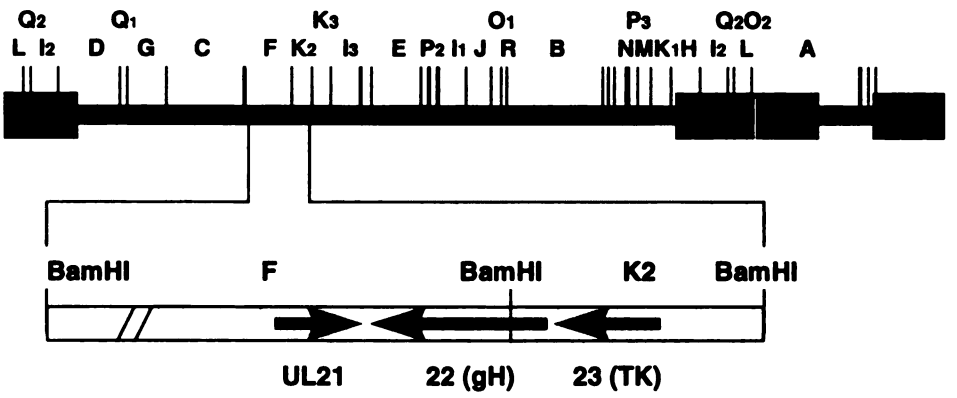

Figure 3. The location of gH and the adjacent genes.

The top portion of the scheme map shows the structure of MDV genomic DNA of MDV and the BamHI digestion map. The BamHI F and K2 fragments were amplified in the low portion of this map. Three potential ORFs are labeled with arrows. The directions of the arrows represent the direction of transcription. The gH ORF was extended from the BamHI K2 to F. The U_L21 ORF was located at 294 bps downstream of the gH stop codon.

Figure 4. The DNA sequence of gH gene of MDV GA strain.

The gH coding region is numbered from 1 to 2439. The stop codon is located at 2440. The TK ORF (partial) was underlined from –319 to –185. The potential poly A sequence was also underlined at 2449.

-319 ACTGGCCTGT CCACAACAAA GTGTCTAGAA TCGTTCATGG ATACTATGTC GGAGAGATTG GTAACACATA GTAGCTGGAA

-239 TGATGCCTTC GAGATTGAAG CTGATGTACT AGCCTATAAT AAAGAGATGG CTATGTAAAA CTACCCATTC ATATCGCGCT

-159 TCTATAATTA GCTTGCCCAC ATCACAATGA TGCGGCAATA TTGACTTATA TTAAGATAGT AATTTGGCGT CCTTAGATCC

-79 AATAAATATC CATGATTTAG TAAGTGTGTT CATACGGATC GTAGCACTTG CAAGTTGCAT TGGATGGCTA CATATCCAAC

1 ATG GGT CTT CCC GGT AGT ATA GTT TTT TTG ATA ATG ATC CAT GCA TTT TGT GCA AAG AAG ACA CCA

67 ACG AAT ACA CTA CCA TCG TTA TTG TCC TTG TTG GGA ATT ACA GAT CTG CCT TCT CTG CGA CTG AAT

133 ATT TTA TCT CTC GAT GGA AGC GCG AAT AAC CAA GGC TCC TGG GTA CGT GAC AAT ACT ACA TTT GTG

199 TAT ATT GGG GCA TCC AGC CCA GCA AAT GGT GTG TTG TTT TAT ATG CCA ACA AGT CAT GTA CAA CAA

265 ATG ACT TTC TAC AAA CGG CCG GTA TCC AAA CTG TTG GCG TCC AAT AAT CTA ATC AAA TTT TTA AAT

331 ACG GGG TCG TAC ATC AAT CAC TCG TTC ATG ACG GCC ATG CCA CCC TAC CGA CGA AAT GTG CAA ATT

397 CCC TCG GAC CGA TCT GGT CTT AAA TTA GAT GAC AAA GAC GAC GCT CAA CCT ACA GGA ACT AAT CCT

463 CCA ACA GAA TTG AAG AAC CTA AAA CCT ATT GAT GTT GTT AAT CCT GAA CAT CGT TTC ATT CTC ACC

529 AGT GAA TTG ACC GGA ACC TAT GTA AAA CAT GTA TGT TTT GTG GAT CCC ATG GAC ATG CTC ATT CCG

595 GTT GAT TAT GCA CAT ATA CGA ACG ATT ATA TTT GGT TCC GAT GGC GCT GAA GTT ATA ATG AAG ATA

661 GGG ATA ACA TTT GCC TCC ATT ACA ATT TCT ATG AAA TCG GCA CCT CCC GTC GAA TTG ATA TTA TCG

727 GAA AGA GCT AGA AAT ATT TCG TTG ATC TGG CCA GCG CTG AAA CCT TAT GAA CCC GTG GAT AAG TTT

793 ACT CGA CGT CCG TAT TTG ATT TAT TTG CTA GGA CCA CAT ATG AAC GCA TCG GAT ATG GAA ATT AAA

859 TCA TAT ATT AAT ATG ATC GAA AGT GTA GAG GAA TCA TCC AAT TAT GAT TTC CAG ATT GCT CAA ACG

925 CAT GCC CAG CTT TTT ATA TTT GCT GCC ACA CCG ATA TCT GAT ATT AAT GAT ATA TAT TGT TTT AGA

991 GTC GTA ACT ACA CGC TTA TTT ATG TCT CTA GTA GCA TCT GTA CGC AAC GCG TTT CAA TCT GGC TAT

1057 ATT TCT TTC GAT GAA ATA ATT AAA ACT GAA GCT AAT ATA AAA ATG ATT ACC GAA ACT CTT TCA ACT

1123 TTT GCC TTG CAT TCA AAC CCT GGT ACA TAC TTC TTG TTA TCT GGA ATG CAT TTG CGG AAT GAA AAT

1189 GCT GAT ATT ATA AAA TCT TTG ATT CGA AAG ACG ATT ATA AAT GCA TCC AAA AAT ACA GCT TCC TTA

1255 TCT ATT CTG CAA CAT CTA TAT GTT TTA AGG TCT GCG TAT GCA TTC AAC ATA TCC CAA GAG AGT GGA

1321 AAT CTG GGG GAG CAT GTT TCG AGC ATT TCA TTA GAG CTC ATT ATA GCT CTT CAC GAG GAA TCC GTC

1387 AGG GAC ACA ATT GCA TGG AAC ACT TCT GTG AGG CAT GCC TTA TAT TAT GCG TTT GCG AGT ATT TTT

1453 CAA CGC CCT CCG AAT GAA TGG GAT GCA TCT CGC ACT GCT CGC AAG GCT CTA TTG TTT GCG TCT TCA

1519 ATG TGT ACT GAA GAA CAT ATC GTA GCT ACG GAA CTG GTC ATA CAG GAA ATG TAT ATC AAA ATC AAT

1585 GTT AAA AAC TCG CCA GTG CAT ATT TTA GAT GTA TAT ACA CCG TGT GTT ACA GCT TTG CGG ATG GAT

1651 ATT TCC GAA CAT CAT CAT AGA CTA TAT GCA ATG TCC GAT GTG ATT TTA CAT CCA GTA ATC GAG AAG

1717 TAT TTG GAA AAT GAT TCC CGT GGT ATC GAT GCT GAA GAA GAA TTA GAA ACA AAA GCA GAA TTG GTA

1783 ATC ACC AAG CTT AAA ACA CCA TTG ATG AGA AGG TTG ACT ATA TAT GCA TCA GAA GTT GTG ACT TGT

1849 TCT GAT GCA GAT ATT TTA GAA GCT ACA GCG CTT TTA GTT TTG CCC ATT TCT GGA CTA GGG AGT TAT

1915 GTT GTG ACA AGA CAA CTT GGA ATA AGA GGT ATT GTC TAT AAT GTG GAT GGT GTT GAC GTG AAC AAT

1981 CAA CTT TAT ATA ACA TAT GTT AGA CTA CCG TGT ACA ACG ACA GCC GGT AAC ATT GTT CCC ATG GTA

2047 CTA CCT AGA CCA CTC GGA AGC GAT TGT CCC TAC TGT GGT TGT GTC CTT TTG AGA TAC TCA ACA AAC

2113 GGA AAC CTT AGA CAC ACC ATT TAT ATT TCA TCA CAA GAT TTG CAG CGA GAA CTG ATT GCA GGA GGG

2179 AAT TCA TCC ATT CGG TAT TTT AAC CCT ACT ATC GCC CAA ATA TAC GGA ACA TCA TTA CTA TTG TAC

2245 CCA AAT GGT ACA ATC GTA CGG ATC TTA GCT TTT GAA TCG GAA CGA GTA ACT ATT ATC TCT GCA ACT

2311 TAC GTC GCA ACT GCT ACA GCC GGG GCT TCG ATA GCT ATA TCA ATA GCA ATT ATT ACT GTA AGA ATG

2377 ATT ATT AAT AAT TTT AGA TAC AAT TAT CAT AGA TAT AAG AAA TTG AGC CTG TAC GAC GAT CTT TAA

2443 TGCAAAAATA AATAAAGAAC CTTTGGGAAT AACAAGCTAT GTATAGAATT TATTTCGCGT GAAGATTTTT CCAAGTCCGA

2523 TCACATTTCA GGTATTACAG CGGTAATAGA TCCATGCATT ATGAGGGTTT GACGTATTAT CTCGATTAAG AACATATTGT

2603 AATACACCCA CTGTTTCTCA AACGAGTGTC TATCAATGAT ATAATACATT GCATGTATCG ACTATAATAC CCCCAATGTT

2683 CAAAGGCTGA TAAAACTGAT ATATCTATGG CCGCGCATAG CAATTCTGCC GTATCTTCTC CCACCGATTC TCGTAACGCG

2763 ACGTCTATGG GATCAATGTC TTTATATAGA CCGTCTAGAA TAAGAGCCAG TTTACGTATC TTGAGGTCCT GTATAGATTT

2843 TGGGTCAG

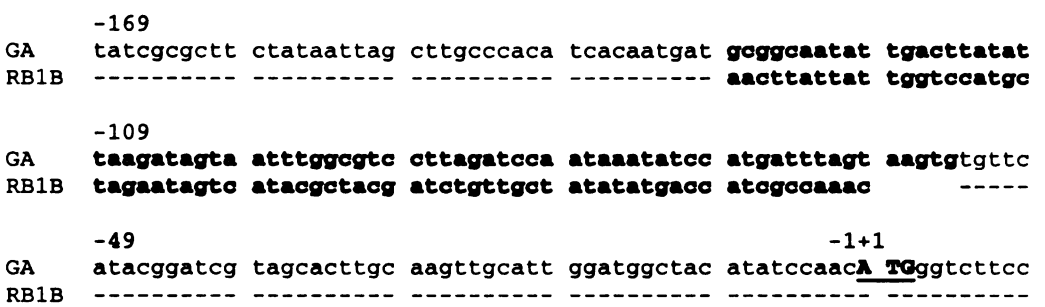

Figure 5. Comparison of the upstream DNA sequences from gH gene between GA and RB1B strains.

GA, DNA sequence from GA strain, RB1B, DNA sequence from RB1B strain. The position of nucleotide residue is referenced to the start codon of gH ORF, counting as +1 at the A of ATG, which is underlined and bolded. The first nucleotide residue to the left of A is numbered as -1. The same nucleotide residue in RB1B as that in GA is represent with -. The different nucleotide residues are aligned and bolded in both GA and RB1B. At least, a "TATA' box at –113 and a "CAT" box at –127 were absent from RB1B sequence.

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1   MGLPGSIVFL IMIHAFCAKK TPTNTLPSLL SLLGITDLPS LRLNILSLDG SANNQGSWVR
61  DNTTFVYIGA SSPANGVLFY MPTSHVQQMT FYKRPVSKLL ASNNLIKFLN TGSYINHSFM
121 TAMPPYRRNV QIPSDRSGLK LDDKDDAQPT GTNPPTELKN LKPIDVVNPE HRFILTSELT
181 GTYVKHVCFV DPMDMLIPVD YAHIRTIIFG SDGAEVIMKI GITFASITIS MKSAPPVELI
241 LSERARNISL IWPALKPYEP VDKFTRRPYL IYLLGPHMNA SDMEIKSYIN MIESVEESSN
301 YDFQIAQTHA QLFIFAATPI SDINDIYCFR VVTTRLFMSL VASVRNAFQS GYISFDEIIK
361 TEANIKMITE TLSTFALHSN PGTYFLLSGM HLRNENADII KSLIRKTIIN ASKNTASLSI
421 LQHLYVLRSA YAFNISQESG NLGEHVSSIS LELIIALHEE SVRDTIAWNT SVRHALYYAF
481 ASIFQRPPNE WDASRTARKA LLFASSMCTE EHIVATELVI QEMYIKINVK NSPVHILDVY
541 TPCVTALRMD ISEHHHRLYA MSDVILHPVI EKYLENDSRG IDAEEELETK AELVITKLKT
601 PLMRRLTIYA SEVVTCSDAD ILEATALLVL PISGLGSYVV TRQLGIRGIV YNVDGVDVNN
661 QLYITYVRLP CTTTAGNIVP MVLPRPLGSD CPYCGCVLLR YSTNGNLRHT IYISSQDLQR
721 ELIAGGNSSI RYFNPTIAQI YGTSLLLYPN GTIVRILAFE SERVTIISAT YVATATAGAS
781 IAISIAIITV RMIINNFRYN YHRYKKLSLY DDL
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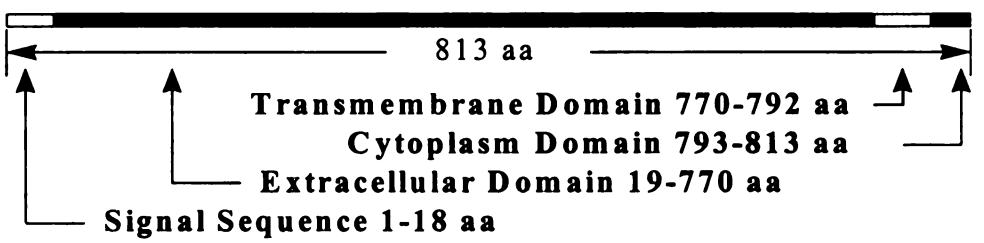
A



B

Figure 1. Characteristics of amino acid sequence of MDV gH precursor

A. Amino acid sequence of gH precursor of MDV GA strain. Four potential transmembrane helices are underlined. The corresponding positions are referenced in Table 2. There are 9 potential N-linked glycosylation sites are boxed. The corresponding positions are at amino acid 62, 116, 247, 279, 410, 434, 469, 727, and 750.

B. Schematic map of gH precursor. The signal sequence, extracellular domain, transmembrane domain and the cytoplasmic domain are indicated, and the related positions are numbered (adapted from Scott et al. 1993).

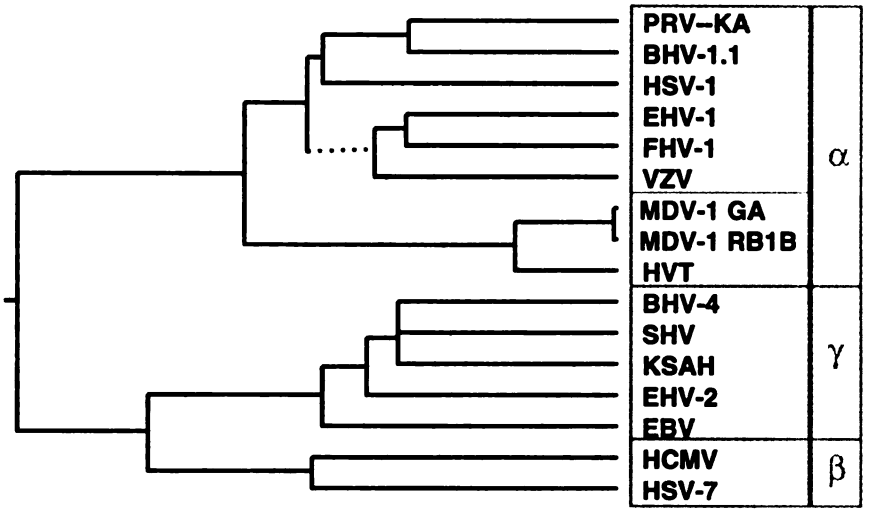


Figure 7. The phylogenic tree of gH homologues of herpesviruses.

A total of 16 gH homologues of herpesviruses were analyzed. The multiple alignment analysis was done by the cluster method of Multiple Sequence Alignment program in DNASTAR package. The rooted phylogenic tree was generated within the same program. The groups of the 16 gHs were exactly matched with the classification of herpesvirus. The MDV gHs with a group were separated from β and γ groups, but MDV gHs were first separated from other α herpesviruses.

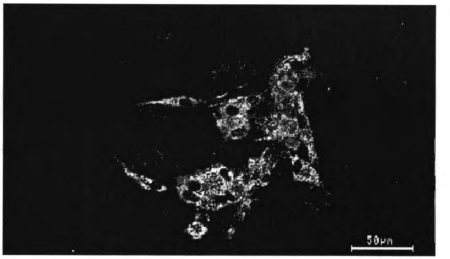


Figure 8. Photograph of gH in DEF infected with MDV GA strain.

The coverslips were prepared as described in Materials and Methods. Mab32 (1:100) was used to detect gH expression in MDV infected DEF. The photograph was taken under laser scanner confocal microscope. One plaque was visualized. The fluorescence was mainly present in the cytoplasm, and the unstained nuclei appeared as black holes. Several multinuclear cells are present in this plaque.

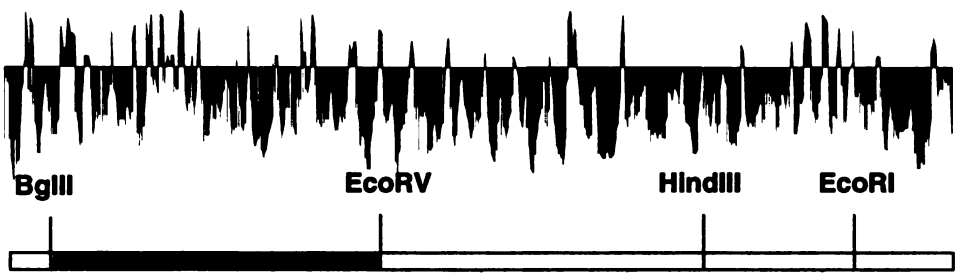


Figure 9. Schematic map of antigenic profile of gH peptide.

In the upper panel, the antigenic profile was generated by MacVector program with predicted gH peptide. Related to the horizontal line in the middle, the up site represents more antigenic than below. The low panel indicates the full length of gH ORF. Several common restriction endonuclease-cutting sites are also indicated. The solid box is the fragment, which was cloned into pGEX-5X-3 vector to generate GSTgH fusion protein. Related to the antigenic profile, this fragment is the main antigenic region in the gH molecule.

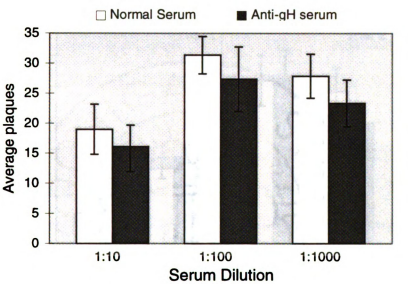


Figure 10. The results of Neutralization tests.

The average plaques were obtained from 8 single observations. The rectangles represent the average plaques. The vertical bars represent the standard deviations. There was no difference between the gH serum and normal serum.

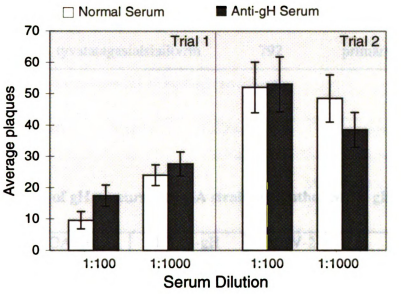


Figure 11. Result of plaque-forming inhibition tests.

Two trails are present. Average plaques (rectangles) were obtained from 8 single observations in trail 1, 4 single observations in trail 2. Vertical bars represent standard deviation. Same results as Figure 10.

Table 2. The locations and the sequences of 4 hydrophobic helices of gH precursor predicted by SOSUI system

No.	N-terminal	Hydrophobic helices	C-terminal	type	length
1	1	mglpgsivflimihafca	18	primary	18
2	619	adileatallvlpisglgsyvvt	641	primary	23
3	660	nqlyityvrlpctttagnivpmv	682	secondary	23
4	770	tyvatatagasiaisiaiitvrm	792	primary	23

Table 3. Comparison of gH precursor of GA strain with other MDV gH homologues

	GA-gH		RB1B-gH		MDV-2-gH		HVT-gH	
	1*	2*	1	2	1	2	1	2
GA-gH	100	100	99.8	99.8	N/A	N/A	66.5	56.0
RB1B-gH			100	100	N/A	57.5	66.4	55.9
MDV-2-gH**					100	100	N/A	50.1
HVT-gH							100	100

*, 1. Percentage of Similarity;
2. Percentage of Identity;
**. The data was directly cited from the publication;
GA and RB1B are MDV-1 strains;
HVT is MDV-3 strain;
N/A, the data were not available.

Table 4. Serological characteristics of anti-gH antibodies

Methods	Mab28	Mab32	Rabbit serum	Antigens used
ELISA*	+	+	+	GSTgH fusion protein
IFA**	+/-	+	+	MDV infected DEF
IP***	ND	ND	+	Truncated gH

*: Total 9 Mabs are ELISA positive against GSTgH fusion protein.
**: Only the listed three antibodies were tested for IFA feature.
***: P, Immunoprecipitation
+: positive
-: negative
ND: Not done.

Table 5. List of database resources of gH homologues of herpesviruses

No.	Organisms	subgroups	Accession No.	References
1	Herpes simplex virus type 1 (HSV-1)	α	P06477	(McGeoch and Davison, 1986)
2	Bovine herpesvirus type 1.1 (BHV-1.1)	α	P27599	Direct Submission
3	Pseudorabies virus (PRV)	α	P27416	(Klupp and Mettenleiter, 1991)
4	Feline herpesvirus type 1 (FHV-1)	α	S64566	(Maeda et al., 1993)
5	Equine herpesvirus typel (EHV-1)	α	P09101	(Telford et al., 1992)
6	Varicella-zoster virus (VZV)	α	P09260	(Davison and Scott, 1986b)
7	Turkey herpesvirus (HVT)	α	P36337	(Scott et al., 1993)
8	MDV-1 GA strain	α		
9	MDV-1 RB1B strain	α	P36336	(Scott et al., 1993)
10	Herpes simplex virus type 7 (HSV-7)	β	P52353	Direct Submission
11	Human cytomegalo-virus (HCMV)	β	P12824	(Cranage et al., 1988)
12	Epstein-Barr virus (EBV)	γ	P03231	(Baer et al., 1984)
13	Equine herpesvirus 2 (EHV-2)	γ	S55616	(Telford et al., 1995)
14	Kaposi's sarcoma-associated herpesvirus (KSAH)	γ	U75698	(Chang et al., 1994)
15	Bovine herpesvirus-4 (BHV-4)	γ	Z79633	(Lomonte et al., 1997)
16	herpesvirus saimiri (SHV)	γ	P16492	(Gompels et al., 1988)

CHAPTER III. ANALYSES OF MDV GH-L INTERACTION IN IN VITRO TISSUE CULTURE EXPRESSION SYSTEM

Abstract

A set of glycoproteins, including gB, gD, gH, and gL have been shown to directly or indirectly mediate membrane fusion events required for both virus entry into host cells and cell-to-cell fusion in herpes simplex virus type I (HSV-1). Glycoproteins H and L form a hetero-oligomeric functional unit (gH-L), which plays an important role in virus infection and cell to cell spread, in most herpesviruses described to date. To investigate the interaction of MDV gH and gL, both gH and gL were expressed in a baculovirus expression system, fowlpox virus (FPV) expression system, and a vaccinia virus MVA/T_7 pol enhanced transient expression system. Indirect immunofluorescence assay was performed using gH and gL antibodies. The results suggested that co-expression of gH and gL in the same cell is required and sufficient for both gH and gL subcellular transportation and cell surface expression in Sf9 cells. The gH requires gL for cell surface expression and the reverse is also true in Sf9 cells. However, gL alone can be detected on the cell surface in DF1 cells and has a small patchy appearance. Co-expression gH and gL in DF1 cells also results in gH-L patch formation on the cell surface, suggesting a fusogenic function of the gH-L complex. Evidence from the FPV expression system indicated that gH is required for protecting gL secretion by providing a membrane anchor for the gL molecule. The results from in vitro translation and transient expression in DF1 cells indicated the amino acids 451-659 (SacI-HindIII fragment) of the gH polypeptide are essential for gH-L complex formation. By co-immunoprecipitation from pulse-chase labeling MDV-infected DEF samples with gL serum, specific and unique bands ranged

from 100 to 110 kDa were precipitated, which reflect the immature and mature from of gH molecules.

Introduction

Previous studies on the molecular mechanisms of MDV infection have focused on two categories predominantly; (1) Oncogenically associated genes and regulative gene products (Bradley et al., 1988; Bradley et al., 1989; Chen et al., 1992; Cui et al., 1991; Cui et al., 1992; Cui et al., 1990; Jones et al., 1992; Li et al., 1994; Maotani et al., 1986; Ono et al., 1994; Peng et al., 1992; Qian et al., 1996; Qian et al., 1995b; Ren et al., 1994; Silva and Witter, 1985; Smith et al., 1995), and (2) Envelope membrane glycoproteins. One of the unusual features of MDV is its strictly cell-associated character in vitro and in vivo. MDV spreading from infected cell to neighboring normal cell is thought to occur by intracellular bridge formation (Kaleta and Neumann, 1977), which also requires the expression of several viral envelope glycoproteins on the infected cell surface. At least 5 glycoproteins (gB, gC, gD, gH and gL) are reported to be directly involved in the initial infection processes and cell to cell spread in HSV-1 infection. gH usually forms a complex with gL as a functional unit. The biological function of gH and gH-L complex appears to be highly conserved in all three groups of herpesviruses, although the processes of maturation and subcellular translocation of gH and/or gL products vary. Appropriate interaction of gH and gL is required for the maturation and subcellular translocation of these two molecules, and is functionally essential for virus entry (penetration) and cell-to-cell spread(Kaye et al., 1992; Hutchinson et al., 1992; Duus et al., 1995; Li et al., 1997b; Li et al., 1995; Yaswen et al., 1993). In HSV-1, gH alone was partially processed, and intracellularly accumulated in rough endocytoplasmic reticulum (RE) with a precursor-like form of the glycoprotein, and was not translocated to the cell surface, unless the cells were super-infected with HSV-1 or HSV-2 (Foa-Tomasi et al.,

1991; Forrester et al., 1991; Gompels and Minson, 1989). This immature gH was unrecognized by the monoclonal antibody, LP11 that efficiently neutralizes HSV-1 infection (Buckmaster et al., 1984; Roberts et al., 1991). The gH co-expressed with gL by using vaccinia virus recombinants was antigenically normal, processed normally, and transported to the cell surface. Similarly, gL was also dependent on gH for proper post-translational processes and cell surface expression. gH and gL form hetero-oligomers which are incorporated into virions, transported to the cell surface, and play a role during entry of virus into cells (Hutchinson et al., 1992). Whereas VZV gL chaperone modulated gH expression via retrograde flow from the Golgi to the ER. The mature gL returns to the ER, where it escorts immature gH from the ER to the Golgi; thereafter, mature gH is transported from the trans-Golgi to the outer cell membrane, where it acts as a major fusogen (Duus and Grose, 1996).

The coding regions of gH genes of MDV-1 RB1B (Scott et al., 1993) and GA strains (see chapter II), and MDV-3 (HVT) have been determined. The primary polypeptides exhibit typical glycoprotein features, including a hydrophobic signal and an anchor sequences (transmembrane domain) and potential N-linked glycosylation sites. Polypeptide sequence comparison to other available gH homologues and phylogenic analysis revealed that MDV gHs are located in between α-herpesvirus subgroup and β- & γ-herpesvirus subgroups, but closer to α-herpesvirus. MDV-2 gH gene was also reported recently (Shimojima et al., 1997). Alignment of the amino acid sequences of MDV gH homologues among three MDV serotypes shows 57.5% (MDV-1 and MDV-2), 56.2% (MDV-1 and HVT), and 50.1% (MDV-2 and HVT) identities (Shimojima et al., 1997). The MDV-1 gL shares 18% identity with the HSV-1 counterpart. An antiserum was

produced which can immunoprecipitate a 25 kDa polypeptide in MDV-infected cells. The gL alone expressed by reFPVgL is highly sensitive to Endo-H, indicating that it may be not properly processed to a mature form. (Yoshida et al., 1994a).

Available information for the interaction and the biological function of MDV gH, gL, and gH-L complex is limited. In order to further understand MDV infection and the cell fusion process, it is essential to study gH and gL expression, as well as their interaction. In this study, the molecular and biologic properties of MDV gH were investigated in vitro in a recombinant baculovirus expression system, a fowlpox expression system, a TNT in vitro translation system, and in a vaccinia virus MVA/T_7 pol enhanced transient expression system.

Materials and methods

Virus and Cells. Duck embryo fibroblasts (DEF) and chicken embryo fibroblasts (CEF) were grown in Leibowitz-McCoy medium (GIBCO Laboratories, Grand island, NY), supplemented with 4% calf serum, penicillin/streptomycin and amphotericin B (growth medium) or 1% calf serum, penicillin/streptomycin and amphotericin B (maintenance medium). Spodoptera frugiperda (Sf9) cells were propagated in complete TNM-FH medium [Grace's insect medium and supplements (Invitrogen corporation, Carlshad, CA) with 10% fetal bovine serum (FBS), Gentamycin, and amphotericin B]. MDV-1 GA strain (Eidson and Schmittle, 1968) was propagated in DEF or CEF. Line O chicken embryo fibroblast cell line, and DF1 cells were propagated in Leibowitz-McCoy medium, supplemented with 5% FBS, penicillin/streptomycin and amphotericin B. Wild type baculovirus was purchased from Invitrogen corporation (Carlshad, CA). Bacterial T_7

RNA polymerase recombinant vaccinia virus, MVA/T_7 pol (Wyatt et al., 1995) was a kind gift from Dr. Bernard Moss (Laboratory of viral disease, NIAID, NIH, Bethesda, MD), which was propagated in CEF. gL and gH+gL recombinant fowlpox virus [reFPV-gL (Yoshida et al., 1994a)), and reFPV-gHL (Lee, unpublished)] were also propagated in CEF.

Constructions of MDV-1 gH and gL recombinant transfer vectors. The gH ORF was amplified from genomic DNA of MDV GA strain by polymerase chain reaction (PCR). The primers used for PCR were 5'-GGG GCT AGC GGA TCC CAA CAT GGG TCT TTC C-3' (forward), and 5'-CCC GTC GAC GGA TCC GCA TTA AAG ATC GTC GT-3' (reverse). The PCR product was cloned into pUC18 vector to produce gH DNA stock, pUCgH. Generally, the DNA fragment and the vector were combined in a ratio of 3:1 and ligation was performed overnight at 14°C with T4 DNA ligase and 1x ligation buffer. Transformation of competent TG1 strain E. coli was conducted via electroporation (Cell-Porator, BRL, Grand Island, NY) at 400 volts, 4 kilo-ohms and a capacitance of 330 microfarads with the ligation mixture and plated on 2xYT agar with ampicillin. Plates were incubated from 16-20 hours at 37°C. Ampicillin resistant colonies were selected and grown in 2ml 2xYT medium containing ampicillin for 5 hours. Colonies were screened for inserts with minipreps. Positive clones were amplified and the DNA was extracted and purified with a Qiagen-tip 500 (Qiagen, Chatsworth, CA). DNA was stained with Hoechst dye and quantitated with a DNA fluorometer (Hoefer Scientific Instruments, San Francisco, CA).

To construct baculovirus transfer vectors, the gH gene was cut out from the pUCgH by BamHI-DraI and DraI-SalI. Two fragments, BamHI-DraI and DraI-SalI,

were cloned into BamHI and SalI sites of baculovirus transfer vector, pBlueBac4 (Invitrogen corporation, Carlshad, CA) to generate a transfer vector, pBacgH. The gL gene was cut out from gL-pbluescript (Yoshida et al., 1994a) with BamHI and XhoI, and then inserted into linearized pBlueBac4 with compatible cohesive ends to generate transfer vector pBacgL. The gL cassette (the gL ORF with the polyhedrin promoter in the upstream, as well as 3' end sequence) was digested from pBacgL with EcoRV-SnaBI (both ends were blunted). This fragment was inserted into pBacgH downstream of the 3' end of the gH gene at SnaBI site to generate transfer vector pBacgHL. The gL gene in pBacgHL has the same orientation as the gH gene. The MDV gH and/or gL genes were placed under control of the baculovirus polyhedrin promoter (Figure 12).

To construct transient expression vector, the gH fragment was cut out from pBacgH with NheI and salI, and cloned into linearized pCDNA3.1zeo vector pBlueBac4 (Invitrogen corporation, Carlshad, CA) at NheI and XhoI sites to generate transfer vector pCDNAgH. The gL fragment was cut out from pBacgL with NheI and PstI, and cloned into pCDNA3.1Zeo vector to generate pCDNAgL. Both gH and gL gene are under control by the T_7 promoter. Three truncated gH fragments were generated by digesting pBacgH with NheI-EcoRI, NheI-HindIII, and NheI-SacI, and cloned into linearized pGEM-7Zf+ vector (Promega corporation, Madison, WI) with compatible cohesive ends to generate transfer vectors pGEMgHe, pGEMgHh and pGEMgHs, respectively. All the three truncated gHs were also under control by the T_7 promoter (Figure 13).

The constructs were conformed free of error by sequencing analysis with an automated sequencer (373A DNA Sequencer, Applied Biosystems, Foster City, CA) and dideoxy sequencing methods (Prism, Applied Biosystems, Foster City, CA).

Developments of gH and gL recombinant baculoviruses. All the reagents used for the transfection were provided in the Bac-N-Blue transfection kit (Invitrogen Corporation, Carlshad, CA). $2x10^6$ log phase Sf9 cells (98% viability) was seeded into a 60mm tissue culture dish in 2ml complete TNM-FH medium. The dish was rocked gently side to side to evenly distribute the cells. The cells were allowed to fully attach to the bottom of the dish to form a monolayer (about 50% confluence) for at least 15 minutes before performing the transfection.

In a 1.5ml sterile microcentrifuge tube, the transfection mixture was set up with 4μl (4μg) pBacgH (pBacgL or pBacgHL), 10μl (1μg) linearized Bac-N-Blue baculovirus DNA, 1ml Grace's Insect media (without supplements, FBS, and antibiotics), and 20μl Insect Liposomes (always added last). The mixture was vortexed vigorously for 10 seconds, then incubated at room temperature for 15 minutes.

While the transfection mixture was incubating, the TNM-FH media was removed from the cells carefully without disrupting the monolayer. The cells were rinsed twice with 2ml Grace's Insect medium without supplements, FBS, and antibiotics. The transfection mixture was directly added onto the cell sheet a drop at a time, and evenly distributed over the monolayer. Following 4 hours incubation at 27°C, the cells was fed with 1ml complete TNM-FH medium, placed in a sealed plastic bag, and incubated at 27°C for 3 days.

Blue plaque assay was used to select and purify the recombinant baculoviruses. The transfected cell culture medium (containing recombinant baculovirus) was collected and diluted to 10^{-2}, 10^{-3}, and 10^{-4} in 3 days post-transfection. Sf9 monolayer in 100mm dish ($5x10^6$ cells) was prepared (2-3 dishes for each viral dilution) in 5ml complete

TNM-FH medium. The confluence of the cells was about 50%. For viral infection, 3ml medium was removed from the cells, 1ml diluted virus was added a drop at a time. The virus was allowed to absorb to Sf9 cells at 27°C for 1 hour. After completely removing the medium, the infected cell sheet was overlapped with 10ml/dish baculovirus agarose [2.5ml pre-warmed (at 47°C) 2.5% agarose solution, and 2.5ml pre-warmed complete TNM-FH medium, mixed with 5ml complete FNM-FH medium with 150μg/ml halogenated indolyl-β-D-galactosidase (bluo-gal)]. The dishes were sealed in a plastic bag, and incubated at 27°C until plaques formed (about 3-5 days).

A single blue plaque was picked, and seeded into 35mm dish with $5x10^5$ Sf9 cells in 2ml complete FNM-FH medium. Three days post-inoculation, 0.75ml cell suspension was harvested, the virus was precipitated with 20% polythylene glycol 8000 (PEG, in 1M sodium chloride), and the DNA was extracted with proteinase K digestion and phenol-chloroform extraction. The DNA was used to check the foreign gene (gH, gL, and gH-L) insertion and the purification of the recombinant virus by PCR. The positive plaques were propagated in Sf9 cells to prepare the high titer recombinant virus stocks. In this way, three recombinant baculoviruses were established, which are rBacgH, rBacgL and rBacgHL (Figure 12).

Polymerase chain reaction. 25μl PCR reaction mixture was setup as described below: 2.5μl DNA (10-100ng/μl), 2.5μl 10x PRC buffer, 2μl 25mM Mg^{++}, 1μl 25mM dNTPs, 1μl PCR primer mixture (5picomol/each), 0.5μl (1unit) Taq polymerase, sterile water to 25μl. The mixture was overlaid with 30μl mineral oil. PCR reaction was executed in MiniCycler (M J Research Inc., Watertown, MA) with the following parameters: Step 1, initial denaturation at 94°C for 2 minutes; step 2, denaturation at 94°C

for 1 minutes; step 3, annealing at 55°C for 2 minutes; step 4, extension at 72°C for 3 minutes; step 5, 30 cycles between step 2 and 4; step 6, final extension at 72°C for 7 minutes; and step 7, holding the reaction at 15°C until analyzed in 1% agarose gel electrophoresis. The primers used in the PCR reaction were 5'-TTT ACT GTT TTC GTA ACA GTT TTG-3' (forward), and 5'-CAA CAA CGC ACA GAA TCT AGC-3' (reverse), which will amplify 839 bps from wild type baculovirus, 2708 bps from rBacgH, and 888 bps from rBacgL.

In vitro translation. In vitro translation was done by using T_NT T_7 coupled reticulocyte lysate system (Promega Corporation, Madison, WI). Five plasmids, pCDNAgH, pGEMgHe, pGEMgHh, pGEMgHs, and pCDNAgL were analyzed. The reaction mixture was setup as described below: 13µl TNT rabbit reticulocyte lysate, 1ul T_NT reaction buffer, 0.5µl T_NT T7 RNA polymerase, 2µl ^{35}S-methionine (1,000Ci/mM at 10mCi/ml, New England Nuclear, Life Science Products, Boston, MA), 0.5µl Rnasin Ribonuclease inhibitor (40u /µl), 2.0µl plasmid DNA templates (1µg), and distill water to 25µl. The reaction mixture was incubated at 30°C for 90 minutes. 2µl reaction product was analyzed with SDS-polyacrylamid gel electrophoresis (SDS-PAGE), whereas 10-15µl samples were analyzed by immunoprecipitation.

Transient expression of gH and gL with MVA/T_7 pol enhancement. DF1 cell were plated onto 35mm culture dishes (10^6 cells/dish) in 2ml Leibowitz-McCoy medium (GIBCO Laboratories, Grand island, NY) with 5% calf serum, penicillin/streptomycin and amphotericin B, and incubated at 37°C overnight. When transfected, the cell confluence was about 80%. The DF1 cells were infected with recombinant vaccinia virus, MVA/T_7 pol, at 10 multiplicity of infection (moi) in 1ml M199 medium (GIBCO

Laboratories, Grand Island, NY) with 5% FBS and, penicillin/streptomycin, without amphotericin B for 1 hour prior to adding the transfection mixture. The transfection mixture was prepared as follows: About 2-10μg plasmid DNA was diluted in 250μl distilled water, slowly adding 250μl 0.5M calcium chloride solution with gentle vortex, slowly adding 500μl 2xHepes solution (140mM sodium chloride, 1.5mM disodium phosphate, 50mM Hepes, pH7.05, stored at –20°C) with gentle vortex. The mixture was held at room temperature for 20 minutes for formation of fine precipitate. Into each 35mm dish 100μl of the transfection mixture was added with large bore pipette tip, and the dish was swirled afterwards to evenly distribute the precipitate. Then the dish was incubated at 37°C, 5% CO_2 for 4 hours. After removing the media, the DF1 cells were shocked with 1ml 15% glycerol solution (fresh prepared with distilled water) for 5 minutes at room temperature. The glycerol was removed, and the DF1 cells were rinsed twice with 2ml phosphate buffered saline (PBS). The dishes were fed with 2ml fresh Leibowitz-McCoy medium with 5% FBS, penicillin/streptomycin, without amphotericin B, and incubated in 37°C, 5% CO_2. After 20 hours incubation, the samples were harvested for further analysis.

Antibodies. Anti-GSTgH monoclonal antibody Mab32 and rabbit serum were developed by using GSTgH fusion protein as an antigen (Chapter II). The anti-gL rabbit serum was reported (Yoshida et al., 1994a).

Indirect immunofluorescence assay (IFA). Samples were taken from recombinant baculovirus infected Sf9 cell coverslips and from gH or gL transiently transforming DF1 cells in 35mm tissue culture dishes. To detect gH or gL expression, samples were fixed for 5 minutes in ice cold acetone (or acetone 60% and ethanol 40%

mixture for tissue culture dishes), and air dried. To detect the cell surface expression of gH and gL, Sf9 cells were fixed with 2% paraformadelhyde solution in PBS for 1 hour. The paraformadelhyde fixed samples were further treated with 0.5% Triton X-100 in PBS for 30 minutes at room temperature for permeabilization of the cells. The fixed samples were stored at -20°C for later use. IFA was performed as previously published (Wu et al., 1997). Briefly, The fixed samples were incubated with either anti-gH Mab32 or rabbit serum (including gH and gL serum) diluted 1:100 in PBS for 30 minutes at 37°C in a humidified incubator. Coverslips were rinsed 15 minutes in PBS then incubated for 30 minutes with either goat anti-mouse or goat anti-rabbit fluorescein isothiocyanate (FITC)-conjugated IgG (Kirkegaard & Perry Laboratories, Gaithersburg, MD). The coverslips were rinsed again, and sealed with 50% glycerol in PBS, before the samples were observed under confocal microscope.

Confocal microscopy. The samples were viewed with a laser scanning confocal microscope (Carl Zeiss, Inc, Thornwood, NY) with 40x oil lane, 488 argon laser line with green (BP521-650) barrier filter, fluorescence operation and confocal modes. The photographs were taken under the same conditions. Some samples were observed under Leica DM IRB microscope (Leica Mikroskopie und system GMbH, Wetzlar, Germany), and photographs were taken with a DEI-750 CE digital camera (Optronics Engineering, Goleta, CA). Adobe Photoshop program (Adobe Systems Incorporated, Mountain view, CA) was used to process the images.

^{35}S-methionine labeling gH and gL. Secondary DEF monolayer in 60mm dish was infected with MDV-1 GA strain. When the cellular pathologic effect (CPE) reached 70-80% (about 72-96 hours post-infection), the infected DEF was incubated with 2ml

methionine free RPMI 1640 medium (Gibco BRL, Life Technologies, Inc., Gaithersburg, MD) for 1 hour. 100μCi ^{35}S-methionine (New England Nuclear, Life Science Products, Boston, MA) was added in 2ml methionine free RPMI 1640 medium, and incubated for another 5 hours. The reFPVgL and reFPVgHL infected CEF (10moi) were labeled in 18 hours post-infection for 5 hours. The transient expressions of gH and gL were directly labeled with 75μCi ^{35}S-methionine per 35mm dish in 1ml methionine free RPMI 1640 medium for 19 hours, right after 15% glycerol treatment of the cells. After labeling, the cells were washed twice with PBS, and lysed in lysis buffer (150mM NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% SDS, 10mM Tris-HCl pH 7.5). For pulse-chase labeling, the cells were treated with ^{35}S-methionine for 10-20 minutes, and washed twice with PBS to remove the trace ^{35}S-methionine, then fed with fresh RPMI 1640 with methionine, and incubated to an indicated time point. The supernatant was collected, and the cells were lysed in lysis buffer,

Immunoprecipitation. Immunoprecipitation was performed in the following way. The cell lysate (200-300μl/sample) was pre-absorbed with pre-bleeding rabbit serum (or NS-1 myeloma ascites) and Sepharose protein A (Pharmacia-Biotech, Uppsala, Sweden) for 1 hour in an ice bath with shacking. The pre-absorbed lysate was mixed with antibody (5μl) and 50μl Sepharose protein A., and incubated for 1 hour in an ice bath with shaking. The Sepharose protein A-antibody-antigen complex was washed 3 times with lysis buffer. 40μl SDS-PAGE loading buffer (1% SDS, 50mM Tris-HCl pH 6.8, 10% glycerol, 2.5% diethylthreotal, 0.01% phenol red in water) was added, the sample was boiled for 5 minutes, centrifuged with a tabletop centrifuge at a maximum speed for 5 minutes and analyzed on 5-15% gradient SDS-PAGE.

The gel was fixed in fixing solution (10% glacial acetic acid, 20% methanol in water) for 45 minutes, incubated for 45 minutes in dimethyl sulfoxide (DMSO) to dehydrate and 45 minutes in 16% 2, 5-diphenyl oxazole (PPO, Sigma Chemical Co., St. Louis, MO) in DMSO, and washed for 10 minutes in tap water. The gel was dried on filter paper with a Speed gel SG 200 gel dryer (Savant, Farmingdale, NY) and exposed to film at -70 C.

Results

gH and gL are expressed in recombinant baculovirus expression system, as well as transiently expressed in MVA/T7 pol expression system

In order to investigate the interaction between MDV gH and gL, these two genes were expressed in a recombinant baculovirus system, as well as transiently expressed in recombinant vaccinia, MVA/T7 pol expression system. The samples were taken from Sf9 cells infected with recombinant baculoviruses, or DF1 cells transfected with pCDNAgH, and pCDNAgL. The samples were fixed with acetone, and strained with anti-gH mab32, anti-gH rabbit serum, and anti-gL rabbit serum. The results were shown in Figure 14A & B, 15A, and 16A & D.

Co-expression of gH and gL is required for gH maturation and translocation in Sf9 cells.

In baculovirus expression system, coverslips of Sf9 were infected with either rBacgH, rBacgHL, or rBacgL, and fixed with paraformadelhyde for cell surface staining. Some of them were permeabilized with Triton X-100. IFA was conducted with gH and gL antibodies. When Sf9 was infected with rBacgH alone, gH was detected only in the permeabilized sample (Figure 15A), but not in the non-permeabilized sample (i.e.

paraformadelhyde fixed sample) (Figure 15 B). When Sf9 was infected with rBacgL alone, similar results were observed. The gL was only detected on the permeabilized samples (Figure 16A), but not in the non-permeabilized sample (Figure 16B). When Sf9 was infected with rBacgHL, the gH and gL were detected in both permeabilized and non-permeabilized samples (Figure 16D and 17A, B, C, & D).

gH and gL expressed in the MVA/T7 pol enhanced expression system in DF1 are similar to that in Sf9, but not the same.

In order to study the interaction of gH and gL in a more native cell of MDV infection, a line O chicken embryo fibroblast cell line, DF1 was used to express the gH and gL products in a MVA/T_7 pol enhanced transient expression system. The MVA/T_7 pol infected DF1 cells were transfected with pCDNAgH, pCDNAgL, and pCDNAgH+ pCDNAgL. The IFA results indicated that gH could be expressed in both pCDNAgH and pCDNAgH+pCDNAgL transfected DF1 cells (Figure 18A & B). However, only co-transfected with pCDNAgL, would the gH be detected on the cell surface (Figure 19A), otherwise, the gH was retained in the cytoplasm (Figure 19B & 18B). The results also indicated that gL was also expressed in DF1 (Figure 18C and D), but gL appears to behave differently in DF1 cells compared to in Sf9 cells. The IFA results indicated that the gL could be detected not only in DF1 cell surface co-transfected with pCDNAgH+pCDNAgL (Figure 19C), but also in DF1 cell surface transfected with pCDNAgL alone (Figure 19D). gL was not detectable on the Sf9 cells surface when the Sf9 cell was infected with rBacgL alone(Figure 16B).

gL alone is consistently secreted from CEF cells

Pulse-chase labeling and immunoprecipitation results revealed that the gL alone expressed in CEF cell was consistently secreted from the cells. The reFPVgL and reFPVgHL infected CEF cells, and MDV-1 GA strain infected DEF cells were labeled with ^{35}S-methionine for 20 minutes, and then chased in time course. The supernatant samples were centrifuged with maximum speed at tabletop centrifuge for 10 minutes to clarify the cell debris before used for immunoprecipitation. Figure 20 shows the results. When gL was expressed in reFPVgL infected CEF, the gL signal from cell lysate was decreased along with the chase time; On the other hand, the gL signal in the supernatant was increased along with the chase time. However, in reFPVgHL infected CEF culture supernatant, there was no significant increase of gL signal in the entire chasing period, although the gL was present in the cell lysate (Figure 21A). This absence might be due to the expression of gH (Figure 22). Moreover, in MDV-1 GA strain infected DEF cells, the gH and gL were also positive by IFA (Figure 8), but in the cell culture supernatant, the gL signal is absent (Figure 21B).

The SacI-HindIII fragment of gH gene is required for gH-L interaction

In order to map the potential domain responsible to the interaction of gH and gL, three truncated gH mutations were constructed in pGEM-7zf+ vector (see Materials and Methods). Three truncated gH were co-translated with gL in T_NT in vitro translation system (Figure 23). The immunoprecipitation results of the translated products with gH serum revealed that the gL was co-immunoprecipitated with gHe, gHh by gH antibody, but not with gHs (Figure 24), suggesting that the SacI-HindIII fragment of gH gene, corresponding to the 451-659 amino acids would be responsible for gL binding. These

results were repeated in DF1 transient expression of gH and gL with MVA/T_7 pol enhancement. The transiently expressed gH, and gL were labeled with ^{35}S-methionine. Immunoprecipitation was performed with both gH and gL serum. Specific truncated gH proteins were precipitated from gH transfected DF1 lysates with gH serum (Figure 25). The gHe and gHh were also co-precipitated with gL from gH and gL co-transfected DF1 samples by gL serum, but gHs was not co-precipitated (Figure 26).

The gH was co-immunoprecipitated with gL antibody from the DEF infected with MDV-1 GA strain.

DEF infected with MDV-1 GA strain was labeled with ^{35}S-Methionine at 96 hours post-infection for 20 minutes, and chased for a different time period. The anti-gL rabbit serum was used to immunoprecipitate the gL, as well as gH products. The results indicated that the gH product was co-immunoprecipitated with gL antibody even at 0 minute chase time. The mobility of the gH product was slowed with the increased chase time. The related molecular weight was ranged from 100 to 110 kDa (Figure 27).

Discussion

The interaction mode and the function of the gH-L complex partners appear to be quite variable. While gH and gL are covalently linked by disulfide bonds in HCMV (Kaye et al., 1992), a noncovalent interaction is present in HSV-1 (Hutchinson et al., 1992) and VZV (Duus et al., 1995). In EBV and HCMV, a third complex partner was detected, represented by a 42 kDa protein and a 125/145 kDa protein, respectively (Li et al., 1997b; Li et al., 1995; Yaswen et al., 1993). Baculovirus expression system has been

successfully used to study gH and gL interaction in several herpesviruses (Ghiasi et al., 1991) (Westra et al., 1997; Pulford et al., 1994a). In this report, the gH and gL gene of MDV-1 GA strain were cloned into baculovirus to generate rBacgH, rBacgL, and rBacgHL. The cell surface immunofluorescence strain was achieved by fixing the Sf9 cells infected with these recombinant baculoviruses with paraformadelhyde solution without permeabilization (Duus et al., 1995). The IFA results revealed the interaction of MDV gH and gL in Sf9 cells and DF1 cells was basically similar to previous reports in other herpesviruses (Westra et al., 1997; Pulford et al., 1994a). The co-expression of MDV gH and gL is required and sufficient for both the gH and gL subcellular translocation and cell surface expression. Expression of gH or gL alone in Sf9 cells will result in the absence of gH or gL from the cell surface, although gL alone is present on the DF1 cell surface.

Although gH is conserved structurally and functionally throughout Herpesviridae, the gL was thought to share homologues among herpesviruses only based on the genomic location and biologic function. VZV gL can be processed to a mature product within the Golgi by itself. Although the full length of gL is required for gH maturation, the subcellular transportation beyond the medial-Golgi of mature gH does not require the gL molecule. The VZV gL is usually retained in ER due to its bearing an ER targeting motif (Duus et al., 1995; Li et al., 1997a). PRV gL bears a neutralization epitope, which is associated with virus infection, but not with cell to cell spread. Therefore, PRV gL is not only a chaperone for gH maturation, but also directly involved in the virus infection processes. MDV gL also appears to behave different in DF1 cells. The gL could be detected in the cell surface with small patch appearance by IFA, when the gH was absent

from the DF1 cells (Figure 19), although the gL molecule may consistently secret into culture medium in FPV expression system in CEF. This process is just like gC, which is a secreting glycoprotein in MDV, but also can be detected on the cell surface (Churchill et al., 1969a; Isfort et al., 1986). On the other hand, with co-expression of gH and gL in FPV expression system in CEF, most of the expressed gL was trapped in the CEF cells, instead of being secreted. The same situation was observed in the MDV infected DEF samples. All these results suggested that gH is an important factor for gL staying in the cells, most likely by providing an anchor.

The gL is normally a simpler form of chaperone protein. However, the diverse gL glycoproteins of EBV and VZV have been reported to be functionally interchangeable, although membrane expression and maturation of gH were separate functions for these two viruses. The domain homologue search indicated that the MDV gL shares homologous domains located in between EBV and VZV gL (Figure 28), suggesting MDV gL might be interchangeable functionally with EBV gL or VZV gL.

There are at least three types of gH-L complex within cells in α-herpesviruses. HSV-1 gL is required for gH maturation and subcellular translocation and both gH and gL are co-expressed on the cell surface (Buckmaster et al., 1984; Foa-Tomasi et al., 1991; Forrester et al., 1991; Gompels and Minson, 1989; Hutchinson et al., 1992; Roberts et al., 1991). The mature VZV gH is expressed on the cell surface alone, and executes the fusogenic function (Duus and Grose, 1996; Duus et al., 1995; Li et al., 1997a). PRV gH is incorporated into the virion in the absence of gL, but virion localization of gH in the absence of gL is not sufficient for infectivity (Klupp et al., 1997). Like HSV gH, MDV gH alone in DF1 cells was retained in the cytoplasm, and absent from the cell surface,

whereas co-expression gH and gL in DF1 results in gH-L patch formation on the cell surface (Figure 19). Similar patches were also observed within the cytoplasm of the DF1 cells transfected with gL alone and gH-L. These cytoplasmic patches may represent transport vesicles, a common phenomenon in secretory and endocytic pathways. The patching or capping in cytoplasmic membrane (a basic mechanism for endocytosis) changes the local environment on the cell surface, which may promote membrane fusion of the enveloped virion (or infected cells) with the adjacent normal cells.

The potential gH-L interaction domain was mapped to SacI-HindIII fragment by serial truncated deletion mutants. The truncated gHe and gHh (with SacI-HindIII fragment) can be co-precipitated with gL from DF1 cells by gL serum. In reverse, the gL is also co-precipitated with gHe and gHh from in vitro translation samples with gH serum. However, gL is unable to be co-precipitated with truncated gHs (without SacI-HindIII fragment) by gH serum. In HSV-1 gH, the N-terminal half of the external domain may be responsible for gH-L interaction, while the C-terminal third of the external domain affected the ability of gH to function in cell-cell fusion and virus entry (Galdiero et al., 1997). According to the domain homologous analysis, MDV gH and HSV-1 gH share 2 homologous domains at the C-terminal 300 amino acids (Figure 29), suggesting the C-terminal 300 amino acid of MDV gH may also function in cell to cell fusion and virus entry, which is the function of the homologous domains of HSV-1 gH (Galdiero et al., 1997).

Difficulty was encountered in immunoprecipitating full length gH protein from Sf9 cells, from MDV infected DEF, and from DF1 cells, although IFA results showed positive reaction to gH antibodies in all cells. However, the truncated gH, including gHe,

gHh, and gHs, was immunoprecipitated from DF1 cells with gH serum, as well as co-precipitated with gL from DF1 cells by gL serum. These results suggest that the C-terminal sequence may affect the properties of the gH molecule significantly, which will protect the protein from immunoprecipitation with gH serum. Since co-precipitation with the truncated gH was detected with gL serum, this feature may be applied to precipitate full length gH from MDV infected DEF cells. The lysates of pulse-chase labeled DEF cells infected with MDV GA strain were precipitated with gL serum. Two specific polypeptides were identified, one with 25 kDa in size, which is the mature form of the gL molecule (Yoshida et al., 1994a) and the other with a molecular weight ranging from 100 to 110 kDa in different chase time (Figure 27). The second polypeptide is in good agreement with the predicted gH (114 kDa) reported by Scott et al. (Scott et al., 1993) based on sequence data, and the 115 kDa gH reported by Yoshida (Yoshida et al., 1994a). The range of the molecular masses may represent the post-translational modification processes. The gH polypeptide binds to gL right after its synthesis. In vitro translation of gH and gL (no post-translational modification element was added) shows similar results, suggesting the appropriate interaction of gH and gL starts at very early stage of gH synthesis. This interaction might occur in the cytosol before gH enters into the ER lumen.

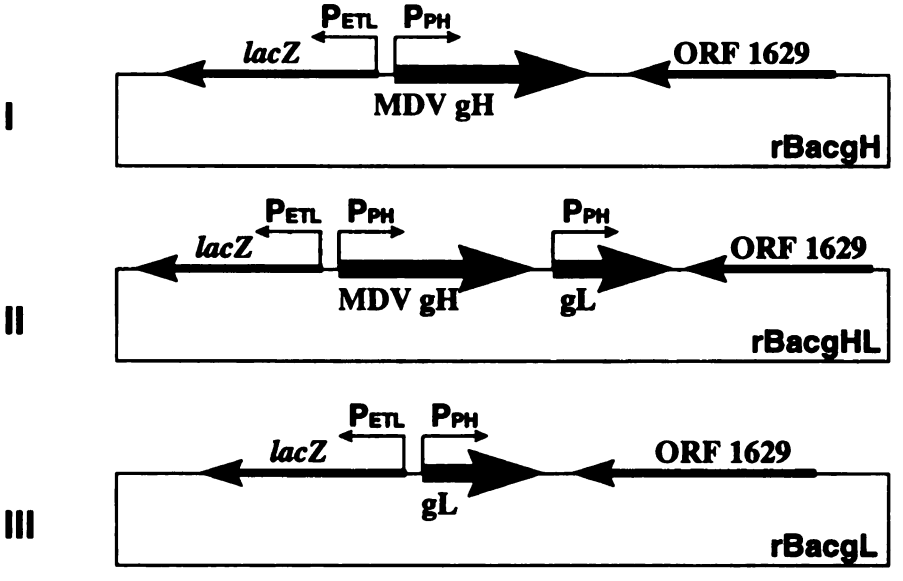


Figure 12. Schematic map of MDV gH, gL, and gHL recombinant baculoviruses

The MDV genes were inserted into the polyhedrin region of baculovirus (AcMNPV) to generate the recombinant viruses. Polyhedren promoter controlled all the MDV genes. I. MDV gH recombinant baculovirus, rBacgH; II. gH and gL recombinant baculovirus, rBacgHL; and III. gL recombinant baculovirus, rBacgL.

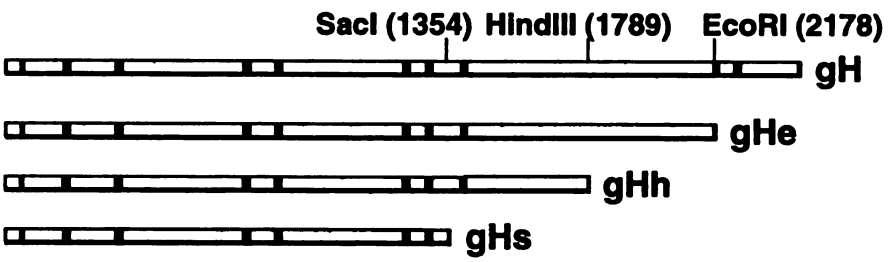


Figure 13. Schematic map of truncated gH fragments.

The gH gene was digested with EcoRI, HindIII, and SactI, respectively, to generate three truncated gH fragments. These fragments were cloned into pGEM-7zf+ vector to generate three truncated gH transfer vectors, which are pGEMgHe for EcoRI truncate; pGEMgHh for HindIII truncate; and pGEMgHs for SacI truncate. The top line represents the whole length of gH molecule. The three restriction endonucleases used for truncating gH were indicated. The related N-linked glycosylation sites were indicated with the vertical bars.

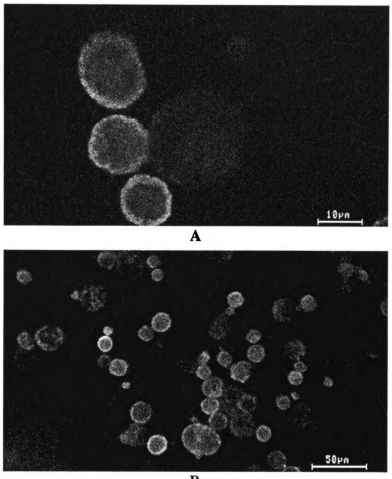
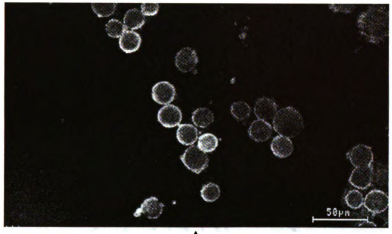
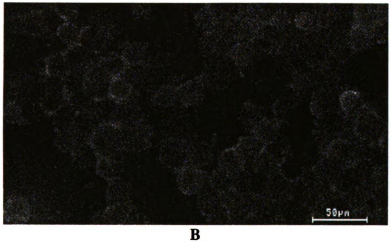


Figure 14. Immunofluorescence assay of gH expression on Sf9 cells.

Sf9 cells infected with rBacgHL, fixed with paraformadelhyde and permeabilized with Triton X-100. A. Straining with anti-gH rabbit serum; B. Staining with Mab32.



A



B

Figure 15. The gH was not expressed on the cell surface when Sf9 cells were infected with rBacgH alone.

The Sf9 cells were infected with rBacgH. The paraformadelhyde fixed samples were permeabilized with Triton X-100 (A) to detect gH expression in the cytoplasm, or without permeabilization for cell surface staining (B). The samples were stained with Mab32. Only the permeabilized sample was positive to Mab32, whereas non-permeabilized sample was negative. These results indicated that gH alone expressed in Sf9 cells was unable to reach the cell surface.

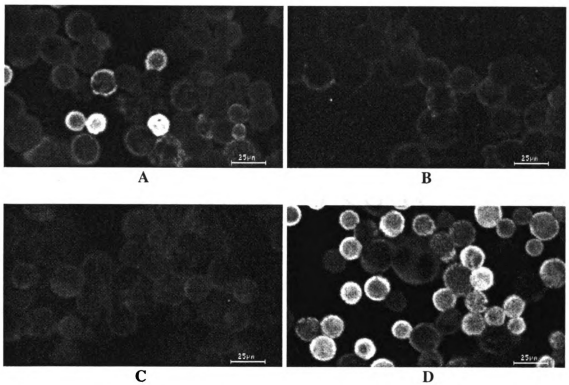


Figure 16. The gL was not expressed on the cell surface when Sf9 cells were infected with rBacgL alone.

Sf9 cells were infected with recombinant baculoviruses: A & B infected with rBacgL, C infected with rBacgH as negative control, D infected with rBacgHL as positive control. The samples were fixed with paraformadelhyde without permeabilization (B) and with permeabilization (A, C, D). All samples were stained with gL antibody. A and D show positive reaction, and B and C are negative response.

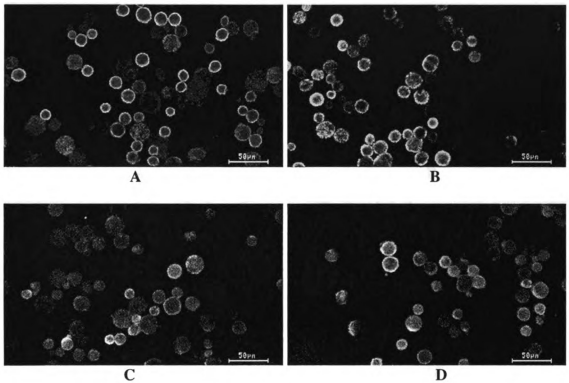


Figure 17. Co-expression of gH and gL in Sf9 cells result in the cell surface expression of both gH and gL.

The Sf9 cells were infected with rBacgHL. The samples were fixed with paraformadelhyde with permeabilization (A and C) and without permeabilization (B and D). A and B were stained with gL antibody, and shown positive reaction in both permeabilized and non-permeabilized conditions. Same results were present in C and D, which were stained with gH Mab32.

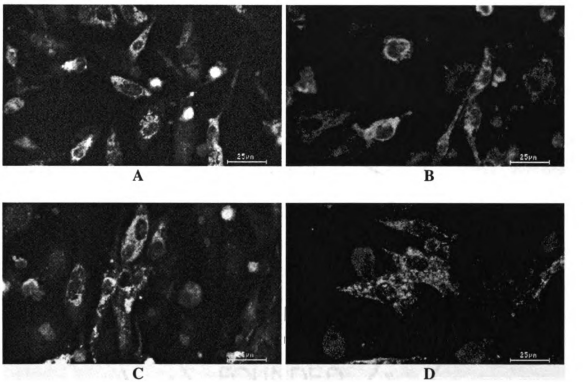


Figure 18. The expressions of gH and gL in DF1 cells transfected with pCDNAgH+pCDNAgL (A & C), pCDNAgH (B), and pCDNAgL (D).

All the samples were fixed with 60% acetone and 40% ethanol at room temperature for 5 minutes. A and B were strained with gH serum, C and D were stained with gL serum. All the samples show fluorescent positive. However, the subcellular distribution patterns were different between gH and gL. gH alone was uniformly distributed all over the cytoplasm (B), while gL alone, as well as co-expressed gH and gL, was unequally distributed (so call patch) (A, C, and D).

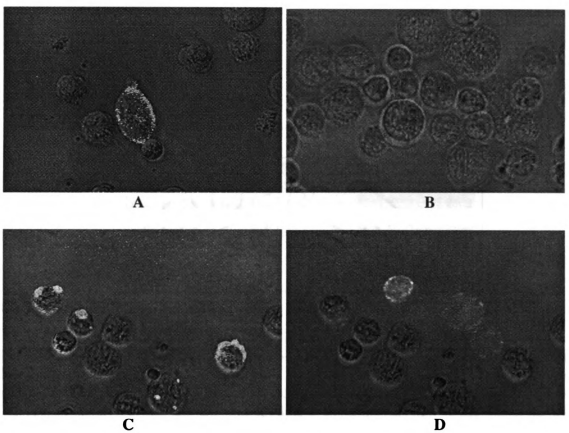


Figure 19. Photographs of gH and gL cell surface expression in DF1 cells.

The DF1 cells were transfected with pCDNAgH+pCDNAgL (A & C), pCDNAgH (B), and pCDNAgL (D). The cells were trypsonized and stained with gH and gL anti-sera. A & B were strained with gH serum, and only A shows positive reaction, B was negative. C & D were strained with gL serum, and both C and D were positive. The gH and gL molecules were forming patches of staining on the cell surface(A, C, and D), which were observed in the cytoplasm, too (See Figure 7).

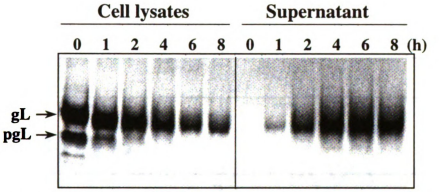


Figure 20. Immunoprecipitation of gL protein from the reFPVgL infected CEF cell lysates and supernatant with gL serum.

The reFPVgL infected CEF cells were pulse-labeled with 35S-methionine for 20 minutes, then chased for 0, 1, 2, 4, 6, and 8 hours. Samples were collected, and immunoprecipitation analysis was conducted as described in the Material and Methods. The results indicated that the gL was gradually decreased in cell lysate samples, and increased in the supernatant samples, suggesting the gL might secrete instantly from the cells. gL, mature form of gL; pgL, gL precursor.

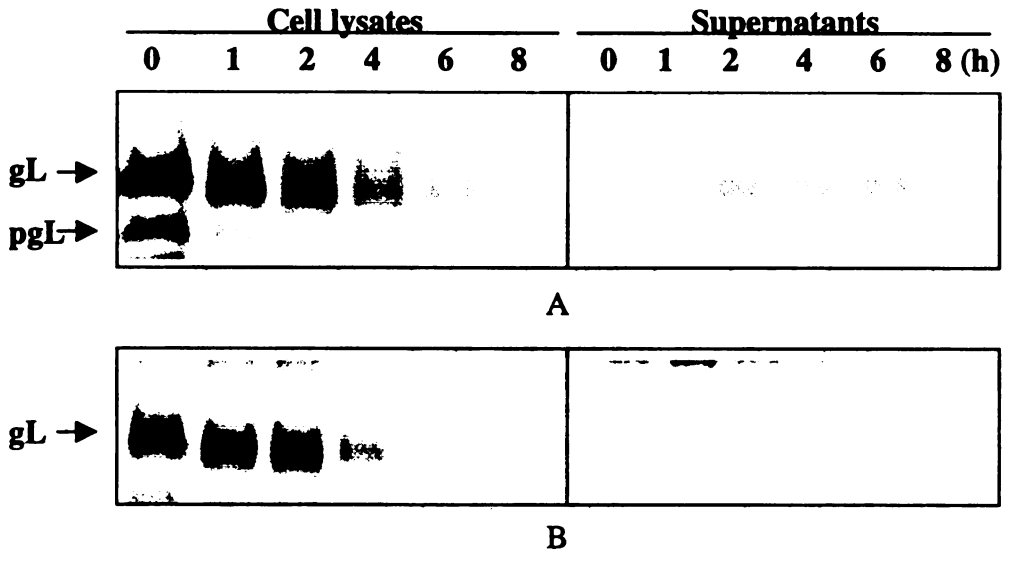


Figure 21. immunoprecipitation of gL from reFPVgH infected CEF (A) and MDV-1 GA strain infected DEF (B).

The infected cells were labeled with ^{35}S-methionine for 10 minutes and chased at indicated time periods. The supernatant and cell lysate were precipitated with gL serum. In the reFPVgHL infected CEF lysate, the gL signal was not significantly changed in the first several hours, whereas the supernatant gL signal was not significantly increased (A). In the GA infected DEFs, the gL signal was absolutely absent from the supernatant (B). gL, mature form gL; pgL, gL precursor.

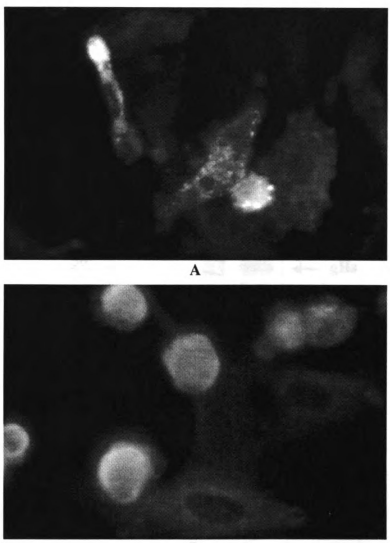


Figure 22. IFA photographs of reFPVgHL infected CEF cells.

The coverslips of reFPVgHL infected CEF were stained with gL (A) and gH (B) serum, respectively. The results were observed with Leica DM IBR microscope, and the photographs were taken in same microscope with DEI-750 digital camera with 40x objective lane.

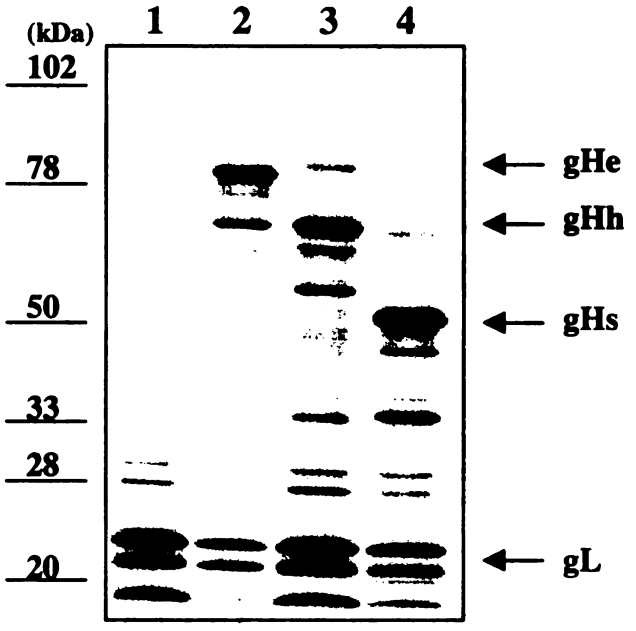


Figure 23. In vitro translation of gH and gL.

The truncated gH were cotranslated in vitro by TNT in vitro translation system. 2μl translation samples were loaded in 5-15% gradient PAGE gel. Lane 1, pCDNAgL alone; Lanes 2 to 4, pGEMgHe, pGEMgHh, and pGEMgHs with pCDNAgL respectively. The standard molecular weight (MW) markers were indicated at the left side. Each truncated gH and gL were indicated with arrow and named. The related MW for gHe is 81 kDa, gHh, 67 kDa, gHs, 50 kDa, and gL, 22 to 23 kDa.

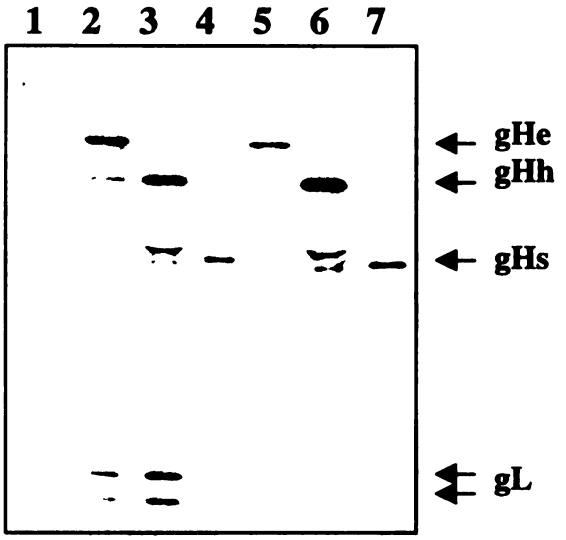


Figure 24. Co-immunoprecipitation of gH and gL from in vitro translation samples with gH serum.

The samples were precipitated with gH serum and analyzed with 5-15% gradient SDS-PAGE. Lane 1, pCDNAgL alone; Lanes 2 to 4, the three truncated gH: pGEMgHe, pGEMgHh, and pGEMgHs with pCDNAgL, respectively; Lanes 5 to 7, three truncated gH only. The gL was co-precipitated with gHe and gHh (Lanes 2 and 3), but not with gHs (Lane 4).

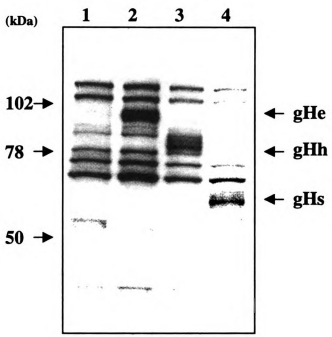


Figure 25. Immunoprecipitation of transiently expressed gH from DF1 cell lysate.

The DF1 cells were transfected with either pGEMgHe, pGEMgHh, pGEMgHs, or pCDNAgL. The cells were labeled with ^{35}S-methionine. The immunoprecipitation was performed with gH serum. Lane 1 was pCDNAgL control, Lanes 2 to 4 were pGEMgHe, pGEMgHh, and pGEMgHs, respectively. The MW markers are indicated at the left, and the truncated gH was indicated at the right with the arrow.

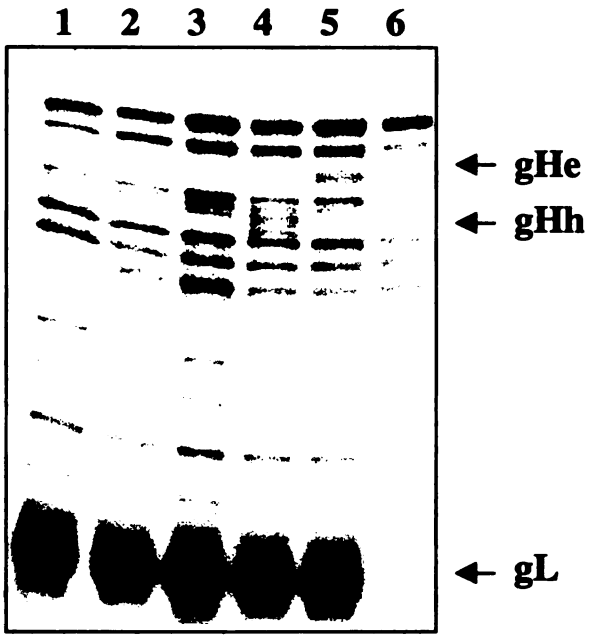


Figure 26. Immunoprecipitation of gL and gH from DF1 cells transiently expressing gH and gL.

The immunoprecipitation was conducted with gL serum. Lane 1, pCDNAgL alone; Lane 2, pCDNAgH+pCDNAgL; Lanes 3 to 5, pGEMgHs, -gHh, and –gHe with pCDNAgL, respectively. Lane 6, MVA/T7 pol control. Only gHe and gHh, but not gHs, were co-precipitated with gL (Lanes 4 and 5). The locations of gHe and gHh and gL were indicated with arrows.

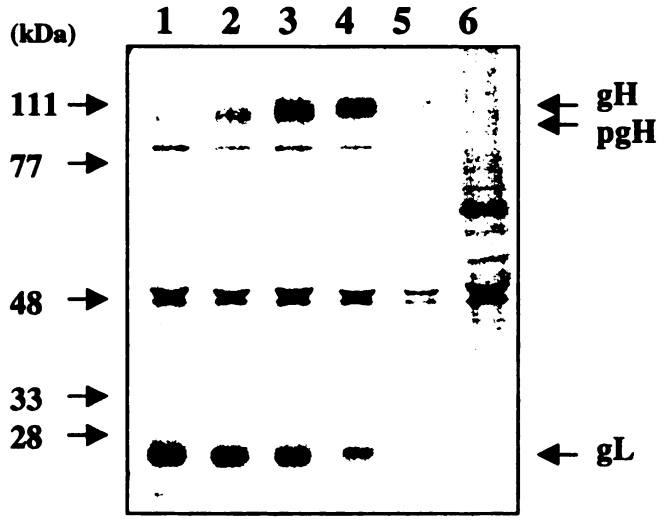


Figure 27. Co-immunoprecipitation of gH and gL with gL serum from MDV-1 GA strain infected DEF cell lysate.

The GA strain infected DEF cells were labeled with ^{35}S-methionine for 20 minutes and chased at the indicated time periods. The precipitation was performed with gL serum. Lanes 1 to 5, different chased time points: 0, 1, 2, 4, and 8 hours. Lane 6, DEF control. The gH was co-precipitated with gL even at 0 hour chasing time point, suggesting the gH binds to gL right after its synthesis. Along with the increase of chasing time, the gH molecule was gradually increased. The molecular weight ranged from 100 to 110 kDa. The MW markers are indicated at the left, and the locations of gH, gH precursor (pgH), and gL are indicated at the right.

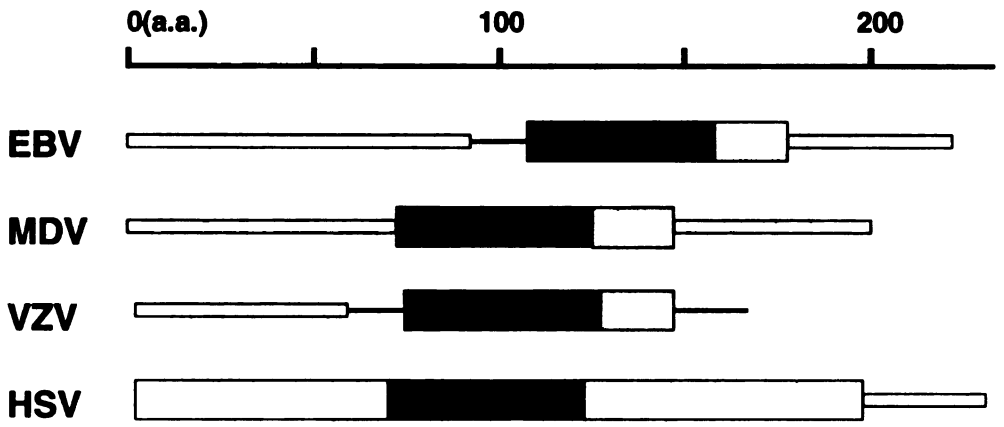


Figure 28. Schematic map of gL domain homologous search results.

MDV gL protein sequence was used to search for the domain homologues within PRODOM protein domain database (http://www.toulouse.inra.fr/prodom/prodom.html). 4 entries were found. The MDV gL has at least 2 domains homologous to EBV and VZV gL , and only 1 domain homologous to HSV-1.

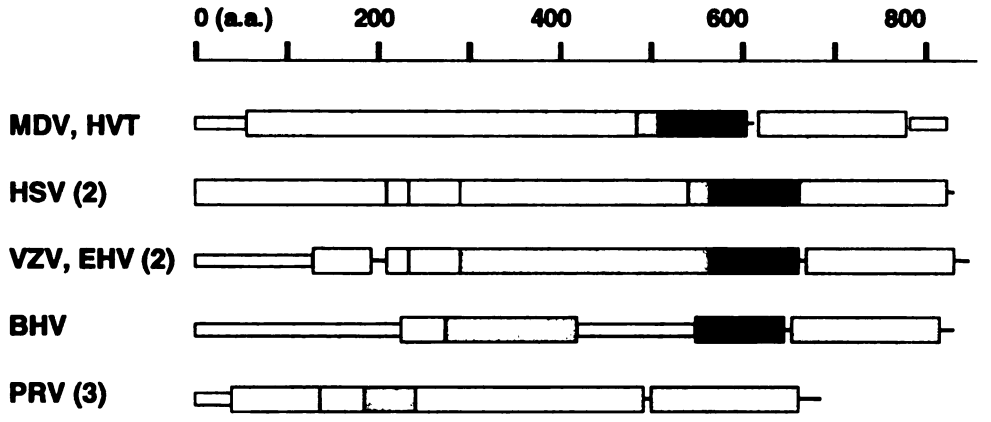


Figure 29. Schematic map of gH domain homologous search results.

MDV gH protein sequence was used to search for domain homologues within the PRODOM protein domain database. Total 11 entries were found. The MDV gHs were closer to HSV-1 gH (at least 3 domain homologues: □, ■, and □). HSV(2), HSV-1 and HSV-2; EHV (2), EHV-1 and EHV-2; PRV (3), three different PRV strains.

CHAPTER IV. CONCLUSION AND FURTHER WORK

One of the unusual features of MDV-1 is its strictly cell-associated character in vitro and in vivo. The mechanism of cell to cell spread has not been characterized for MDV but is thought to occur by intracellular bridge formation which may require the expression of several MDV glycoproteins on the surface of the infected cell. A set of glycoproteins, including gB, gD, gH, and gL have been shown to directly or indirectly mediate membrane fusion events required both for virus entry of host cells and cell fusion in herpes simplex virus type I (HSV-1). The gH usually forms a complex with gL as a functional unit. The biological function of gH and gH-L complex appears to be highly conserved in all three groups of herpesviruses. The appropriate interaction of gH and gL is required for maturation and subcellular translocation of these two molecules, which is functionally essential for virus entry (penetration) and cell-to-cell spread (Kaye et al., 1992; Hutchinson et al., 1992; Duus et al., 1995; Li et al., 1997b; Li et al., 1995; Yaswen et al., 1993). The objective of this research project was to identify the gH gene in a genomic DNA library of the MDV GA strain, and analyze its potential biological functions. Several expression systems were applied to investigate the MDV gH, gL, and their interaction. We concluded:

1. A 2439 bps ORF was identified from the DNA sequence of BamHI F and K2 fragments of MDV GA strain, which predicts a 813 amino acid polypeptide. The predicted molecular weight of gH precursor is 90.1 kDa based on the polypeptide sequence, whereas the mature form of gH is 110kDa in size based on immunoprecipitation and SDS-PAGE results.

2. This peptide is homologous to HSV-1 gH, and has typical glycoprotein features: 1) a single signal sequence, 2) a large extracellular domain, 3) transmembrane domains, and 4) a small cytoplasmic domain. There are 9 potential N-linked glycosylation sites within the extracellular domain. The gH homologues of three serotypes of MDVs share a high percentage of identity and similarity. The evolutionary location of MDV gH is in between α-herpesvirus and γ & β herpesviruses, closer to but beyond α-herpesvirus group, although MDV is classified as an α-herpesvirus based on its genomic structure and gene arrangement.
3. Mabs and serum were developed by using GSTgH fusion protein as an antigen. These antibodies can be used to detect gH expression by several serological methods, including Immunoprecipitation, IFA, and ELISA.
4. There was no evidence of neutralization and plaque-forming inhibition of rabbit serum against the GSTgH fusion protein. The GSTgH fusion protein only contains a small portion of the gH gene (BglII-EcoRV fragment), although this portion includes the most antigenic fragment of gH protein. Therefore, the serological characteristics of the antibody developed from the GSTgH fusion protein may not be a good representation of real biological activity of the entire gH molecule. On the other hand, the functional unit is the gH-L complex, so the best way to evaluate the biological function of gH or gL is to study the gH-L complex, not gH or gL alone. Therefore, to evaluate the biological function of the gH-L complex could be a direction for follow-up research. These studies could lead to a better understand of the biological function of gH-L complex, and thus potentially lead to a better understanding of the mechanism of MDV infection.

5. Co-expression of gH and gL in the same cells is required and sufficient for both gH and gL subcellular transportation and cell surface expression in Sf9 cells. The gH requires gL for its cell surface expression, the reverse is also true in Sf9 cells. gL alone can be detected on the cell surface of DF1 cells with the small patch appearance. Co-expression gH and gL in DF1 cells results in gH-L patch formation on the cell surface, suggesting the fusogenic function of gH-L complex. The post-translational modification and the subcellular traffic of gH and gL appear to be very important for gH-L interaction and final complex formation. To date, the knowledge we have about the gH-L subcellular trafficking mechanism is only end point information. The middle processes to reach the end point are missing. The recently introduced life color protein, green fluorescence protein (GFP), appears to be a good candidate for follow-up study on protein synthesis, modification, subcellular translocation, and cell surface expression. gH or gL GFP fusion protein may provide more detail information about gH and gL subcellular processes, as well as gH-L interaction. Therefore, using gH and gL GFP fusion protein as tools to study the subcellular processes of gH and gL may be the other direction for follow-up research. The results of these studies could provide the inside information about virus replication, envelope formation, as well as virus egression.
6. Results from FPV expression system studies indicated that gH is required for blocking gL secretion by providing a membrane anchor for the gL molecule.
7. Amino acids 451-659 (SacI-HindIII fragment) of the gH polypeptide are essential for gH-L complex formation, whereas the C-terminal 300 amino acid of the gH polypeptide may be involved in membrane fusion processes.

LIST OF REFERENCES

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Adldinger, H.K. and Calnek, B.W. 1973. Pathogenesis of Marek's disease: Early distribution of virus and viral antigens in infected chickens. J.Natl.Cancer Inst. **50**: 1287-1298.

Anderson, A.S., Francesconi, A., and Morgan, R.W. 1992. Complete nucleotide sequence of the Marek's disease virus ICP4 gene. Virology **189**: 657-667.

Anderson, A.S., Parcells, M.S., and Morgan, R.W. 1998. The glycoprotein D (US6) homolog is not essential for oncogenicity or horizontal transmission of Marek's disease virus. J.Virol. **72**: 2548-2553.

Babic, N., Klupp, B.G., Makoschey, B., Karger, A., Flamand, A., and Mettenleiter, T.C. 1996. Glycoprotein gH of pseudorabies virus is essential for penetration and propagation in cell culture and in the nervous system of mice. J Gen Virol. **77**: 2277-2285.

Bacon, L.D. 1987. Influence of the major histocompatibility complex on disease resistance and productivity. Poult.Sci. **66**: 802-811.

Baer, R., Bankier, A.T., Biggin, M.D., Deininger, P.L., Farrell, P.J., Gibson, T.J., Hatfull, G., Hudson, G.S., Satchwell, S.C., and Seguin, C. 1984. DNA sequence and expression of the B95-8 Epstein-Barr virus genome. Nature **310**: 207-211.

Balan, P., Davis-Poynter, N., Bell, S., Atkinson, H., Browne, H., and Minson, T. 1994. An analysis of the in vitro and in vivo phenotypes of mutants of herpes simplex virus type 1 lacking glycoproteins gG, gE, gI or the putative gJ. J Gen Virol. **75**: 1245-1258.

Ball, R.F., Hill, J.F., Lyman, J., and Wyatt, A. 1971. The resistance to Marek's disease of chicks from immunized breeders. Poult.Sci. **50**: 1084-1090.

Basu, S., Dubin, G., Nagashunmugam, T., Basu, M., Goldstein, L.T., Wang, L., Weeks, B., and Friedman, H.M. 1997. Mapping regions of herpes simplex virus type 1 glycoprotein I required for formation of the viral Fc receptor for monomeric IgG. J.Immunol. **158**: 209-215.

Beasley, J.N., Patterson, L.T., and McWade, D.H. 1970. Transmission of Marek's disease by poultry house dust and chicken dander. Amer.J.Vet.Res. **31**: 339-344.

Becker, Y., Asher, Y., Tabor, E., Davidson, I., Malkinson, M., and Weisman, Y. 1992. Polymerase chain reaction for differentiation between pathogenic and non-pathogenic serotype 1 Marek's disease viruses (MDV) and vaccine viruses of MDV-serotypes 2 and 3. J.Virol.Methods **40**: 307-322.

Beninga, J., Kalbacher, H., and Mach, M. 1996. Analysis of T helper cell response to glycoprotein H (gpUL75) of human cytomegalovirus: evidence for strain-specific T cell determinants. J.Infect.Dis. **173**: 1051-1061.

Biggs, P.M. 1961. A discussion on the classification of the avian leucosis complex and fowl paralysis. British Veterinary Journal **117**: 326-334.

Biggs, P.M., Milne, B.S., de-The, G., and Payne, L.N. 1972. Biological properties of a number of Marek's disease virus isolates, p.88-94. Biggs, P.M., (Ed.) Oncogenesis and Herpesviruses. International Agency for Research on Cancer, Lyon, France.

Biggs, P.M. and Payne, L.N. 1963. Transmission experiments with Marek's disease (fowl paralysis). Vet.Rec. **75**: 177-179.

Borysiewicz, L.K., Hickling, J.K., Graham, S., Sinclair, J., Cranage, M.P., Smith, G.L., and Sissons, J.G. 1988. Human cytomegalovirus-specific cytotoxic T cells. Relative frequency of stage-specific CTL recognizing the 72-kD immediate early protein and glycoprotein B expressed by recombinant vaccinia viruses. J Exp.Med **168**: 919-931.

Boyle, K.A. and Compton, T. 1998. Receptor-binding properties of a soluble form of human cytomegalovirus glycoprotein B. J Virol. **72**: 1826-1833.

Bradley, G., Hayashi, M., Lancz, G., Tanaka, A., Nonoyama, M., Horiuchi, T., Mikami, T., and Hirai, K. 1988. Structure of the Marek's disease virus BamHI-H gene family: A gene potentially important for tumor induction, p.69-75. Kato, S., (Ed.) Advances in Marek's disease research. Japanese Association on Marek's Disease, Osaka, Japan.

Bradley, G., Lancz, G., Tanaka, A., and Nonoyama, M. 1989. Loss of Marek's disease virus tumorigenicity is associated with truncation of RNAs transcribed within BamHI-H. J.Virol. **63**: 4129-4135.

Breathnach, R. and Chambon, P. 1981. Organization and expression of eucaryotic split genes coding for proteins. Annu.Rev.Biochem. **50**: 349-383.

Briles, W.E., Briles, R.W., Taffs, R.E., and Stone, H.A. 1983. Resistance to a malignant lymphoma in chickens is mapped to subregion of major histocompatibility B complex. Science **219**: 977-979.

Briles, W.E., Stone, H.A., and Cole, R.K. 1977. Marek's disease: Effects of B histocompatibility alloalleles in resistant and susceptibile chicken lines. Science **195**: 193-195.

Britt, W.J., Vugler, L., Butfiloski, E.J., and Stephens, E.B. 1990. Cell surface expression of human cytomegalovirus (HCMV) gp55-116 (gB): use of HCMV-recombinant

vaccinia virus-infected cells in analysis of the human neutralizing antibody response. J Virol. **64**: 1079-1085.

Britt, W.J. and Vugler, L.G. 1992. Oligomerization of the human cytomegalovirus major envelope glycoprotein complex gB (gp55-116). J Virol. **66**: 6747-6754.

Browne, H., Baxter, V., and Minson, T. 1993. Analysis of protective immune responses to the glycoprotein H-glycoprotein L complex of herpes simplex virus type 1. J.Gen.Virol. **74**: 2813-2817.

Browne, H., Bell, S., Minson, T., and Wilson, D.W. 1996. An endoplasmic reticulum-retained herpes simplex virus glycoprotein H is absent from secreted virions: evidence for reenvelopment during egress. J.Virol. **70**: 4311-4316.

Brunovskis, P. and Velicer, L.F. 1995. The Marek's disease virus (MDV) unique short region: alphaherpesvirus- homologous, fowlpox virus-homologous, and MDV-specific genes. Virology **206**: 324-338.

Buckmaster, A.E., Scott, S.D., Sanderson, M.J., Boursnell, M.E., Ross, N.L., and Binns, M.M. 1988. Gene sequence and mapping data from Marek's disease virus and herpesvirus of turkeys: implications for herpesvirus classification. J.Gen.Virol. **69**: 2033-2042.

Buckmaster, E.A., Gompels, U., and Minson, A. 1984. Characterisation and physical mapping of an HSV-1 glycoprotein of approximately 115 X 10(3) molecular weight. Virology **139**: 408-413.

Bulow, V.v. and Biggs, P.M. 1975. Differentiation between strains of Marek's disease virus and turkey herpesvirus by immunofluorescence assays. Avian Pathol. **4**: 133-146.

Bumstead, N., Sillibourne, J., Rennie, M., Ross, N., and Davison, F. 1997. Quantification of Marek's disease virus in chicken lymphocytes using the polymerase chain reaction with fluorescence detection. J.Virol.Methods **65**: 75-81.

Buranathai, C., Rodriguez, J., and Grose, C. 1997. Transformation of primary chick embryo fibroblasts by Marek's disease virus. Virology **239**: 20-35.

Buscaglia, C. and Calnek, B.W. 1988a. Maintenance of Marek's disease herpesvirus latency in vitro by a factor found in conditioned medium. J.Gen.Virol. **69**: 2809-2818.

Buscaglia, C., Calnek, B.W., and Schat, K.A. 1988b. Effect of immunocompetence on the establishment and maintenance of latency with Marek's disease herpesvirus. J.Gen.Virol. **69**: 1067-1077.

Byerly, J.L. and Dawe, D.L. 1972. Delayed hypersensitivity reactions in Marek's disease virus-infected chickens. Amer.J.Vet.Res. **33**: 2267-2273.

Bzik, D.J., DebRoy, C., Fox, B.A., Pederson, N.E., and Person, S. 1986. The nucleotide sequence of the gB glycoprotein gene of HSV-2 and comparison with the corresponding gene of HSV-1. Virology **155**: 322-333.

Bzik, D.J., Fox, B.A., DeLuca, N.A., and Person, S. 1984. Nucleotide sequence specifying the glycoprotein gene, gB, of herpes simplex virus type 1. Virology **133**: 301-314.

Cai, W.Z., Person, S., DebRoy, C., and Gu, B.H. 1988. Functional regions and structural features of the gB glycoprotein of herpes simplex virus type 1. An analysis of linker insertion mutants. J Mol.Biol **201**: 575-588.

Calnek, B.W. 1985. Genetic Resistance, p.293-328. Payne, L.N., (Ed.) Marek's disease. Martinus Nijhoff, Boston.

Calnek, B.W. 1986. Marek's disease - A model for herpesvirus oncology. Crit.Rev.Microbiol. **12**: 293-320.

Calnek, B.W., Alexander, A.M., and Kahn, D.E. 1970. Feather follicle epithelium: a source of enveloped and infectious cell-free herpesvirus from Marek's disease. Avian Dis. **14**: 219-233.

Calnek, B.W., Carlisle, J.C., Fabricant, J., Murthy, K.K., and Schat, K.A. 1979. Comparative pathogenesis studies with oncogenic and nononcogenic Marek's disease viruses and turkey herpesvirus. Am.J.Vet.Res. **40**: 541-548.

Calnek, B.W., de-The, G., and Payne, L.N. 1972. Antibody development in chickens exposed to Marek's disease virus, p.129-136. Biggs, P.M., (Ed.) Oncogenesis and Herpesviruses. International Agency Research on Cancer, Lyon.

Calnek, B.W., Hitchner, S.B., and Adldinger, H.K. 1970. Lyophilization of cell-free Marek's disease herpesvirus and a herpesvirus from turkeys. Appl.Microbiol. **20**: 723-726.

Calnek, B.W., Schat, K.A., Fabricant, J., Todaro, G., and zur, H. 1980. Modification of Marek's disease pathogenesis by in ovo infection or prior vaccination, p.185-197. Essex, M., (Ed.) Viruses in Naturally Occurring Cancers, Cold Spring Harbor Conferences on Cell Proliferation. Cold Spring Harbor Laboratory, New York.

Calnek, B.W., Shek, W.R., and Schat, K.A. 1981. Latent infections with Marek's disease virus and turkey herpesvirus. J.Natl.Cancer Inst. **66**: 585-590.

Calnek, B.W. and Spencer, J.L. 1985. Pathogenesis of Marek's Disease, p.374-390. Calnek, B.W., (Ed.) Proceedings of the international symposium on Marek's disease. American Association of Avian Pathologists, Kennett Square, PA.

Calnek, B.W. and Witter, R.L. 1997. Marek's disease, p.369-413. Calnek, B.W., Barnes, H.J., Beard, C.W., McDougald, L.R., and Saif, Y.M., (Eds). Disease of Poultry. Iowa state University Press, Ames, Iowa, USA.

Campadelli-Fiume, G., Arsenakis, M., Farabegoli, F., and Roizman, B. 1988. Entry of herpes simplex virus 1 in BJ cells that constitutively express viral glycoprotein D is by endocytosis and results in degradation of the virus. J.Virol. **62**: 159-167.

Campadelli-Fiume, G., Stirpe, D., Boscaro, A., Avitabile, E., Foa-Tomasi, L., Barker, D., and Roizman, B. 1990. Glycoprotein C-dependent attachment of herpes simplex virus to susceptible cells leading to productive infection. Virology **178**: 213-222.

Cauchy, L. 1974. The detection of viral antigens in Marek's disease by immunoperoxidase, p.77-88. Krustak, E., (Ed.) Viral Immunodiagnosis. Academic Press, Inc., New York.

Cebrian, J., Kaschka-Dierich, C., Berthelot, N., and Sheldrick, P. 1982. Inverted repeat nucleotide sequence in the genomes of Marek disease virus and the herpesvirus of the turkey. Proc.Natl.Acad.Sci.USA **79**: 555-558.

Chang, Y., Cesarman, E., Pessin, M.S., Lee, F., Culpepper, J., Knowles, D.M., and Moore, P.S. 1994. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science **266**: 1865-1869.

Chase, C.C., Carter-Allen, K., Lohff, C., and Letchworth, G.J. 1990. Bovine cells expressing bovine herpesvirus 1 (BHV-1) glycoprotein IV resist infection by BHV-1, herpes simplex virus, and pseudorabies virus. J.Virol. **64**: 4866-4872.

Chen, X. and Velicer, L.F. 1992. Expression of the Marek's disease virus homolog of herpes simplex virus glycoprotein B in Escherichia coli and its identification as B antigen. J Virol. **66**: 4390-4398.

Chen, X.B., Sondermeijer, P.J., and Velicer, L.F. 1992. Identification of a unique Marek's disease virus gene which encodes a 38-kilodalton phosphoprotein and is expressed in both lytically infected cells and latently infected lymphoblastoid tumor cells. J.Virol. **66**: 85-94.

Cho, B.R. and Kenzy, S.G. 1972. Isolation and characterization of an isolate (HN) of Marek's disease virus with low pathogenicity. Appl.Microbiol. **24**: 299-306.

Chubb, R.C. and Churchill, A.E. 1968. Precipitating antibodies associated with Marek's disease. Vet.Rec. **82**: 4-7.

Churchill, A.E. and Biggs, P.M. 1967. Agent of Marek's disease in tissue culture. Nature **215**: 528-530.

Churchill, A.E., Chubb, R.C., and Baxendale, W. 1969a. The attenuation with loss of oncogenicity of the herpes-type virus of Marek's disease (strain HPRS-16) on passage in cell culture. J.Gen.Virol. **4**: 557-564.

Churchill, A.E., Payne, L.N., and Chubb, R.C. 1969b. Immunization against Marek's disease using a live attenuated virus. Nature **221**: 744-747.

Confer, A.W. and Adldinger, H.K. 1980. Cell-mediated immunity in Marek's disease: Cytoxic responses in resistant and susceptible chickens and relation to disease. Amer.J.Vet.Res. **41**: 307-312.

Cook, M.K. and Sears, J.F. 1970. Preparation of infectious cell-free herpestype virus associated with Marek's disease. J.Virol. **5**: 258-261.

Corden, J., Wasylyk, B., Buchwalder, A., Sassone-Corsi, P., Kedinger, C., and Chambon, P. 1980. Promoter sequences of eukaryotic protein-coding genes. Science **209**: 1406-1414.

Coussens, P.M. and Velicer, L.F. 1988. Structure and complete nucleotide sequence of the Marek's disease herpesvirus gp57-65 gene. J.Virol. **62**: 2373-2379.

Cranage, M.P., Kouzarides, T., Bankier, A.T., Satchwell, S., Weston, K., Tomlinson, P., Barrell, B., Hart, H., Bell, S.E., and Minson, A.C. 1986. Identification of the human cytomegalovirus glycoprotein B gene and induction of neutralizing antibodies via its expression in recombinant vaccinia virus. EMBO J **5**: 3057-3063.

Cranage, M.P., Smith, G.L., Bell, S.E., Hart, H., Brown, C., Bankier, A.T., Tomlinson, P., Barrell, B.G., and Minson, T.C. 1988. Identification and expression of a human cytomegalovirus glycoprotein with homology to the Epstein-Barr virus BXLF2 product, varicella-zoster virus gpIII, and herpes simplex virus type 1 glycoprotein H. J.Virol. **62**: 1416-1422.

Crittenden, L.B., Muhm, R.L., and Burmester, B.R. 1972. Genetic control of the susceptibility to the avian leukosis complex. 2. Marek's disease. Poult.Sci. **51**: 261-267.

Cui, Z.Z., Lee, L.F., Liu, J.L., and Kung, H.J. 1991. Structural analysis and transcriptional mapping of the Marek's disease virus gene encoding pp38, an antigen associated with transformed cells. J.Virol. **65**: 6509-6515.

Cui, Z.Z., Qin, A., and Lee, L.F. 1992. Expression and processing of Marek's disease virus PP38 gene in insect cells and immunological characterization of the gene product, p.123-126. Anonymous 4th International Symposium on Marek's Disease, 19th World's Poultry Congress, Vol. 1. World's Poultry Science Assn., Amsterdam.

Cui, Z.Z., Yan, D., and Lee, L.F. 1990. Marek's disease virus gene clones encoding virus-specific phosphorylated polypeptides and serological characterization of fusion proteins. Virus Genes **3**: 309-322.

Davidson, I., Becker, Y., and Malkinson, M. 1991. Monospecific antibodies to Marek's disease virus antigen B dimer (200 kDa) and monomer (130 and 60 kDa) glycoproteins neutralize virus infectivity and detect the antigen B proteins in infected cell membranes. Arch.Virol. **121**: 125-139.

Davidson, I., Malkinson, M., Strenger, C., and Becker, Y. 1988. An improved ELISA method, using a streptavidin-biotin complex, for detecting Marek's disease virus antigens in feather-tips of infected chickens. J.Virol.Methods **14**: 237-241.

Davidson, I., Yang, H., Witter, R.L., and Malkinson, M. 1996. The immunodominant proteins of reticuloendotheliosis virus. Vet.Microbiol. **49**: 273-284.

Davison, A.J. and McGeoch, D.J. 1986a. Evolutionary comparisons of the S segments in the genomes of herpes simplex virus type 1 and varicella-zoster virus. J Gen Virol. **67**: 597-611.

Davison, A.J. and Scott, J.E. 1986b. The complete DNA sequence of varicella-zoster virus. J Gen Virol. **67**: 1759-1816.

Dean, H.J. and Cheung, A.K. 1993. A 3' coterminal gene cluster in pseudorabies virus contains herpes simplex virus UL1, UL2, and UL3 gene homologs and a unique UL3.5 open reading frame. J Virol. **67**: 5955-5961.

Dean, H.J., Terhune, S.S., Shieh, M.T., Susmarski, N., and Spear, P.G. 1994. Single amino acid substitutions in gD of herpes simplex virus 1 confer resistance to gD-mediated interference and cause cell-type-dependent alterations in infectivity. Virology **199**: 67-80.

Desai, P., Schaffer, P., and Minson, A. 1988. Excretion of non-infectious virus particles lacking glycoprotein H by a temperature-sensitive mutant of herpes simplex virus type 1: evidence that gH is essential for virion infectivity. J Gen Virol. **69 (Pt 6)**: 1147-1156.

Dingwell, K.S., Brunetti, C.R., Hendricks, R.L., Tang, Q., Tang, M., Rainbow, A.J., and Johnson, D.C. 1994. Herpes simplex virus glycoproteins E and I facilitate cell-to-cell spread in vivo and across junctions of cultured cells. J Virol. **68**: 834-845.

Dingwell, K.S., Doering, L.C., and Johnson, D.C. 1995. Glycoproteins E and I facilitate neuron-to-neuron spread of herpes simplex virus. J.Virol. **69**: 7087-7098.

Duus, K.M. and Grose, C. 1996. Multiple regulatory effects of varicella-zoster virus (VZV) gL on trafficking patterns and fusogenic properties of VZV gH. J Virol. **70**: 8961-8971.

Duus, K.M., Hatfield, C., and Grose, C. 1995. Cell surface expression and fusion by the varicella-zoster virus gH:gL glycoprotein complex: analysis by laser scanning confocal microscopy. Virology **210**: 429-440.

Eidson, C.S. and Schmittle, S.C. 1968. Studies on acute Marek's disease. I. Characteristics of isolate GA in chickens. Avian Dis. **12**: 467-476.

Eisenberg, R.J., Cerini, C.P., Heilman, C.J., Joseph, A.D., Dietzschold, B., Golub, E., Long, D., Ponce, d.L., and Cohen, G.H. 1985. Synthetic glycoprotein D-related peptides protect mice against herpes simplex virus challenge. J Virol. **56**: 1014-1017.

Eisenberg, R.J., Ponce, d.L., Friedman, H.M., Fries, L.F., Frank, M.M., Hastings, J.C., and Cohen, G.H. 1987. Complement component C3b binds directly to purified glycoprotein C of herpes simplex virus types 1 and 2. Microb.Pathog. **3**: 423-435.

Else, R.W. 1974. Vaccinal immunity to Marek's disease in bursectomized chickens. Vet.Rec. **95**: 182-187.

Emini, E.A., Luka, J., Armstrong, M.E., Keller, P.M., Ellis, R.W., and Pearson, G.R. 1987. Identification of an Epstein-Barr virus glycoprotein which is antigenically homologous to the varicella-zoster virus glycoprotein II and the herpes simplex virus glycoprotein B. Virology **157**: 552-555.

Fadly, A.M. 1997. Avian retrovoruses. Food animal retroviruses **13**: 71-85.

Foa-Tomasi, L., Avitabile, E., Boscaro, A., Brandimarti, R., Gualandri, R., Manservigi, R., Dall'Olio, F., Serafini-Cessi, F., and Fiume, G.C. 1991. Herpes simplex virus (HSV) glycoprotein H is partially processed in a cell line that expresses the glycoprotein and fully processed in cells infected with deletion or ts mutants in the known HSV glycoproteins. Virology **180**: 474-482.

Forrester, A., Farrell, H., Wilkinson, G., Kaye, J., Davis-Poynter, N., and Minson, T. 1992. Construction and properties of a mutant of herpes simplex virus type 1 with glycoprotein H coding sequences deleted. J Virol. **66**: 341-348.

Forrester, A.J., Sullivan, V., Simmons, A., Blacklaws, B.A., Smith, G.L., Nash, A.A., and Minson, A.C. 1991. Induction of protective immunity with antibody to herpes simplex virus type 1 glycoprotein H (gH) and analysis of the immune response to gH expressed in recombinant vaccinia virus. J Gen Virol. **72**: 369-375.

Friedman, H.M., Cohen, G.H., Eisenberg, R.J., Seidel, C.A., and Cines, D.B. 1984. Glycoprotein C of herpes simplex virus 1 acts as a receptor for the C3b complement component on infected cells. Nature **309**: 633-635.

Fries, L.F., Friedman, H.M., Cohen, G.H., Eisenberg, R.J., Hammer, C.H., and Frank, M.M. 1986. Glycoprotein C of herpes simplex virus 1 is an inhibitor of the complement cascade. J.Immunol. **137**: 1636-1641.

Fukuchi, K., Sudo, M., Lee, Y.S., Tanaka, A., and Nonoyama, M. 1984. Structure of Marek's disease virus DNA: Detailed restriction enzyme map. J.Virol. **51**: 102-109.

Fukuchi, K., Sudo, M., Tanaka, A., and Nonoyama, M. 1985a. Map location of homologous regions between Marek's disease virus and herpesvirus of turkey and the absence of detectable homology in the putative tumor-inducing gene. J.Virol. **53**: 994-997.

Fukuchi, K., Tanaka, A., Schierman, L.W., Witter, R.L., and Nonoyama, M. 1985b. The structure of Marek's disease virus DNA: The presence of unique expansion in nonpathogenic viral DNA. Proc.Natl.Acad.Sci.USA **82**: 751-754.

Fukuchi, K., Tanaka, A., Schierman, L.W., Witter, R.L., and Nonoyama, M. 1985c. The structure of Marek's disease virus DNA: The presence of unique expansion in nonpathogenic viral DNA. Proc.Natl.Acad.Sci.USA **82**: 751-754.

Fuller, A.O. and Spear, P.G. 1985. Specificities of monoclonal and polyclonal antibodies that inhibit adsorption of herpes simplex virus to cells and lack of inhibition by potent neutralizing antibodies. J.Virol. **55**: 475-482.

Galdiero, M., Whiteley, A., Bruun, B., Bell, S., Minson, T., and Browne, H. 1997. Site-directed and linker insertion mutagenesis of herpes simplex virus type 1 glycoprotein H. J Virol. **71**: 2163-2170.

Ghiasi, Nesburn, A., and Wechsler, S. 1991. Cell surface expression of herpes simplex virus type 1 glycoprotein H in recombinant baculovirus-infected cells. Virology **185**: 187-194.

Ghiasi, H., Kaiwar, R., Nesburn, A.B., and Wechsler, S.L. 1992. Baculovirus-expressed glycoprotein H of herpes simplex virus type 1 (HSV-1) induces neutralizing antibody and delayed type hypersensitivity responses, but does not protect immunized mice against lethal HSV-1 challenge. J Gen Virol. **73**: 719-722.

Ghosh-Choudhury, N., Butcher, M., and Ghosh, H.P. 1990. Expression from cloned DNA of biologically active glycoprotein C of herpes simplex virus type 1 in mammalian cells. J.Gen.Virol. **71**: 689-699.

Gibbs, C.P., Nazerian, K., Velicer, L.F., and Kung, H. 1983. Extensive homology exists between Marek's disease herepsvirus and its vaccine virus, herpesvirus of turkeys. Proc.Natl.Acad.Sci.USA **81**: 3365-3369.

Gompels, U. and Minson, A. 1986. The properties and sequence of glycoprotein H of herpes simplex virus type 1. Virology **153** : 230-247.

Gompels, U.A., Craxton, M.A., and Honess, R.W. 1988. Conservation of glycoprotein H (gH) in herpesviruses: nucleotide sequence of the gH gene from herpesvirus saimiri. J.Gen.Virol. **69**: 2819-2829.

Gompels, U.A. and Minson, A.C. 1989. Antigenic properties and cellular localization of herpes simplex virus glycoprotein H synthesized in a mammalian cell expression system. J Virol. **63**: 4744-4755.

Goodchild, W.M. 1969. Some observations on Marek's disease (fowl paralysis). Vet.Rec. **84**: 87-89.

Gretch, D.R., Gehrz, R.C., and Stinski, M.F. 1988a. Characterization of a human cytomegalovirus glycoprotein complex (gcI). J.Gen.Virol. **69**: 1205-1215.

Gretch, D.R., Kari, B., Gehrz, R.C., and Stinski, M.F. 1988b. A multigene family encodes the human cytomegalovirus glycoprotein complex gcII (gp47-52 complex). J.Virol. **62**: 1956-1962.

Gretch, D.R., Kari, B., Rasmussen, L., Gehrz, R.C., and Stinski, M.F. 1988c. Identification and characterization of three distinct families of glycoprotein complexes in the envelopes of human cytomegalovirus. J.Virol. **62**: 875-881.

Groot, A.J. and Albers, G.A. 1992. The effect of MHC on resistance to Marek's disease in White Leghorn crosses, p.185-188. 4th International Symposium on Marek's Disease, 19th World's Poultry Congress, Vol. 1. World's Poultry Science Assn., Amsterdam.

Grose, C. 1980. The synthesis of glycoproteins in human melanoma cells infected with varicella-zoster virus. Virology **101**: 1-9.

Gupta, M.K., Chauhan, H.V., Jha, G.J., and Singh, K.K. 1989. The role of the reticuloendothelial system in the immunopathology of Marek's disease. Vet.Microbiol. **20**: 223-234.

Haddad, R.S. and Hutt, F. 1989. Depletion of glycoprotein gp85 from virosomes made with Epstein-Barr virus proteins abolishes their ability to fuse with virus receptor-bearing cells. J.Virol. **63**: 4998-5005.

Haffer, K., Sevoian, M., and Wilder, M. 1979. The role of the macrophages in Marek's disease: In vitro and in vivo studies. Intl.J.Cancer **23**: 648-656.

Haider, S.A., Lapen, R.F., and Kenzy, S.G. 1970. Use of feathers in a gel precipitation test for Marek's disease. Poult.Sci. **49**: 1654-1657.

Hamdy, F., Sevoian, M., and Holt, S.C. 1974. Biogenesis of Marek's disease (Type II leukosis) virus in vitro: Electron microscopy and immunological study. Infect.Immun. **9**: 740-761.

Hansen, M.P., Van Zandt, J.N., and Law, G.R. 1967. Differences in susceptibility to Marek's disease in chickens carrying two different B locus blood group alleles. Poult.Sci. **46**: 1268

Harris, S.L., Frank, I., Yee, A., Cohen, G.H., Eisenberg, R.J., and Friedman, H.M. 1990. Glycoprotein C of herpes simplex virus type 1 prevents complement- mediated cell lysis and virus neutralization. J.Infect.Dis. **162**: 331-337.

Heineman, T., Gong, M., Sample, J., and Kieff, E. 1988. Identification of the Epstein-Barr virus gp85 gene. J Virol. **62**: 1101-1107.

Hepkema, B.G., Blankert, J.J., Albers, G.A., Tilanus, M.G., Egberts, E., van der Zijpp, A.J., and Hensen, E.J. 1993. Mapping of susceptibility to Marek's disease within the major histocompatibility (B) complex by refined typing of white leghorn chickens. Anim.Genet. **24**: 283-287.

Herold, B.C., Gerber, S.I., Belval, B.J., Siston, A.M., and Shulman, N. 1996. Differences in the susceptibility of herpes simplex virus types 1 and 2 to modified heparin compounds suggest serotype differences in viral entry. J Virol. **70**: 3461-3469.

Herold, B.C. and Spear, P.G. 1994. Neomycin inhibits glycoprotein C (gC)-dependent binding of herpes simplex virus type 1 to cells and also inhibits postbinding events in entry. Virology **203**: 166-171.

Herold, B.C., WuDunn, D., Soltys, N., and Spear, P.G. 1991. Glycoprotein C of herpes simplex virus type 1 plays a principal role in the adsorption of virus to cells and in infectivity. J.Virol. **65**: 1090-1098.

Herrold, R.E., Marchini, A., Fruehling, S., and Longnecker, R. 1996. Glycoprotein 110, the Epstein-Barr virus homolog of herpes simplex virus glycoprotein B, is essential for Epstein-Barr virus replication in vivo. J Virol. **70**: 2049-2054.

Higgins, D.A. and Calnek, B.W. 1976. Some effects of silical treatment on Marek's disease. Infect.Immun. **13**: 1054-1060.

Highlander, S.L., Sutherland, S.L., Gage, P.J., Johnson, D.C., Levine, M., and Glorioso, J.C. 1987. Neutralizing monoclonal antibodies specific for herpes simplex virus glycoprotein D inhibit virus penetration. J.Virol. **61**: 3356-3364.

Hirai, Ikuta, and Kato 1979. Comparative studies on Marek's disease virus and herpesvirus of turkey DNAs. Journal.of.General.Virology (UK). 131

Hirai, K., Horiuchi, T., and Mikami, T. 1988. Molecular biology of Marek's disease virus, p.21-42. Kato, S., (Ed.) Advances in Marek's disease research. Japanese Association on Marek's Disease, Osaka.

Hirai, K., Ikuta, K., and Kato, S. 1979. Comparative studies on Marek's disease virus and herpesvirus of turkey DNAs. J.Gen.Virol. **45**: 119-131.

Hlozanek, I. 1970. The influence of ultraviolet-inactivated Sendai virus on Marek's disease virus infection in tissue culture. J.Gen.Virol. **9**: 45-50.

Holland, M.S., Silva, R.F., Mackenzie, C.D., Bull, R.W., and Witter, R.L. 1994. Identification and localization of glycoprotein B expression in lymphoid tissues of chickens infected with turkey herpesvirus. Avian.Dis. **38**: 446-453.

Honess, R.W. and Roizman, B. 1974. Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. J Virol. **14**: 8-19.

Hong, C.C. and Sevoian, M. 1971. Interferon production and host resistance to type II avian (Marek's) leukosis virus (JM strain). Appl.Microbiol. **22**: 818-820.

Huber, M.T. and Compton, T. 1997. Characterization of a novel third member of the human cytomegalovirus glycoprotein H-glycoprotein L complex. J.Virol. **71**: 5391-5398.

Hudson, L. and Payne, L.N. 1973. An analysis of the T and B cells of Marek's disease lymphomas of the chicken. Nature New Biol.Nature New Biology J1 - Nature New Biology J2 - Nature New Biology **241**: 52-53.

Hutchinson, L., Browne, H., Wargent, V., Davis-Poynter, N., Primorac, S., Goldsmith, K., Minson, A., and Johnson, D. 1992. A novel herpes simplex virus glycoprotein, gL, forms a complex with glycoprotein H (gH) and affects normal folding and surface expression of gH. J Virol. **66**: 2240-2250.

Igarashi, T., Takahashi, M., Donovan, J., Jessip, J., Smith, M., Hirai, K., Tanaka, A., and Nonoyama, M. 1987. Restriction enzyme map of herpesvirus of turkey DNA and its collinear relationship with Marek's disease virus DNA. Virology **157**: 351-358.

Ikuta, K., Honma, H., Maotani, K., Ueda, S., and Kato, S. 1982. Monoclonal antibodies specific to and cross-reactive with Marek's disease virus and herpesvirus of turkeys. Biken J. **25**: 171-175.

Ikuta, K., Nakajima, K., Naito, M., Ann, S.H., Ueda, S., Kato, S., and Hirai, K. 1985a. Identification of Marek's disease virus-specific antigens in Marek's disease lymphoblastoid cell lines using monoclonal antibody against virus-specific phosphorylated polypeptides. Int.J.Cancer **35**: 257-264.

Ikuta, K., Nakajima, K., Ueda, S., Kato, S., and Hirai, K. 1985b. Differences in the processing of secreted glycoprotein A induced by Marek's disease virus and herpesvirus of turkeys. J.Gen.Virol. **66**: 1131-1137.

Ikuta, K., Nishi, Y., Kato, S., and Hirai, K. 1981. Immunoprecipitation of Marek's disease virus-specific polypeptides with chicken antibodies purified by affinity chromatography. Virology **114**: 277-281.

Isfort, R.J., Kung, H.J., and Velicer, L.F. 1987. Identification of the gene encoding Marek's disease herpesvirus A antigen. J.Virol. **61**: 2614-2620.

Isfort, R.J., Qian, Z., Jones, D., Silva, R.F., Witter, R., and Kung, H.J. 1994. Integration of multiple chicken retroviruses into multiple chicken herpesviruses: herpesviral gD as a common target of integration. Virology **203**: 125-133.

Isfort, R.J., Stringer, R.A., Kung, H.J., and Velicer, L.F. 1986. Synthesis, processing, and secretion of the Marek's disease herpesvirus A antigen glycoprotein. J.Virol. **57**: 464-474.

Jacobs, L. 1994. Glycoprotein E of pseudorabies virus and homologous proteins in other alphaherpesvirinae. Arch Virol. **137**: 209-228.

Jang, H.K., Kitazawa, T., Ono, M., Kawaguchi, Y., Meada, K., Yokoyama, N., Tohya, Y., Niikura, M., and Mikami, T. 1996a. Protection studies against Marek's disease using baculovirus-expressed glycoproteins B and C of Marek's disease virus type 1. Avian Pathol. **25** : 5-24.

Jang, H.K., Ono, M., Kato, Y., Tohya, Y., Niikura, M., and Mikami, T. 1996b. Identification of a potential Marek's disease virus serotype 2 glycoprotein D gene with homology to herpes simplex virus glycoprotein D. Arch Virol. **141**: 2207-2216.

Jilg, W., Bogedain, C., Mairhofer, H., Gu, S.Y., and Wolf, H. 1994. The Epstein-Barr virus-encoded glycoprotein gp 110 (BALF 4) can serve as a target for antibody-dependent cell-mediated cytotoxicity (ADCC). Virology **202**: 974-977.

Johnson, D.C., Burke, R.L., and Gregory, T. 1990. Soluble forms of herpes simplex virus glycoprotein D bind to a limited number of cell surface receptors and inhibit virus entry into cells. J.Virol. **64**: 2569-2576.

Johnson, D.C. and Feenstra, V. 1987. Identification of a novel herpes simplex virus type 1-induced glycoprotein which complexes with gE and binds immunoglobulin. J.Virol. **61**: 2208-2216.

Johnson, R.M. and Spear, P.G. 1989. Herpes simplex virus glycoprotein D mediates interference with herpes simplex virus infection. J.Virol. **63**: 819-827.

Jones, D., Lee, L., Liu, J.L., Kung, H.J., and Tillotson, J.K. 1992. Marek disease virus encodes a basic-leucine zipper gene resembling the fos/jun oncogenes that is highly expressed in lymphoblastoid tumors. Proc.Natl.Acad.Sci.USA **89**: 4042-4046.

Kaashoek, M.J., Moerman, A., Madic, J., Rijsewijk, F.A., Quak, J., Gielkens, A.L., and van Oirschot, J.T. 1994. A conventionally attenuated glycoprotein E-negative strain of bovine herpesvirus type 1 is an efficacious and safe vaccine. Vaccine **12**: 439-444.

Kaleta, E.F. and Bankowski, R.A. 1972. Production of interferon by the CAL-1 and turkey herpesvirus strains associated with Marek's disease. Amer.J.Vet.Res. **33**: 567-571.

Kaleta, E.F. and Neumann, U. 1977. Investigations on the mode of transmission of the herpesvirus of turkeys in vitro. Avian Pathol. **6**: 33-39.

Kanamori, A., Ikuta, K., Ueda, S., Kato, S., and Hirai, K. 1987. Methylation of Marek's disease virus DNA in chicken T-lyphoblastoid cell lines. J.Gen.Virol. **68**: 1485-1490.

Karlin, S., Mocarski, E.S., and Schachtel, G.A. 1994. Molecular evolution of herpesviruses: Genomic and protein sequence comparisons. J.Virol. **68**: 1886-1902.

Kawamura, H., King, D.J., and Anderson, D.P. 1969. A herpesvirus isolated from kidney cell culture of normal turkeys. Avian Dis. **13**: 853-863.

Kawamura, M., Hayashi, M., Furuichi, T., Nonoyama, M., Isogai, E., and Namioka, S. 1991. The inhibitory effects of oligonucleotides, complementary to Marek's disease virus mRNA transcribed from the BamHI-H region, on the proliferation of transformed lymphoblastoid cells, MDCC-MSB1. J.Gen.Virol. **72**: 1105-1111.

Kaye, J.F., Gompels, U.A., and Minson, A.C. 1992. Glycoprotein H of human cytomegalovirus (HCMV) forms a stable complex with the HCMV UL115 gene product. J.Gen.Virol. **73**: 2693-2698.

Keller, P.M., Davison, A.J., Lowe, R.S., Bennett, C.D., and Ellis, R.W. 1986. Identification and structure of the gene encoding gpII, a major glycoprotein of varicella-zoster virus. Virology **152**: 181-191.

Keller, P.M., Davison, A.J., Lowe, R.S., Riemen, M.W., and Ellis, R.W. 1987. Identification and sequence of the gene encoding gpIII, a major glycoprotein of varicella-zoster virus. Virology **157**: 526-533.

Keller, P.M., Neff, B.J., and Ellis, R.W. 1984. Three major glycoprotein genes of varicella-zoster virus whose products have neutralization epitopes. J Virol. **52**: 293-297.

Kino, Y., Eto, T., Nishiyama, K., Ohtomo, N., and Mori, R. 1986. Immunogenicity of purified glycoprotein gB of herpes simplex virus. Arch Virol. **89**: 69-80.

Klupp, B.G., Baumeister, J., Karger, A., Visser, N., and Mettenleiter, T.C. 1994. Identification and characterization of a novel structural glycoprotein in pseudorabies virus, gL. J Virol. **68**: 3868-3878.

Klupp, B.G., Fuchs, W., Weiland, E., and Mettenleiter, T.C. 1997. Pseudorabies virus glycoprotein L is necessary for virus infectivity but dispensable for virion localization of glycoprotein H. J Virol. **71**: 7687-7695.

Klupp, B.G. and Mettenleiter, T.C. 1991. Sequence and expression of the glycoprotein gH gene of pseudorabies virus. Virology **182**: 732-741.

Knapp, A.C. and Enquist, L.W. 1997a. Pseudorabies virus recombinants expressing functional virulence determinants gE and gI from bovine herpesvirus 1.1. J Virol. **71**: 2731-2739.

Knapp, A.C., Husak, P.J., and Enquist, L.W. 1997b. The gE and gI homologs from two alphaherpesviruses have conserved and divergent neuroinvasive properties. J Virol. **71**: 5820-5827.

Kodama, H., Mikami, T., Inoue, M., and Izawa, H. 1979. Inhibitory effects of macrophages against Marek's disease virus plaque formation in chicken kidney cell cultures. J.Natl.Cancer Inst. **63**: 1267-1271.

Kozak, M. 1986. Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. Cell **44**: 283-292.

Lee, L.F. 1979. Macrophage restriction of Marek's disease virus replication and lymphoma cell proliferation. J.Immunol. **123** : 1088-1091.

Lee, L.F. 1993. Characterization of a monoclonal antibody against a nuclear antigen associated with serotype-1 Marek's disease virus-infected and transformed cells. Avian Dis. **37**: 561-567.

Lee, L.F., Armstrong, R.L., and Nazerian, K. 1972. Comparative studies of six avian herpesviruses. Avian Dis. **16**: 799-805.

Lee, L.F., Kieff, E.D., Bachenheimer, S.L., Roizman, B., Spear, P.G., Burmester, B.R., and Nazerian, K. 1971. Size and composition of Marek's disease virus deoxyribonucleic acid. J.Virol. **7**: 289-294.

Lee, L.F., Liu, X., and Witter, R.L. 1983a. Monoclonal antibodies with specificity for three different serotypes of Marek's disease viruses in chickens. J.Immunol. **130**: 1003-1006.

Lee, L.F., Liu, X., and Witter, R.L. 1983b. Monoclonal antibodies with specificity for three different serotypes of Marek's disease viruses in chickens Monoclonal antibodies with specificity for three different serotypes of Marek's disease viruses in chickens. J.Immunol. **130**: 1003-1006.

Lee, S.K. and Longnecker, R. 1997. The Epstein-Barr virus glycoprotein 110 carboxy-terminal tail domain is essential for lytic virus replication. J Virol. **71**: 4092-4097.

Lesnik, F., Chudy, D., Bogdan, J., Vrtiak, O.J., and Rudic, M. 1978. Testing the immunogenicity of the dermal antigen of Marek's disease virus. Vet.Med.(Praha) **23**: 421-430.

Li, D.S., Pastorek, J., Zelnik, V., Smith, G.D., and Ross, L.J. 1994. Identification of novel transcripts complementary to the Marek's disease virus homologue of the ICP4 gene of herpes simplex virus. J.Gen.Virol. **75**: 1713-1722.

Li, L., Nelson, J.A., and Britt, W.J. 1997. Glycoprotein H-related complexes of human cytomegalovirus: identification of a third protein in the gCIII complex. J Virol. **71**: 3090-3097.

Li, Q., Buranathai, C., Grose, C., and Hutt-Fletcher, L.M. 1997a. Chaperone functions common to nonhomologous Epstein-Barr virus gL and Varicella-Zoster virus gL proteins. J Virol. **71**: 1667-1670.

Li, Q., Spriggs, M.K., Kovats, S., Turk, S.M., Comeau, M.R., Nepom, B., and Hutt-Fletcher, L.M. 1997b. Epstein-Barr virus uses HLA class II as a cofactor for infection of B lymphocytes. J Virol. **71**: 4657-4662.

Li, Q., Turk, S.M., and Hutt-Fletcher, L.M. 1995. The Epstein-Barr virus (EBV) BZLF2 gene product associates with the gH and gL homologs of EBV and carries an epitope critical to infection of B cells but not of epithelial cells. J Virol. **69**: 3987-3994.

Little, S.P. and Schaffer, P.A. 1981. Expression of the syncytial (syn) phenotype in HSV-1, strain KOS: genetic and phenotypic studies of mutants in two syn loci. Virology **112**: 686-702.

Litwin, V., Jackson, W., and Grose, C. 1992. Receptor properties of two varicella-zoster virus glycoproteins, gpI and gpIV, homologous to herpes simplex virus gE and gI. J Virol. **66**: 3643-3651.

Liu, J.L., Lee, L.F., Ye, Y., Qian, Z., and Kung, H.J. 1997. Nucleolar and nuclear localization properties of a herpesvirus bZIP oncoprotein, MEQ. J Virol. **71**: 3188-3196.

Liu, J.L., Ye, Y., Lee, L.F., and Kung, H.J. 1998. Transforming potential of the herpesvirus oncoprotein MEQ: morphological transformation, serum-independent growth, and inhibition of apoptosis. J Virol. **72**: 388-395.

Liu, Y.N., Klaus, A., Kari, B., Stinski, M.F., Eckhardt, J., and Gehrz, R.C. 1991. The N-terminal 513 amino acids of the envelope glycoprotein gB of human cytomegalovirus stimulates both B- and T-cell immune responses in humans. J Virol. **65**: 1644-1648.

Lomonte, P., Filee, P., Lyaku, J.R., Bublot, M., Pastoret, P.P., and Thiry, E. 1997. Analysis of the biochemical properties of, and complex formation between,

glycoproteins H and L of the gamma2 herpesvirus bovine herpesvirus-4. J.Gen.Virol. **78**: 2015-2023.

Longenecker, B.M., Pazderka, F., Gavora, J.S., Spencer, J.L., and Ruth, R.F. 1976. Lymphoma induced by herpesvirus: Resistance associated with a major histocompatibility gene. Immunogenetics **3**: 401-407.

Maeda, K., Kawaguchi, Y., Kamiya, N., Ono, M., Tohya, Y., Kai, C., and Mikami, T. 1993. Identification and nucleotide sequence of a gene in feline herpesvirus type 1 homologous to the herpes simplex virus gene encoding the glycoprotein H. Arch.Virol. **132**: 183-191.

Makimura, K., Peng, F.Y., Tsuji, M., Hasegawa, S., Kawai, Y., Nonoyama, M., and Tanaka, A. 1994. Mapping of Marek's disease virus genome: Identification of junction sequences between unique and inverted repeat regions. Virus Genes **8**: 15-24.

Mallory, S., Sommer, M., and Arvin, A.M. 1997. Mutational analysis of the role of glycoprotein I in varicella-zoster virus replication and its effects on glycoprotein E conformation and trafficking. J Virol. **71**: 8279-8288.

Mannini-Palenzona, A., Moschella, A., Costanzo, F., Manservigi, R., and Monini, P. 1995. Cell-type dependent sensitivity of herpes simplex virus 1 mutants to plaque development inhibition by an anti-gD monoclonal antibody. New Microbiol **18**: 351-358.

Manservigi, R., Spear, P.G., and Buchan, A. 1977. Cell fusion induced by herpes simplex virus is promoted and suppressed by different viral glycoproteins. Proc Natl Acad Sci U S A **74**: 3913-3917.

Maotani, K., Kanamori, A., Ikuta, K., Ueda, S., Kato, S., and Hirai, K. 1986. Amplification of a tandem direct repeat within inverted repeats of Marek's disease virus DNA during serial in vitro passage. J.Virol. **58**: 657-660.

Maray, T., Malkinson, M., and Becker, Y. 1988. RNA transcripts of Marek's disease virus (MDV) serotype-1 in infected and transformed cells. Virus Genes **2**: 49-68.

Marek, J. 1907. Multiple Nervenentzuendung (Polyneuritis) bei Huehnern. Dtsch Tierarztl Wochenschr **15**: 417-421.

Marshall, G.S., Rabalais, G.P., Stout, G.G., and Waldeyer, S.L. 1992. Antibodies to recombinant-derived glycoprotein B after natural human cytomegalovirus infection correlate with neutralizing activity. J Infect Dis **165**: 381-384.

Massaer, M., Haumont, M., Place, M., Bollen, A., and Jacobs, P. 1993. Induction of neutralizing antibodies by varicella-zoster virus gpII glycoprotein expressed from recombinant vaccinia virus. J.Gen.Virol. **74**: 491-494.

McGeoch, D. and Davison, A. 1986. DNA sequence of the herpes simplex virus type 1 gene encoding glycoprotein gH, and identification of homologues in the genomes of varicella-zoster virus and Epstein-Barr virus. Nucleic-Acids-Res. **14**: 4281-4292.

McGeoch, D.J., Dalrymple, M.A., Davison, A.J., Dolan, A., Frame, M.C., McNab, D., Perry, L.J., Scott, J.E., and Taylor, P. 1988. The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1. J Gen Virol. **69**: 1531-1574.

McKie, E.A., Ubukata, E., Hasegawa, S., Zhang, S., Nonoyama, M., and Tanaka, A. 1995. The transcripts from the sequences flanking the short component of Marek's disease virus during latent infection form a unique family of 3'-coterminal RNAs. J.Virol. **69**: 1310-1314.

Miller, N. and Hutt-Fletcher, L.M. 1988. A monoclonal antibody to glycoprotein gp85 inhibits fusion but not attachment of Epstein-Barr virus. J.Virol. **62**: 2366-2372.

Minson, A.C., Hodgman, T.C., Digard, P., Hancock, D.C., Bell, S.E., and Buckmaster, E.A. 1986. An analysis of the biological properties of monoclonal antibodies against glycoprotein D of herpes simplex virus and identification of amino acid substitutions that confer resistance to neutralization. J.Gen.Virol. **67**: 1001-1013.

Miriagou, V., Argnani, R., Kakkanas, A., Georgopoulou, U., Manservigi, R., and Mavromara, P. 1995. Expression of the herpes simplex virus type 1 glycoprotein E in human cells and in Escherichia coli: protection studies against lethal viral infection in mice. J.Gen.Virol. **76**: 3137-3143.

Mitaku Laboratory. Classification and Secondary Structure Prediction of Membrane Proteins. [online] http://www.tuat.ac.jp/~mitaku/adv_sosui/. 5-12-1998

Moffat, J.F., Zerboni, L., Kinchington, P.R., Grose, C., Kaneshima, H., and Arvin, A.M. 1998. Attenuation of the vaccine Oka strain of varicella-zoster virus and role of glycoprotein C in alphaherpesvirus virulence demonstrated in the SCID-hu mouse. J Virol. **72**: 965-974.

Montalvo, E.A. and Grose, C. 1987. Assembly and processing of the disulfide-linked varicella-zoster virus glycoprotein gpII(140). J Virol. **61**: 2877-2884.

Montalvo, E.A., Parmley, R.T., and Grose, C. 1985. Structural analysis of the varicella-zoster virus gp98-gp62 complex: posttranslational addition of N-linked and O-linked oligosaccharide moieties. J.Virol. **53**: 761-770.

Morimura, T., Hattori, M., Ohashi, K., Sugimoto, C., and Onuma, M. 1995. Immunomodulation of peripheral T cells in chickens infected with Marek's disease virus: involvement in immunosuppression. J.Gen.Virol. **76**: 2979-2985.

Mulder, W.A., Jacobs, L., Priem, J., Kok, G.L., Wagenaar, F., Kimman, T.G., and Pol, J.M. 1994. Glycoprotein gE-negative pseudorabies virus has a reduced capability to infect second- and third-order neurons of the olfactory and trigeminal routes in the porcine central nervous system. J Gen Virol. **75**: 3095-3106.

Naito, M., Nakajima, K., Iwa, N., Ono, K., Yoshida, I., Konobe, T., Ikuta, K., Ueda, S., Kato, S., and Hirai, K. 1986. Demonstration of a Marek's disease virus-specific antigen in tumour lesions of chickens with Marek's disease using monoclonal antibody against a virus phosphorylated protein. Avian Pathol. **15**: 503-510.

Nakajima, K., Ikuta, K., Naito, M., Ueda, S., Kato, S., and Hirai, K. 1987. Analysis of Marek's disease virus serotype-1 specific phosphorylated polypeptides in virus-infected cells and Marek's disease lymphoblastoid cells. J.Gen.Virol. **68**: 1379-1390.

Nakajima, K., Shibayama, T., Yokota, M., Ikuta, K., Kato, S., and Hirai, K. 1989. Identification of virus-specific polypeptides by monoclonal antibodies against serotype-2 Marek's disease virus. J.Gen.Virol. **70**: 2563-2572.

Navarro, D., Lennette, E., Tugizov, S., and Pereira, L. 1997. Humoral immune response to functional regions of human cytomegalovirus glycoprotein B. J Med Virol. **52**: 451-459.

Navarro, D., Paz, P., and Pereira, L. 1992. Domains of herpes simplex virus I glycoprotein B that function in virus penetration, cell-to-cell spread, and cell fusion. Virology **186**: 99-112.

Navarro, D., Paz, P., Tugizov, S., Topp, K., La Vail, J., and Pereira, L. 1993. Glycoprotein B of human cytomegalovirus promotes virion penetration into cells, transmission of infection from cell to cell, and fusion of infected cells. Virology **197**: 143-158.

Nazerian, K. 1968. Electron microscopy of a herpesvirus isolated from Marek's disease in duck and chicken embryo fibroblast cultures. Proc.Electron Micro.Soc. 222-223.

Nazerian, K. 1970. Attenuation of Marek's disease virus and study of its properties in two different cell cultures. J.Natl.Cancer Inst. **44**: 1257-1267.

Nazerian, K. 1974. DNA configuration in the core of Marek's disease virus. J.Virol. **13**: 1148-1150.

Nazerian, K., Lee, L.F., Witter, R.L., and Burmester, B.R. 1971. Ultrastructural studies of a herpesvirus of turkeys antigenically related to Marek's disease virus. Virology **43**: 442-452.

Nazerian, K., Lee, L.F., Yanagida, N., and Ogawa, R. 1992a. Protection against Marek's disease by a fowlpox virus recombinant expressing the glycoprotein B of Marek's disease virus. J.Virol. **66**: 1409-1413.

Nazerian, K. and Purchase, H.G. 1970. Combined fluorescent-antibody and electron microscopy study of Marek's disease virus-infected cell culture. J.Virol. **5**: 79-90.

Nazerian, K., Sharma, J.M., and Spencer, J.L. 1985. Pathogenesis of Marek's disease in turkeys, p.262-267. Calnek, B.W., (Ed.) Proceedings of the international symposium on Marek's disease. American Association of Avian Pathologists, Kennett Square, PA.

Nazerian, K., Solomon, J.J., Witter, R.L., and Burmester, B.R. 1968. Studies on etiology of Marek's disease. II. Finding of a herpesvirus in cell culture. Proc.Soc.Exp.Biol.Med. **127**: 177-182.

Nazerian, K., Witter, R.L., Lee, L.F., and Yanagida, N. 1996. Protection and synergism by recombinant fowl pox vaccines expressing genes from Marek's disease virus. Avian Dis **40**: 368-376.

Neidhardt, H., Schroder, C.H., and Kaerner, H.C. 1987. Herpes simplex virus type 1 glycoprotein E is not indispensable for viral infectivity. J.Virol. **61**: 600-603.

Nemeckova, S., Ludvikova, V., Maresova, L., Krystofova, J., Hainz, P., and Kutinova, L. 1996. Induction of varicella-zoster virus-neutralizing antibodies in mice by co-infection with recombinant vaccinia viruses expressing the gH or gL gene. J Gen Virol. **77**: 211-215.

Nicola, A.V., Peng, C., Lou, H., Cohen, G.H., and Eisenberg, R.J. 1997. Antigenic structure of soluble herpes simplex virus (HSV) glycoprotein D correlates with inhibition of HSV infection. J Virol. **71**: 2940-2946.

Nicola, A.V., Willis, S.H., Naidoo, N.N., Eisenberg, R.J., and Cohen, G.H. 1996. Structure-function analysis of soluble forms of herpes simplex virus glycoprotein D. J.Virol. **70**: 3815-3822.

Nicolson, L., Cullinane, A.A., and Onions, D.E. 1990. The nucleotide sequence of an equine herpesvirus 4 gene homologue of the herpes simplex virus 1 glycoprotein H gene. J.Gen.Virol. **71**: 1793-1800.

Niikura, M., Matsuura, Y., Hattori, M., Onuma, M., and Mikami, T. 1991. Expression of the A antigen (gp57-65) of Marek's Disease Virus by a Recombinant Baculovirus. J.Gen.Virol. **72**: 1099-1104.

Oei, H.L. and de Boer, G.F. 1986. Comparison of intramuscular and subcutaneous administration of Marek's disesae vaccine. Avian Pathol. **15**: 569-579.

Okada, K., Fujimoto, Y., and Mikami, T. 1974. Electron microscopic autoradiography in the development of herpesvirus of turkey. Archives fur die gesamte Virusforschung **46**: 111-126.

Okada, K., Fujimoto, Y., Mikami, T., and Yonohara, K. 1972. The fine structure of Marek's disease virus and herpesvirus of turkey in cell culture. Jap.J.Vet.Res. **20**: 57

Okada, K., Fujimoto, Y., Nakanishi, Y.H., Onuma, M., and Mikami, T. 1980. Cohelical arrangement of the DNA strand in the core of Marek's disease virus particles. Arch.Virol. **64**: 81-85.

Okazaki, W., Purchase, H.G., and Burmester, B.R. 1970. Protection against Marek's disease by vaccination with a herpesvirus of turkeys. Avian Dis. **14**: 413-429.

Olson, J.K., Bishop, G.A., and Grose, C. 1997a. Varicella-zoster virus Fc receptor gE glycoprotein: serine/threonine and tyrosine phosphorylation of monomeric and dimeric forms. J Virol. **71**: 110-119.

Olson, J.K. and Grose, C. 1997b. Endocytosis and recycling of varicella-zoster virus Fc receptor glycoprotein gE: internalization mediated by a YXXL motif in the cytoplasmic tail. J.Virol. **71**: 4042-4054.

Olson, J.K. and Grose, C. 1998. Complex formation facilitates endocytosis of the varicella-zoster virus gE:gI Fc receptor. J Virol. **72**: 1542-1551.

Omar, A.R. and Schat, K.A. 1997. Characterization of Marek's disease herpesvirus-specific cytotoxic T lymphocytes in chickens inoculated with a non-oncogenic vaccine strain of MDV. Immunology **90**: 579-585.

Ono, K., Takashima, M., Ishikawa, T., Hayashi, M., Yoshida, I., Konobe, T., Ikuta, K., Nakajima, K., Ueda, S., and Kato, S. 1985. Partial protection against Marek's disease in chickens immunized with glycoproteins gB purified from turkey-herpesvirus-infected cells by affinity chromatography coupled with monoclonal antibodies. Avian.Dis. **29**: 533-539.

Ono, M., Katsuragi-Iwanaga, R., Kitazawa, T., Kamiya, N., Horimoto, T., Niikura, M., Kai, C., Hirai, K., and Mikami, T. 1992. The restriction endonuclease map of Marek's disease virus (MDV) serotype 2 and collinear relationship among three serotypes of MDV. Virology **191**: 459-463.

Ono, M., Kawaguchi, Y., Maeda, K., Kamiya, N., Tohya, Y., Kai, C., Niikura, M., and Mikami, T. 1994. Nucleotide sequence analysis of Marek's disease virus (MDV) serotype 2 homolog of MDV serotype 1 pp38, an antigen associated with transformed cells. Virology **201**: 142-146.

Otaki, Y., Nunoya, T., Tajima, M., Kato, S., and Nomura, Y. 1988. Depression of vaccinal immunity to Marek's disease by infection with chicken anaemia agent. Avian Pathol. **17**: 333-347.

Papworth, M.A., Van Dijk, A.A., Benyon, G.R., Allen, T.D., Arrand, J.R., and Mackett, M. 1997. The processing, transport and heterologous expression of Epstein-Barr virus gp110. J Gen Virol. **78**: 2179-2189.

Parcells, M.S., Anderson, A.S., and Morgan, R.W. 1994. Characterization of a Marek's disease virus mutant containing a lacZ insertion in the US6 (gD) homologue gene. Virus Genes **9**: 5-13.

Payne, L.N. 1985. Pathology, p.43-75. Payne, L.N., (Ed.) Marek's disease. Martinus Nijhoff, Boston.

Payne, L.N. and Biggs, P.M. 1967. Studies on Marek's disease. II. Pathogenesis. J.Natl.Cancer Inst. **39**: 281-302.

Payne, L.N. and de-The, G. 1972. Pathogenesis of Marek's disease - A review, p.21-37. Biggs, P.M., (Ed.) Oncogenesis and Herpesviruses. IARC Scientific Publications, Lyon.

Payne, L.N., Frazier, J.A., and Powell, P.C. 1976. Pathogenesis of Marek's disease. Intl.Rev.Exp.Biol. **16**: 59-154.

Payne, L.N., Powell, P.C., Rennie, M., and Ross, L.J. 1978a. Vaccination of bursectomised chicken with inactivated Marek's disease virus-specific antigens. Avian Pathol. **7**: 427-432.

Payne, L.N., Rennie, M., Powell, P.C., and Rowell, J.G. 1978b. Transient effect of cyclophosphamide on vaccinal immunity to Marek's disease. Avian Pathol. **7**: 295-304.

Payne, L.N. and Roszkowski, J. 1973. The presence of immuologically uncommitted bursa and thymus dependent lymphoid cells in the lymphomas of Marek's disease. Avian Pathol. **1**: 27-34.

Peeters, B., de Wind, N., Broer, R., Gielkens, A., and Moormann, R. 1992. Glycoprotein H of pseudorabies virus is essential for entry and cell-to- cell spread of the virus. J Virol. **66**: 3888-3892.

Pellett, P.E., Biggin, M.D., Barrell, B., and Roizman, B. 1985. Epstein-Barr virus genome may encode a protein showing significant amino acid and predicted secondary structure homology with glycoprotein B of herpes simplex virus 1. J Virol. **56**: 807-813.

Peng, F., Bradley, G., Tanaka, A., Lancz, G., and Nonoyama, M. 1992. Isolation and characterization of cDNAs from BamHI-H gene family RNAs associated with the tumorigenicity of Marek's disease virus. J.Virol. **66**: 7389-7396.

Peng, Q., Zeng, M., Bhuiyan, Z.A., Ubukata, E., Tanaka, A., Nonoyama, M., and Shirazi, Y. 1995. Isolation and characterization of Marek's disease virus (MDV) cDNAs

mapping to the BamHI-I2, BamHI-Q2, and BamHI-L fragments of the MDV genome from lymphoblastoid cells transformed and persistently infected with MDV. Virology **213**: 590-599.

Peng, T., Ponce-de-Leon, M., Jiang, H., Dubin, G., Lubinski, J.M., Eisenberg, R.J., and Cohen, G.H. 1998. The gH-gL complex of herpes simplex virus (HSV) stimulates neutralizing antibody and protects mice against HSV type 1 challenge. J Virol. **72**: 65-72.

Pereira, L., Hoffman, M., Tatsuno, M., and Dondero, D. 1984. Polymorphism of human cytomegalovirus glycoproteins characterized by monoclonal antibodies. Virology **139**: 73-86.

Petrovskis, E.A., Meyer, A.L., and Post, L.E. 1988. Reduced yield of infectious pseudorabies virus and herpes simplex virus from cell lines producing viral glycoprotein gp50. J.Virol. **62**: 2196-2199.

Phillips, P.A. and Biggs, P.M. 1972. Course of infection in tissues of susceptible chickens after exposure to strains of Marek's disease virus and turkey herpesvirus. J.Natl.Cancer Inst. **49**: 1367-1373.

Powell, P.C. 1976. Studies on Marek's disease lymphoma-derived cell lines. Biblio.Haemat.Biblioteca Haematalogica J1 - Biblioteca Haematalogica J2 - Biblioteca Haematalogica **43**: 348-350.

Powell, P.C. and Davison, T.F. 1986. Induction of Marek's disease in vaccinated chickens by treatment with betamethasone or corticosterone. Israel Vet.Med.Assn. **42**: 73-78.

Powell, P.C., Howes, K., Lawn, A.M., Mustill, B.M., Payne, L.N., Rennie, M., and Thompson, M.A. 1984. Marek's disease in turkeys: The induction of lesions and the establishment of lymphoid cell lines. Avian Pathol. **13**: 201-214.

Powell, P.C. and Rowell, J.G. 1977. Dissociation of antiviral and antitumor immunity in resistance to Marek's disease. J.Natl.Cancer Inst. **59**: 919-924.

Pratt, W.D., Morgan, R.W., and Schat, K.A. 1992. Characterization of reticuloendotheliosis virus-transformed avian T- lymphoblastoid cell lines infected with Marek's disease virus. J.Virol. **66**: 7239-7244.

Pulford, D., Lowrey, P., and Morgan, A.J. 1994a. Expression of the Epstein-Barr virus envelope fusion glycoprotein gp85 gene by a recombinant baculovirus. J.Gen.Virol. **75**: 3241-3248.

Pulford, D., Lowrey, P., and Morgan, A.J. 1994b. Expression of the Epstein-Barr virus envelope fusion glycoprotein gp85 gene by a recombinant baculovirus. J Gen Virol. **75**: 3241-3248.

Pulford, D.J., Lowrey, P., and Morgan, A.J. 1995. Co-expression of the Epstein-Barr virus BXLF2 and BKRF2 genes with a recombinant baculovirus produces gp85 on the cell surface with antigenic similarity to the native protein. J Gen Virol. **76**: 3145-3152.

Purchase, H.G. 1985. Clinical disease and its economic impact, p.17-42. Payne, L.N., (Ed.) Marek's disease - Scientific Basis and Methods of Control. Martinus Nijhoff Publishing, Boston.

Purchase, H.G. and Biggs, P.M. 1967. Characterization of five isolates of Marek's disease. Res.Vet.Sci. **8**: 440-449.

Purchase, H.G., Okazaki, W., and Burmester, B.R. 1972. The minimum protective dose of the herpesvirus turkeys vaccine against Marek's disease. Vet.Rec. **91**: 79-84.

Qian, Z., Brunovskis, P., Lee, L., Vogt, P.K., and Kung, H.J. 1996. Novel DNA binding specificities of a putative herpesvirus bZIP oncoprotein. J.Virol. **70**: 7161-7170.

Qian, Z., Brunovskis, P., Rauscher, F., Lee, L., and Kung, H.J. 1995a. Transactivation activity of Meq, a Marek's disease herpesvirus bZIP protein persistently expressed in latently infected transformed T cells. J Virol. **69**: 4037-4044.

Qian, Z., Brunovskis, P., Rauscher, F., III, Lee, L., and Kung, H.J. 1995b. Transactivation activity of Meq, a Marek's disease herpesvirus bZIP protein persistently expressed in latently infected transformed T cells. J.Virol. **69**: 4037-4044.

Quere, P. and Dambrine, G. 1988. Development of anti-tumoral cell-mediated cytotoxicity during the course of Marek's disease in chickens. Ann.Rech.Vet.Ann.Rech.Vet.J1 - Ann.Rech.Vet.J2 - Ann.Rech.Vet. **19** : 193-202.

Rasmussen, L.E., Nelson, R.M., Kelsall, D.C., and Merigan, T.C. 1984. Murine monoclonal antibody to a single protein neutralizes the infectivity of human cytomegalovirus. Proc.Natl.Acad.Sci.U.S.A. **81**: 876-880.

Ren, D., Lee, L.F., and Coussens, P.M. 1994. Identification and characterization of Marek's disease virus genes homologous to ICP27 and glycoprotein K of herpes simplex virus-1. Virology **204**: 242-250.

Rennie, M., Powell, P.C., and Mustill, B.M. 1980. The effect of bursectomy on vaccination against Marek's disease with the herpesvirus of turkeys. Avian Pathol. **9**: 557-566.

Rispens, B.H., Van Vloten, H., Mastenbroek, N., Maas, H.J., and Schat, K.A. 1972a. Control of Marek's disease in the Netherlands. I. Isolation of an avirulent Marek's disease virus (strain CVI 988) and its use in laboratory vaccination trials. Avian Dis. **16**: 108-125.

Rispens, B.H., Van Vloten, H., Mastenbroek, N., Maas, J.L., and Schat, K.A. 1972b. Control of Marek's disease in the Netherlands. II. Field trials on vaccination with an avirulent strain (CVI 988) of Marek's disease virus. Avian.Dis. **16**: 126-138.

Roberts, S.R., Ponce, d.L., Cohen, G.H., and Eisenberg, R.J. 1991. Analysis of the intracellular maturation of the herpes simplex virus type 1 glycoprotein gH in infected and transfected cells. Virology **184**: 609-624.

Robertson, G.R., Scott, N.A., Miller, J.M., Sabine, M., Zheng, M., Bell, C.W., and Whalley, J.M. 1991. Sequence characteristics of a gene in equine herpesvirus 1 homologous to glycoprotein H of herpes simplex virus. DNA Seq. **1**: 241-249.

Rodriguez, J.E., Moninger, T., and Grose, C. 1993. Entry and egress of varicella virus blocked by same anti-gH monoclonal antibody. Virology **196**: 840-844.

Roizman, B., Desrosiers, R.C., Fleckenstein, B., Lopez, C., Minson, A.C., and Studdert, M.J. 1992. The family Herpesviridae: An update. Arch.Virol. **123**: 425-449.

Roizman, B. and Sears, A.E. 1995. Herpes simplex viruses ans their replication, p.2231-2296. Fields, B.N., Knipe, D.M., and Howley, P.M., (Eds). Virology. Lippincott-Raven, Philadelphia, New York.

Rong-Fu, W., Beasley, J.N., Cao, W.W., Slavik, M.F., and Johnson, M.G. 1993. Development of PCR method specific for Marek's disease virus. Mol.Cell Probes. **7**: 127-131.

Rosenberger, J.K. 1983. Reovirus interference with Marek's disease vaccination. Proc.West.Poult.Dis.Conf. 50-51.

Ross, L.J. 1977. Antiviral T cell-mediated immunity in Marek's disease. Nature **268**: 644-646.

Ross, L.J. 1985. Molecular Biology of the Virus, p.113-150. Payne, L.N., (Ed.) Marek's Disease - Scientific Basis and Methods of Control. Martinus Nijhoff Publishing, Boston.

Ross, L.J. and Binns, M.M. 1991a. Properties and evolutionary relationships of the Marek's disease virus homologues of protein kinase, glycoprotein D and glycoprotein I of herpes simplex virus. J Gen Virol. **72**: 939-947.

Ross, L.J., Binns, M.M., and Pastorek, J. 1991b. DNA sequence and organization of genes in a 5.5 kbp EcoRI fragment mapping in the short unique segment of Marek's disease virus (strain RB1B). J.Gen.Virol. **72**: 949-954.

Ross, L.J., Binns, M.M., and Pastorek, J. 1991c. DNA sequence and organization of genes in a 5.5 kbp EcoRI fragment mapping in the short unique segment of Marek's disease virus (strain RB1B). J Gen Virol. **72**: 949-954.

Ross, L.J., Binns, M.M., Tyers, P., Pastorek, J., Zelnik, V., and Scott, S. 1993. Construction and properties of a turkey herpesvirus recombinant expressing the Marek's disease virus homologue of glycoprotein B of herpes simplex virus. J.Gen.Virol. **74**: 371-377.

Ross, L.J., Milne, B.S., and Biggs, P.M. 1983. Restriction endonuclease analysis of Marek's disease virus DNA and homology between strains. J.Gen.Virol. **64**: 2785-2790.

Ross, L.J., Sanderson, M., Scott, S.D., Binns, M.M., Doel, T., and Milne, B. 1989. Nucleotide sequence and characterization of the Marek's disease virus homologue of glycoprotein B of herpes simplex virus. J Gen Virol. **70**: 1789-1804.

Ross, N.L., DeLorbe, W., Varmus, H.E., Bishop, J.M., Brahic, M., and Haase, A. 1981. Persistence and expression of Marek's disease virus DNA in tumour cells and peripheral nerves studied by in situ hybridization. J.Gen.Virol. **57**: 285-296.

Roudier, J., Petersen, J., Rhodes, G.H., Luka, J., and Carson, D.A. 1989. Susceptibility to rheumatoid arthritis maps to a T-cell epitope shared by the HLA-Dw4 DR beta-1 chain and the Epstein-Barr virus glycoprotein gp110. Proc Natl Acad Sci U S A **86**: 5104-5108.

Rouse, B.T., Wells, R.J., and Warner, N.L. 1973. Proportion of T and B lymphocytes in lesions of Marek's disease: Theoretical implications for pathogenesis. J.Immunol. **110**: 534-539.

Sarmiento, M., Haffey, M., and Spear, P.G. 1979. Membrane proteins specified by herpes simplex viruses. III. Role of glycoprotein VP7(B2) in virion infectivity. J Virol. **29**: 1149-1158.

Schat, K.A., Buckmaster, A., and Ross, L.J. 1989. Partial transcription map of Marek's disease herpesvirus in lytically infected cells and lymphoblastoid cell lines. Int.J.Cancer **44**: 101-109.

Schat, K.A. and Calnek, B.W. 1978a. Characterization of an apparently nononcogenic Marek's disease virus. J.Natl.Cancer Inst. **60**: 1075-1082.

Schat, K.A. and Calnek, B.W. 1978b. In vitro inactivation of cell-free Marek's disease herpesvirus by immune peripheral blood lymphocytes. Avian.Dis. **22**: 693-697.

Schat, K.A., Calnek, B.W., and Fabricant, J. 1982. Characterization of two highly oncogenic strains of Marek's disease virus. Avian Pathol. **11**: 593-605.

Schat, K.A., Calnek, B.W., Fabricant, J., and Graham, D.L. 1985. Pathogenesis of infection with attenuated Marek's disease virus strains. Avian Pathol. **14**: 127-146.

Schat, K.A., Chen, C.H., Shek, W.R., and Calnek, B.W. 1982. Surface antigens on Marek's disease lymphoblastoid tumor cell lines. J.Natl.Cancer Inst. **69**: 715-720.

Schat, K.A., Chen, C.L., Calnek, B.W., and Char, D. 1991. Transformation of T-lymphocyte subsets by Marek's disease herpesvirus. J.Virol. **65**: 1408-1413.

Schat, K.A., Schultz, R.D., Calnek, B.W., Hilgers, J., and Yohn, D.S. 1978. Marek's disease: Effect of virus pathogenicity and genetic susceptibility on response of peripheral blood lymphocytes to concanavalin-A, p.183-186. Bentvelzen, P., (Ed.) Advances Comparative Leukosis Research. Elsevier, Amsterdam.

Scholten, R., Hilgers, L.A., Jeurissen, S.H., and Weststrate, M.W. 1990. Detection of Marek's disease virus antigen in chickens by a novel immunoassay. J.Virol.Methods **27**: 221-226.

Scott, S.D., Ross, N.L., and Binns, M.M. 1989. Nucleotide and predicted amino acid sequences of the Marek's disease virus and turkey herpesvirus thymidine kinase genes; comparison with thymidine kinase genes of other herpesviruses. J.Gen.Virol. **70**: 3055-3065.

Scott, S.D., Smith, G.D., Ross, N.L., and Binns, M.M. 1993a. Identification and sequence analysis of the homologues of the herpes simplex virus type 1 glycoprotein H in Marek's disease virus and the herpesvirus of turkeys. J.Gen.Virol. **74**: 1185-1190.

Sears, A.E., McGwire, B.S., and Roizman, B. 1991. Infection of polarized MDCK cells with herpes simplex virus 1: two asymmetrically distributed cell receptors interact with different viral proteins. Proc.Natl.Acad.Sci.U.S.A. **88**: 5087-5091.

Sevoian, M. and Chamberlain, D.M. 1962a. Avian lymphomatosis. II. Experimental reproduction of the ocular form. Veterinary Medicine **57**: 608-609.

Sevoian, M., Chamberlain, D.M., and Counter, F.T. 1962b. Avian lymphomatosis. I. Experimental reproduction of the neural and visceral forms. Veterinary Medicine **57**: 500-501.

Sharma, J.M. 1981. Fractionation of Marek's disease virus-induced lymphoma by velocity sedimentation and association of infectivity with cellular fractions with and without tumor antigen expression. Amer.J.Vet.Res. **42**: 483-486.

Sharma, J.M. 1984. Effect of infectious bursal disease virus on protection against Marek's disease by turkey herpesvirus vaccine. Avian Dis. **28**: 629-640.

Sharma, J.M. 1989. In situ production of interferon in tissues of chickens exposed as embryos to turkey herpesvirus and Marek's disease virus. Am.J.Vet.Res. **50**: 882-886.

Sharma, J.M. and Burmester, B.R. 1982. Resistance to Marek's disease at hatching in chickens vaccinated as embryos with the turkey herpesvirus. Avian Dis. **26**: 134-149.

Sharma, J.M. and Cooper, E.L. 1978. Immunopathology of Marek's Disease, p.132-142. Gershwin, M.E., (Ed.) Animal Models of Comparative and Developmental Aspects of Immunity and Disease. Pergamon Press,

Sharma, J.M. and Coulson, B.D. 1977. Cell-mediated cytotoxic response to cells bearing Marek's disease tumor-associated surface antigen in chickens infected with Marek's disease virus. J.Natl.Cancer Inst. **58**: 1647-1651.

Sharma, J.M. and Stone, H.A. 1972. Genetic resistance to Marek's disease. Delineation of the response of genetically resistant chickens to Marek's disease virus infection. Avian.Dis. **16**: 894-906.

Sharma, J.M. and Witter, R.L. 1975a. The effect of B-cell immunosuppression on age-related resistance of chickens to Marek's disease. Cancer Res. **35**: 711-717.

Sharma, J.M., Witter, R.L., and Purchase, H.G. 1975b. Absence of age-resistance in neonatally thymectomized chickens as evidence for cell-mediated immune surveillance in Marek's disease. Nature **253**: 477-479.

Shek, W.R., Calnek, B.W., Schat, K.A., and Chen, C.-L.H. 1983. Characterization of Marek's disease virus-infected lymphocytes: Discrimination between cytolytically and latently infected cells. J.Natl.Cancer Inst. **70**: 485-492.

Shieh, M.T., Wudunn, D., Montgomery, R.I., Esko, J.D., and Spear, P.G. 1992. Cell Surface Receptors for Herpes Simplex Virus Are Heparan Sulfate Proteoglycans. J.Cell Biol. **116**: 1273-1281.

Shimojima, Y., Jang, H.K., Ono, M., Kai, C., and Mikami, T. 1997. Identification and DNA sequence analysis of the Marek's disease virus serotype 2 gene homologous to the herpes simplex virus type 1 glycoprotein H. J Vet Med Sci **59**: 629-634.

Showalter, S.D., Zweig, M., and Hampar, B. 1981. Monoclonal antibodies to herpes simplex virus type 1 proteins, including the immediate-early protein ICP 4. Infect.Immun. **34**: 684-692.

Silva, R.F. 1992. Differentiation of pathogenic and non-pathogenic serotype 1 Marek's disease viruses (MDVs) by the polymerase chain reaction amplification of the tandem direct repeats within the MDV genome. Avian Dis. **36**: 521-528.

Silva, R.F. and Barnett, J.C. 1991. Restriction endonuclease analysis of Marek's disease virus DNA: differentiation of viral strains and determination of passage history. Avian Dis. **35**: 487-495.

Silva, R.F. and Witter, R.L. 1985. Genomic expansion of Marek's disease virus DNA is associated with serial in vitro passage. J.Virol. **54**: 690-696.

Smith, G.D., Zelnik, V., and Ross, L.J. 1995. Gene organization in herpesvirus of turkeys: identification of a novel open reading frame in the long unique region and a truncated homologue of pp38 in the internal repeat. Virology **207**: 205-216.

Smith, M.W. and Calnek, B.W. 1973. Effect of virus pathogenicity on antibody production in Marek's disease. Avian Dis. **17**: 727-736.

Smith, M.W. and Calnek, B.W. 1974. High-virulence Marek's disease virus infection in chickens previously infected with low-virulence virus. J.Natl.Cancer Inst. **52**: 1595-1603.

Solomon, J.J., Witter, R.L., Nazerian, K., and Burmester, B.R. 1968. Studies on the etiology of Marek's disease. I. Propagation of the agent in cell culture. Proc.Soc.Exp.Biol.Med. **127**: 173-177.

Spaete, R.R., Saxena, A., Scott, P.I., Song, G.J., Probert, W.S., Britt, W.J., Gibson, W., Rasmussen, L., and Pachl, C. 1990. Sequence requirements for proteolytic processing of glycoprotein B of human cytomegalovirus strain Towne. J Virol. **64**: 2922-2931.

Spaete, R.R., Thayer, R.M., Probert, W.S., Masiarz, F.R., Chamberlain, S.H., Rasmussen, L., Merigan, T.C., and Pachl, C. 1988. Human cytomegalovirus strain Towne glycoprotein B is processed by proteolytic cleavage. Virology **167**: 207-225.

Spear, P.G. 1993. Membrane fusion induced by herpes simplex virus, p.201-232. Bentz, J., (Ed.) Viral fusion mechanisms. CRC Press, Inc., Boca Raton, Fla.

Spear, P.G., Shieh, M.T., Herold, B.C., WuDunn, D., and Koshy, T.I. 1992. Heparan sulfate glycosaminoglycans as primary cell surface receptors for herpes simplex virus. Adv.Exp.Med.Biol. **313**: 341-353.

Spencer, J.L. and Calnek, B.W. 1970. Marek's disease: Application of immunofluorescence for detection of antigen and antibody. Amer.J.Vet.Res. **31**: 345-358.

Strnad, B.C., Schuster, T., Klein, R., Hopkins, R.F., Witmer, T., Neubauer, R.H., and Rabin, H. 1982. Production and characterization of monoclonal antibodies against the Epstein-Barr virus membrane antigen. J.Virol. **41**: 258-264.

Sugaya, K., Bradley, G., Nonoyama, M., and Tanaka, A. 1990. Latent transcripts of Marek's disease virus are clustered in the short and long repeat regions. J.Virol. **64**: 5773-5782.

Sui, D., Wu, P., Kung, H.J., and Lee, L.F. 1995. Identification and characterization of a Marek's disease virus gene encoding DNA polymerase. Virus Res. **36**: 269-278.

Svennerholm, B., Jeansson, S., Vahlne, A., and Lycke, E. 1991. Involvement of glycoprotein C (gC) in adsorption of herpes simplex virus type 1 (HSV-1) to the cell. Arch.Virol. **120**: 273-279.

Tal-Singer, R., Peng, C., Ponce, d.L., Abrams, W.R., Banfield, B.W., Tufaro, F., Cohen, G.H., and Eisenberg, R.J. 1995. Interaction of herpes simplex virus glycoprotein gC with mammalian cell surface molecules. J.Virol. **69**: 4471-4483.

Tal-Singer, R., Seidel-Dugan, C., Fries, L., Huemer, H.P., Eisenberg, R.J., Cohen, G.H., and Friedman, H.M. 1991. Herpes simplex virus glycoprotein C is a receptor for complement component iC3b. J.Infect.Dis. **164**: 750-753.

Telford, E.A., Watson, M.S., Aird, H.C., Perry, J., and Davison, A.J. 1995. The DNA sequence of equine herpesvirus 2. J.Mol.Biol. **249**: 520-528.

Telford, E.A., Watson, M.S., McBride, K., and Davison, A.J. 1992. The DNA sequence of equine herpesvirus-1. Virology **189**: 304-316.

Tillotson, J.K., Lee, L.F., Kung, H.J., Horiuchi, T., Mikami, T., and Hirai, K. 1988. Accumulation of viral transcripts coding for a DNA binding protein in Marek's disease tumor cells, p.128-134. Kato, S., (Ed.) Advances in Marek's disease research. Japanese Assoc. on Marek's Dis., Osaka.

Tugizov, S., Navarro, D., Paz, P., Wang, Y., Qadri, I., and Pereira, L. 1994. Function of human cytomegalovirus glycoprotein B: syncytium formation in cells constitutively expressing gB is blocked by virus-neutralizing antibodies. Virology **201**: 263-276.

Turner, A., Bruun, B., Minson, T., and Browne, H. 1998. Glycoproteins gB, gD, and gHgL of herpes simplex virus type 1 are necessary and sufficient to mediate membrane fusion in a Cos cell transfection system. J Virol. **72**: 873-875.

Urban, M., Klein, M., Britt, W.J., Hassfurther, E., and Mach, M. 1996. Glycoprotein H of human cytomegalovirus is a major antigen for the neutralizing humoral immune response. J.Gen.Virol. **77**: 1537-1547.

Van Zaane, D., Brinkhof, J.M., and Gielkens, A.L. 1982a. Molecular-biological characterization of Marek's disease virus II. Differentiation of various MDV and HVT strains. Virology **121**: 133-146.

Van Zaane, D., Brinkhof, J.M., Westenbrink, F., and Gielkens, A.L. 1982b. Molecular-biological characterization of Marek's disease virus. I. Identification of virus-specific polypeptides in infected cells. Virology **121**: 116-132.

Vengris, V.E. and Mare, C.J. 1973. Protection of chickens against Marek's disease virus JM-V strain with statolon and exogenous interferon. Avian.Dis. **17**: 758-767.

Volpini, L.M., Calnek, B.W., Sekellick, M.J., and Marcus, P.I. 1995. Stages of Marek's disease virus latency defined by variable sensitivity to interferon modulation of viral antigen expression. Vet.Microbiol. **47**: 99-109.

Wakenell, P.S., Miller, M.M., Goto, R.M., Gauderman, W.J., and Briles, W.E. 1996. Association between the Rfp-Y haplotype and the incidence of Marek's disease in chickens. Immunogenetics **44**: 242-245.

Wang, X. and Hutt-Fletcher, L.M. 1998. Epstein-Barr virus lacking glycoprotein gp42 can bind to B cells but is not able to infect. J Virol. **72**: 158-163.

Weeks, B.S., Sundaresan, P., Nagashunmugam, T., Kang, E., and Friedman, H.M. 1997. The herpes simplex virus-1 glycoprotein E (gE) mediates IgG binding and cell-to-cell spread through distinct gE domains. Biochem.Biophys.Res.Commun. **235**: 31-35.

Westra, D.F., Glazenburg, K.L., Harmsen, M.C., Tiran, A., Jan, S., Welling, G.W., Hauw, T., and Welling-Wester, S. 1997. Glycoprotein H of herpes simplex virus type 1 requires glycoprotein L for transport to the surfaces of insect cells. J.Virol. **71**: 2285-2291.

Whealy, M.E., Card, J.P., Robbins, A.K., Dubin, J.R., Rziha, H.J., and Enquist, L.W. 1993. Specific pseudorabies virus infection of the rat visual system requires both gI and gp63 glycoproteins. J Virol. **67**: 3786-3797.

Whitbeck, J.C., Knapp, A.C., Enquist, L.W., Lawrence, W.C., and Bello, L.J. 1996. Synthesis, processing, and oligomerization of bovine herpesvirus 1 gE and gI membrane proteins. J Virol. **70**: 7878-7884.

Whitbeck, J.C., Peng, C., Lou, H., Xu, R., Willis, S.H., Ponce, d.L., Peng, T., Nicola, A.V., Montgomery, R.I., Warner, M.S., Soulika, A.M., Spruce, L.A., Moore, W.T., Lambris, J.D., Spear, P.G., Cohen, G.H., and Eisenberg, R.J. 1997. Glycoprotein D of herpes simplex virus (HSV) binds directly to HVEM, a member of the tumor necrosis factor receptor superfamily and a mediator of HSV entry. J Virol. **71**: 6083-6093.

Wilson, M.R., Southwick, R.A., Pulaski, J.T., Tieber, V.L., Hong, Y., and Coussens, P.M. 1994. Molecular analysis of the glycoprotein C-negative phenotype of attenuated Marek's disease virus. Virology **199**: 393-402.

Witter, R.L. 1979. A proposed method for designating avian cell lines and transplantable tumours. Avian Pathol. **8**: 487-498.

Witter, R.L. 1982. Protection by attenuated and polyvalent vaccines against highly virulent strains of Marek's disease virus. Avian Pathol. **11**: 49-62.

Witter, R.L. 1983. Characteristics of Marek's disease viruses isolated from vaccinated commercial chicken flocks: Association of viral pathotype with lymphoma frequency. Avian Dis. **27**: 113-132.

Witter, R.L. 1985. Principles of vaccination, p.203-250. Payne, L.N., (Ed.) Marek's disease. Martinus Nijhoff, Boston.

Witter, R.L. 1987. New serotype 2 and attenuated serotype 1 Marek's disease vaccine viruses: comparative efficacy. Avian Dis. **31**: 752-765.

Witter, R.L. 1989. Very virulent Marek's disease viruses: Importance and control. World's Poult.Sci.J. **45**: 60-75.

Witter, R.L. 1991. Attenuated Revertant Serotype 1 Marek's Disease Viruses: Safety and Protective Efficacy. Avian Dis. **35**: 877-891.

Witter, R.L. 1992. Influence of serotype and virus strain on synergism between Marek's disease vaccine viruses. Avian Pathol. **21**: 601-614.

Witter, R.L. 1997a. Avian tumor viruses: persistent and evolving pathogens. Acta Vet.Hung. **45**: 251-266.

Witter, R.L. 1997b. Increased virulence of Marek's disease virus field isolates. Avian.Dis. **41**: 149-163.

Witter, R.L., Burgoyne, G.H., and Solomon, J.J. 1969. Evidence for a herpesvirus as an etiologic agent of Marek's disease. Avian Dis. **13**: 171-184.

Witter, R.L. and Burmester, B.R. 1979. Differential effect of maternal antibodies on efficacy of cellular and cell-free Marek's disease vaccines. Avian Pathol. **8**: 145-156.

Witter, R.L., Nazerian, K., Purchase, H.G., and Burgoyne, G.H. 1970. Isolation from turkeys of a cell-associated herpesvirus antigenically related to Marek's disease virus. Am.J.Vet.Res. **31**: 525-538.

Witter, R.L., Sharma, J.M., and Fadly, A.M. 1980. Pathogenicity of variant Marek's disease virus isolates in vaccinated and unvaccinated chickens. Avian Dis. **24**: 210-232.

Witter, R.L., Solomon, J.J., Champion, L.R., and Nazerian, K. 1971. Long term studies of Marek's disease infection in individual chickens. Avian Dis. **15**: 346-365.

Wu, C.A., Nelson, N.J., McGeoch, D.J., and Challberg, M.D. 1988. Identification of herpes simplex virus type 1 genes required for origin-dependent DNA synthesis. J.Virol. **62**: 435-443.

Wu, P., Lee, L.F., and Reed, W.M. 1997. Serological characteristics of a membrane glycoprotein gp82 of Marek's disease virus. Avian Dis **41**: 824-831.

Wu, P., Sui, D., and Lee, L.F. 1996. Nucleotide sequence analysis of a 9-kb region of Marek's disease virus genome exhibits a collinear gene arrangement with the UL29 to UL36 genes of herpes simplex virus., p.219-224. Silva, R.F., Cheng, H.H., Coussens, P., Lee, L.F., and Velicer, L.F., (Eds). Proceedings of the 5th international symposium on Marek's disease. American Association of Avian Pathologist, East Lansing, Michigan, USA.

Wu, T.F., Sun, W., Boussaha, M., Southwick, R., and Coussens, P.M. 1996. Cloning and sequence analysis of a Marek's disease virus origin binding protein (OBP) reveals strict conservation of structural motifs among OBPs of divergent alphaherpesviruses. Virus Genes **13**: 143-157.

Wudunn, D. and Spear, P.G. 1989. Initial interaction of herpes simplex virus with cells is binding to heparan sulfate. J.Virol. **63**: 52-58.

Wyatt, L.S., Moss, B., and Rozenblatt, S. 1995. Replication-deficient vaccinia virus encoding bacteriophage T7 RNA polymerase for transient gene expression in mammalian cells. Virology **210**: 202-205.

Yanagida, N., Lee, L.F., Yoshida, S., and Nazerian, K. 1992a. Nucleotide sequence and predicted amino acid sequence of Marek's disease virus homologs of UL49, UL48 (Vp16), UL47, and UL46 of herpes simplex virus, p.44-48. 4th International Symposium on Marek's Disease, 19th World's Poultry Congress, Vol. 1. World's Poultry Science Assn., Amsterdam.

Yanagida, N., Ogawa, R., Li, Y., Lee, L.F., and Nazerian, K. 1992b. Recombinant fowlpox viruses expressing the glycoprotein B homolog and the pp38 gene of Marek's disease virus. J Virol. **66**: 1402-1408.

Yao, Z., Jackson, W., Forghani, B., and Grose, C. 1993. Varicella-zoster virus glycoprotein gpI/gpIV receptor: expression, complex formation, and antigenicity within the vaccinia virus-T7 RNA polymerase transfection system. J Virol. **67**: 305-314.

Yaswen, L.R., Stephens, E.B., Davenport, L.C., and Hutt-Fletcher, L.M. 1993. Epstein-Barr virus glycoprotein gp85 associates with the BKRF2 gene product and is incompletely processed as a recombinant protein. Virology **195**: 387-396.

Yoshida, S., Lee, L.F., Yanagida, N., and Nazerian, K. 1994a. Identification and characterization of a Marek's disease virus gene homologous to glycoprotein L of herpes simplex virus. Virology **204**: 414-419.

Yoshida, S., Lee, L.F., Yanagida, N., and Nazerian, K. 1994b. Mutational analysis of the proteolytic cleavage site of glycoprotein B (gB) of Marek's disease virus. Gene **150**: 303-306.

Yoshida, S., Lee, L.F., Yanagida, N., and Nazerian, K. 1994c. The glycoprotein B genes of Marek's disease virus serotypes 2 and 3: identification and expression by recombinant fowlpox viruses. Virology **200**: 484-493.

Yuasa, N., Imai, K., Horiuchi, T., Mikami, T., and Hirai, K. 1988. Efficacy of Marek's disease vaccine, herpesvirus of turkeys, in chickens affected with chicken anemia agent, p.358-363. Kato, S., (Ed.) Advances in Marek's disease research. Japanese Assoc. on Marek's Dis., Osaka.

Zelnik, V., Darteil, R., Audonnet, J.C., Smith, G.D., Riviere, M., Pastorek, J., and Ross, L.J. 1993. The complete sequence and gene organization of the short unique region of herpesvirus of turkeys. J Gen Virol. **74**: 2151-2162.

Zelnik, V., Ross, L.J., Smith, G.D., Ramsay, L.A., and Pastorek, J. 1992. Comparison of glycoprotein D (gD) genes of Marek's disease virus and herpesvirus of turkeys, p.114-117. 4th International Symposium on Marek's Disease, 19th World's Poultry Congress, Vol. 1. World's Poultry Science Assn., Amsterdam.

Ziemann, K., Mettenleiter, T.C., and Fuchs, W. 1998. Gene arrangement within the unique long genome region of infectious laryngotracheitis virus is distinct from that of other alphaherpesviruses. J.Virol. **72**: 847-852.

Zsak, L., Zuckermann, F., Sugg, N., and Ben-Porat, T. 1992. Glycoprotein gI of pseudorabies virus promotes cell fusion and virus spread via direct cell-to-cell transmission. J Virol. **66**: 2316-2325.

Zuckermann, F.A., Mettenleiter, T.C., Schreurs, C., Sugg, N., and Ben-Porat, T. 1988. Complex between glycoproteins gI and gp63 of pseudorabies virus: its effect on virus replication. J Virol. **62**: 4622-4626.