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INFLUENCE OF MAREK'S DISEASE VIRUS ON SUBGROUP J AVIAN LEUKOSIS VIRUS INFECTION IN MEAT-TYPE CHICKENS

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ABSTRACT

INFLUENCE OF MAREK'S DISEASE VIRUS ON SUBGROUP J AVIAN LEUKOSIS VIRUS INFECTION IN MEAT-TYPE CHICKENS

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The objective of this study was to determine the influence of various serotypes of Marek's disease viruses (MDV) on avian leukosis virus subgroup J (ALV-J)-induced viremia, cloacal shedding and tumors, in meat-type chickens. Chickens from two different breeders were placed in six different groups and infected with ALV-J at hatch; chickens were also inoculated with various strains of MDV vaccines at hatch and challenged with very virulent plus (vv+) MDV at two weeks of age.

Data from this study suggest that bivalent MD vaccines containing serotype 2 and 3 MDV, namely turkey herpesvirus and SB-1 (HVT+SB-1) may reduce viremia and cloacal shedding of ALV-J infection in one of the two strains of chickens used. Chickens infected with ALV-J and bivalent MDV vaccine had lower percentage of viremia and cloacal shedding than chickens inoculated with serotype 1 MDV vaccine (Rispens) or vv+MDV (strain 648A). In the second experiment, commercial broiler breeder chickens infected with serotype 1 MDV vaccine had the highest incidence of viremia and cloacal shedding. There was no influence of strain of MDV vaccine on ALV-J tumors.

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ABBREVIATIONS

ALV-J = Subgroup J avian leukosis virus

MD = Marek's disease

MDV = Marek's disease virus

vv+MDV = very virulent plus Marek's disease virus

CPE = cytopathic effect

ELISA = enzyme-linked immunosorbant assay

VN = virus neutralization

V = viremia

S = cloacal shedding

INTRODUCTION

Most neoplasms of lymphoid and other hematopoietic cells in commercial poultry are caused by viruses, which belong to one of four distinct groups: a) Marek's disease virus (MDV), an oncogenic herpesvirus; b) avian leukosis virus (ALV); c) reticuloendotheliosis virus (REV); and lymphoproliferative disease virus of turkey (LPDV) (Witter, 1997b).

ALVs infect primarily chickens, whereas REV infects chickens, turkeys and other avian species. ALVs are members of the leukosis/sarcoma (L/S) group of avian retroviruses. Based on properties of viral envelope glycoproteins, members of the L/S group including ALVs from chickens are classified into five exogenous subgroups: A, B, C, D and J and one endogenous subgroup E (Payne and Fadly, 1997). Among the structural polypeptides (p27, p19, p15, p12 and p10) shared by all members of L/S group of avian retroviruses including endogenous and exogenous ALVs, p27 is the most abundant. Exogenous ALVs are capable of inducing a variety of neoplasms, but endogenous ALVs are rarely oncogenic. Under natural conditions, lymphoid leukosis, a B-cell lymphoma that originates in the bursa of Fabricius and metastasizes to various visceral organs, is the most common neoplasm induced by exogenous ALVs (Fadly, 1997).

Exogenous ALVs spread by vertical and horizontal transmission, a process that requires a fully infectious virus. In contrast, endogenous viral (*ev*) genes are inherited as host genes and may or may not be expressed (Payne and Fadly, 1997).

Chickens infected with exogenous ALV shed virus into the albumen of eggs and vaginal and cloacal secretions and congenitally transmit virus to the next generation. In contrast, endogenous ALVs are believed not to be shed or shed at a very low level (Smith at al., 1986; Crittenden, 1991). The oviduct which is the source of virus for shedding of ALV contains the highest viral titers, compared to other visceral organs. Congenital transmission of ALV is more frequent in hens with low antibody titer and can occur in chickens with no detectable shedding of group specific (gs) antigen p27 (Payne and Fadly, 1997).

Congenital transmission from dam to progeny is the major method of maintaining infection in flocks. Most congenitally infected chicks become immunologically tolerant to the virus and remain viremic throughout their lifetime without developing neutralizing antibody. Such females transmit virus to nearly 100% of their progeny. Males do not appear to transmit virus (Fadly and Crittenden, 1987). Viremic chicks that are in close contact with other chicks early in life transmit the infection through feces, which contaminates drinking water. Transfer of infection can also occur via vaccination needles or by vent sexing in the hatchery (Fadly et al., 1981).

The frequency of birds exposed to ALV early in life that become permanently viremic and lack neutralizing antibody is highly variable as in the frequency of birds that develop transient viremia with development of neutralizing antibody. Some antibody positive hens can transmit the infection to the next generation, but the rate is usually lower than with viremic tolerant hens. The frequency with which horizontal transmission occurs depends on genetic

makeup, concurrent exposure to immuno-depressive agents, and the strain of ALV (Fadly et al.,1985; Fadly et al., 1987).

The most common neoplasm induced by ALV is lymphoid leukosis. In this disease, the *c-myc* gene in a bursal lymphoid cell (B cell) is activated. B cell lymphomagenesis is a multi-stage event, with activation of other cellular oncogenes leading to tumor progression and metastasis. The first event can be seen in the bursa a few weeks after infection by ALV in which the normal B cell components are replaced by proliferating transformed B lymhoblasts. Some of the transformed follicles regress, but one or more may progress over a period of several months to neoplasia with metastasis to other organs such as liver and spleen, leading to death of the chicken. The cells arising from one follicle are clonal but progression to neoplasia in several follicles leads to polyclonal tumors (Payne and Fadly, 1997).

A less common expression of infection by ALV is erythroid leukosis (erythroblastosis), a disease in which the *c-erbB* gene in an erythroid cell is activated. The bone marrow is largely replaced by proliferating erythroblasts, leukemia develops, and there is localization in the liver, spleen and other organs which become enlarged due to accumulation of intravascular erythroblasts. Erythroid leukosis has a shorter latent period than LL.

Another disease caused by ALV is myeloid leukosis (myelobastosis and myelocytomatosis). Cases generally occur sporadically in adult birds. A myeloid cell becomes transformed, with the development of a severe leukemia. The liver,

spleen and other organs become infiltrated by intravascular and extravascular myeloid cells.

A variety of other tumors are also induced by the various strains of ALV. The most common are myxosarcoma, histiocytic sarcoma, osteosarcoma, chondrosarcoma, hemangioma and osteopetrosis (Payne, 1998).

Endogenous and exogenous ALVs can be differentiated on the basis of host range determined in gp85 by biological assays in which selective chicken embryo fibroblasts (CEFs) are used. A number of biological assays can be used for the detection of endogenous and exogenous ALVs. Most ALVs produce no visible morphologic changes in culture. Thus, indirect biologic assay such as complement fixation (COFAL), Enzyme linked immunosorbent assay (ELISA), phenotypic mixing (PM), resistance inducing factor (RIF), and non producer (NP) cell activation tests are used for detection. These procedures are cumbersome and require the use of selective cell culture. The most sensitive procedure for differentiating between endogenous and exogenous ALV is the virus isolation. Samples are inoculated on C/E (cell line resistant to endogenous ALV) and C/O (cell line susceptible to both endogenous and exogenous ALVs) CEF; 7 to 9 days later cell lysates are tested for the presence of ALV gs antigen by ELISA. If a positive ELISA is obtained from C/O but not C/E CEFs the sample is positive for endogenous ALV; positive ELISA on both C/E and C/O indicates exogenous ALV. Currently, the most specific test for detection of antibody to ALVs is the virus neutralization test. Both virus isolation and virus neutralization tests require genetically susceptible chicken embryo fibroblasts (Fadly and Witter, 1998).

AVIAN LEUKOSIS VIRUS SUBGROUP J:

Avian Leukosis Virus (ALV) subgroup J (ALV-J) was first reported in the United Kingdom in 1991 (Payne, 1991). ALV strain HPRS-103, the prototype of ALV-J, appears to be a recombinant between an exogenous ALV and ancient endogeneous avian retroviral envelope (E51) sequences (Benson et al., 1998 a,b).

ALV-J was isolated from commercial meat-type chickens (Payne et al., 1991a,b). The virus induces predominantly myelocytic myeloid leukosis and nephromas (Arshad et al., 1999). Domestic fowl, red jungle fowl, Sonnerat's jungle fowl and turkey are susceptible to infection (Payne et al., 1992).

The most frequent gross lesion in chickens with myeloid leukosis (ML) is moderate to massive enlargement of the liver caused by diffuse or miliary tumor infiltration. A common occurrence is the development of skeletal myelocytomas, which are creamy in color and can be seen on the inner surface of the sternum, ribs, vertebrae and synsacrum. ML tumors can also be seen in the spleen, thymus, gonads and kidney. Bursal involvement has not been reported. Microscopically, the presence of immature myelocytes with characteristic eosinophilic cytoplasmic granules in the affected tissues is considered pathogenomonic for ML (Payne et al., 1992,1993). Strain HPRS-103, the prototype ALV-J, lacks a viral oncogene and presumably induces myelocytomatosis by insertional mutagenesis (Bai et al., 1995).

During the last several years, several strains of ALV-J were isolated from broiler breeder and commercial broiler flocks experiencing a relatively high incidence of ML in the United States, and many other countries around the world (Nakamuro, 2000). In the United States, ALV-J induced ML was diagnosed in affected flocks at 4 weeks of age or older. Strain ADOL-Hc1 of ALV-J had been designated as the US prototype of ALV-J (Fadly and Smith, 1999).

Field observations suggest that flocks which experience a relatively high rate of Marek's Disease outbreaks also develop a high incidence of ALV-J-induced tumors. In addition, field observations suggest that ALV-J is gaining virulence, as losses in affected flocks are occurring earlier and at a higher incidence. Most recently, ALV-J infection and tumors have been diagnosed on two occasions in flocks other than meat-type chickens (Fadly, 1998).

The current program for control of ALV-J infection in broiler breeder chickens is based on the elimination of dams that test positive for ALV-J by virus isolation tests or by direct ELISA, a test that presently does not discriminate between endogenous and exogenous ALV (Fadly and Witter, 1998).

The ALV infection status of birds is determined by the presence (+) or absence (-) of viremia (V), viral shedding (S) and serum antibodies (A). In most cases horizontal transmission of ALV, occurs through close contact with infected chicks leads to immune non-shedders infection status (V-S-A+). However, ALV-J infection status in meat-type chickens, post-hatch infected can result either in tolerant viremic infection (V+S+A-) similar to congenitally infected chickens, or in an immune status after showing a transient viremia. A proportion

of the immune chickens may also shed (V-S+A+) the virus. Chickens exposed to the virus at an early age after hatch develop tolerant viremia (V+S+A-), whereas chickens exposed to the virus at an older age develop immunity and may or may not shed the virus (Venugopal, 1999).

MAREK'S DISEASE

Marek's Disease (MD) is the most common virus induced lymphoproliferative disease of chickens, characterized by mononuclear cell infiltration of peripheral nerves, gonad, iris, various viscera, muscle and skin (Payne, 1985). The disease was first described in 1907 in roasters with paresis due to mononuclear infiltration of peripheral nerves and spinal roots (Marek, 1907).

MD is caused by a cell-associated herpesvirus, Marek's disease virus (MDV). Molecular structure and genomic organization of MDV are similar to those of alpha herpesviruses. The DNA of MDV is linear, double stranded, and 166-184 kb in size. Strains of MDV that induce disease in chickens are classified as serotype 1. However, two additional classes of MDV, serotype 2 and 3, occur naturally, in chickens and turkeys, respectively. Both are non-oncogenic, but share antigenic determinants as determined by immunological assays and cross protection tests (Schat, 1985). Because of considerable variation in disease induction among isolates of pathogenic serotype 1 MDV, isolates are further classified as mild (m), virulent (v), and very virulent (vv) and very virulent plus

(vv+) (Witter, 1997). This classification is based on ability of serotype 1 MDV to induce lesions in vaccinated chickens (Witter, 1998).

MDV spreads by direct or indirect contact between birds, primarily by the airborne route. Epithelial cells in the keratinizing layer of the feather follicle is the site of MDV replication. Fully infectious virus in these cells serve as a source of MDV in the environment. Many apparently normal birds are carriers that can transmit the infection. Continual shedding of virus by infected birds and hardiness of the virus results in a high prevalence of infection. There is no vertical transmission of MDV (Calnek and Witter, 1997).

Nerve lesions is a frequent finding in affected birds. Macroscopic changes are found in one or more peripheral nerves and in spinal roots and ganglia. Celiac, cranial, mesenteric, brachial and sciatic plexuses can be affected and characterized by loss of cross-striations, gray or yellow discoloration and sometimes with an edematous appearance (Payne, 1985; Calnek and Witter, 1997).

MDV-induced lymphoid tumors may occur in one or more of a variety of organs. Lymphomatous lesions can be found in the gonad, lung, heart, mesentery, kidney, liver, spleen, bursa, thymus, adrenal gland, pancreas, proventriculus, intestine, iris, skeletal muscle and skin. Visceral tumors are especially common in more acute forms of the disease, and may be found in the absence of gross nerve lesions. Severe outbreaks induce a high prevalence of visceral lesions (62-89%), while in others the prevalence is low (5-7%) (Calnek and Witter, 1997).

Diffuse infiltration of the liver causes loss of normal lobule architecture and often gives the surface a course granular appearance. Nodular tumors can be seen in the liver. Also, the proventriculus becomes thickened and firm, with small to large focal areas of infiltration within and between glands. Muscle lesions may be both superficial and in deep layers, and are most common in pectoral muscles. Gross changes vary from tiny whitish streaks to nodular tumors.

Microscopically there are two types of lesions that can be seen in peripheral nerves (Calnek and Witter, 1997). Type A lesions consist of masses of proliferating lymphoblastic cells; demyelination and Shwann cell proliferation. Type B lesions are characterized by diffuse light-to-moderate infiltration by small lymphocytes and plasma cells, usually with edema, and sometimes, with demyelination and Schwann cell proliferation. A few macrophages can be found.

Visceral tumors in MD often appear to be infiltrating tissue and consist of a pleomorphic population of small to medium lymphocytes, lymphoblasts, large reticular cells, large basophilic cells with large, dark nuclei. Plasma cells can be seen.(Pope, 1996; Calnek and Witter, 1997). Irregularity of the pupil and loss of pigmentation is the result of mononuclear cell infiltration of the iris.

Very virulent plus (vv+) MDV causes early mortality with 77-100% of the affected birds showing bursal and thymic atrophy. Visceral lesions with heart affected more often than other organs, and a high percentage of ocular lesion can be seen in chickens infected with vv+ MDV (Witter, 1997a).

Lymphoid organs from chickens infected with vv+MDV show little evidence of recovery between 8 and 14 days post infection (DPI), whereas the chickens infected with vMDV have a significant return towards normal by 14 DPI. Chickens infected with vv MDV had evidence of intermediate degrees of bursal and thymic atrophy and recovery (Calnek et al., 1998).

MDV can be isolated from blood lymphocytes, heparinized whole blood, and isolated tumor cells or feather tips. Chicken kidney cell and duck embryo fibroblast cultures are preferred for isolation of serotype 1 MDV, whereas CEF are used for isolation of serotype 2 and 3 MDV viruses. On the basis of plague Serotype 1, 2 and 3 MDV can be distinguished. But Immunofluorescent (IFA) staining with using specific monoclonal antibodies provides a more accurate differentiation. Both viral antigens and antibodies can be detected by IFA, immunoperoxidase, agar gel precipitation (AGP), and ELISA tests (Calnek and Witter, 1997).

Epidemiological, pathological, molecular and immunohistochemical assays can be used in differential diagnosis of MDV induced lymphomas, and those induced by ALV and REV. Microscopically, LL tumor cells are primarily immature lymphocytes, which are characterized by poorly defined cytoplasmic membranes, abundant cytoplasm and vesicular nuclei. If typical LL bursal lesions are not present, LL and MD can not be distinguished by gross examination. In such cases, the history, symptoms, gross and microscopic lesions, and cytology should all be considered before a diagnosis of LL or MD can be made. The demonstration by immunofluorescence of specific cell surface antigenic markers

on the tumor cells (MATSA and T-cell for MD and surface IgM for LL) is the best way to establish a definite diagnosis of field cases that can not be readily diagnosed on the basis of clinical signs and gross and microscopic lesions (Fadly and Witter,1998).

MD has been well controlled in most flocks by the use of live vaccine viruses. Commercial vaccines have been prepared with all three serotypes of MDV. Serotype 1 MD vaccines are attenuated mild MDV, represented by the Rispens strain (CVI-988) and MD11/75 (Witter, 1991). Serotype 2 MD vaccines are derived from naturally occurring non-virulent chicken herpesvirus, represented by SB-1 and 301B/1. Serotype 3 MD vaccines are derived from non-virulent Turkey herpesvirus (HVT). There is a synergetic effect when viruses from two or more types are combined together, especially between serotypes 2 and 3. (Calnek et al., 1983; Calnek and Witter, 1997).

Losses have decreased dramatically since the advent of vaccination in the early 1970s to early 1980s due to efficient vaccination with HVT. However, because of the emergence of vvMDV, HVT alone did not provide adequate protection. In late 1980s, bivalent vaccines were developed and provided good protection for over ten years before more virulent forms of field virus emerged (Witter, 1997b). More virulent field MDV, now termed vv+MDV, emerged in the early 1990s and is not well protected against by bivalent HVT and SB-1 vaccines. Results of four consecutive trials by Witter (1996) and Kreager (1998) indicated that Rispens, whether given alone or in bivalent or trivalent combinations, provides better protection against these vv+MDV than other

licensed vaccine strains. Vaccines are administered by inoculation of newly hatched chicks or 18-day-old embryos.

From several reports, it is known that there are interactions between Retrovirus and MDV. Bacon et al. (1989) Reported serotype 2 MDV vaccine increased ALV-A-induced LL in certain lines of chickens and serotype 3 MDV vaccine did not augment LL incidence. Pulaski et al. (1992) reported that SB-1 has been increasing the production of ALV virus in vitro. Marsh et al. (1995) reported chickens inoculated with serotype 2 MDV and subgroup A avian leukosis virus had higher ALV-induced hyperplastic follicles in bursa. Aly et al. (1996) reported that serotype 2 MDV also augmented both ALV- and REV-induced bursal lymphoma. Salter et al. (1999) reported that serotype 2 MDV vaccine virus, strain SB-1, a component of the bivalent MD vaccine increased the bursal lymphoma incidence in chickens carrying ALVA6 (subgroup A ALV envelope glycoprotein).

Clearly, interactions between subgroup A ALV and serotype 2 MDV is well documented, but it is not known whether various strains of MDV influence subgroup J ALV-induced infection.

The objective of this study was to determine the influence of various MDV serotypes on ALV-J-induced infection and tumors in two different lines of chickens.

MATERIAL AND METHODS

VIRUSES:

Strain ADOL- Hc1, of ALV-J; stock titer of $10^{5.38}$ tissue culture infectious units (TCIU)/ml

Strain RAV-1, of ALV-A

Serotype 1 vv+ MDV, strain 648A

Serotype 2 MDV, strain SB-1

Serotype 3 MDV, strain HVT (FC-126)

Dr. R.L. Witter, Avian Disease and Oncology Laboratory (ADOL) East Lansing, Michigan kindly provided all Marek's disease viruses.

CHICKENS: Fertile eggs were obtained from: 1) White Rock breeder flock maintained at USDA-ARS Southeast Poultry Research Laboratory, in Athens, Georgia, and 2) commercial meat-type broiler breeder flock. The White Rock breeders are known to be free from infection with ALV subgroups A, B, J as well as MDV.

All embryonated eggs were hatched at ADOL. In both experiments, chickens were maintained in isolators provided with filtered air under positive pressure from the day of hatch until the end of the experiments. Chickens in each treatment group were housed in separate isolators.

VIROLOGIC ASSAYS FOR ALV-J: At various ages, samples of blood and cloacal swabs were collected from chickens in all groups. Blood was obtained from jugular or radial vein and collected into sterile tubes containing heparin as

anticoagulant. Samples of blood and cloacal swabs were centrifuged at 1500 rpm for 10 minutes at 4 C and tested for ALV as previously described (Fadly and Witter, 1998). Briefly, samples were inoculated on Line 0 CEF in 35 mm plates; 7-9 days later cell lysates were tested for the presence of ALV group specific antigen (p27) by an enzyme-linked immunosorbent assay (ELISA) (Smith et al., 1979).

SEROLOGIC ASSAYS FOR ALV-J: The virus neutralization (VN) test was used to test samples for antibody to ALV-J as described by Fadly and Witter (1998). Plasma samples were inactivated at 56 C for 30 minutes and diluted 1:5 in tissue culture medium without serum. Fifty microliter of strain ADOL-Hc1 of ALV-J, with a titer of 10^3 infectious unit/ml was added to each well of a 96 well Dynatech Immulon I U-bottom microtiter plate. Fifty microliter of plasma samples were added to each well. After incubation for 45 minutes at 37C, $5X10^4$ of line 0 CEF cells (150 microliter) were added to each well. Plates were incubated for 7-9 days. After freezing and thawing for two times, samples were tested for p27 with ELISA. Samples that tested negative by ELISA were considered positives for antibody. Positive and negative controls were included for each test.

VIROLOGIC ASSAYS FOR MDV: Whole blood was collected from the jugular vein into sterile tubes using heparin as anticoagulant. Samples were centrifuged at low (700) rpm. Peripheral blood monocytes were collected and inoculated on line 0 CEF cell culture. Cultures were incubated for 5-7 days and examined for MDV-induced plaque. Samples with plaques were scored positive for MD infection.

EXPERIMENTAL DESIGN:

The experimental design for both experiment 1 and 2 is shown in Table 1.

EXPERIMENT 1: Day-old progeny chicks of White Rock breeders were divided into 8 groups; 20 chicks in each group. Chicks in groups 1, 2, 3, 4, 5, 6 were inoculated with 2X 10^3 infectious unit of strain ADOL-Hc1 at one day of age. Chicks in groups 7 and 8 were inoculated with $2X10^3$ infectious unit of strain RAV-1 of ALV subgroup A. Also at hatch, chicks in groups 1, 4, and 7 were inoculated with a mixture of HVT, 1500 PFU/0.1ml, and SB-1, 1500 PFU/ 0.1ml. Chicks in groups 2 and 5 were inoculated with 500 PFU/ 0.2ml of Rispens, strain of MDV. At 2 weeks of age, chickens in groups 1, 2, 3 were challenged with 500 PFU/ 0.1ml of vv+ MDV strain 648A. Chickens in all treatment groups were inoculated with respective inoculum via intra-abdominal route.

All chickens in experiments 1 and 2 were tested for ALV-J at hatch. In addition, at two weeks of age, immediately before challenge with vv+ MDV chickens in groups 1, 2 and 3 were tested for the presence of various strains of MDV vaccines, HVT, SB-1 and Rispens.

At 4, 8, 12 and 24 weeks of age, all chickens were tested for ALV-J induced viremia, cloacal shedding and antibody (Fadly and Witter, 1998).

EXPERIMENT 2: Day-old progeny chicks from a commercial broiler flock were divided into 8 groups; 20 chicks in each group. The experimental design for experiment 2 was the same as that in experiment 1, except the experiment was terminated at 17 weeks of age.

PATHOLOGY: In both experiments, chickens that died and those that survived the experiment (24 weeks, experiment 1; 17 weeks, experiment 2) were necropsied. Chickens were examined for ALV-J- and MDV-induced tumors. Tissues were fixed in 10% formalin buffer and paraffin embedded, sections were stained with hematoxilyn and eosin.

STATISTICS: The SAS Institute Inc. (1999), SAS/STAT Software NC: SAS Institute Inc. was used in chi-square tests to analyze the differences between chickens in groups for viremia, cloacal shedding and antibody levels. Significance was assumed at the 0.05 level of probability (Allison, 1999).

Table 1: Experimental design for experiments 1 and 2

	Treatment					
	1st day of age					2 weeks of age
Group	ALV-J	ALV-A	HVT	SB-1	Rispens	vv+MDV
1	+	-	+	+	-	+
2	+	-	-	-	+	+
3	+	-	-	-	-	+
4	+	-	+	+	-	-
5	+	-	-	-	+	-
6	+	-	-	-	-	-
7	-	+	+	+	-	-
8	-	+	-	-	-	-

Strain ADOL-Hc1 of Subgroup J ALV
Strain RAV-1 of Subgroup A ALV
Serotype 3 MDV vaccine, strain FC-126
Serotype 2 MDV vaccine, strain SB-1
Serotype 1 MDV vaccine, strain Rispens
Serotype 1 vv+MDV challenge ,strain 648A

RESULTS

Experiment 1:

Tables 2A and 2B show the incidence and percentage of ALV-J-induced viremia, antibody and cloacal shedding in White Rock chickens used in experiment 1. At 4 weeks of age, there was no significant difference in incidences of viremia among chickens in various groups. At 8 weeks of age, the incidence of viremia in chickens in groups 1 and 4 that had been inoculated with bivalent vaccine (HVT+SB-1) were significantly lower than that in other groups. At 12 weeks of age, chickens in group 1 that received HVT+ SB-1 at hatch had the lowest level of viremia, and chickens in group 2 that received Rispens at hatch and vv+ MDV at 2 weeks of age had the highest level of viremia. There was no significant difference among chickens in groups 5 and 6.

At 24 weeks of age, chickens in groups 1 and 4 had no or little evidence of viremia. Again the highest level of viremia was noted in chickens in group 2 that had been vaccinated with Serotype 1 vaccine at hatch and challenged with vv+MDV at 2 weeks of age.

As viremia levels decreased in chickens in groups infected with ALV-J and vaccinated with bivalent MDV vaccine (from 4 weeks through 24 weeks), the antibody levels were increasing. Chickens in groups 1 and 4 had the highest antibody levels at 24 weeks of age.

Cloacal shedding was detected at 4, 8, 12 and 24 weeks of age. At 4 weeks and 8 weeks of age, the incidence of cloacal shedding in chickens in all

groups was very low if any. At 12 weeks of age the incidence of shedding in chickens in groups 2 and 6 was higher than that in chickens in other groups. At 24 weeks of age, the highest shedding incidence was detected in chickens in group 2, whereas, the lowest incidence was in chickens in groups 1 and 4. Due to MDV-induced mortality, only one chicken survived to 24 week in group 3.

The very low incidence of viremia, shedding and antibody of ALV-A in White Rock chickens suggest that the breeder flock is genetically resistant to infection with ALV-A.

Table 2 A. ALV-J- induced viremia, shedding and antibody in White Rock strain of chickens (Experiment 1)

		Age (weeks)											
		4			8			12			24		
Group	Treatment	V	S	A	V	S	A	V	S	A	V	S	A
1	ALV-J / HVT - SB-1/ vv+ MDV	15/18	0/18	1/18	5/16	0/16	9/16	1/13	1/14	14/14	0/11	1/11	11/11
2	ALV-J / Rispens / vv+MDV	19/19	3/19	2/19	17/19	1/19	7/19	13/18	11/18	10/17	11/12	8/12	7/14
3	ALV-J / vv+MDV	15/18	1/18	4/18	6/7	0/7	0/7	1/2	0/2	1/2	1/1	0/1	1/1
4	ALV-J / HVT - SB-1	18/20	1/20	1/20	7/20	0/20	15/20	6/20	3/20	18/20	2/19	1/19	17/19
5	ALV-J / Rispens	18/19	0/19	0/19	14/19	1/19	10/19	9/19	7/19	15/19	9/19	8/19	11/19
6	ALV-J	19/19	2/19	3/19	17/19	0/19	10/19	11/19	13/19	15/19	3/3	1/3	2/3
7	ALV-A / HVT- SB-1	2/19	0/19	2/19	2/19	0/19	0/19	2/19	1/19	0/19	1/18	0/18	0/18
8	ALV-A	1/19	1/20	4/18	0/20	0/20	0/17	4/16	0/16	0/16	NA	NA	NA

V= viremia S= cloacal shedding A= neutralizing antibody

Chickens were observed for 24 weeks. No + / No total chickens

NA = Not applicable

Table 2 B. Percentage of ALV-J- induced viremia, shedding and antibody in White Rock chickens (Experiment 1)

		Age (week)											
		4			8			12			24		
Group	Treatment	V	S	A	V	S	A	V	S	A	V	S	A
1	ALV-J / HVT-SB-1/ vv+MDV	83 a	0 a	5 a	31 a	0 a	56 a	8 a	7 a	100 a	0 a	9 a	100 a
2	ALV-J / Rispens / vv+MDV	100 a	15 a	10 a	89 b	5 a	36 a	72 b	61 b	59 b	91 b	66 b	50 b
3	ALV-J / vv+MDV	83 a	5 a	22 ab	85 b	0 a	0	NA	NA	NA	NA	NA	NA
4	ALV-J / HVT - SB-1	90 a	5 a	5 a	35 a	0 a	75 ab	30 ac	15 a	90 a	10 a	5 a	89 a
5	ALV-J / Rispens	94 a	0 a	0 a	73 b	5 a	53 a	47 bc	37 b	79 ab	47 c	42 b	58 b
6	ALV-J	100 a	10 a	15 a	89 b	0 a	53 a	57 bc	68 b	79 ab	NA	NA	NA

V= viremia S= cloacal shedding
A = neutralizing antibody
For each age at testing, percentages within a column followed by different letters are significantly different ($p<0.05$)
NA = not applicable

Experiment 2:

Table 3A and 3B shows the incidence and percentage of ALV-J-induced viremia, antibody and cloacal shedding, respectively in commercial broiler breeder chickens used in experiment 2. In this experiment commercial broiler breeders were tested for viremia, shedding and antibody at 4, 8 and 12 weeks of age. The experiment was terminated at 17 weeks of age.

At 4 weeks of age, there was no significant difference in incidences of viremia among chickens in various groups. At 8 weeks of age, the incidence of viremia for chickens in groups 1 and 4, inoculated with bivalent vaccine (HVT+SB-1) was significantly lower than that in any of the other groups.

At 12 weeks of age, except for chickens in group 2 that were inoculated with Rispens/ vv+ MDV there was no statistically significant difference in ALV-J viremia among chickens in various groups. Chickens in group 2 had the highest frequency of viremia. At 17 weeks of age, chickens in groups 1 and 4 that were inoculated with bivalent vaccine had the lowest frequency of viremia. Although there was no statistical difference among chickens in groups inoculated with ALV-J/ Rispens/vv+MDV and only ALV-J, the viremia levels were highest in the former group.

At 17 weeks of age, cloacal shedding was significantly higher in chickens in groups inoculated with ALV-J/Rispens/vv+MDV compared to with chickens in other groups.

At 4 weeks of age, antibody was not detected in chickens in any group, but there was a low level of shedding and the incidence of antibody positive birds

was increasing in all groups. Antibody was not detected in chickens used in experiment 2. At 17 weeks of age, there was no significant difference in antibody among chickens in all groups. Also, at 17 weeks of age, cloacal shedding was highest in chickens in group 2, and there was difference in antibody frequency between chickens in group 2 and chickens in groups 1, 4, 5, and 6. Due to MDV-induced mortality in group 3 only one chicken survived to 17 weeks of age. Commercial broiler breeder chickens used in this experiment were also found to be resistant to infection with subgroup A -ALV.

Tumor incidence in experiments 1 and 2:

Table 4 shows the incidence of ALV-J and MD-induced tumors in MD vaccinated and unvaccinated chickens. In both experiments chickens in groups infected with only ALV-J or ALV-J and Rispens MD vaccine developed the highest frequency of ALV-J -induced tumors. Only 3 birds developed ML in White Rock chickens. Commercial broiler breeder chickens most of the ALV-J-induced tumors were LL. Several other type of tumors such as myxosarcoma, erythroblastosis and hemangioma were detected in both experiments. In White Rock chickens although the groups infected with ALV-J/Rispens/vv+MDV and ALV-J/Rispens had the highest tumor incidence, there was no significant difference among other groups.

In commercial broiler breeder chickens there was significant difference between group 5 and groups 1, 2, 4. Although there was no significant difference, the ALV-J-induced tumor incidence was highest in chickens infected

with ALV-J and vaccinated with Rispens compared with all other groups in broiler breeder progeny.

Influence of MDV on ALV-J- induced tolerant infection:

Table 5 shows the influence of various strains of MDV on the incidence of ALV-J tolerant infection in meat-type chickens. Because the results from experiments, 1 and 2 were similar therefore, the data were pooled. Chickens inoculated with HVT + SB-1 had the highest incidence of immunity against ALV-J infection. Whereas chickens inoculated with serotype 1 MDV had the highest incidence of tolerantly infected chickens.

Table 3 A. ALV-J- induced viremia, shedding and antibody in commercial broiler breeder chickens (Experiment 2)

		Age (week)											
		4			8			12			17		
Group	Treatment	V	S	A	V	S	A	V	S	A	V	S	A
1	ALV-J/ HVT- SB-1/ vv+MDV	14/19	3/19	0/19	12/19	10/19	4/19	10/18	11/18	10/18	6/16	8/16	9/16
2	ALV-J / Rispens / vv+MDV	18/19	1/18	0/18	17/17	16/17	0/17	14/16	15/16	6/16	13/16	14/16	6/16
3	ALV-J / vv+MDV	14/18	4/18	0/18	1/3	1/3	2/3	0/1	0/1	1/1	0/1	0/1	1/1
4	ALV-J / HVT- SB-1	15/20	5/20	0/20	10/20	10/20	5/20	8/17	11/17	10/17	6/17	10/17	11/17
5	ALV-J / Rispens	19/20	4/20	0/20	15/20	9/20	4/20	13/20	8/20	9/20	7/16	9/16	9/16
6	ALV-J	15/20	5/20	0/20	14/20	8/20	9/20	12/19	8/19	13/19	9/18	9/18	10/18
7	ALV-A/ HVT-SB-1	1/10	0/10	0/10	2/10	0/10	0/10	1/9	0/9	0/9	0/9	0/9	0/9
8	ALV-A	1/8	0/8	0/8	1/8	1/8	0/8	0/8	1/8	4/8	0/7	2/7	4/7

V= viremia S= cloacal shedding
A= neutralizing antibody
Chickens were observed 17 weeks.
No +/ No total chickens

Table 3 B. Percentage of ALV-J-induced viremia, shedding and antibody in commercial broiler breeder chickens

Group	Treatment	Age (week)											
		4			8			12			17		
		V	S	A	V	S	A	V	S	A	V	S	A
1	ALV-J/ HVT-SB-1/ vv+MDV	74 A	16 A	0 A	63 A	53 A	21 A	55 A	61 A	55 A	35 A	50 A	56 A
2	ALV-J / Rispens / vv+MDV	95 B	5 A	0 A	100 B	94 B	0 B	88 B	93 B	38 A	81 B	88 B	38 A
3	ALV-J / vv+MDV	78 A	22 A	0 A	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	ALV-J / HVT-SB-1	75 A	25A	0 A	50 A	50 A	25 A	47 A	65 A	59 AB	35 A	59 A	65 A
5	ALV-J / Rispens	95 B	20 A	0 A	75 AB	45 A	20 A	65 A	40 A	45 A	44 A	56 A	56 A
6	ALV-J	75 A	25 A	0 A	70 AB	40 A	45 A	63 A	42 A	68 AB	50 AB	50 A	56 A

V= viremia S= cloacal shedding
A= neutralizing antibody
For each age at testing, percentages within a column followed by different letters are significantly different ($P<0.05$)
NA = not applicable

Table 4: Tumor incidence in White Rock and commercial broiler breeder chickens

Treatment[A]	Chicken Line: White Rock						Commercial broiler breeder					
	No at risk[B]	MD (%)	ALV-J ML	LL	other[C]	total(%)	No at risk	MD (%)	ALV-J ML	LL	other	total(%)
ALV-J/HVT+SB-1/vv+MDV	14	4(28)	0	0	1	1(7)	17	3(18)	0	1	1	2(12)
ALV-J/Rispens/vv+MDV	16	5(31)	0	2	1	3(19)	17	7(41)	0	1	0	1(6)
ALV-J/vv+MDV	11	9(81)	0	0	1	1(9)	14	9(64)	0	4	0	4(28)
ALV-J/HVT+SB-1	19	0	1	1	1	3(16)	17	0	0	1	0	1(6)
ALV-J/Rispens	19	0	1	3	0	4(21)	17	0	0	6	1	7(41)
ALV-J	5	0	1	3	0	4(80)	19	0	0	4	0	4(21)

[A]Chickens were inoculated with ADOL-Hc1 strain of ALV-J and with various serotypes of MD vaccines (HVT, SB-1, or Rispens) at hatch; chickens were challenged with strain 648A of MDV at two weeks of age.

[B]No at risk = no. of chickens died with tumors + no. of chickens survived the experiment

[C]Other = myxosarcoma, erythroblastosis, hemangioma

ML = Myeloid leukosis

LL = Lymphoid leukosis

Table 5: Influence of virulent and vaccine strains of MDV and ALV-J tolerant infection in 17-24 week- old meat-type chickens[A]

Lot #	Treatment					Status of ALV-J infection	
	ALV-J	HVT	SB-1	Rispens vv+MDV		V+S+A-[B]	V-S-A+[C]
1	+	+	+	-	+	6/27 (22) a	16/27 (59) a
2	+	-	-	+	+	16/30 (53) b	2/30 (6) b
3	+	-	-	-	+	NA [D]	NA
4	+	+	+	-	-	5/26 (19) a	22/26 (85) c
5	+	-	-	+	-	14/25 (56) b	7/25 (28) b
6	+	-	-	-	-	7/21 (33) ab	7/21 (33) ab

[A]At hatch chickens were inoculated with strain ADOL-Hc1 of ALV-J and with MDV vaccines (HVT, SB-1, or Rispens) at hatch; chickens were challenged with strain 648A of MDV at 2 weeks of age. Because virological and serological results at 24 weeks of age (experiment 1) and at 17 weeks of age (experiment 2) were similar, the data were pooled.

[B]Tolerantly infected chickens (viremic, shed virus in cloacal sections and lacked antibody).

[C]Immune chickens (non-viremic, non-shedder and antibody positive).

[D]NA = Not applicable, all chickens died from MDV within 8 weeks of age.
For each age at testing percentages within a column followed by different letters are significantly different ($P<0.05$)

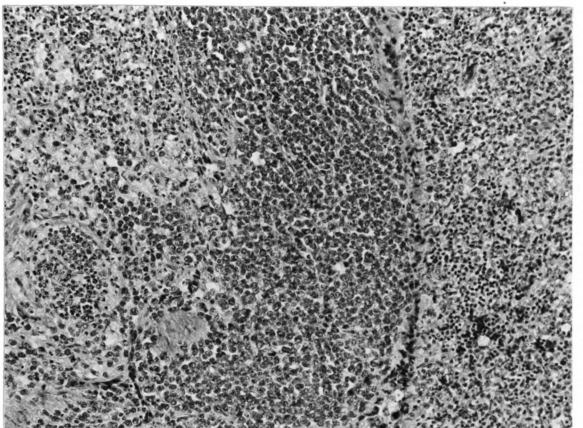

Figure 1. Photomicrograph of liver from 24-weeks-old White Rock chicken with ML infected with ADOL-Hc1 strain of ALV-J at hatch. Note typical lesions of ML, namely accumulated myelocytes in parenchyma. H&E. X261

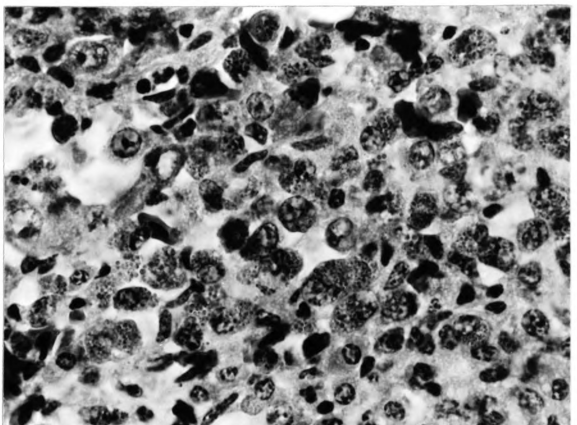

Figure 2. Photomicrograph of liver from 24-weeks-old chicken inoculated with strain ADOL-Hc1 at hatch. Note myelocytes with eosinophilic granules. H&E. X1305.

DISCUSSION

Data from experiments 1 and 2 demonstrate that chickens from both White Rock and commercial broiler breeder flocks are susceptible to infection with strain ADOL-Hc1 of ALV-J. The data from this study indicate that bivalent MDV vaccines containing serotype 2 and 3 (HVT/SB-1) may influence the response of chickens to infection with ALV-J. Viremia results from experiment 1 suggest that the bivalent MDV vaccine used in this study significantly reduced the incidence ALV-J viremia in White Rock chickens. At 8 and 24 weeks of age, the incidence of chickens with ALV-J viremia was the lowest in groups inoculated with bivalent MDV vaccine (groups 1 and 4), regardless of challenge with vv+MDV suggesting that bivalent vaccine can also reduce ALV-J viremia in chickens exposed to virulent virus. Chickens in group 1 that developed viremia cleared the virus by 24-weeks of age when antibody levels were detected in 100% of the chickens.

In both experiments chickens received ALV-J/Rispens and challenged with 648A strain of vv+MDV had the highest incidence of cloacal shedding. Cloacal shedding rates of ALV-J in chickens in groups infected with ALV-J and vaccinated with bivalent MDV vaccine, was very low, compared to the other groups, suggesting that there is also some influence of bivalent vaccine on ALV-J shedding. In contrast the chickens in groups infected with ALV-J and vaccinated with serotype 1 MD had the highest frequency of cloacal shedding whether challenged or not with vv+MDV.

Bacon et. al. (1989) reported that serotype 2 MDV, but not HVT, may augment the development of subgroup A ALV-induced lymphoma in certain lines of chickens. Pulaski et. al. (1992), Coussens (1994), reported that there is a direct relationship between serotype 2 MDV and subgroup A-ALV, which results in enhanced retroviral gene transcription. In the present study, bivalent vaccine containing serotype 2 and 3 did not enhance the viremia of ALV-J, but appeared to enhance the clearance of viremia of ALV-J in birds inoculated with these two serotypes in the White Rock strain.

Data from experiment 2 confirmed data from experiment 1, indicating that serotype 1 MD vaccine with vv+MDV increased susceptibility of chickens to ALV-J-induced infection. There had been an enhancement of ALV-J-induced viremia levels in chickens, which received ALV-J and serotype 1, Rispens strain of MD vaccine and challenged with 648A strain of vv+MDV. Although there was no statistically significant difference between chickens in group 1 and chickens in groups 4, 5 and 6 in experiment 2, viremia levels were low (35%) at 17 weeks of age. These birds in both groups were inoculated with bivalent serotype 2 and 3 MDV vaccine and whether challenged or not with vv+MDV.

There are reports that the incidence of tumor-associated mortality is widely variable between ALV-J infected flocks, and may involve additional factors such as the presence of immunosuppressive agents, concurrent infections, and vaccination against other diseases (Venugopal, 1999). In the present study, it was demonstrated that serotype1 of MDV may enhance the strain ADOL-Hc1 of ALV-J infection, but not tumor development in commercial broiler breeder

chickens used in Experiment 2. In the same experiment there was no difference between chickens infected with only ALV-J or ALV-J/Rispens strain of MDV for ALV-J induced viremia. The enhancement of viremia probably due to 648A strain of vv+MDV.

ALV-J induced tumors in meat-type chickens are primarily ML (Arshad et al.,1999). In our study only 3 ML were seen in White Rock chickens. These were in groups infected with ALV-J alone and various vaccine strains of MD. The tumors in all groups in both experiments were mainly LL. This difference in type of tumors can be probably explained by strain of chickens and virus used in the current experiments. Myeloid tumors were same as described by Payne et al., (1992, 1993).

Payne et al. (1992), reported that there were type B like MDV nerve lesions in chickens infected with ALV-J. In both experiments in this study, the type B nerve lesions were seen only in chickens that were infected with 648A strain of vv+MDV, isolated from layers which causes 100% MD and bursal thymic atrophy in non-vaccinated chickens and 98% nerve lesions in bivalent MD vaccinated chickens (Witter, 1996). Strain HPRS-103 of ALV-J showes a lower propensity to replicate in the medullary region of the lymphoid follicles of the bursa of Fabricius, in comparison to subgroup A (Venugopal, 1999). Supporting the same suggestions in our study in both experiments bursal tumors were not seen in chickens which were infected with strain ADOL-Hc1 of ALV-J. Due to vv+MDV strain 648A there were bursal thymic atrophy in groups infected with 648A strain.

In meat-type chickens, post-hatch infection of chickens with ALV-J can result either in tolerant viremic infection (V+S+A-), similar to congenitally infected chickens, or in an immune status after showing a transient viremia. A proportion of the immune chickens may also shed (V-S+A+) the virus. Chickens exposed to the virus shortly after hatch tend to develop tolerant viremia (V+S+ A-), while exposure at an older age leads to immune chickens that may or may not shed the virus (Venugopal, 1999). In the current study, chickens infected with strain ADOL-Hc1 of ALV-J were mostly tolerant viremic and immune shedders; these results are in agreement with those reported by Venugopal (1999). Chickens in groups infected with ALV-J/Rispens and challenged with vv+MDV were mostly tolerant viremic (V+S+A-), in contrast chickens infected with bivalent vaccine (HVT/SB-1) which were mostly non-viremic, non-shedder and antibody positive (V-S-A+).

Chickens infected with ALV-J/Rispens/vv+MDV were tolerant viremic (V+S+A-) and chickens infected with ALV-J/Rispens were both tolerant viremic (V+S+A-) and immune non-shedders (V-S-A+), suggesting that vv+MDV may influence the infection status of ALV-J infected chickens. Chickens infected with ALV-J/Rispens were both V+S+A- and V-S-A+.

MD-induced tumors were more frequently encountered in chickens infected with ALV-J/Rispens/vv+MDV than in those infected with ALV-J/HVT+SB-1/vv+MDV, regardless of strain of chickens used. Results reported in this study are interesting and warrant further investigations.

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