FURTHER STUDIES OF THE PATHOGENESIS OF INFECTIOUS BURSAL DISEASE IN CHICKENS

By

Nader Mounir Ismail

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ABSTRACT

FURTHER STUDIES OF THE PATHOGENESIS OF INFECTIOUS BURSAL DISEASE IN CHICKENS

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White Leghorn chickens of various lines were resistant to clinical manifestations of infectious bursal disease (IBD) following inoculation with IBD virus (IBDV) at 1 day of age. In contrast, chickens inoculated with IBDV at 6 weeks of age were susceptible to IBD, regardless of line of chicken used.

Neonatally chemically bursectomized chickens inoculated with at least 62 X 10^6 bursa cells were as susceptible to clinical manifestations of IBD as intact chickens following inoculation with IBDV at 5 weeks of age. In contrast, chemically bursectomized chickens inoculated with 2.5 X 10^6 or less bursa cells were refractory to clinical manifestations of IBD.

Chickens inoculated with IBDV following surgical bursectomy at 5 weeks of age did not develop IBD. The concentration of IBDV in spleens of surgically bursectomized chickens was about 100 times lower than that in intact chickens.

These results suggest that the susceptibility of chickens to clinical manifestations of IBD is dependent on the number of target bursa cells available. Further, the data indicate that age at infection but not the genetic constitution of chickens may influence the response of chickens to infection with IBDV.

INTRODUCTION

Infectious bursal disease (IBD) is an acute contagious disease of young chickens caused by a Diplorna virus (Hirai and Shimakura, 1974; Harkness, et al., 1975; Pattison, et al., 1975). The disease affects primarily the bursa of Fabricius (BF); other lymphoid organs are also affected but to a lesser degree (Cheville, 1967; Lukert and Hitchner, 1984). The disease is considered to be of economic importance not only because of the morbidity and mortality it can inflict on growing chickens but also because of its immunosuppressive effects. Losses in conventional flocks are primarily due to reduced weight gain and condemnation of the carcasses because of marked hemorrhage in the skeletal muscles. Chickens are most susceptible to IBD between the ages 3-6 weeks (Lukert and Hitchner, 1984). However, an IBD outbreak in 14- 15-week-old White Leghorn chickens has been reported (Ley, et al., 1979a). One of the earliest signs of IBD virus (IBDV) infection in chickens is the tendency for some chickens to pick at their own vents (Cosgrove, 1962). Affected chickens show soiled vent feathers, whitish or watery diarrhea, anorexia, depression, ruffled feathers, trembling, severe prostration, dehydration and finally death (Lukert and Hitchner, 1984). In susceptible chickens the

disease appears suddenly and there is a high morbidity rate, usually approaching 100% (Lukert and Hitchner, 1984). Actual mortality may be nil but can be as high as 20-30% (Lukert and Hitchner, 1984). Mortality in specific pathogen free (SPF) chickens may reach as high as 100% (Muller, et al., 1979). The most characteristic lesion of IBD is necrosis of the BF with subsequent atrophy of that organ (Cheville, 1967). An early age resistance to clinical manifestations of IBD, regardless of maternal antibody status, has been proposed (Hitchner, 1971). However, infection of chickens with IBDV during the first week after hatching may lead to severe defects of humoral immune response as a consequence of the early destruction of the BF (Faragher, et al., 1974; Ivanyi and Morris, 1976; Hirai, et al., 1979b). Older chickens with regressed bursa do not show signs of illness upon infection with IBDV (Hitchner, 1976). However, an IBD outbreak in 14- and 15-week-old chickens has been reported (Ley, et al., 1979a). Severity of clinical signs of IBD in chickens infected with virus at various ages has been studied by several workers. Skeeles et al. (1979a) suggested that mortality and many of the clinical signs seen with IBD are associated with a depletion in circulating level of hemolytic complement. Immune complexes have been detected in the renal glomeruli of susceptible chickens infected with IBDV (Ley, et al, 1979b). In a later study, injection of chickens with IBD hyperimmune serum during IBDV infection,

in an attempt to produce immune complexes, did not increase severity of disease in either 2- or 6-week-old susceptible chickens (Skeeles et al., 1979b). Those investigators also reported that IBDV neutralizing antibody rose rapidly at 3-5 days postinfection (PI) in groups of chickens inoculated with virus at either 2 or 8 weeks of age. However, the mean titer of IBDV neutralizing antibody in chickens inoculated with virus at 8 weeks was higher than in those inoculated at 2 weeks of age. Skeeles et al. (1980) noted a clotting abnormality in chickens that developed severe IBD (inoculated with IBDV at 42 days of age), but not in chickens that remained clinically normal (inoculated with IBDV at 17 days of age). Apparently, infection of chickens with IBDV at 42 days of age results in more damage to bursal tissue and more tissue thromboplastin is released to circulation, thus influencing the coagulation scheme.

Evidence that the BF is the primary target organ for IBD is well documented (Cheville, 1967; Fadly, et al., 1976; Hitchner, 1978; Kaufer & Weiss, 1980). Fadly, et al. (1976) demonstrated that neonatal treatment with cyclophosphamide (CY) rendered chickens resistant to clinical IBD. However, whether IBDV replicated in such CY-treated chickens was not determined. Recently IBDV-induced clinical signs and mortality were shown to be more evident in chickens inoculated with virus at 5 weeks than in those inoculated at 1 or 11 weeks of age (Fadly & Nazerian, 1983). However, the rate of

virus replication, as determined by virus isolation and immunofluorescence test, was the same in various groups of chickens. Kaufer and Weiss (1980) found that surgical bursectomy of chickens at 4 weeks of age prevented the development of IBD lesions. Further, the concentration of IBDV in the spleen, thymus, and liver of bursectomized chickens was about 1,000 times lower than that of intact chickens. Schat, et al. (1981) found that embryonal bursectomy of chickens delayed but did not prevent the appearance of IBD-infected cells. Moreover, the BF or antibody-producing cells were not required to initiate and sustain infection with IBDV. Review of the literature revealed no report of the relationship between the number of target bursa cells available and the susceptibility of chickens to clinical manifestations of IBD.

The objectives of this investigation were to 1) compare pathogenesis of IBD in various lines of White Leghorn chickens inoculated with virus at 1 or 6 weeks of age; 2) compare the pathogenesis of IBD in chickens neonatally chemically bursectomized and subsequently inoculated with various numbers of bursa cells with that in intact chickens; and 3) determine whether IBDV can induce disease in surgically bursectomized chickens.

REVIEW OF THE LITERATURE

Etiology:

A specific disease entity affecting the BF of chickens was reported by Cosgrove in 1962. The disease was called "avian nephrosis" because of the extreme kidney damage in chickens that succumbed to infection. Winterfield and Hitchner (1962) described an isolate (Gray) of infectious bronchitis virus obtained from the kidney of a field case of avian nephrosis. A small percentage of the chicks experimentally inoculated with this virus died and exhibited nephrosis not unlike that seen in the newly reported syndrome. Because of the similarity between the kidney lesions induced by the Gray virus and those seen in avian nephrosis as described by Cosgrove (1962), it was first believed that the Gray virus was the causative agent of IBD. Later studies, however, revealed that chickens immune to the Gray virus could still be infected with IBDV and would develop changes in the BF specific for that disease. In subsequent studies with chickens affected with IBD, Winterfield, et al. (1962) succeeded in isolating an agent in chicken embryos. The mortality pattern was irregular and the agent was difficult to maintain on serial passages. The isolate was referred to as "infectious bursal agent" and was

shown to be the true cause of IBD. Original filteration of a bursal homogenate (Benton, et al., 1967b) indicated that the IBDV is between 10 and 50 mu in size. Further, electron microscopy has indicated that the IBDV is a naked icosahedral virus with a diameter of 55-60 nm (Hirai and Shimakura, 1974; Nick, et al., 1976). The RNA genome of IBDV is double stranded and segmented, having only 2 segments (Dobos, 1979; Muller, et al., 1979). Dobos, et al. (1979) proposed that IBDV and other similar viruses be included in a new family called birna viruses. The capsid of the IBD virion consists of four structural proteins (Nick, et al., 1976; Dobos, 1979); these proteins are arranged to give the capsid 32 capsomers (Hirai, et al., 1979a). It has not been determined which of these proteins might induce protection to the disease. McFerran, et al. (1980) classified several isolates of IBDV from chickens, turkeys and ducks into two serotypes using the neutralization test. They proposed that the cell culture adapted vaccine strain from chickens should be the prototype virus for serotype I and that the TY 89 isolate from a turkeys should be the prototype for serotype II. The isolates in serotype II consisted of an antigenically homogenous group of viruses from turkeys and chickens. However, within serotype I, which represented isolates from chickens and ducks, some isolates showed only a 30% cross reaction with the prototype strain. Similar findings were made using several strains of viruses isolated

from chickens and turkeys (Lukert & Hitchner, 1984). Isolates of IBDV from turkeys were shown to belong to a new serotype designated serotype II, and the previously known IBDV of chicken was designated serotype I (Lukert, et al., 1981; Nusbaum, et al., 1981; Jackwood, et al., 1982). Many strains of IBDV have been adapted to propagate in chicken embryo cell culture. Virus-induced cytopathic effect (CPE) has been characterized by aggregates of small refractile cells, followed by cytonecrotic areas that eventually spread to the entire cell sheet (Cho, et al., 1979). Cell culture adapted virus may be quantified by plaque assay or microtiter techniques (Lukert & Hitchner, 1984).

Rinaldi, et al. (1972) and Petek, et al. (1973) were able to culture egg-adapted strains of IBDV in chicken embryo fibroblasts (CEF). These cells proved more sensitive to the virus than either chicken embryos or suckling mice. Lukert and Davis (1974) successfully adapted wild type virus obtained from BF of infected chickens in cells derived from embryonic bursa. After 4 serial passages in embryo bursa cells the virus grew in chicken embryo kidney cells and produced plaques under agar. This virus was subsequently propagated in CEF and used as an attenuated live virus vaccine (Skeeles & Lukert, 1980). Turkey and duck embryo cells have also been shown to be susceptible to IBDV (McNulty, et al., 1979). Mammalian cell lines derived from rabbit kidneys (RK-13) (Rinaldi, et al., 1972), and green

monkey kidneys (vero) (Lukert, et al., 1975) are susceptible to the virus. Hirai and Calnek (1979) propagated virulent IBDV in normal chicken lymphocytes and in a B-cell lymphoblastoid cell line derived from an avian leukosis virus-induced tumor. The virus failed to replicate in six T-cell lymphoblastoid cell lines derived from Marek's disease tumors. Those authors concluded that the IgM-bearing B lymphocytes were probably the target cells for IBDV. This conclusion received verification in a subsequent study in which normal lymphocytes (B-, T- and null cells) of chickens were infected with IBDV; of all three types of cells, only the IgM-bearing cells were susceptible to infection with the virus (Nakai & Hirai, 1981). Isolation of IBDV from field cases of the disease may be very difficult, since the virus does not always grow well in chicken embryos (Lukert & Hitchner, 1984). McFerran, et al. (1980) reported difficulty in isolating and passing IBDV in cell cultures of chicken embryo origin.

Benton, et al. (1967b) found that IBDV resisted treatment with ether and chloroform, and was inactivated at pH 12, but unaffected at pH 2. IBDV has been shown to resist heat, treatment for 5 hours at 56C (Benton, et al., 1967b). Further, the virus was also shown to be unaffected by exposure for 1 hour, at 30C, to 0.5% phenol or 0.125% Thimerosal. Marked reduction in virus infectivity was noted after treatment with 0.5% formalin for 6 hours (Benton, et

al., 1967b). Those investigators also reported that of three disinfectants (an iodine complex, a quarternary ammonium compound, a phenolic derivative) tested, only the iodine complex was effective against IBDV. Landgraf, et al. (1967) found that the virus survived 60 but not 70C for 30 minutes, and 0.5% chloramine inactivated the virus after 10 minutes. The hardy nature of this virus may explain its long persistent survival in poultry houses.

Host range:

For many years the chicken was considered the only species in which natural infection with IBDV occurred. All breeds appear to be susceptible to IBD. White Leghorns are thought to exhibit the most severe reactions and the highest mortality rate (Lukert and Hitchner, 1984). However, Morez (1966) found no difference in mortality between heavy and light breeds in a survey of 700 outbreaks of the disease. Demonstration of IBDV and antibody in turkeys and ducks have confirmed the existence of IBDV in these species (Page, et al., 1978; McNulty, et al., 1979; Johnson, et al., 1980; McFerran, et al., 1980). Giambrone, et al. (1978) found that IBDV infections of turkeys were subclinical in 3- to 6-week old poults, although microscopic lesions in the BF were seen. Those authors demonstrated virus infected cells in the bursa by immunofluorescence. Further, neutralizing antibody was detected at 12 days PI, and the virus could be

reisolated after five passages in chicken embryos. Weisman and Hitchner (1978) could not isolate IBDV from 6- to 8-week-old poults following infection with virus. Further, infection was subclinical and no damage to the bursa was evident. However, the neutralizing antibody was observed at 9 days PI. Jackwood, et al. (1983) studied the effect of serotype II IBDV isolate from turkeys on the humoral immune response of turkey poults. In all experiments, the humoral immune response of poults to different antigens was not affected by infection with two serotype II IBD viruses. Although no clinical IBD was observed in any poult, the serotype II IBDV isolates were infectious and transmissible in poults. Furthermore, the response of turkeys to infection with IBDV at various ages was recently studied (Chui and Thorsen, 1983). Pathological signs of infection were detected only in turkeys infected with virus at 1 day of age. However, age at infection did not influence the rate of detecting IBDV or antibody. Further, both cellular and humoral immune responses were suppressed in turkeys infected with virus at 1 day of age. The effects varied with virus strain. Weisman & Hitchner (1978) were unable to infect corturnix quail with a chicken strain of IBDV.

Disease prevalence:

IBD is prevalent in most concentrated poultry-producing areas of the world (Lukert and Hitchner, 1984). Initial

outbreaks were first observed on farms in the neighborhood of Gumboro, Delaware; hence, "Gumboro disease" became synonymous for the condition. Lukert and Hitchner (1984) reported that the incidence of IBD is now considered to be very high. Further, infection with IBDV can be expressed as an early subclinical before 3 weeks of age or a mild subclinical to severe clinical disease from 3 to 6 weeks of age. In many cases IBD is modified by presence of maternal immunity at time of infection. Early in the history of this disease, incidence was considered to be low with economic losses resulting only from mortality and decreased growth rate (Lukert and Hitchner, 1984). It is now recognized that the great economic losses of IBD result from the immunosuppressive effects of virus infection during the first or second week of life (Lukert & Hitchner, 1984). Current emphasis is on control of early infection via maternal immunity.

Pathogenesis:

Clinical IBD is most commonly recognized in susceptible 3- to 6-week-old chickens (Lukert and Hitchner, 1984). In both field outbreaks and experimental infections, newly hatched chicks seldom show any clinical signs or experience significant mortality (Hitchner, 1978). An early age resistance to IBDV infection regardless of maternal antibody has been proposed (Hitchner, 1971). However, infection of

chickens with IBDV during the first week after hatching may lead to severe defects of the humoral immune response as a consequence of the early destruction of the BF (Faragher, et al., 1974; Ivanyi and Morris, 1976; Hirai et al., 1979b). Older chickens with regressed bursae also show no signs of illness upon infection with IBDV (Hitchner, 1976). This upper age limit of resistance to clinical IBD may be due to natural involution of the BF which begins at 10 weeks of age (Glick, 1956). However, an IBD outbreak in 14- and 15-week-old White Leghorn chickens has been reported (Ley, et al., 1979a).

Studies on transmission of IBDV (Benton, et al., 1967a) revealed that the disease is highly contagious. IBDV is known to spread readily by direct contact. Organic materials, feed and water from an infected environment were shown to be capable of initiating infection in susceptible chickens (Lukert & Hitchner, 1984). Furthermore, there is no evidence that IBDV is transmitted through the egg or if a true carrier state exists in recovered chickens. Resistance of the virus to heat and to the most commonly used disinfectants is sufficient to account for virus durability in the environment between outbreaks of the disease.

The incubation period for IBD is very short and clinical signs of the disease are seen within 2-3 days PI (Lukert and Hitchner, 1984). Helmboldt and Garner (1964) detected histologic evidence of IBD infection in the BF

within 24 hours PI with virus. Muller, et al. (1979), using immunofluorescence techniques detected IBDV-infected gut-associated macrophages and lymphoid cells, particularly of the caecum, within 4-5 hours after oral exposure to the virus. Following replication in these cells the virus is thought to spread via the portal vein and main blood stream to various organs and tissues. In the BF, the virus finds optimal conditions and replicates rapidly and massively. Already at 11 hours PI, viral antigen is found in large amounts in the BF (Muller, et al., 1979). In contrast, at this early period, virus antigen is not detectable by immunofluorescent methods in other organs.

The earliest sign of infection in a flock is the tendency for some chickens to pick at their own vents. Cosgrove (1962), in his original report, described soiled vent feathers, whitish or watery diarrhea, anorexia, depression, ruffled feathers, trembling, severe prostration, dehydration and, finally, death. Striking features of this disease are the sudden and high morbidity rate, spiking death curve, and rapid flock recovery (Lukert and Hitchner, 1984). Initial outbreaks on a farm are usually the most acute (Lukert and Hitchner, 1984), whereas recurrent outbreaks in succeeding broods are less severe and frequently go undetected. Many infections are silent, owing to age of birds, infection with avirulent field strains, or infection in presence of maternal antibody (Lukert and

Hitchner, 1984). The reason for the apparent age susceptibility of chickens to IBDV (3 to 6 weeks of age) has been the subject of several research investigations regarding the pathogenesis of IBDV infections.

Fadly, et al. (1976) found that neonatal treatment of chickens with CY--which destroyed the lymphoid elements of the BF and abrogated humoral immunity--rendered them refractory to IBDV-induced clinical signs and lesions following infection with virus at 4 weeks of age. Kaufer and Weiss (1980) demonstrated that surgical bursectomy protected chickens against clinical IBD. However, the titer of IBDV in the liver, spleen and thymus of bursectomized chickens was 1000 times less than that of intact chickens. Further, IBDV infection in these chickens resulted in very discrete and transient necrosis of lymphatic tissues. Schat, et al. (1981) studied the pathogenesis of IBD in chickens that had been embryonally bursectomized and subsequently inoculated with IBDV at 2 or 6 weeks of age. Results from those studies indicated that the BF or antibody producing cells were not required to initiate and sustain infection with IBDV. Moreover, hemorrhagic lesions were observed more frequently in embryonally bursectomized chickens than in intact chickens. Skeeles, et al. (1979a) attempted to show that IBD lesions were a result of formation of immune complexes as proposed by Ivanyi and Morris (1976). Histological lesions in the BF resemble an Arthus reaction (necrosis, hemorrhage,

large number of polymorphonuclear cells). This reaction is a type of immunologic injury induced by antigen-antibody complexes and complement. The hypothesis is that either antibody or complement is deficient early in the life of the chicken and therefore the Arthus reaction would not occur. Skeeles, et al. (1979a) demonstrated that 2-week-old chicks produce antibody just as fast as 8-week-old chicks; however, the former had very little complement. They also showed that complement was depleted in IBDV-infected chickens at 3, 5, and 7 days PI. Immune complexes have been detected in the renal glomeruli of susceptible chickens infected with IBDV (Ley, et al., 1979b). In a later study, injection of chickens with IBD hyperimmune serum during IBDV infection, in an attempt to produce immune complexes, did not increase severity of disease in either 2- or 6-week-old susceptible chickens (Skeeles, et al., 1979b). Those investigators also reported that IBDV neutralizing antibody rose rapidly at 3-5 days PI in groups of chickens inoculated with virus at either 2 or 8 weeks of age. However, the mean titer of IBDV neutralizing antibody in chickens inoculated with virus at 8 weeks was higher than in those inoculated at 2 weeks of age. Skeeles, et al. (1980) noted a clotting abnormality in chickens that developed severe IBD (inoculated with IBDV at 42 days of age), but not in chickens that remained clinically normal (inoculated with IBDV at 17 days of age). Apparently infection of chickens with IBDV at 42 days of age

results in more damage to bursal tissue and more tissue thromboplastin is released to circulation, thus influencing the coagulation scheme.

Gross lesions of IBD have recently been reviewed (Lukert & Hitchner, 1984). IBDV-infected chickens are usually dehydrated, with marked discoloration of pectoral muscles. Frequently, hemorrhages are present in the thigh and pectoral muscles (Cosgrove, 1962). There is increased mucus in the intestine, enlargement of the BF and renal changes (Cosgrove, 1962) may be prominent in chickens that die or are in advanced stages of the disease. Such lesions are most probably a consequence of severe dehydration (Lukert & Hitchner, 1984). In chickens killed and examined during the course of infection, kidneys appear normal (Lukert & Hitchner, 1984). There is considerable evidence that the BF is the primary target organ for the IBDV (Cheville, 1967; Fadly, et al., 1976; Hitchner, 1978; Kaufer & Weiss, 1980). Cheville (1967) studied the effects of IBDV on bursal weights. At 3 days PI the BF begins to increase in size and weight because of edema and hyperemia. By the fourth day it usually doubles its normal weight and then begins to recede in size. By the fifth day it has returned to normal weight, but the BF continues to atrophy and from the eighth day on it is approximately one-third its original weight. By the second or third PI day, the BF has a gelatinous yellowish transudate covering the serosal surface.

Longitudinal striations on the surface become prominent, and the normal white color turns to cream. The transudate disappears as the bursa returns to its normal size and becomes gray during and following the period of atrophy. Infected bursa often show necrotic foci and at times petechial or ecchymotic hemorrhages on the mucosal surface. Occasionally, extensive hemorrhage throughout the entire bursa has been observed (Lukert & Hitchner, 1984). Also, the spleen may be slightly enlarged and very often has small gray foci uniformly dispersed on the surface. Occasionally, hemorrhages are observed in the mucosa at the juncture of the proventriculus and gizzard.

Microscopic pathology of IBD has been well documented (Helmboldt & Garner, 1964; Cheville, 1967; Lukert and Hitchner, 1984). The most striking lesions are usually present in the BF. Degeneration and necrosis of lymphocytes particularly in the medullary area of the bursal follicles are usually seen at 3 or 4 days PI. Inflammatory cell infiltration, pyknotic debris, hyperplasia of reticuloendothelial cells and hemorrhages are often seen, but are not a consistent feature. As the inflammatory reaction declines, cystic cavities develop in medullary areas of follicles due to necrosis and phagocytosis of heterophils and plasma cells. Fibroplasia of interfollicular connective tissue is a common lesion of IBD. Proliferation of the bursal epithelial layer that produced a glandular structure

of columnar epithelial cells containing globules of mucin has been described in the BF of IBDV-inoculated chickens (Lukert & Hitchner, 1984). In the spleen, lymphoid necrosis in the germinal follicles and periarteriolar lymphoid sheath is a common lesion in IBDV-inoculated chickens (Lukert & Hitchner, 1984). However, the spleen recovers from the infection rather rapidly, with no sustained damage to the germinal follicles (Lukert & Hitchner, 1984). Lesions in the thymus and cecal tonsils are less extensive compared with those in the BF. IBDV-induced lesions in the harderian gland consist primarily of a 5- to 10-fold reduction in plasma cell population (Dohms, et al., 1981). Microscopic lesions of the kidney are nonspecific and probably occur because of severe dehydration of affected chickens. Helmboldt and Garner (1964) found large casts of homogenous material infiltrated with heterophils in the kidneys of only 5% of chickens examined.

The importance of the BF in the ontogeny of the humoral immune response in the chicken was first studied by Chang, et al. (1955). The role of humoral immunity in disease resistance has been investigated by studying the response of bursectomized chickens to various infection. Chang, et al. (1959) demonstrated that bursectomized chickens were far less resistant to Salmonella typhimurium than unbursectomized control chickens. Baluda (1967) found that hormonal bursectomy interfered with development of normal resistance

of avian myeloblastosis virus. Bursectomized chickens were more responsive to avian encephalomyelitis than were normal control chickens (Cheville, 1970). Challey (1962) observed that death rates from Eimeria tenella were higher among bursectomized chickens. Interference with the BF by CY or by IBDV enhanced the pathogenicity of inclusion body hepatitis virus IBHV (Fadly, et al., 1976). In contrast, interference with normal development of the BF decreases the susceptibility of chickens to diseases in which the BF is the target organ. Peterson, et al. (1964; 1966) indicated that surgical bursectomy during the first month of life protected chickens from lymphoid leukosis. Treatment of chickens with CY protected chickens against IBD (Fadly, et al., 1976). Purchase and Gilmour (1975) indicated that CY which destroyed the lymphoid elements of the BF and abrogated humoral immunity, prevented lymphoid leukosis.

The immunosuppressive effects of IBDV were first reported by Allan, et al. (1972), and Faragher, et al. (1972, 1974). Suppression of the antibody response to Newcastle disease (ND) virus was greatest in chicks inoculated with IBDV at 1 day of age; whereas moderate or negligible suppression were noted in chickens inoculated with IBDV at 7 or 14 days of age, respectively (Faragher, et al., 1974). Hirai, et al. (1974) also demonstrated decreased humoral antibody response to killed ND vaccine and Hemophilus gallinarum (HG) vaccine in chickens inoculated

with IBDV at 2, 4 or 6 weeks of age. Early infection with IBDV was also shown to render chickens more susceptible to IBH (Fadly, et al., 1976), coccidiosis (Anderson, et al., 1977), Marek's disease (Sharma, 1984), hemorrhagic-aplastic anemia and gangrenous dermatitis (Rosenberger, et al., 1975), infectious bronchitis (Winterfield et al., 1978) and salmonellosis and colibacillosis (Wyeth, 1975). The effect of IBDV on the cellular immunity is less obvious than on the humoral response. Panigrahy, et al. (1977) reported that early IBDV infections caused a prolonged skin graft rejection. Other workers (Giambrone, et al., 1977; Hudson, et al., 1975) found no effect from early IBD infections on skin graft rejection or tuberculin-delayed hypersensitivity reaction. Sivanandan and Maheswaram (1981) found that maximal depression of cellular immunity occurred at 6 weeks post-inoculation. A significant suppression of T-cell response of poults to the mitogen conconavalin A from 3 days up to 4 weeks PI was found (Lukert & Hitchner, 1984). There was no reduction, however, in tuberculin reactions in IBDV-inoculated poults. Sharma and Lee (1983) found that reduced mitogenic response of lymphocytes in IBDV-infected chickens was not due to a lack of functional T-cells, but was due to macrophage-like suppressor cells.

Both active and passive immunity are required for protection against IBD. Of the two types, passive immunity is of prime concern because early infections are the most

immunosuppressive (Lukert & Hitchner, 1984). As with many diseases, maternal antibody can interfere with stimulation of an active immune response (Lukert & Hitchner, 1984). Antibody transmitted from the dam to progeny via the yolk of the egg can protect such progeny against early infections with IBDV, which usually leads to immunosuppression. The half-life of maternal antibodies to IBDV is 3-5 days (Skeeles, et al., 1979c). Therefore, if the antibody titer of the progeny is known, the time that chicks will become susceptible can be predicted. Lucio and Hitchner (1979) found that all chicks with maternal antibody titers below 1:100 were susceptible to infection with IBDV; whereas 40% of chicks with maternal antibody titers ranging from 1:100 to 1:600 were protected against challenge with the virus. Skeeles, et al. (1979c) reported that maternal antibody titers must fall below 1:64 before chicks can be vaccinated effectively with an attenuated strain of IBDV. Use of killed vaccines in oil emulsions to stimulate high levels of maternal immunity is extensive in the field. Lucio and Hitchner (1979) demonstrated that oil emulsion IBD vaccines can stimulate adequate maternal immunity to protect chicks for 4-5 weeks after hatching. In contrast, progeny from breeders vaccinated with live virus vaccines were shown to be protected for 1-3 weeks of age. Active immunity to IBD may be stimulated by field exposure to the virus or by vaccination (Lukert & Hitchner, 1984). Antibody levels are

normally very high after field exposure or vaccination, and virus neutralizing titers greater than 1:1000 are common (Lukert & Hitchner, 1984). Immunization of breeder flocks, to confer parental immunity to their progeny, is the principle method used to control IBD in chickens (Lukert & Hitchner, 1984). The major problem with active immunization of maternally immune chicks is determining the proper time for vaccination (Lukert & Hitchner, 1984). Further, the levels of maternal antibody, route of vaccination and virulence of vaccine virus are important factors that should be considered in determining the proper time for vaccination against IBD. There are three types of live IBDV vaccines: relatively virulent, attenuated avirulent, and intermediate strains (Lukert & Hitchner, 1984). Intermediate strains are intended for use in maternally immune chickens because these strains can overcome higher levels of maternal antibody. The vaccine virus replicates in the thymus, spleen and BF and persists there for at least 2 weeks. Once the maternal antibody has been catabolized there is a primary antibody response to virus that persists (Lukert & Hitchner, 1984). Further, attenuated vaccine virus strains were found to replicate in the maturer lymphocytes at the periphery of the bursal follicles, not in the immature central lymphocytes. Oil adjuvant vaccines work best in chickens that have been primed with live virus either in form of vaccine or field exposure to the virus (Lukert and Hitchner, 1984).

MATERIALS AND METHODS

1. Solutions, chemicals and media

Phosphate-buffered saline solution

Phosphate-buffered saline solution (PBS), pH 7.4, was prepared as listed below.

Salt	Grams per liter[a]
NaCl	8
KCl	0.2
KH_2Po_4	0.2
Na_2HPO_4	1.2
phenol red	0.3 ml

Versine

Versine solution was prepared by using 0.2 gm of Ethylenediaminetetracetate (EDTA) in 1 liter of PBS, of pH 7.4. Versine was used as a diluent for trypsin in the preparation of chicken embryo fibroblast (CEF) and transfer of primary cell cultures.

Trypsin[b]

Trypsin stock solution, 2.5%, was stored frozen at -20°C. The stock solution was diluted 1:10 with versine prior to use in the preparation or transfer of primary cell cultures.

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[a]Dissolved in deionized distilled water.

[b]Trypsin 1:250, Gibco Laboratories, Chagrin Falls, Ohio 44022.

Cyclophosphamide (Cytoxan)[a]

Cyclophosphamide is a synthetic antineoplastic drug. It causes destruction to bursal lymphoid cells without affecting the epithelium or stroma.

Heparin (Lipo-Hepin)[b]

Heparin was used as an anticoagulant in collecting blood samples, 60 units heparin/ml blood. Also, it was added to the media used in preparation of bursa single cell suspension.

Thiobarbiturate anesthesia (Bio-Tal)[c]

Thiobarbiturate anesthesia was used to anesthetize chickens for surgical bursectomy. It was used at a dose of 1.25 mg per 400 gm body weight.

F10-199 mixed media

F10-199 mixed media was used for all cell cultures work in this study, media formulation is shown below:

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[a]Cytoxan; Mead Johnson & Co., Evansville, Indiana 47721.

[b]Lipo-Hepin; Riker Laboratories, Inc., Northridge, California.

[c]Bio-Tal; Bio-Ceutic Laboratories, Inc., St. Joseph, Missouri.

Component	Grams per liter[d]
F10 media[a]	4.9
199 media[a]	5.5
$NaHCo_3$	0.85
TPB[b]	1.48
Pen/Strep[c]	1 ml
Inactivated calf serum[a]	4%

RPMI 1640 media

RPMI 1640 media, pH 8.1, was used for preparation of bursa single cell suspension. Media formulation is shown below.

Component	Grams per liter[d]
RPMI 1640	10.4
$NaHCo_3$	1.5
Pen/Strep	1.0 ml
DNase[e]	0.01
BFS[f]	20 ml
Heparin	2500 IU

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[a]Gibco Laboratories, Grand Island Biological Company, Grand Island, New York 14072.

[b]Tryptose-Phosphate-Broth (TPB), Difco Laboratories, Detroit, Michigan.

[c]Penicillin: Sigma Chemical Company, St. Louis, Missouri 63178.
Streptomycin: Pfizer Laboratories Division, New York, New York 10017.
(Pen/Strept): 20 X 10^6 units penicillin and 20 grams streptomycin dissolved in 200 ml sterile water.

[d]Dissolved in deionized distilled water.

[e]Deoxyribonuclease 1 (pancrease) DNase, lyophilized, B grade. Calbiochem, Los Angeles, California 90054.

[f]Bovine Fetal Serum (BFS), Grand Island Biological Company, Grand Island, New York 14072.

2. Chickens and chicken embryos

In all experiments, chickens were from crosses between males of the Regional Poultry Research Laboratory (RPRL) line 15I, subline 5 ($15I_5$) and females of line 7, subline 1 (7_1). In addition, chickens of lines 7_1, 15B, O, P, 6_3 and N were also used in experiment 1. The breeder flocks are known to be free from many pathogens and have been proven to be free from IBD antibody by the virus neutralization test. All chickens were maintained in negative pressure, stainless-steel metal isolators from the day of hatch until the end of the experiment.

Chicken embryos derived from the RPRL's specific pathogen free (SPF) breeder flocks of lines $15I_5$ x 7_1, or 15B were used for virus recovery tests.

3. Cell-cultures

CEF cultures were prepared as described by Solomon (1975) and Purchase (1980). Primary cell cultures were grown in 150 X 25 mm tissue culture dishes* in 5% Co_2 incubator at 37°C. Secondary cell cultures were prepared by trypsinizing the primary culture for 5 minutes at 37°C. Secondary CEF cultures were used in propagation and titration of the cell culture adapted IBDV and in the virus-neutralization test.

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*Falcon Div., Becton, Dickinson and Co., Oxnard, California 93030.

4. Viruses

a. A virulent strain of IBDV (IM strain) was used to inoculate chickens (Winterfield et al., 1972). It was propagated and titrated in chicken embryos. The stock virus, a 20% suspension in infected allantoamnionic fluids of the chorioallantoic membrane (CAM), livers, and spleens harvested 5-7 days PI of 10 day-old embryonated eggs, had a titer of 5×10^3 50% embryo-infectious doses/ml. Each chicken was inoculated by eye drop with 500 EID_{50} of the IM strain of IBDV.

b. Two cell culture-adapted strains (D-78 and RP) were used in the virus neutralization tests. The D-78 isolate was propagated in QT-35 cells (Cowen, 1984). The RP isolate (originally obtained from P.D. Lukert, University of Georgia, Athens, Georgia) was propagated and titrated in CEF.

5. Collection of samples

Individual blood samples were collected before and after IBDV inoculation from the wing vein or by heart puncture at termination. Blood or spleen samples were collected for virus recovery tests. Samples were stored at $-20^{\circ}C$ until used.

For serum collection, blood samples were allowed to clot at room temperature, then placed in a $37^{\circ}C$ incubator until the clot contracted. The serum was removed and clarified by centrifugation at 2500 rpm for 10 minutes. All

serum samples were inactivated in 56°C water bath for 30 minutes prior to testing. Serum samples were stored at -20°C until used.

Samples of the BF or spleens were also fixed in 10% buffered-formalin for microscopic evaluation.

6. Virus recovery tests

Chicken embryos of two lines ($15I_5$ X 7_1, or 15B) from the RPRL's pathogen free breeder flocks were inoculated with 0.2 ml of a 1:10 weight/volume suspension of spleen (10% dilution in case of blood) in F10-199 media supplemented with antibiotics (Pen/Strep), 100,000 IU; Gentamicin[a] 10,000 ug and Fungizone,[b] 5,000 ug). Spleen samples were macerated with the blunt end of a sterile 1.0 ml pipette. Tissue suspension or blood was inoculated into the CAM of 9-11 day-old chicken embryos. Each sample was inoculated into five embryos. Embryos dying during the initial 48 hours were considered non-specific. Subsequent deaths within 3-8 days PI were regarded as IBDV-induced mortality as described previously (Lukert and Hitchner, 1984). Positive virus isolation was recorded only if at least two embryos died in the post 48-hour time period.

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[a]GRS Garamycin, Schering Corporation, Kenilworth, New Jersey 07033.

[b]Fungizone, E. R. Squibb & Sons, Inc., Princeton, New Jersey 08540.

7. Virus neutralization (VN) tests

Sterile plastic microplates having 96 flat-bottomed wells arranged in 12 rows of 8 each were used in the VN tests. The microtiter system for the VN tests in studies of IBD has been described by Skeeles et al. (1979a). The test consisted of making duplicate two-fold serial dilutions of serum in a constant amount of virus. A 0.05 ml volume of the virus dilution containing 1000 $TCID_{50}$ (D78 strain) or 250 $TCID_{50}$ (RPL strain) was placed in each well of the microtiter plates except the last well in each row, which was used as a cell control. The serum was serially diluted using a 0.05 ml microdiluter. After incubation for at least 30 minutes 50,000 CEF in 0.2 ml of F10-199 medium containing 4% calf serum was added to each well. A virus control was maintained on each row of the microtiter plates. After the CEF were added the plates were covered by plastic sterile covers and placed in a high-humidity, 5% CO_2-incubator at 37°C for 4-5 days. After incubation the covers were removed and the medium poured off. The cell monolayers were finally stained with 1:5 mixture of 1% basic fuchsin and 1% crystal violet for 5 minutes. Endpoints were determined to be the reciprocal of the highest dilution of serum that protected a monolayer from destruction by the virus. A titer of 20 or less was considered negative for virus neutralizing antibody.

8. Tests for humoral immune function

At 4 weeks of age, chickens were intravenously (IV) inoculated with 2.5×10^9 organisms of Brucella abortus

(BA) strain 119-3 (USDA, animal and plant health service, veterinary service laboratories, Ames, Iowa) and 2.5 X 10^9 sheep red blood cells (SRBC) (Gibco Laboratories, Madison, WI). Seven days later, inoculated chickens were tested for agglutinins against SRBC (Wegman and Smithies, 1966) and BA (Fadly and Witter, 1983) by microagglutination tests. Plastic microplates having 96 round-bottomed wells arranged in 12 rows of 8 each were used in agglutination tests. A 0.05 ml volume of clear PBS was placed in each well of the microtiter plates. The serum was serially diluted (two-fold dilution) using a 0.05 ml microdiluter from well number 1 to 11. A constant amount (0.05 ml) of a 1% SRBC suspension or a 1:16 dyed BA suspension was placed in each well of the microtiter plates. The last well in each row was considered as a SRBC or BA control. Plates were refrigerated undisturbed and read within 24 hours. The agglutinin titer to SRBC or BA in the serum was determined as the reciprocal of the highest dilution in which complete agglutination occurred. A titer of 2 or less was considered negative for SRBC or BA agglutinins.

9. Pathology

Chickens that died and those that survived the experimental period of 11 days were necropsied and examined for gross and microscopic lesions of IBD. Samples of the BF or spleen were fixed in 10% buffered-formalin and stained with hematoxylin and eosin (H & E).

10. Preparation of bursa cell suspension

BF from 5-week-old $15I_5$ X 7_1 chickens (donors) were aseptically removed and immediately placed in ice-cold medium. Minced BF were suspended in RPMI 1640 media with 0.001% wt/vol DNase, 2% vol/vol BFS and 2.5 U preservative-free heparin/ml. The clumps were broken up by using 5 or 10 ml syringe with long wide blunt end needle. The cell suspension was placed consecutively through 4 layers of sterile gauze, a 100- and a 250-mesh sieve (Cistron Corp., Elmsford, NY), and washed three times in the media by centrifugation at 1,000 rpm for 10 minutes. The total number of mononuclear cells was counted by the method of Natt and Herrick (1952), and the viable cells were determined by trypan blue exclusion. An additional 5 U heparin/ml was added to the inoculum prior to inoculation of the recipients (7 day-old $15I_5$ X 7_1 chicks that had been neonatally treated with CY) (Toivanen, et al., 1972a; 1972b; 1974a; 1974b; Purchase & Gilmour, 1975; Fadly, et al., 1981).

11. Experimental design

Experiment 1: In experiment 1, the pathogenesis of IBD in various lines of White Leghorn chickens inoculated with virus at 1 or 42 days of age was examined. Chickens of various White Leghorn lines 15B, O, P, $15I_5$ X 7_1, 6_3 and N were inoculated with the IM strain (Winterfield, et al., 1972) of IBDV at 1 or 42 days of age. Chickens of line 7_1 were also included, but were inoculated with virus at 1 or

28 days of age. Uninoculated hatchmates were also maintained as controls. Table 1 presents the number of chickens used in each group. Chickens were examined daily for IBDV-induced clinical signs and mortality. At 5 and 11 days PI samples of blood and serum from two to five chickens from each group were tested for IBDV and antibody, respectively. Samples of BF and spleen were also collected for histological evaluation.

Experiment 2: In experiment 2, the pathogenesis of IBD in chickens neonatally chemically bursectomized by CY and subsequently inoculated with various numbers of bursa cells (obtained from 5-week-old syngenic donor chickens) was studied. One-day-old $15I_5 \times 7_1$ chicks (150) were divided into nine groups; each group consisted of 15 to 25 chicks. The various treatments and the number of chickens per treatment are shown in Table 6. Chicks in seven groups were treated with CY for 3 days post hatch. Each chick received a total dose of 10 mg of CY. At 7 days of age, chicks in five of the seven CY-treated groups were inoculated with bursa cell suspension. The number of bursa cells inoculated ranged from 0.5×10^6 to 10×10^7, in a volume of 0.1-0.2 ml of medium. At 4 weeks of age, the response of CY-treated, CY-treated and subsequently inoculated with bursa cells, and control untreated chickens to sensitization with SRBC and BA was examined. At 5 weeks of age, BF from 3-5 chickens from each group were weighed and examined histologically for

structural restoration before inoculation with IBDV. Chickens in all groups were inoculated with 500 EID_{50} of the IM strain of IBDV via eye drop. Chickens were examined daily for clinical signs and mortality of IBD. At 4 days PI, sera were collected from 4-5 chickens from each group for antibody determination. The chickens were killed and bursae and spleens were collected for microscopic evaluation; spleens were also tested for IBDV. At 11 days PI sera from all chickens were tested for IBDV neutralizing antibody.

Experiment 3: In experiment 3, the influence of surgical bursectomy on the pathogenesis of IBD was examined. Five-week-old $15I_5$ X 7_1 chickens were divided into 4 groups. Chickens in groups 2 and 3 were surgically bursectomized at 5 weeks of age. Chickens in groups 1 and 4 remained as intact controls. Two days following surgical bursectomy, chickens in groups 1 and 3 were inoculated with 500 EID_{50} of the IM strain of IBDV via eye drop. Table 9 shows the number of chickens used in each group. Chickens were examined daily for clinical signs and mortality of IBD. At 4 days PI spleens and sera from 5 chickens from each group were tested for IBDV and antibody, respectively. At 11 days PI sera were tested for IBDV neutralizing antibody.

RESULTS

IBDV-induced clinical signs and mortality in various lines of White Leghorn chickens inoculated with IBDV at 1 day or 42 days of age (Expt. 1).

Table 1 shows clinical signs and mortality in various lines of White Leghorn chickens inoculated with IBDV at 1 day or 42 days of age. At 4 days PI, clinical signs of IBD such as severe depression and ruffled feathers were seen only in groups inoculated with the virus at 42 days of age (28 days for line 7_1 chickens). In contrast, no clinical signs were seen in chickens inoculated with virus at 1 day of age, regardless of line of chicken used. IBDV-induced mortality was higher in chickens inoculated with the virus at 42 days of age (40-80%) than in those inoculated with virus at 1 day of age (10-30%).

Gross and microscopic bursal lesions in White Leghorn chickens inoculated with IBDV at 1 day or 42 days of age (Expt. 1).

Gross examination of chickens that died and those that survived the experiment revealed typical gross lesions of IBD (Cosgrove, 1962). All chickens were affected, regardless of age at inoculation. The BF was atrophied, gelatinous and cream colored or gray. Further, in some

chickens the mucosal surface of the BF exhibited necrotic focci and petechial hemorrhage.

Microscopic examination of bursal tissues confirmed the gross pathology. The lesions in the BF consisted primarily of lymphoid necrosis, lymphoid depletion, reticuloendothelial hyperplasia, hemorrhage, cyst formation and heterophilic infiltration. The severity of bursal damage was of the same degree in chickens of all groups, regardless of age at inoculation or line of chicken used.

•Concentration of IBDV in blood of various lines of White Leghorn chickens at 5 days PI with virus at 1 day or 42 days of age (Expt. 1).

Data in Table 2 show the rate of recovery and titer of IBDV in various lines of White Leghorn chickens at 5 days PI with virus at 1 day or 42 days of age. No considerable difference was detected in the rate of recovery or titer of IBDV in blood of chickens inoculated with virus at 1 day of age ($5 \times 10^{1.63}$ - $5 \times 10^{2.9}$ EID_{50}/ml), regardless of line of chicken used. Also, there was no considerable difference in the rate of recovery or titer of virus in blood of chickens inoculated with IBDV at 42 days of age ($5 \times 10^{1.63}$ - $5 \times 10^{2.2}$ EID_{50}/ml), regardless of line of chicken used. However, the titer of IBDV in blood of line N chicken was $5 \times 10^{4.5}$ EID_{50}/ml.

•Titers of IBDV neutralizing antibody in various lines of White Leghorn chickens at 5 and 11 days PI with virus at 1 day or 42 days of age (Expt. 1).

Table 3 shows titers of IBDV neutralizing antibody in various lines of White Leghorn chickens at 5 and 11 days PI with virus at 1 day or 42 days of age. At 5 days PI, IBDV neutralizing antibody was not detected in the majority of sera of chickens inoculated with virus at 1 day of age. In contrast, the average titer of IBDV neutralizing antibody in chickens inoculated with virus at 42 days of age (28 days for line 7_1 chickens) varied from 80-266. At 11 days PI, the average titer of IBDV neutralizing antibody in sera of chickens inoculated with virus at 1 day of age varied from 80 to 140; whereas the average titer of IBDV neutralizing antibody in chickens inoculated with virus at 42 days of age (28 days for line 7_1 chickens) varied from 2200 to 3200.

Because data from chickens inoculated with IBDV at 1 day of age were similar, regardless of line of chicken used, the data were pooled. Similarly the data from chickens inoculated with virus at 42 days of age were pooled. Figure 1 illustrates the influence of age at inoculation on the frequency of detecting IBDV from blood of chickens at 5 days PI with virus. It also illustrates influence of age at inoculation on the rate of detecting IBDV neutralizing antibody at 5 and 11 days PI. At 5 days PI, the frequency of detecting IBDV in blood was significantly ($p \leq 0.05$) higher in chickens inoculated with virus at 1 day of age than in chickens inoculated at 42 days of age (86% vs. 43%). In contrast, the rate of detecting IBDV

neutralizing antibody was significantly ($p \leq 0.05$) higher in chickens inoculated with virus at 42 days of age than in chickens inoculated at 1 day of age (90% vs. 7% at 5 days PI and 100% vs. 31% at 11 days PI).

Further, at 5 and 11 days PI, the titers of IBDV neutralizing antibody in chickens inoculated with virus at 1 day of age were considerably lower than that in chickens inoculated at 42 days of age (Figure 2). At 5 days PI, the average titer of IBDV neutralizing antibody in chickens inoculated with virus at 1 day of age was 20 compared with average titer of 136 in chickens inoculated with virus at 42 days of age. Also, at 11 days PI, the average titers of IBDV neutralizing antibody in chickens inoculated with virus at 1 day or 42 days of age were 93 and 2904, respectively.

Table 1. IBDV-induced clinical signs and mortality in various lines of White Leghorn chickens inoculated with IBDV at 1 day or 42 days of age.[a]

Line	Age at inoculation (days)	No. of chickens inoculated	Clinical signs 4 days PI	No. died within 7 days PI	Mortality %
7_1	1	10	-	1	10
	28	15	+	9	60
15_B	1	10	-	0	0
	42	13	+	6	46
0	1	10	-	0	0
	42	15	+	7	47
P	1	10	-	1	10
	42	15	+	0	0
$15I_5X7_1$	1	10	-	3	30
	42	15	+	6	40
6_3	1	10	-	1	10
	42	15	+	11	73
N	1	10	-	1	10
	42	15	+	12	80

[a]Each chicken was inoculated with 500 EID_{50} of the IM strain of IBDV via eye drop.

Table 2. Concentration of IBDV in blood of various lines of White leghorn chickens at 5 days PI with virus at 1 day or 42 days of age.[a]

Line	Age at inoculation (days)	No. +/no. tested	Average titer EID-50/ml
7_1	1	2/2	$5X10^{2.9}$
	28	2/3	$5X10^{1.8}$
15_B	1	2/2	$5X10^{1.63}$
	42	1/3	$5X10^{1.63}$
0	1	2/2	$5X10^{2}$
	42	1/3	$5X10^{1.63}$
P	1	1/2	$5X10^{1.63}$
	42	2/3	$5X10^{2.2}$
$15I_5X7_1$	1	2/2	$5X10^{2.6}$
	42	1/3	$5X10^{2}$
6_3	1	1/2	$5X10^{1.75}$
	42	1/3	$5X10^{1.63}$
N	1	2/2	$5X10^{1.93}$
	42	1/3	$5X10^{4.5}$

[a]Chickens were inoculated with 500 EID_{50} of the IM strain of IBDV via eye drop.

Table 3. IBDV neutralizing antibody titers in White leghorn chickens of various lines at 5 and 11 days post-inoculation with virus at 1 day or 42 days of age.[a]

Line	Age at inoculation[d] (days)	Neutralizing antibody			
		5 days PI		11 days PI	
		No. +/no. tested	Average titer[b]	No. +/no. tested	Average titer[b]
7_1	1	0/2	--[c]	0/5	--
	28	2/3	240	5/5	3200
15_B	1	0/2	--	0/5	--
	42	3/3	80	5/5	2240
0	1	0/2	--	3/5	133
	42	3/3	80	5/5	3200
P	1	0/2	--	4/5	140
	42	3/3	106	5/5	3200
$15I_5X7_1$	1	1/2	80	2/5	80
	42	2/3	120	5/5	3200
6_3	1	0/2	--	0/5	--
	42	3/3	266	4/4	2200
N	1	0/2	--	2/5	120
	42	3/3	160	3/3	3200

[a]Five chickens from each line were tested before inoculation for IBDV antibody; all were negative.

[b]Reciprocal of highest serum dilution that neutralized virus in a microtiter serum neutralization test.

[c]A titer of 20 or less was considered negative.

[d]Chickens were inoculated with 500 EID_{50} of the IM strain of IBDV via eye drop.

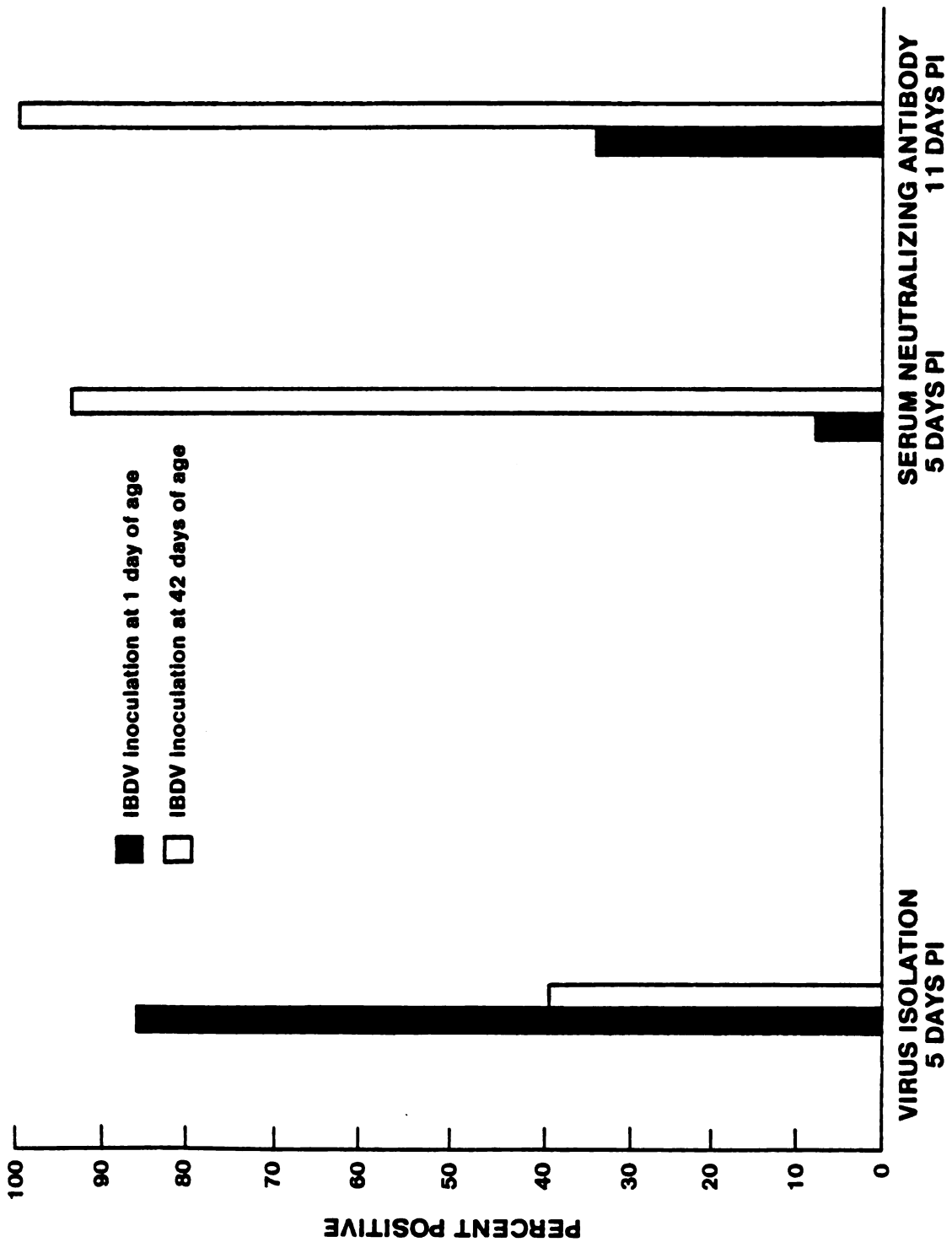

Figure 1. IBDV and antibody in White Leghorn chickens at 5 and 11 days PI with virus at 1 day or 42 days of age.

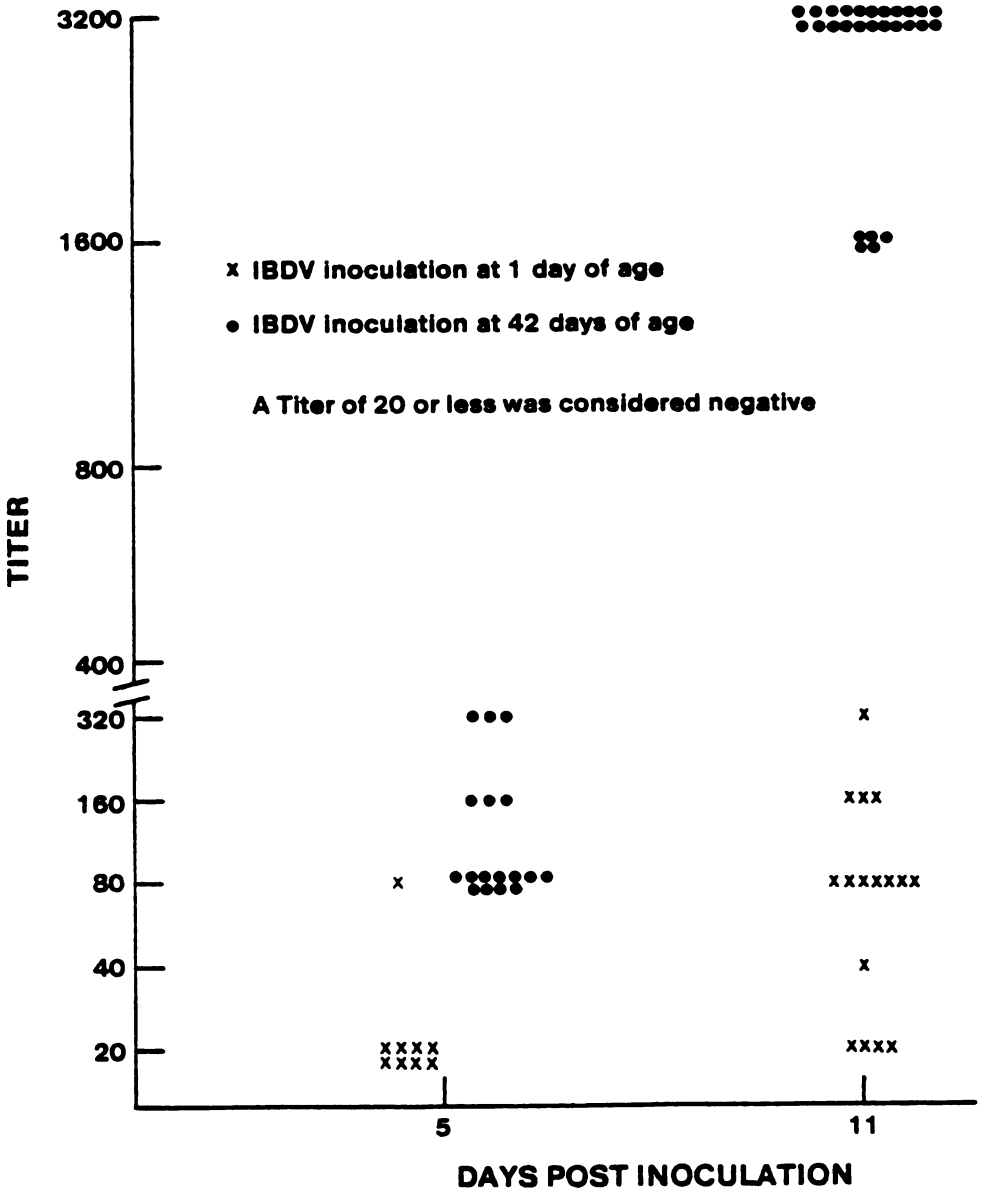

Figure 2. IBDV neutralizing antibody titers in White Leghorn chickens at 5 and 11 days PI with virus at 1 day or 42 days of age.

•Response of chickens neonatally treated with CY and subsequently inoculated with various numbers of bursa cells to sensitization with SRBC and BA (Expt. 2).

Table 4 shows the response of chickens neonatally treated with CY and subsequently inoculated with various numbers of bursa cells to sensitization with SRBC and BA. Inoculation of CY-treated chicks with at least 12.5×10^6 bursa cells restored their humoral immune function. The percentage of chickens that responded to sensitization with SRBC and BA in groups inoculated with 12.5×10^6 or more bursa cells varied from 89%-100%. In contrast, the percentage of chickens that responded to sensitization with SRBC and BA in groups inoculated with 2.5×10^6 or less varied from 0%-5.5%. The titers of agglutinins to SRBC and BA in groups inoculated with 12.5×10^6 bursa cells or more were significantly ($p<0.05$) higher than in those inoculated with 2.5×10^6 bursa cells or less. The titers of agglutinins in groups received 12.5×10^6 bursa cells or more were equivalent to or slightly higher than those in intact chickens.

•Partial restoration of bursal structure in chickens neonatally treated with CY and subsequently inoculated with various numbers of bursa cells (Expt. 2).

Table 5 shows the degree of restoration of bursal structure in chickens neonatally treated with CY and subsequently inoculated with various numbers of bursa cells. Bursa weights were drastically decreased in CY-treated

chickens (0.06% of body weight) compared with intact chickens (0.68% of body weight). Bursa weights in CY-treated chickens and subsequently inoculated with bursa cells ranged from 0.08% to 0.22% of body weight. The increase in bursa weight was directly related to the number of bursa cells inoculated. However, even inoculation of 100×10^6 bursa cells did not completely restore bursal weight to normal (0.22% of body weight). To evaluate whether reconstitution of bursal structure was achieved by bursa cell inoculation, the number of developed bursal lymphoid follicles (follicles with normal histological architecture), per 100 follicles counted, was determined (Hashimoto and Sugimura, 1976). The percentage of developed bursal follicles was directly related to the number of bursa cells inoculated. The degree of restoration of bursal follicles in chickens neonatally treated with CY and subsequently inoculated with various numbers of bursa cells are illustrated in Figures 5-9. The new developed bursal follicles possess the same architecture of normal bursal follicles (Figure 3). The new developed follicles are full of bursal lymphocytic cells reforming follicular cortex and medulla. In contrast, only atrophied bursal follicles were seen in control CY-treated chickens (Figure 4).

Response of 5-week-old chickens that had been neonatally treated with CY and subsequently inoculated with various numbers of bursa cells to infection with IBDV (Expt. 2).

Chickens neonatally chemically bursectomized by CY and subsequently inoculated with at least 62 x 10^6 bursa cells were as susceptible to IBDV-induced clinical manifestations as intact chickens. Clinical manifestations of IBD such as severe depression and ruffled feathers were observed 3-4 days following inoculation with virus at 5 weeks of age. In contrast, chemically bursectomized chickens inoculated with 12.5 X 10^6 or less bursa cells were refractory to the IBD clinical manifestations as compared with intact chickens or chickens inoculated with 62 X 10^6 or more bursa cells. At 4 days PI, typical IBD gross lesions, such as gelatinous, yellowish transudate covering the BF, and petechial hemorrhages on the mucosal surface were observed in intact chickens, but not in those neonatally treated with CY (Figure 10). Only CY-induced atrophy of BF was noted in this group. Moderate gross lesions of IBD were also observed in chemically bursectomized chickens inoculated with 12.5 X 10^6 bursa cells or more. The BF was yellowish, gelatinous and showed, in some cases, petechial hemorrhage (Figures 11 & 12).

Chemically bursectomized chickens with CY which received no bursa cells, showed no microscopic lesions of IBD following inoculation of IBDV at 5 weeks of age. However, bursal follicles were atrophied with complete depletion of lymphocytes and interfollicular fibrosis as a result of CY-treatment (Figure 13). In contrast, normal follicles from uninoculated hatchmates were large in size,

full of lymphocytes and with minimal interfollicular connective tissue (Figure 14). Typical microscopic lesions of IBD were observed 4 days PI of virus in 5-week-old control chickens. Atrophy of bursal follicles, lymphoid cell depletion, lymphoid necrosis and cyst formation were observed (Figure 15). Thickened interfollicular connective tissue, reticuloendothelial hyperplasia and inflammatory cells infiltration especially heterophils were prominent in the bursal follicles and in the interfollicular spaces (Figure 16). Microscopic examination of BF of chemically bursectomized chickens inoculated with various numbers of bursa cells and inoculated with IBDV at 5 weeks of age, showed IBD specific lesions (Figures 17-21). These IBD lesions involved only the newly developed or restored bursal follicles. Bursal lesions were scored according to severity of damage (Fadly and Nazerian, 1983) on a scale of zero-3. Zero, no histologic lesions; 1, mild lesions (scattered cell depletion); 2, moderate (atrophy of follicles/moderate cell depletion); 3, severe (necrosis/ severe depletion and cyst formation). It was evident that the bursal damage in CY-treated chickens inoculated with 62 X 10^6 or 10 X 10^7 bursa cells was as severe as that of intact chickens following inoculation with IBDV at 5 weeks of age (Table 6). At 11 days PI, the BF from chickens of all groups were atrophied. Severe depletion of lymphocytes, infiltration with heterophils and fibroblasts were also seen. At that time, it was difficult to distinguish

the follicular damage induced by CY from that induced by IBDV.

Data in Table 7 show the influence of the number of bursa cells inoculated on the frequency of isolation and titer of IBDV in spleens, at 4 days PI, of 5-week-old chickens that had been neonatally chemically bursectomized. At 4 days PI, the concentration of IBDV in spleens of chemically bursectomized chickens inoculated with 12.5 X 10^6 or more bursa cells was comparable to that in spleens of intact chickens. Further, the concentration of IBDV in spleens of these chickens was about 100 times higher than that in spleens of chemically bursectomized chickens inoculated with 2.5 X 10^6 bursa cells or less. The rate of recovery of IBDV from spleens of chemically bursectomized chickens inoculated with various numbers of bursa cells ranged from 60-100%. In contrast, IBDV was recovered from only 25% of chemically bursectomized chickens which received no bursa cells. All spleens collected from intact uninfected chickens were negative for IBDV.

Data in Table 8 show the influence of the number of bursa cells inoculated on the development and titer of IBDV neutralizing antibody, at 4 and 11 days PI, in 5-weekold chickens that had been neonatally chemically bursectomized by CY. At 4 days PI, IBDV neutralizing antibody was not detected in chickens of all groups. At 11 days PI, the titers of IBDV neutralizing antibody in chemically bursectomized chickens inoculated with 12.5 X 10^6, 62.5

X 10^6 or 100 X 10^6 bursa cells were 240, 2000 and 2830, respectively. Whereas in intact IBDV-inoculated chickens the titer of virus neutralizing antibody was as high as 3200. At 11 days PI, IBDV neutralizing antibody was not detected in chemically bursectomized chickens inoculated with 2.5 X 10^6 bursa cells or less. Further, at 11 days PI, the rate of detecting IBDV neutralizing antibody was significantly ($p \leq 0.05$) higher in chemically bursectomized chickens inoculated with 12.5 X 10^6 bursa cells or more than in those inoculated with 2.5 X 10^6 bursa cells or less (78-100% vs. 0%). No IBDV neutralizing antibody was detected in sera of intact uninfected chickens.

Table 4. Response of chickens neonatally treated with CY and subsequently inoculated with various numbers of bursa cells to sensitization with SRBC and BA

Treatment		Agglutinin[C]					
		SRBC			BA		
CY[A]	No.of B.cells[B] X 10^6	No. Responder/ No. Tested	% Responder	$\bar{X}$ Log_2	No. Responder/ No. Tested	% Responder	$\bar{X}$ Log_2
-	0	17/17	100	9±0.2 ad	17/17	100	7.4±0.1 a
+	0	0/15	0	0	0/15	0	0
+	0.5	0/15	0	0	0/15	0	0
+	2.5	1/18	5.5	4±0.0	4/18	22	2.3±0.4 b
+	12.5	16/18	89	8.3±0.6 ad	16/18	89	7.5±0.4 ca
+	62.5	19/19	100	9.6±0.4 ac	19/19	100	8.9±0.2 df
+	100	23/23	100	10.5±0.1 bc	23/23	100	9.1±0.1 ef

[A]Day old $15I_5$ X 7_1 chicks were neonatally chemically bursectomized by cyclophosphamide (CY); each chick received a total dose of 10 mg given in 3 daily doses of 4, 4 and 2 mg each.

[B]CY-treated, 7-day-old $15I_5$ X 7_1 chicks were intravenously inoculated with single cell suspension of bursa obtained from 5-week-old $15I_5$ X 7_1 chickens.

[C]Sera were tested at 7 days post-sensitization with sheep red blood cells (SRBC) and *Brucella abortus* (BA) at 4 weeks of age.

Values, within a column, followed by different letters are significantly ($p<0.05$) different.

Table 5. Partial restoration of bursal structure in chickens neonatally treated with CY and subsequently inoculated with various numbers of bursa cells.

Treatment		Relative bursal weight[c]	Average no. of developed bursal follicles %[d]
CY[a]	No.of B.cells[b] X 10^6		
+	0	60	2[e]
+	0.5	83	4
+	2.5	86	4
+	12.5	110	15
+	62	170	38
-	100	220	57
-	-	680	100

[a]Day-old $15I_5$ X 7_1 chicks were neonatally chemically bursectomized by CY; each chick received a total dose of 10mg given in 3 daily doses of 4, 4, and 2 mg each.

[b]CY-treated, 7-day-old $15I_5$ X 7_1 chicks were intravenously inoculated with single cell suspension of bursa obtained from 5-week-old $15I_5$ X 7_1 chickens.

[c]Relative bursal weight = weight of bursa (mg)/100 gm of body weight.

[d]Number of developed bursal lymphoid follicles with normal architecture per 100 follicles counted.

[e]The bursa is completely atrophied except for 2 follicles in one cross section.

3-5 chickens were examined in each treatment.

Table 6. Summary of IBDV-induced microscopic lesions in bursa of Fabricius of 5-week-old chickens that had been neonatally treated with CY and subsequently inoculated with various numbers of bursa cells.

Treatment			IBDV-induced microscopic lesions[d]	
CY[a]	No. of B. cells[b] X 10^6	IBDV[c]	no. of bursa with lesions/ no. tested	Average score[e] index
-	0	-	0/3	0.0[f]
+	0	-	0/5	0.0
+	0	+	0/5	0.0
+	0.5	+	2/6	0.3
+	2.5	+	5/6	1.2
+	12.5	+	4/4	1.8
+	62.5	+	4/4	3.0
+	100.0	+	5/5	3.0
-	-	+	4/4	3.0

[a]Day-old $15I_5 \times 7_1$ chicks were neonatally chemically bursectomized by CY; each chick received a total dose of 10 mg given in 3 daily doses of 4, 4 and 2 mg each.

[b]CY-treated, 7-day-old $15I_5 \times 7_1$ chicks were intravenously inoculated with single cell suspension of bursa obtained from 5-week-old $15I_5 \times 7_1$ chickens.

[c]Chickens were inoculated by eye drop with 500 EID_{50} of the IM strain of IBDV at 5 weeks of age.

[d]Bursal tissues were examined by 4 days PI with virus at 5 weeks of age.

[e]Average score index = no. with lesions X mean score/no. examined.

[f]Bursal lesions scored according to severity of damage on a scale of: 0=no histologic lesion; 1=mild; 2=moderate; 3=severe; by the following criteria: lymphoid necrosis, lymphoid depletion, reticuloendothelial hyperplasia, fibrosis, hemorrhage, cyst formation and heterophilic inflammation.

Table 7. Concentration of IBDV in spleens at 4 days PI of 5-week-old chickens that had been neonatally treated with CY and subsequently inoculated with various numbers of bursa cells.

Treatment			Virus recovery from spleen	
CY[a]	No. of B. cells[b] X 10^6	IBDV[c]	no. positive/ no. tested	Average titer/ml
-	0	-	0/5	0
+	0	-	0/4	0
+	0	+	1/4	$5 \times 10^{1.63}$
+	0.5	+	3/5	5×10^{2}
+	2.5	+	5/5	$5 \times 10^{2.2}$
+	12.5	+	5/5	$5 \times 10^{3.6}$
+	62	+	5/5	$5 \times 10^{3.86}$
+	100.0	+	4/4	$5 \times 10^{3.8}$
-	-	+	4/4	$5 \times 10^{4.24}$

[a]Day-old $15I_5 \times 7_1$ chicks were neonatally chemically bursectomized by CY; each chick received a total dose of 10 mg given in 3 daily doses of 4, 4 and 2 mg each.

[b]CY-treated, 7-day-old $15I_5 \times 7_1$ chicks were intravenously inoculated with single cell suspension of bursa obtained from 5-week-old $15I_5 \times 7_1$ chickens.

[c]Chickens were inoculated by eye drop with 500 EID_{50} of the IM strain of IBDV at 5 weeks of age.

-Spleen samples were assayed for IBDV by inoculation of 0.2 ml of serial ten-fold dilutions of spleen (made on a W/V basis), in F10-199 media supplemented with antibiotics and Fungizone on the CAM of 10-day-old SPF chicken embryos.

-Deaths within 48 hours PI were considered nonspecific; subsequent deaths within 3-8 days PI were considered as IBDV induced.

Table 8. IBDV neutralizing antibody, at 4 and 11 days PI, in 5-week-old chickens that had been neonatally treated with CY and subsequently inoculated with various numbers of bursa cells.

Treatment			4 DPI		11 DPI	
CY[a]	No. of B cells[b] X 10^6	IBDV[c]	No. positive/ no. tested	Average titer[d]	No. positive/ no. tested	Average titer[d]
-	0	-	0/5	≤20[e]	0/5	≤20
+	0	-	0/5	≤20	0/5	≤20
+	0	+	0/5	≤20	0/9	≤20
+	0.5	+	0/5	≤20	0/6	≤20
+	2.5	+	0/5	≤20	0/9	≤20
+	12.5	+	0/5	≤20	7/9	240
+	62.0	+	0/5	≤20	8/9	2000
+	100.0	+	0/5	≤20	13/13	2830
-	-	+	0/5	≤20	12/12	>3200

[a]Day-old $15I_5 \times 7_1$ chicks were neonatally chemically bursectomized by CY; each chick received a total dose of 10 mg given in 3 daily doses of 4, 4 and 2 mg each.

[b]CY-treated, 7-day-old $15I_5 \times 7_1$ chicks were intravenously inoculated with single cell suspension of bursa obtained from 5-week-old $15I_5 \times 7_1$ chickens.

[c]Chickens were inoculated by eye drop with 500 EID_{50} of the IM strain of IBDV at 5 weeks of age.

[d]Two-fold serial dilutions of each serum sample in a constant amount (1000 TCID) of IBDV (D78 RPL_1), the end point was the reciprocal of the highest dilution of serum that protected a monolayer of CEF from IBDV-induced CPE.

[e]A titer of 20 or less was considered negative.

5 chickens from each group tested before infection were IBDV antibody negative.

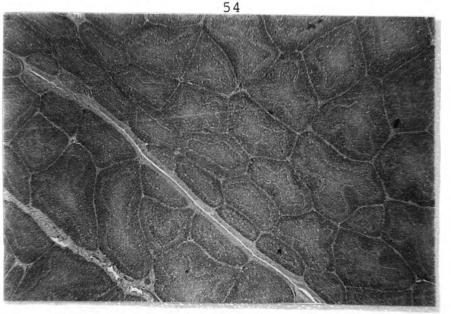

Figure 3. Photomicrograph of BF from 5 week-old control uninoculated chicken. Note normal appearance of lymphoid follicles. Active follicles consist of lymphoid cells that form discrete follicles with little interfollicular tissue. H & E . X 31.

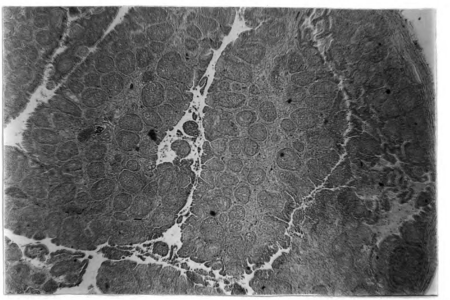

Figure 4. Photomicrograph of BF from CY-treated chicken killed prior to inoculation with IBDV at 5 weeks of age. Note the CY-induced atrophy of the bursal follicles and relative prominence of stromal elements. H & E . X 31.

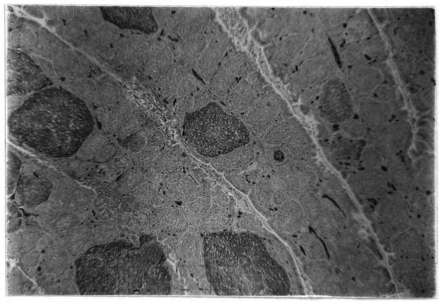

Figure 5. Photomicrograph of BF from CY-treated chicken, inoculated with 0.5 X 10^6 bursa cells and killed prior to inoculation with IBDV at 5 weeks of age. Note the CY-induced atrophy of the bursal follicles with few developed follicles. H & E . X 31.

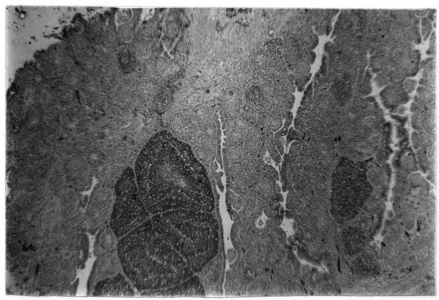

Figure 6. Photomicrograph of BF from CY-treated chicken, inoculated with 2.5 X 10^6 bursa cells and killed prior to inoculation with IBDV at 5 weeks of age. Note the CY-induced atrophy at the bursal follicles with few developed follicles. H & E . X 31.

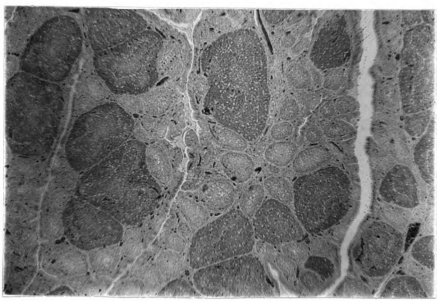

Figure 7. Photomicrograph of BF from CY-treated chicken, inoculated with 12.5 X 10^6 bursa cells and killed prior to inoculation with IBDV at 5 weeks of age. Note moderate number of developed follicles. H & E . X 31.

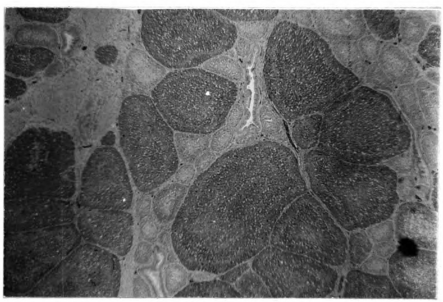

Figure 8. Photomicrograph of BF from CY-treated chicken, inoculated with 62 X 10^6 bursa cells and killed prior to inoculation with IBDV at 5 weeks of age. Note several well-developed follicles and fewer number of atrophied follicles compared with sections in Figures 4, 5, 6, 7. H & E . X 31.

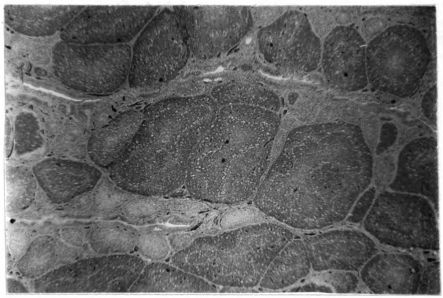

Figure 9. Photomicrograph of BF from CY-treated chicken, inoculated with 100 X 10^6 bursa cells and killed prior to inoculation with IBDV at 5 weeks of age. Note several well-developed follicles and fewer number of atrophied follicles compared with sections in Figures 4, 5, 6, 7. H & E . X 31.

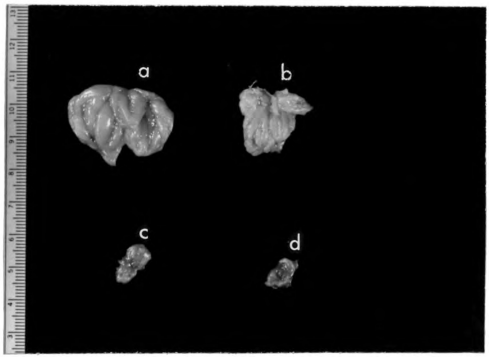

Figure 10. Photograph of BF from 5-week-old chickens, at 4 days PI with IBDV. (a) Uninfected control, note BF normal size and structure; (b) IBDV infected, note virus-induced atrophy; (c) CY-treated, note complete atrophy; (d) CY-treated and IBDV infected, note complete atrophy.

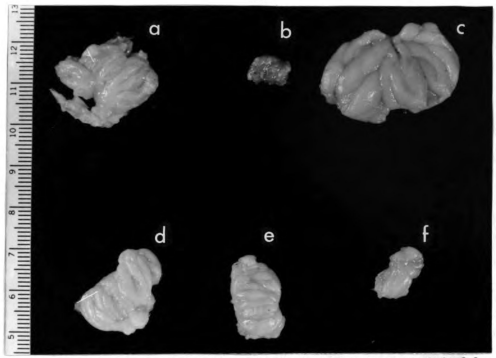

Figure 11. Photograph of BF from CY-treated chickens that had been inoculated with various numbers of bursa cells and killed 4 days PI with IBDV at 5 weeks of age. (a) IBDV infected, note virus induced atrophy; (b) CY-treated, note complete atrophy; (c) Uninfected control, note normal size and structure. (d), (e), (f) CY-treated, inoculated with 12.5×10^6, 2.5×10^6 or 0.5×10^6 bursa cells, respectively.

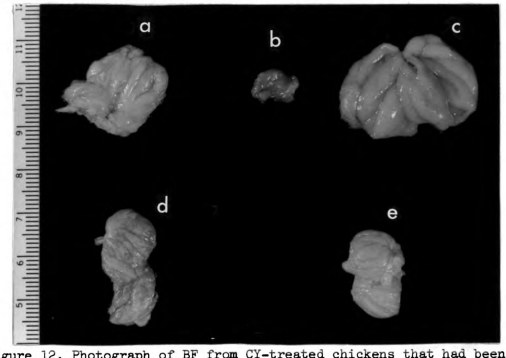

Figure 12. Photograph of BF from CY-treated chickens that had been inoculated with various numbers of bursa cells and killed 4 days PI with IBDV at 5 weeks of age. (a) IBDV infected, note virus induced atrophy; (b) CY-treated, note complete atrophy; (c) Uninfected control, note normal size and structure; (d) CY-treated, inoculated with 100×10^6 bursa cells and IBDV infected, note petechial hemorrhage in bursal follicles; (e) CY-treated, inoculated with 62×10^6 bursa cells and IBDV infected.

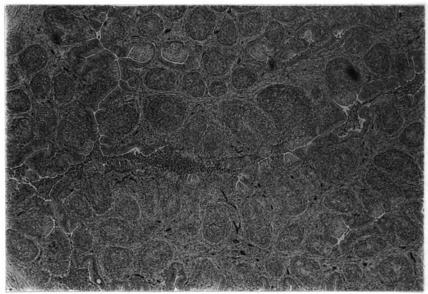

Figure 13. Photomicrograph of BF from CY-treated chicken killed 4 days PI with IBDV at 5 weeks of age. Note the CY-induced atrophy of the bursal follicles and relative prominence of stromal elements. H & E . X 50.

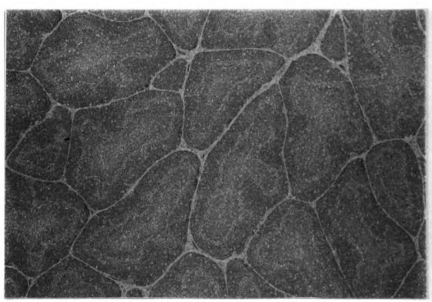

Figure 14. Photomicrograph of BF from 39-day-old control uninoculated chicken. Note normal appearance of lymphoid follicles. Lymphoid cells form discrete follicles with minimal stromal elements. H & E . X 50.

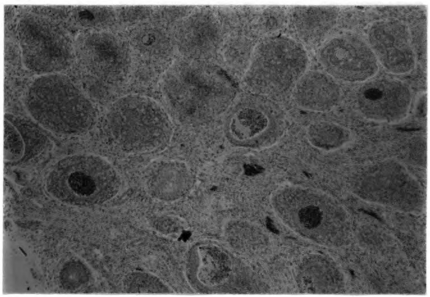

Figure 15. Photomicrograph of BF from a chicken killed 4 days PI with IBDV at 5 weeks of age. Follicles are degenerated, atrophied, with inflammatory cells infiltration and cysts formation. H & E . X 50.

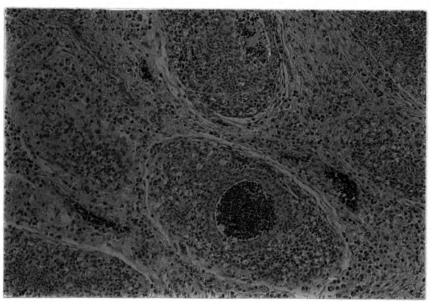

Figure 16. Photomicrograph of BF from a chicken killed 4 days PI with IBDV at 5 weeks of age. Note fibroplasia and inflammatory cells infiltration. Also note lymphoid necrosis, depletion and cyst formation. H & E . X 125.

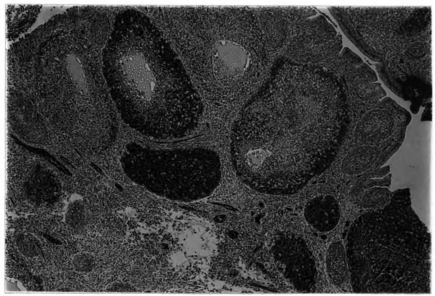

Figure 17. Photomicrograph of BF from CY-treated chicken that had been inoculated with 12.5 X 10^6 bursa cells and killed 4 days PI with IBDV at 5 weeks of age. Note lymphoid necrosis, cysts formation in newly developed follicles. H & E . X 50.

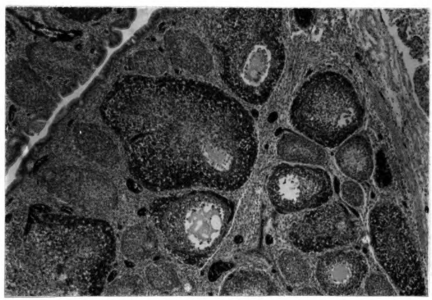

Figure 18. Photomicrograph of BF from CY-treated chicken that had been inoculated with 62 X 10^6 bursa cells and killed 4 days PI with IBDV at 5 weeks of age. Note lymphoid necrosis, cysts formation in newly developed follicles. H & E . X 50.

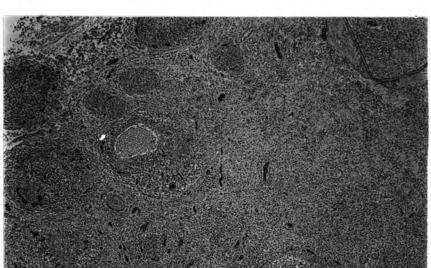

Figure 19. Photomicrograph of BF from CY-treated chicken that had been inoculated with 100 X 10^6 bursa cells and killed 4 days PI with IBDV at 5 weeks of age. Note severe infection, only ghosts of follicles remain. H & E . X 50.

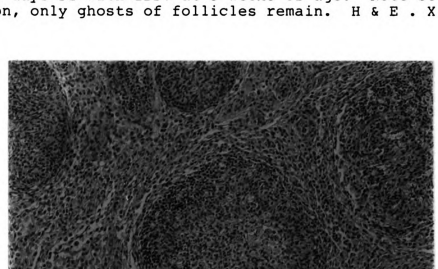

Figure 20. Photomicrograph of BF from CY-treated chicken that had been reconstituted with 100 X 10^6 bursa cells and killed 4 days PI with IBDV at 5 weeks of age. Note severe inflammatory cells infiltration, lymphocytic depletion and necrosis in newly developed follicle. H & E . X 125.

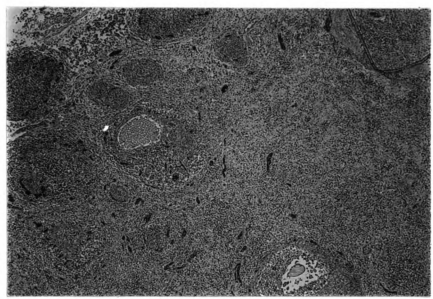

Figure 19. Photomicrograph of BF from CY-treated chicken that had been inoculated with 100 X 10^6 bursa cells and killed 4 days PI with IBDV at 5 weeks of age. Note severe infection, only ghosts of follicles remain. H & E . X 50.

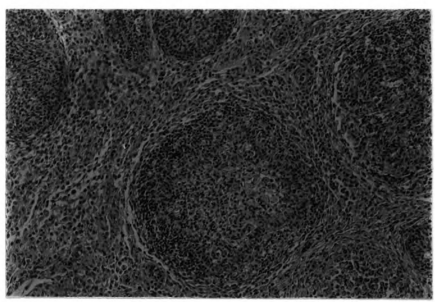

Figure 20. Photomicrograph of BF from CY-treated chicken that had been reconstituted with 100 X 10^6 bursa cells and killed 4 days PI with IBDV at 5 weeks of age. Note severe inflammatory cells infiltration, lymphocytic depletion and necrosis in newly developed follicle. H & E . X 125.

Influence of surgical bursectomy at 5 weeks of age on response of chickens to infection with IBDV (Expt. 3).

Data in Table 9 show the influence of surgical bursectomy at 5 weeks of age on the development of IBDV-induced clinical manifestations, viremia and antibody. In this experiment intact chickens developed severe clinical IBD. At 3 days PI, severe depression and ruffled feathers were seen. Further, 40% of these chickens died between 4 and 5 days PI. Typical gross and histological lesions of IBD were noted in these chickens. Grossly the BF was atrophied, gelatinous, creamy yellow in color. Microscopically the bursal follicles were atrophied. Necrosis, severe depletion of lymphocytes and infiltration with inflammatory cells were detected. In contrast, surgically bursectomized chickens did not develop clinical IBD within 11 days PI.

At 4 days PI, the concentration of IBDV in spleens of intact chickens was 100 times higher than that in bursectomized chickens. IBDV neutralizing antibodies were not detected in bursectomized chickens at either 4 or 11 days PI with IBDV. In contrast, the average titers of IBDV neutralizing antibodies in intact chickens were 48 and 3200 at 4 and 11 days PI with virus, respectively. All spleens collected from intact uninfected chickens were negative for IBDV. Further, all sera collected from intact uninoculated chickens were negative IBDV antibody.

Table 9. Influence of surgical bursectomy at 5 weeks of age on response of chickens to infection with IBDV

Treatment					IBDV recovery[d]		IBDV neutralizing antibody			
Surgical bursectomy[a]	IBDV[b]	No. of chickens	IBD[c] clinical signs	Mortality %	no. positive/tested	Average titer/ml	no. positive/tested 4 days PI	no. positive/tested 11 days PI	Average titer[e] 4 days PI	Average titer[e] 11 days PI
-	+	20	+	40	5/5	$5 \times 10^{3.7}$	5/5	7/7	48	>3200
+	-	19	-	0	0/5	0	0/5	0/14	$\leq$20	<20
+	+	20	-	0	1/5	$5 \times 10^{1.63}$	0/5	0/14	$\leq$20	$\leq$20
-	-	5	-	0	0/5	0	0/5	0/14	<20	<20

[a] 15, 5 X 7, chickens were surgically bursectomized at 5 weeks of age, for anesthesia Biotal was used at rate of 1.25 mg/400 gm body weight.

[b] Each chicken was inoculated by eye drop with 500 EID_{50} of the IM strain of IBDV.

[c] Clinical signs such as severe depression and ruffled feathers were observed at 3-4 days PI.

[d] Spleen tissues were examined at 4 days PI with virus at 5 weeks of age. Tissues were assayed for IBDV by inoculation of a 10% suspension on CAM of 10-day-old embryos at a rate of 0.2ml/embryo.

[e] Two-fold serial dilutions of each serum sample in a constant amount (1000 T CID) of IBDV, the end point was the reciprocal of the highest dilution of serum that protected a monolayer of CEF from IBDV-induced cytopathic effect (CPE).

DISCUSSION

Data from this study provide further evidence for the important role of the BF in the pathogenesis of IBD in chickens. The data indicate that age at infection, but not the genetic constitution of White Leghorn chickens may influence the response of chickens to infection with IBDV. An early and upper age resistance, regardless of status of antibody, to IBDV-induced clinical manifestations has been proposed (Hitchner, 1971; Ley, et al., 1979a). Further, data from this study (experiment 2) reveal for the first time, the relationship between the number of target bursa cells available and the susceptibility of chickens to IBDV-induced clinical signs and lesions.

Results from experiment 1 indicate that chickens inoculated with IBDV at 1 day of age were considerably less susceptible to clinical manifestations of IBD than those inoculated with virus at 42 days of age, regardless of line of chicken used. However, bursal damage induced by IBDV in chickens inoculated at 1 day of age was of the same degree as in chickens inoculated with virus at 42 days of age. Chickens inoculated with IBDV at 1 or 11 weeks of age were shown to be less susceptible to clinical manifestations of IBD than chickens inoculated at 5 weeks of age (Fadly and

Nazerian, 1983). Analysis of viremia and antibody results indicate that the frequency of detecting IBDV in blood was significantly ($p \leq 0.05$) higher in chickens inoculated with virus at 1 day of age than in those inoculated at 42 days of age. In contrast, the frequency and titer of IBDV antibody were higher in chickens inoculated with virus at 42 days of age than in those inoculated at 1 day of age. These results clearly indicate that 42-day-old chickens are capable of mounting a stronger and quicker immune response to IBDV than are 1-day-old chickens. The relatively high titer IBDV antibody in chickens inoculated with virus at 42 days of age is probably the reason for the low frequency of detecting IBDV in blood of these chickens. IBDV antibody has been shown to rise more rapidly in chickens inoculated with IBDV at 8 weeks of age than in those inoculated with virus at 2 weeks of age (Skeeles, et al., 1979a). In the present study, mortality induced by IBDV in chickens inoculated with virus at 42 days of age ranged from 40%-80%. None of line P chickens died within 7 days following inoculation with IBDV at 42 days of age, although severe gross and microscopic IBD lesions were seen in all inoculated chickens of this line. Susceptibility of line P chickens to IBDV-induced lesions and mortality has been reported previously (Fadly, et al., 1985). Thus, although none of line P chickens died in this experiment, it is clear that these chickens are as susceptible to IBD as chickens of other lines following inoculation with virus at 1 or 42 days of age.

Data from experiment 2 suggest that the development of IBDV-induced lesions, viremia and antibody in chickens is dependent on the number of target bursa cells available. Inoculation of 0.5 or 2.5 X 10^6 bursa cells did not restore the structure or function of the bursa-dependent lymphoid system. Complete restoration of the bursa-dependent immune function and partial restoration of the bursal structure were seen only in CY-treated chickens which received 12.5 X 10^6 or more bursa cells. Toivanen et al. (1972b) reported that bursal cells from 3 1/2-10-week-old donors were capable of restoring the humoral immune function, but not the bursal morphology. In another study Toivanen et al. (1974a) reported that transplantation of histocompatible bursal stem cells from 3-day-old donors resulted in a complete reconstitution of the bursa-dependent lymphoid system both in function and in morphology. Further, Purchase and Gilmour (1975), have shown that hemagglutination response to SRBC was restored in all CY-treated chickens that received bursa cells from 7-8-day-old donors. In another study, Fadly, et al. (1981) found that restoration of the humoral immune response in CY-treated chickens inoculated with bursa cells from 7- or 13-week-old chickens infected with avian leukosis virus was less effective than that had been achieved previously with normal donors (Toivanen and Toivanen, 1973). In the present study, the relative bursa weights were considerably higher in CY-treated chickens that received 12.5 X 10^6 or more bursa cells than in CY-treated control chickens.

Relative bursa weights have been shown to be a reliable indicator of the morphological restoration of the lymphoid follicles of BF (Toivanen and Toivanen, 1973). Also, data from this experiment indicate that the degree of restoration of bursal lymphoid follicles was directly related to the number of bursa cells inoculated.

Results of gross and microscopic evaluation of BF of chickens at 4 days PI with IBDV at 5 weeks of age revealed prominent gross lesions of IBD in CY-treated chickens inoculated with 62×10^6 or 10×10^7 bursa cells. Gross lesions in these two groups of chickens were as severe as those in intact chickens inoculated with IBDV at 5 weeks of age. Microscopic evaluation of bursal tissues revealed that the degree of IBDV-induced lesions is dependent on the number of bursa cells inoculated. In contrast, no IBD lesions were seen in chickens treated only with CY or in CY-treated chickens inoculated with 0.5×10^6 bursa cells. These data support the results reported by Fadly, et al. (1976), who showed that CY-treated chickens were refractory to IBDV-induced clinical signs and lesions. In the present study, restoration of the susceptibility of CY-treated chickens to IBDV-induced lesions was achieved by inoculation of 62×10^6 - 10×10^7 bursa cells. Restoration of susceptibility of CY-treated chickens to lymphoid leukosis, a disease in which the BF is the target organ, has also been achieved by inoculation of susceptible bursa cells (Purchase and Gilmour, 1975).

Analysis of results obtained from virus isolation tests indicate that the concentration of IBDV in spleens of CY-treated chickens was directly related to the number of bursa cells inoculated. At 4 days PI, the lowest concentration of IBDV was seen in spleens of CY-treated chickens inoculated with 2.5×10^6 or less bursa cells. The concentration of IBDV in spleens of CY-treated chickens inoculated with 12.5×10^6 or more bursa cells was about 100 times higher than that in spleens of CY-treated chickens inoculated with 2.5×10^6 or less bursa cells. These results agree in part with that of Kaufer and Weiss (1980), who found that the titer of IBDV in the spleen, thymus and liver of surgically bursectomized chickens was about 1,000 times lower than in the intact chickens. Obviously, the presence of enough number of bursa cells is a crucial point in virus replication and pathogenesis of the disease. Schat, et al. (1981) indicated that embryonal bursectomy delayed but did not prevent the appearance of IBDV-infected cells. These authors demonstrated IBD viral antigen in the spleen, thymus, caecal tonsils and kidneys in embryonally bursectomized chickens.

Analysis of results obtained from the virus neutralization tests suggests that the frequency of detection and titer of IBDV antibody were also dependent on the number of bursa cells inoculated. At 11 days PI, the titer of IBDV neutralizing antibody in CY-treated chickens inoculated with 12.5×10^6, 62×10^6 or 10×10^7 bursa cells were 240, 2000 or 2830, respectively. In intact chickens inoculated with

IBDV at 5 weeks of age, the virus neutralizing antibody was as high as 3200. In contrast, IBDV neutralizing antibody was not detected in chickens treated only with CY or in those inoculated with 2.5×10^6 or less bursa cells. Schat, et al. (1981) found that embryonally bursectomized chickens inoculated with IBDV at 6 weeks of age were negative for virus-neutralizing antibodies. In the present study, the rate of detecting IBDV neutralizing antibody at 11 days PI was significantly ($p \leq 0.05$) higher in CY-treated chickens inoculated with 12.5×10^6 or more bursa cells than in those inoculated with 2.5×10^6 or less bursa cells (78%-100% vs. 0%).

Data from experiment 3 indicate that chickens surgically bursectomized prior to inoculation with IBDV at 5 weeks of age were resistant to clinical IBD. Similar results have been reported in chickens surgically bursectomized prior to inoculation with IBDV at 4 weeks of age (Kaufer & Weiss, 1980). The virus concentration in spleens of surgically bursectomized chickens was about 100 times lower than that in intact chickens. These findings agree with those of Kaufer and Weiss (1980) who found that the concentration of IBDV in spleen, thymus and liver of 4-week-old surgically bursectomized chickens was about 1,000 times lower than in intact chickens. Further, in the present study the IBDV neutralizing antibody in surgically bursectomized chickens was considerably lower than that in intact chickens. Because surgical bursectomy after 3 weeks of age does not signif-

icantly impair antibody formation (Peterson, 1964; Warner and Szenberg, 1964; Cooper et al., 1969; Glick, 1977), the low IBD antibody response seen in surgically bursectomized chickens is probably due to insufficient number of target cells necessary to establish infection.

Data from this study support the hypothesis that the bursa cells are the major target in IBDV infection. However, IBDV has been shown to replicate in macrophages and granulocytes (Kaufer & Weiss, 1976; Muller, et al., 1979). Hirai and Calnek (1979) clearly indicated that the target cells for IBDV infection are the B-cells and that T-cells are either totally refractory or have very low susceptibility to IBDV. Furthermore, those authors have postulated that the IgM-bearing cells are the specific target cells for IBDV infection. In contrast, Schat, et al. (1981) indicated that the BF or the antibody-producing cells were not required to initiate and sustain infection with IBDV in embryonally bursectomized chickens inoculated with virus at 2 or 6 weeks of age.

Data reported in this study furnish further proof that the BF plays an important role in the pathogenesis of IBD. In addition, the data suggest that availability of a large number of highly susceptible cells is a crucial point in the development of clinical IBD. These data also suggest that there is little effect, if any, of the genetic constitution on the response of White Leghorn chickens to infection with IBDV.

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