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Immunosuppression by Avian Infectious Bursal Disease Virus and Mouse Hepatitis Virus

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1. INTRODUCTION

This chapter briefly discusses two viruses that infect diverse species of animals but that share an important similarity in that both viruses are lymphotropic and cause profound immunosuppression in their respective hosts. Infectious bursal disease (IBD) of chickens, also referred to as Gumboro disease, is an economically important disease of commercial chickens. In unprotected chickens, the IBD virus (IBDV) rapidly destroys the lymphocyte population in the bursa of Fabricius, the principal organ that regulates humoral immunity in the chicken. Continued economic loss due to IBD in the field and recent general interest in viral immunosuppression have stimulated renewed efforts in understanding the characteristics of the immunosuppressive effects of this disease. The mouse

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hepatitis virus (MHV), also a common infection in laboratory mouse colonies, causes a debilitating disease accompanied by severe immunosuppression. The influence of MHV on immune functions of the host seems to be related to a close interaction between virus particles and host lymphoid cells.

This discussion is not intended to be a comprehensive review of IBDV and MHV. Only the important features of the infections are discussed, with emphasis on the influence these viruses have on the immune capabilities of the host.

2. IMMUNOSUPPRESSION BY AVIAN INFECTIOUS BURSAL DISEASE VIRUS

2.1. A Characterization of the Virus and the Disease

Infectious bursal disease virus is widespread in the environment and infects most commercial populations of chickens early in life. The virus nucleocapsid is a naked icosahedron with 32 capsomeres and a diameter of 55–63 nm.[1] The IBDV genome is double-stranded RNA.[2] Recent molecular cloning studies with an Australian isolate of IBDV have demonstrated that the genome has a large segment of 3400 bp and a small segment of 2900 bp.[3] The large segment codes for five proteins of molecular weights 52, 41, 32, 28, and 16, kDa, respectively, whereas the small segment codes for a single protein of 90 kDa. Several viral structural proteins identified from purified virus preparations have been examined for their immunogenic potential.[4]

In the laboratory, IBDV can be propagated in embryonated chicken eggs. Best virus yields may be obtained by inoculating 9- to 10-day-old embryos from IBDV-free flocks by the dropped chorioallantoic membrane route.[5] Some isolates of IBDV have been adapted to cell cultures of avian and mammalian origin. Chick embryo fibroblast cells are used most frequently for *in vitro* studies with cell-culture-adapted IBDV.[6]

The chicken is the most common natural host of IBDV, although natural infection may also occur in other avian species, particularly turkeys. The IBDV isolates may be classified into serotypes 1 and 2. The two serotypes cross-react by the immunofluorescent test but not by the virus neutralization test.[7,8] Most isolates of chicken origin fall into serotype 1 and most isolates of turkey origin into serotype 2, although there is no strict species restriction of the two serotypes. Under natural conditions, turkeys exposed to IBDV do not develop clinical disease or detectable immunosuppression.

Cosgrove[9] first reported the disease in chickens. Chickens acquire infection from contaminated premesis; there is no evidence for vertical transmission. The virus replicates in B lymphocytes and one of the first detectable lesions is necrosis of lymphoid elements in the bursa of Fabricius.[10] Bursal necrosis is accompanied by inflammatory changes. Other lymphoid organs such as spleen and thymus also experience transient lymphoid cell depletion. Bursal degeneration is permanent. Predilection of IBDV for B lymphocytes was also demonstrated *in vitro*. Established lymphoid cell lines of B but not of T cells were susceptible to infection with IBDV.[11]

The age of the chicken at the time of infection with IBDV seems to determine the nature of the ensuing disease. In chickens younger than 3 weeks, IBDV does not cause high mortality, although the chickens develop bursal atrophy and severe immunosuppression. In older chickens, clinical disease occurs and is characterized by sudden onset, variable but often high mortality, and rapid recovery of survivors.

The principal economic concern with IBDV in commercial chicken flocks is the effect of this disease on immune competence of young chickens. Because infection occurs soon after hatching, under natural conditions the infected chickens respond poorly to vaccines used routinely to protect against common viral infections. Infected chickens also become vulnerable to opportunistic infections. The nature of IBDV-induced immunosuppression in humoral and cellular responses is described below.

2.2. Influence on Circulating B and T Lymphocytes

Several attempts have been made to study the effect of IBDV on circulating B and T lymphocytes.[12–14] In general, infection with IBDV reduced the number of circulating B cells. The depression in B-cell numbers was more pronounced in chickens exposed to the virus *in ovo* or at the time of hatching than in those in which exposure was delayed until the birds were 3 weeks of age or older. The reduction in circulating B cells was detected within 1 week after virus inoculation and persisted through the observation period of 8 weeks.

The influence of IBDV on circulating T lymphocytes was variable. In one study,[14] T-cell numbers were reduced below control levels if infection occurred at the time of hatching but were increased if the infection was delayed until birds were 3 weeks of age. In another study,[13] this relationship of age at the time of infection with numbers of circulating T cells was the reverse of the previous findings.

2.3. Influence on Antibody Production

Infectious bursal disease virus severely compromises the ability of chickens to mount antibody responses against a variety of infectious and noninfectious antigens including viral, bacterial, and protozoan antigens.[13,15–21] The age at which chickens become exposed to the virus has a profound effect on the degree of B-cell immunosuppression. Infection during the first 2 weeks of age results in much more severe immunodepression than does infection at older ages.[22] Both primary and secondary antibody responses may be reduced.[13,16] B-cell immunosuppression following infection with IBDV during the early posthatching period is probably persistent, although the duration of immunosuppression has not been well established.

Infection with IBDV also affects serum immunoglobulin (Ig) levels. Serum IgM levels generally dropped following IBDV infection; whereas IgG levels varied depending on the age of the chicken at the time of infection.[13,16] The IgG levels measured at eight weeks of age were lower in virus infected chickens

than in age-matched control chickens if infection occurred before or at the time of hatching but the levels were elevated if infection occurred at one week of age or older.[13] IBDV also caused a defect in the IgM that was produced. Ivanyi and Morris [16] noted that chickens infected with IBDV exclusively produced IgM as a 7S monomer. Further, the IgM of infected chickens lost the MI^a allotypic marker normally present on chicken IgM.[23]

2.4. Influence on Cellular Immune Functions

Circumstantial evidence strongly indicates that IBDV may compromise cell-mediated immune functions. For example, infection with IBDV results in (1) extensive histologic lesions in the thymus and the virus replicates to high titers in the thymus [10,24]; (2) reduction in circulating T cells[14]; (3) poor efficacy of Marek disease vaccine that appears to protect mainly via cell-mediated immunity[25]; and (4) exacerbation of disease conditions in which defense by cellular immune mechanisms if important.[19,26] Despite compelling indications that IBDV may influence cell-mediated immunity, relatively meager efforts have been devoted to study this influence.

There have been conflicting reports on the ability of young chickens exposed to IBDV to reject allogeneic skin grafts; in one study, the rejection was delayed,[27] while in the others it was not.[12,18] The evidence that IBDV may influence cellular immunity comes from *in vitro* studies. Most efforts have been directed toward delineating the mitogenic response of T cells,[28–31] although other cellular functions have also been examined.[28,31,32]

Preparations of T cells obtained from IBDV-exposed chickens respond poorly to mitogens such as phytohemagglutinin (PHA) and concanavalin A (Con A). Infection at the time of hatching as well as at 3–4 weeks of age influenced mitogenic response. The depression in the mitogenic response was transient, although the time when it occurred following viral inoculation varied. When whole blood cultures were used, the T-cell responsiveness was reduced during the first 2 weeks postinfection,[28,30,31] although in one study,[28] maximum reduction occurred 6–7 weeks after viral infection. In assays conducted with peripheral blood leukocytes fractionated on Ficoll-Hypaque[30] or spleen cells,[31] the mitogenic hyporesponsiveness was consistently transient and occurred during the first 1–2 weeks of viral infection followed by complete recovery of responsiveness.

Peripheral blood leukocytes from IBDV-infected chickens were also deficient in mounting a mixed lymphocyte reaction when cocultured with allogeneic stimulator cells.[28] A reduced mixed lymphocyte response was detected in chickens exposed to IBDV at the time of hatching or at 3 weeks of age; chickens infected at the time of hatching were more severely affected than were those infected at 3 weeks. Interestingly, unlike the mitogenic response that was affected transiently, the defect in mixed lymphocyte reaction was persistent and was detectable until the birds were 10 weeks of age, the longest interval between infection and testing. The ability of cells from virus-infected chickens to serve as stimulator cells in the mixed lymphocyte reaction assay has not been examined.

The natural killer (NK) cell activity of spleen effector cells from virus-exposed chickens was compared with that of the effector cells obtained from age-matched normal chickens.[31] No consistent differences in the activity between the two groups were noted. Similarly, IBDV did not cause detectable alteration of phagocytic activity of circulating phagocytes.[32]

2.5. Influence on Soluble Immune Factors

Little is known about the effect of IBDV on soluble mediators of immunity. Interferon-β (IFN_β) was detected in a variety of tissues and serum following inoculation with IBDV at 1 day or 3 weeks of age.[33] The role played by IFN_β in regulating immune functions is unknown. Currently, avian lymphokines are being actively studied; it should be of interest to examine possible modulation of these by IBDV.

2.6. Mechanism of Immunosuppression

2.6.1. B-Cell Immunity

It is likely that one of the major reasons for depressed antibody synthesis in chickens exposed to IBDV is that the virus selectively infects and lyses B lymphocytes. The observation that infection during the early posthatching period is more immunodepressive than infection after 3 weeks of age indicates that B-cell precursors within the confines of the bursa may be more susceptible to the cytopathic effects of IBDV than are mature B cells in circulation. Indeed, when Ivanyi and Morris[16] delayed infection with IBDV from less than 6 up to 42 hr after hatching, they noted progressively decreasing proportions of birds with immune deficiency in anti-sheep erythrocyte (anti-SRBC) antibody responses. Decreased number of circulating B cells following IBDV infection of neonates[12–14] may also indicate intrabursal destruction of B-cell precursors resulting in reduced peripheralization of B cells to the circulation. Selective susceptibility of B cells with IgM but not IgG receptors[11] further suggests that the virus is more cytopathic for B cells during early stages of differentiation before the switch from IgM to IgG expression occurs.

The mechanism by which IBDV induces the production of altered IgM, i.e., monomeric IgM that fails to polymerize and loses allotypic marker MI^a, is not known.[16] The virus may destroy IgM-producing cells that may be replaced by a population of B cells with functional impairment expressed by production of altered IgM.

Other mechanisms may also be involved in the suppression of B-cell function. For example, IBDV may compromise antibody production by damaging helper T cells or other accessory cells such as macrophages that play an important role in generating B-cell responses to certain antigens. In addition to destroying B cells, IBDV may also stimulate the appearance of suppressor cells that may participate in inhibiting antibody responses.[34]

2.6.2. T-Cell Immunity

The mechanism of hyporesponsiveness of T cells to mitogen stimulation has been examined.[29,31] We noted that spleen cells of chickens undergoing acute infection with IBDV responded poorly to PHA but that their response was restored to near normal levels if the responder cells were pretreated with carbonyl iron.[31] Thus, the spleen cell response was being inhibited by suppressor cells that could be removed by carbonyl iron treatment. The suppressor cells shared several characteristics with macrophages, i.e., the suppressor cells were adherent to plastic, were phagocytic, and resisted treatment with antithymocyte and antibursa cell sera. Suppressor cells isolated from spleen of IBDV-infected chickens were able to inhibit the mitogenic response of spleen cells of normal virus-free chickens. We recently confirmed the presence of suppressor cells in IBDV spleens using Con A as a T-cell mitogen and have shown that addition of exogenous conditioned medium with high interleukin-2 (IL-2) activity was ineffective in restoring the mitogenic response of spleen cells of IBDV-infected chickens.[35]

The above observations suggested that reduced mitogenic response of lymphocytes in IBDV-infected chickens was not due to lack of functional T cells but to the presence of suppressor cells. Other mechanisms of T-cell immunosuppression may be involved as well. Confer and MacWilliams[29] suggested that the mitogenic hyporesponsiveness of whole blood cells from IBDV-infected chickens was associated with increased number of circulating large immature lymphocytes incapable of mitogen-induced blastogenesis.

3. IMMUNOSUPPRESSION BY MOUSE HEPATITIS VIRUS

3.1. Biology of the Virus

Mouse hepatitis viruses are classified as coronaviruses.[36] They are pleomorphic or rounded enveloped particles with a diameter of 60–220 nm, surrounded by a fringe or layer of typical club-shaped spikes. Their genome consists of single-stranded polyadenylated RNA of positive polarity. Viruses are released by internal budding into cytoplasmic vesicles derived from the endoplasmic reticulum.

The antigenicity of coronaviruses is related to three major antigens.[37,38] Surface glycoproteins of murine coronaviruses are responsible for the induction of neutralizing, complement-fixing, and hemagglutination-inhibiting antibodies.[39–41] Hybridization with MHV-specific complementary DNA (cDNA) showed a close relationship among murine strains MHV-A59, MHV-3, and JHM.[42] Oligofingerprinting demonstrated genomic variations in MHV strains that could be related to neurovirulence.[42–44] These variations did not seem to correlate with the serologic relationships of these viruses.

The coronavirus genome is a positive single-stranded infectious molecule

of RNA containing about 18,000 nucleotides.[45] T_1 oligonucleotide mapping indicated that no extensive sequence reiteration occurred in the coronavirus genome[43,46,47] and that the 3′ end of the genomic RNA was polyadenylated and formed a 3′-coterminal nested sequence.[48,49] Synthesis of each of the intracellular RNAs is initiated independently and is not processed from a large precursor protein.[50,51] Subgenomic RNAs, as well as genomic RNA, contained the 5′-cap structure.[52] These RNA molecules were also found to act as individual mRNAs and to be translated into single proteins of a size corresponding to the coding capacity of the unique 5′-terminal sequences not present in the next smallest RNA.[53–55]

The nucleocapsid protein possesses a molecular weight of 50–60kDa and is nonglycosylated.[56] A cyclic adenosine monophosphate(cAMP)-independent protein kinase is associated with the virion, but it is not yet known whether the enzyme is virally coded or is a sequestered host cell enzyme.[57] A high homology of amino acid sequences has been found between nucleocapsid proteins from neuropathogenic JHM and nonpathogenic A59, although two regions of lower homology are present.[58]

The virion possesses a lipid envelope containing matrix and peplomer proteins. All coronavirions have a glycoprotein of 20–30kDa. A small glycosylated portion of the molecule is peripheral to the lipid membrane,[59] whereas a second strong hydrophobic domain is thought to correspond to a portion of the molecule integrated into the lipid membrane.[60] A stable complex between this protein and the viral RNA could be formed *in vitro*, suggesting a third domain in the protein which is internal to the lipid membrane and responsible for the interaction with viral nucleocapsids.[60,61]

Peplomer proteins are glycoproteins of molecular weight 80–200 kDa with one or two major species derived from a single primary translation product but modified by post-translational cleavage.[62] Glycosylation of the MHV peplomer protein was inhibited by tunicamycin. The lack of reabsorption and cell fusion observed in tunicamycin-treated cells suggested that the peplomer protein plays a role in the reception of virions on cell surfaces and in the induction of cell fusion.[60] In addition, trypsin treatment of A59 virus, which cleaves the 180 kDa to two 90-kDa subunit polypeptides, greatly increases the capacity of the virus to cause cell fusion.[63]

3.2. Pathogenesis of Mouse Coronaviruses

The MHV-JHM strain, the first murine coronavirus to be isolated,[64] is a neurotropic virus causing acute and chronic demyelinating diseases. Infection with other mouse hepatitis viruses results in hepatitis, encephalomyelitis and/or enteritis. It is impossible, however, to classify virus strains according to target organs since several organs can be affected. The virulent strains MHV_2 and MHV_3 and the less virulent MHV_1, MHV_5 and MHV_{A59} can cause hepatitis in newborns and adult mice, whereas MHV_S induces enteritis in newborns.

MHV_3 is the most virulent strain of MHV. The severity and the type of infection however are related to age, immune resistance, and genetic factors.

Most strains of mice display a full susceptibility, leading to death within a few days. The A/J strain is unique, as it is the only full resistant strain with 100% survival of infected adult animals. Other mouse strains are semisusceptible, and animals surviving the acute disease develop chronic manifestations with progressive neurologic involvement.[65]

3.2.1. Acute MHV_3 Infection

In some strains of mice, e.g., C57BL/6, DBA/2, BALB/c, or NZB, parenteral administration of 10 LD_{50} of MHV_3 always leads to fulminant hepatitis and death. Peritoneal macrophages, and liver Kupffer cells are the major sites of viral replication. Infectious virions are disseminated to all organs during the viremic phase.[66] By contrast, full resistance to MHV_3 is observed in the A/J mouse strain even after the administration of large doses of virus (10^7 LD_{50}). Histopathologic studies showed an absence of lesions. During the first 4 days of infection, virus was recovered from the liver of resistant as well as susceptible strains of mice. In A/J strain mice, viral titers were consistently $<10^3$ LD_{50}, whereas in susceptible DBA/2 mice, titers greater than 10^4 were always found. In the resistant mouse strain, infectious virus was cleared from the liver, brain and serum within 7 days, whereas virus continued to replicate in susceptible animals until death.[65]

3.2.2. Persistent MHV_3 Infection

In contrast to full susceptibility or resistance, other mouse strains, such as C3H or hybrid animals resulting from a cross between susceptible and resistant parents, exhibit an intermediate sensitivity. In this type (semisusceptibility), MHV_3 causes a chronic disease with paralysis, virus persistence, and immunodepression. The virus could be recovered from brain, liver, spleen, lymph nodes, or peritoneal macrophages for several months.[67] This type of disease and the clinical outcome vary greatly, however, with age, immune resistance, and genetic factors. Immunologic immaturity as observed in mice during the first 2 weeks of life, or T-cell deprivation following neonatal thymectomy or treatment with antilymphocyte antiserum, induce a full susceptibility to MHV_3 infection in mice of the normally resistant A/J strain.[68] Similarly, genetic factors are of a primary importance in MHV infection. The first evidence of an association of host genes with resistance was reported for MHV_2.[69] Genetic study of MHV_3 infection indicated that at least two recessive genes are involved in resistance to acute and chronic diseases and showed that the genes involved in both diseases are different. The capacity to resist the development of paralysis is conferred to heterozygote as well as to homozygote mice by $H\text{-}2^f$ or $H\text{-}2^q$ alleles, indicating that resistance to paralysis is H-2 linked.[70] It was also shown that such an action was mostly mediated through the expression of mouse class I antigens of the major histocompatibility complex.[71]

3.3. MHV_3-Induced Immunosuppression

MHV_3 infection in mice induces a marked nonspecific immunosuppression during the acute as well as the chronic phase of the disease. Sequential determination of Ig levels in chronically infected mice revealed a progressive decrease of all Igs during the first 3 months of infection. At the end of that period, most animals were severely hypogammaglobulinemic, and some suffered from infections.[72] In addition, a marked decrease of the antibody response against T-cell-dependent and -independent antigens was observed in semisusceptible mice chronically infected with MHV_3. In these experiments, it was observed that, when tested 40 and 80 days postinfection, primary and secondary plaque-forming cell (PFC) responses and serum antibody titers against SRBC T-dependent antigen and lipopolysaccharide (LPS) T-independent antigen were markedly diminished in paralyzed as well as in nonparalyzed mice.[72]

3.4. Immune Functions of MHV_3-Infected Mice

It was noteworthy that chronically infected mice, in spite of their hypogammaglobulinemia, developed anti-MHV_3 complement-fixing antibodies. Such antibodies were detectable 2–4 weeks following viral infection, showed an important rise by day 60 and reached a maximum level by days 100–130 after which titers decreased in surviving animals. No correlation was observed, however, between the onset of paralysis and the increase of MHV_3 antibody, in spite of the fact that bound Igs associated with antigens were found in chronic plexus vessels.[73] In addition, most of the anti-MHV_3 antibody produced in chronically infected mice was of the IgM class.

Antiviral antibodies are capable of modifying a lytic acute infection into a subacute and persistent infection or even to prevent the induction of the chronic disease.[74] The effectiveness of humoral immunity seems to depend on the titer, avidity, and neutralizing capacity of the antibody. In rats infected with the strain JHM, cerebrospinal antibodies seem to occur only in animals expressing a JHM-induced disease with viral replication in the central nervous system (CNS). Nevertheless, localized production of IgG was unable to protect rats from either the acute or the chronic neurologic disease.[75]

Specific cell-mediated immune reactions against MHV_3 antigens were detected in chronically infected mice and protection, using lymphoid cells, could be transferred from MHV_3-paralyzed animals into susceptible newborns. Similarly, a specific cellular immunity was observed during the course of MHV-JHM infection in rats. Wege *et al.*[76] showed that spleen cells obtained from diseased animals not only proliferated in the presence of basic myelin proteins *in vitro* but adoptive transfer of such cells was followed by the occurrence of experimental allergic encephalomyelitis-like lesions in the CNS. Such results suggest that MHV-JHM replication in the CNS may lead to alteration of myelin, and/or to cell membrane changes by insertion of viral proteins that may trigger, in turn, an immune response against myelin.

3.5. Mechanism of Immunosuppression

The nonspecific immunodepression always observed in MHV_3-infected animals after antigenic challenge could be related to a direct effect of the virus on one or several components of the immune response. This was tested by studying the PFC response in lethally X-irradiated normal F_1 hybrids reconstituted with T, B, or spleen cells originating from MHV_3-infected or -noninfected syngeneic animals. These experiments clearly showed that the immunocompetence of T and B lymphocytes originating from MHV_3-infected mice was normal. In addition, macrophage functions, as tested by phagocytosis of yeast particles *in vitro* and by the *in vivo* uptake of radiolabelled SRBC, were not different in infected and control animals.[77]

3.5.1. Lymphocyte Depletion

A striking feature always observed during the chronic phase of mouse hepatitis, associated or not with paralysis, is the marked decrease of cell numbers in the lymphoid organs. This is observed in bone marrow, spleen, thymus, lymph nodes, peripheral blood and peritoneal exudates. Differential enumeration, however, of T and B lymphocytes in the spleen at different times postinfection did not demonstrate important variations.

The mechanism whereby MHV_3 infection exerts a suppressive effect is related neither to a quantitative deficiency of lymphocytes nor to a dietary insufficiency for the following reasons: (1) healthy virus carrier mice are immune deficient in spite of normal body weight and a lymphocyte count similar to that of noninfected control mice; (2) T-cell functions appeared normal when tested in MHV_3-paralyzed mice; (3) T cells, B cells, or macrophages, originating from MHV_3-infected paralyzed mice, have the capacity to reconstitute immune responses fully in lethally irradiated syngeneic animals[78]; (4) in spite of a decrease in the total number of bone marrow cells, the number of spleen colony-forming cells increased sharply after infection and reached maximum levels by day 50; and (5) LPS-stimulated bone marrow or spleen B cells obtained from MHV_3-infected mice, synthesized *in vitro* IgM and IgG in amounts comparable to that produced by B cells originating from noninfected controls. In these experiments, it was not possible to detect suppressor cells or factors.[72]

3.5.2. Viral Replication in Immunocompetent Cells

Inhibition of lymphocyte activation and/or proliferation by MHV_3 seems to be a possible factor in alteration of immune functions. Previous work indicated indeed that lymphocytes supported MHV_3 replication, which in turn interfered with cellular metabolism. Viral replication in macrophage-depleted T lymphocytes was demonstrated by a progressive increase of viral titers in culture supernatants and further evidenced by dot immunobinding analysis.[77] Infection of lymphocytes with virulent MHV_3, however, results in a

strong inhibition of the lymphoproliferative response of cells stimulated with mitogens or with allogeneic cells. Viral infection of either stimulator cells or responder cells in mixed lymphocyte reactions indicated that the inhibitory effect was due to a direct contact of infectious viral particles with the proliferating cells. Since virus can be regularly recovered from brain, liver, spleen, lymph nodes, and peritoneal macrophages of MHV_3-infected mice during the evolution of the disease (up to 7 months postinfection), it is likely that persistent viral replication in lymphocytes and macrophages is responsible, at least in part, for immunosuppression and for some of the immunopathologic effects seen during the course of the chronic disease.

3.5.3. Genetic Influence

Since the direct interaction between MHV_3 and lymphoid cells appears to be a major factor for immunosuppression, the intrinsic capacity of cells to restrict viral replication was studied. Persistent MHV_3 infection can readily be induced *in vitro* in fibroblast and lymphocyte cultures.[79,80] Therefore, work was performed to see whether genetically controlled virus persistency could be detected at the *in vitro* level. A carrier state was established *in vitro* using short-term progeny passages in cells originating from various mouse strains exhibiting different sensitivities. Results showed a correlation between pehnotypic expression of *in vivo* sensitivity and the capacity, which was not H-2 linked, of macrophages or lymphocytes to restrict viral replication. This indicates that resistance to MHV_3 may be the result of restriction of viral replication in macrophages and lymphoid cells. Such restriction of MHV replication in macrophages from resistant strain mice also has been observed with other serotypes.[69,81] Macrophages genetically resistant to MHV_2 were converted *in vitro* to susceptible macrophages by lymphokines present in the supernatant fluid from allogeneic mixed lymphocyte cultures,[82] by spleen cells from cortisone-treated mice,[83] or by silica treatment.[84] By contrast, genetically susceptible macrophages were converted into resistant cells by Con A administration.[85]

In addition, resistance to viral infection displayed by other target cells, such as hepatocytes[86] and fibroblasts,[79] should minimize pathologic damage and therefore ensure the survival of infected mice and the development of an adequate immune response, leading to total elimination of virus. By contrast, susceptible mice infected with the virus can neither restrict viral replication nor resist virus-induced cellular injuries. Dissemination of the infection thus leads to extensive pathologic lesions and death. The intermediate behavior, displayed by infected cells originating from semisusceptible mouse strains, seems to be related to an incomplete restriction of viral replication leading to virus persistence, cell lysis, and subsequent immunodepression. Resistance mechanisms, genetically determined and expressed at macrophage and lymphocyte levels, by controlling or not controlling viral replication, therefore seem to play a major role in MHV_3-induced immunosuppression.

4. SUMMARY

Two viral diseases with strong immunosuppressive effects were discussed. Avian IBDV replicates in B lymphocytes and causes clinical disease and associated immunosuppression. Infected chickens fail to produce antibody against a variety of antigens and show reduced T-cell response to mitogenic stimulation *in vitro*. The mechanism of immunosuppression is not entirely clear, although lysis of B cells or B-cell precursors by IBDV is likely the cause of failure of the antibody response. The mitogenic hyporesponsiveness of T cells appears to be mediated by virus-activated suppressor macrophage-like cells.

Mouse hepatitis virus, a coronavirus that causes an acute or chronic disease in laboratory mice, also induces immunosuppression. Infected animals develop persistent hypogammaglobulinemia and show decreased antibody response against T-dependent and T-independent antigens. The virus replicates in lymphocytes and macrophages, and the reduced immune responsiveness likely results from direct interaction of the virus with cells of the immune system. Suppressor cells or factors do not seem to be involved.

REFERENCES

1. Hirai, K., N. Kato, A. Fujiura, and S. Shimakura, Further morphological characterization and structural proteins of infectious bursal disease virus, *J. Virol.* **32**:323–328 (1979).
2. Muller, H., C. Scholtissek, and H. Becht, The genome of infectious bursal disease virus consists of two segments of double stranded RNA, *J. Vtrol.* **31**:584–589 (1979).
3. Azad, A. A., S. A. Barrett, and K. J. Fahey, The characterization and molecular cloning of the double-stranded RNA genome of an Australian strain of infectious bursal disease virus, *Virology* **143**:35–44 (1985).
4. Fahey, K. J., I. J. O'Donnell, and A. A. Azad, Characterization by Western blotting of the immunogens of infectious bursal disease virus, *J. Gen. Virol.* **66**:1479–1488 (1985).
5. Hitchner, S. B., Infectivity of infectious bursal disease virus for embryonating eggs, *Poultry Sci.* **49**:511–516 (1970).
6. Lukert, P. D., and R. B. Davis, Infectious bursal disease virus: Growth and characterization in cell cultures, *Avian Dis.* **18**:243–250 (1974).
7. McFerran, J. B., M. S. McNutty, E. R. McKillop, T. J. Conner, R. M. McCracken, D. S., Collins, and G. M. Allan, Isolation and serological studies with infectious bursal disease virus from fowl, turkeys and ducks: Demonstration of a second serotype, *Avian Pathol.* **9**:395–403 (1980).
8. Jackwood, D. J., Y. M. Saif, and J. H. Hughes, Characteristics and serologic studies of two serotypes of infectious bursal disease virus in turkeys, *Avian Dis.* **26**:871–882 (1982).
9. Cosgrove, A. S., An apparently new disease of chickens—Avian nephrosis, *Avian Dis.* **6**:385–389 (1962).
10. Cheville, N. F., Studies on the pathogenesis of Gumboro disease in the bursa of Fabricius, spleen and thymus of the chicken, *Am. J. Pathol.* **51**:527–551 (1967).
11. Hirai, K., and B. W. Calnek, In vitro replication of infectious bursal disease virus in established lymphoid cell lines and chicken B lymphocytes, *Infect. Immun.* **25**:964–970 (1979).
12. Hudson, L., M. Pattison, and N. Thantrey, Specific B lymphocyte suppression by infectious bursal agent (Gumboro virus) in chickens, *Eur. J. Immunol.* **5**:675–679 (1975).

13. Hirai, K., K. Kunihiro, and S. Shimakura, Characterization of immunosuppression in chickens by infectious bursal disease virus, *Avian Dis.* **23**:950–965 (1979).
14. Sivanandan, V., and S. K. Maheswaran, Immune profile of infectious bursal disease. I. Effect of infectious bursal disease virus on peripheral blood T and B lymphocytes of chickens, *Avian Dis.* **24**:715–725 (1980).
15. Allan, W. H., J. T. Farraghar, and G. A. Cullen, Immunosuppression by infectious bursal agent in chickens immunized against Newcastle disease, *Vet. Rec.* **90**:511–512 (1972).
16. Ivanyi, J., and R. Morris, Immunodeficiency in the chicken. IV. An immunological study of infectious bursal disease, *Clin. Exp. Immunol.* **23**:154–165 (1976).
17. Giambrone, J. J., C. S. Eidson, and S. H. Kleven, Effect of infectious bursal disease on the response of chickens to *Mycoplasma synoviae,* Newcastle disease virus and infectious bronchitis virus, *Am. J. Vet. Res.* **38**:251–253 (1977).
18. Giambrone, J. J., J. P. Donahoe, D. L. Dawe, and C. S. Eidson, Specific suppression of the bursa-dependent immune system of chicks with infectious bursal disease virus, *Am. J. Vet. Res.* **38**:581–583 (1977)
19. Anderson, W., W. M. Reid, and P. D. Lukert, Influence of infectious bursal disease on the development of immunity to *Eimeria tenella, Avian Dis.* **21**:637–641 (1978).
20. Hopkins, I. G., K. R. Edwards, and D. H. Thornton, Measurement of immunosuppression in chickens caused by infectious bursal disease vaccines using *Brucella abortus* strain 19, *Res. Vet. Sci.* **27**:260–261 (1979).
21. Pejkovski, C., F. G. Develaar, and B. Kouwenhoven, Immunosuppressive effect of infectious bursal disease virus on vaccination against infectious bronchitis, *Avian Pathol.* **8**:95–106 (1979).
22. Faragher, J. T., W. H. Allen, and C. J. Wyeth, Immunosuppressive effect of infectious bursal agent on vaccination against Newcastle disease, *Vet. Rec.* **95**:385–388 (1974).
23. Ivanyi, J., Polymorphism of chicken serum allotypes, *J. Immunogenet.* **2**:87–107 (1975).
24. Kaufer, I., and E. Weiss, Significance of bursa of Fabricius as target organ in infectious bursal disease of chickens, *Infect. Immun.* **27**:364–367 (1980).
25. Sharma, J. M., Effect of infectious bursal disease virus on protection against Marek's disease by turkey herpesvirus vaccine, *Avian Dis.* **28**:629–640 (1984).
26. McDonald, L. R., T. Karlson, and W. M. Reid, Interaction of infectious bursal disease and coccidiosis in layer replacement chickens, *Avian Dis.* **24**:999–1005 (1980).
27. Panigraphy, B., L. K. Misra, S. A. Naqi, and C. F. Hall, Prolongation of skin graft survival in chickens with infectious bursal disease, *Poultry Sci.* **56**:1745 (1977).
28. Sivanandan, V., and S. K. Maheswaran, Immune profile of infectious bursal disease. III. Effect of infectious bursal disease on the lymphocyte responses to phytomitogens and on mixed lymphocyte reaction of chickens, *Avian Dis.* **25**:112–121 (1981).
29. Confer, A., and P. S. MacWilliams, Correlation of hematological changes and serum and monocyte inhibition with the early suppression of phytohemagglutinin stimulation of lymphocytes in experimental infectious bursal disease, *Can. J. Comp. Med.* **46**:169–175 (1982).
30. Confer, A. W., W. T. Springer, S. M. Shane, and J. F. Donovan, Sequential mitogen stimulation of peripheral blood lymphocytes from chickens inoculated with infectious bursal disease virus, *Am. J. Vet. Res.* **452**:2109–2113 (1981).
31. Sharma, J. M., and L. F. Lee, Effect of infectious bursal disease on natural killer cell activity and mitogenic response of chicken lymphoid cells: Role of adherent cells in cellular immune suppression, *Infect. Immun.* **42**:747–754 (1983).
32. Santivatr, D. S. K. Maheswaran, J. A. Newman, and B. S. Pomeroy, Effect of infectious bursal disease virus infection on the phagocytosis of *Staphylococcus aureus* by mononuclear phagocytic cells of susceptible and resistant strains of chickens, *Avian Dis.* **25**:303–311 (1981).
33. Gelb, J., C. S. Eidson, O. J. Fletcher, and S. H. Kleven, Studies on interferon induction by

infectious bursal disease virus (IBDV). II. Interferon production in White Leghorn chickens infected with an attenuated or pathogenic isolant of IBDV, *Avian Dis.* **23**:634–645 (1979).
34. Blaese, R. M., A. V. Muchmore., I. Koski., and N. J. Dooley, Infectious agammaglobulinemia: Suppressor T cells with specificity for individual immunoglobulin classes, *Adv. Exp. Biol.* **88**:155–159 (1977).
35. Sharma, J. M., and T. Fredericksen, Mechanism of T cell immunosuppression by infectious bursal disease virus of chickens, in: *Avian Immunology.* Vol. II W. T. Weber and D. L. Ewert, eds., pp. 283–294, Liss, New York (1987).
36. Tyrrell, D. A. J., J. D. Almeida, D. M. Berry, C. H., Hamre, M. S. Hofstad, L. Malluci, and K. McIntosh, Coronaviruses, *Nature (Lond.),* **220**:650 (1968)
37. Hajer, I., and J. Storz, Antigens of bovine coronavirus strain LY-138 and their diagnostic properties, *Am. J. Vet. Res.* **39**:441–444 (1978).
38. Yaseen, S. A., and M. Johnson-Lussenburg, Antigenic studies on coronavirus. I. Identification of the structural antigens of human coronavirus strain 229E, *Can. J. Microbiol.* **27**:334–342 (1981).
39. Garwes, D. I., M. H. Lucas, D. A. Higgins, B. V. Pike, and S. F. Cartwright, Antigenicity of structural components from porcine transmissible gastroenteritis virus, *Vet. Microbiology* **3**:179–190 (1979).
40. McNaughton, M. R., H. J. Hasony, and S. Reed, Antibody to virus components in volunteers experimentally infected with human coronaviruses 229E group viruses, *Infect. Immun.* **31**:845–849 (1981).
41. Schmidt, O. W., and G. E. Kenny, Immunogenicity and antigenicity of human coronavirus 229E and OC43, *Infect. Immun.* **32**:1000–1006 (1981).
42. Weiss, S. R., and J. L. Leibowitz, Comparison of the RNAs of murine human coronaviruses, in: *Biochemistry and Biology of Coronaviruses* (V. Ter Meulen, S. Siddell, and H. Wege, eds.), pp. 245–259, Plenum, New York (1981).
43. Lai, M. M. C., and S. A. Stohlman, Comparative analysis of RNA genome of mouse hepatitis virus, *J. Virol.* **38**:661–670 (1981).
44. Wege, H., J. R. Stephenson, M. Koga, and V. Ter Meulen, Genetic variation of neurotropic and non-neurotropic murine coronaviruses, *J. Gen. Virol.* **54**:67–74 (1981).
45. Robb, J. A., and C. W. Bond, Coronaviridae, in: *Comprehensive Virology* (H. Fraenkel-Conrat and R. R. Wagner, eds.), pp. 193–247, Plenum, New York (1979).
46. Lomniczi, B., and I. Kennedy, Genome of infectious bronchitis virus, *J. Virol.* **24**:99–107 (1977).
47. Lai, M. M. C., and S. A. Stohlman, Genomic structure of mouse hepatitis virus: Comparative analysis by oligonucleotide mapping, in: *Biochemistry and Biology of Coronaviruses* (V. Ter Meulen, S. Siddell, and H. Wege, eds.), pp. 69–82, Plenum, New York (1981).
48. Yogo, Y., N. Hirano, H. Shibuta, and M. Matumoto, Polyadenylate in the virion RNA of mouse hepatitis virus, *J. Biochem. (Tokyo)* **82**:1103–1108 (1977).
49. McNaughton, M. R., and M. H. Madge, The genome of human coronavirus strain 229E, *J. Gen. Virol.* **39**:497–504 (1978).
50. Jacobs, L., W. J. M. Spaan, M. E. Horzinek, and B. A. M. Van der Zeijst, Synthesis of subgenomic mRNA of mouse hepatitis virus is initiated independently: Evidence from UV transcription mapping, *J. Virol.* **39**:401–406 (1981).
51. Stern, D. F., and B. M. Sefton, Synthesis of coronavirus mRNAs: Kinetics of inactivation of infectious bronchitis virus RNA synthesis by UV light, *J. Virol.* **42**:755–759 (1982).
52. Lai, M. M. C., C. D. Patton, and S. A. Stohlman, Replication of mouse hepatitis virus: Negative-stranded RNA and replicative form RNA are a genome length, *J. Virol.* **44**:487–492 (1982).
53. Rottier, P. J. M., M. C. Horzinek, and B. A. M. Van der Zeijst, Viral protein synthesis in mouse hepatitis virus strain A59-infected cells: Effect of Tunicamycin, *J. Virol.* **40**:350–357 (1981).

54. Leibowitz, J. L., S. R. Weiss, E. Paavola, and C. W. Bond, Cell-free translation of murine coronavirus RNA, *J. Virol.* **43:**905–913 (1982).
55. Siddell, S. G., Coronavirus JHM: Coding assignment of subgenomic mRNAs, *J. Gen. Virol.* **64:**113–125 (1983).
56. Siddell, S. G., H. Wege, and V. Ter Meulen, The structure and replication of coronaviruses, *Curr. Topics Microbiol. Immunol.* **99:**131–163 (1982).
57. Siddell, S. G., S. G. Barthel, and V. Ter Meulen, Coronavirus JHM: A virion-associated protein kinase, *J. Gen. Virol.* **52:**235–243 (1981).
58. Armstrong, J., S. Smeekens, and P. Rottier, Sequence of the nucleocapsid gene from murine coronavirus MHV-A59, *Nucleic Acid Res.* **11:**883–891 (1983).
59. Sturman, L. S., Characterization of a coronavirus. I. Structural proteins' effects on preparative conditions on the migration of protein in polyacrylamide gels, *Virology.* **77:**637–649 (1977).
60. Sturman, L. S, The structure and behaviour of coronavirus A59 glycoprotein, in: *Biochemistry and Biology of Coronaviruses* (V. Ter Meulen, S. Siddell, and H. Wege, eds.), pp. 1–18, Plenum, New York (1981).
61. Sturman, L. S., K. V. Holmes, and J. Behnke, Isolation of coronavirus envelope glycoproteins and interaction with the viral nucleocapsids, *J. Virol.* **33:**449–462 (1980).
62. Sturman, L. S., and K. V. Holmes, Characterization of a coronavirus. II. Glycoproteins of the viral envelope: Tryptic peptide analysis, *Virology* **77:**650–660 (1977).
63. Sturman, L. S., and K. V. Holmes, Proteolytic cleavage of peplomeric glycoprotein E2 of MHV yields two 90K subunits and activates fusion, in: *Molecular Biology and Pathogenesis of Coronaviruses* (P. M. J. Rottier, B. A. M. Van der Zeijst, W. J. M. Spaan, and M. C. Horzinek, eds.), pp. 25–35, Plenum, New York (1984).
64. Cheever, F. S., J. B. Daniels, A. M. Pappenheimer, and O. T. Bailey, A murine virus (JHM) causing disseminated encephalomyelitis with extensive destruction of myelin, *J. Exp. Med.* **90:**181–194 (1949).
65. Le Prevost, C., E. Levy-Leblond, J. L. Virelizier, and J. M. Dupuy, Immunopathology of mouse hepatitis virus type 3 infection. I. Role of humoral and cell-mediated immunity in resistance mechanisms, *J. Immunol.* **114:**221–225 (1975).
66. Piazza, M., G. Pane, and F. De Ritis, The fate of MHV-3 after intravenous injection into susceptible mice, *Arch. Ges. Virusforsch.* **22:**472–475 (1967).
67. Le Prevost, C., J. L. Virelizier, and J. M. Dupuy, Immunopathology of mouse hepatitis virus type 3 infection. III. Clinical and mitogenic observations of a persistant virus infection, *J. Immunol.* **115:**640–643 (1975).
68. Dupuy, J. M., E. Levy-Leblond, and C. Le Prevost, Immunopathology of mouse hepatitis virus type 3 infection. II. Effect of immunosuppression in resistant mice, *J. Immunol.* **114:**226–230 (1975).
69. Bang, F. B., and A. Warwick, Mouse macrophages as host cells for the mouse hepatitis virus and the genetic basis of their susceptibility, *Proc. Natl. Acad. Sci. USA* **46:**1065–1075 (1960).
70. Levy-Leblond, E., D. Oth, and J. M. Dupuy, Genetic study of mouse sensitivity to MHV-3 infection: Influence of the H-2 complex, *J. Immunol.* **112:**1359–1362 (1979).
71. Oth, D., D. Pekovic, V. Cainelli-Gebara, and J. M. Dupuy, Expression of H-2K antigens in brain lesions, and influence of H-2K gene on susceptibility to paralysis, in MHV_3 infected mice, in: *Genetic Control of Host Resistance to Infection and Malignancy* (E. Skamene and P. Kongshavn, eds.), pp. 135–140, Liss, New York (1985).
72. Leray, D., C. Dupuy, and J. M. Dupuy, Immunopathology of mouse hepatitis virus type 3 infection. IV. MHV3-induced immunosuppressions, *Clin. Immunol. Immunopathol.* **23:**1457–1465 (1982).
73. Virelizier, J. L., A. D. Dayan, and A. C. Allison, Neuropathological effects of persistent infection of mice by mouse hepatitis virus, *Infect. Immun.* **12:**1127–1140 (1975).
74. Levy, G., R. Shaw, J. L. Leibowitz, and E. Cole, The immune response to mouse hepatitis

virus infection: Genetic variation in antibody response and disease, in: *Molecular Biology and Pathogenesis of Coronaviruses* (P. J. M. Rottier, B. A. M. Van der Zeijst, W. J. M. Spaan, and M. C. Horzinek, eds), pp. 345–364, Plenum, New York (1984).

75. Sorensen, O., S. Beushausen, S. Puchalski, S. Cheley, R. Anderson, M. Coulter-Mackie, and S. Dales, *In vivo* and *in vitro* models of demyelinating disease. VIII. Genetic, immunologic and cellular influences on JHM virus infection of rats, in: *Molecular Biology and Pathogenesis of Coronaviruses* (P. J. M. Rottier, B. A. M. Van der Zeijst, W. J. M. Spaan, and M. Horzinek, eds.), pp. 279–298, Plenum, New York (1984).
76. Wege, H., R. Watanabe, and V. Ter Meulen, Virological and immunological aspects of coronavirus induced subacute demyelinating encephalomyelitis in rats, in: *Molecular Biology and Pathogenesis of Coronaviruses* (P. J. M. Rottier, B. A. M. Van der Zeijst, W. J. M. Spaan, and M. Horzinek, eds.), pp. 259–270, Planum, New York (1984).
77. Krzystyniak, K., and J. M. Dupuy, Immunodepression of lymphocyte response in mouse hepatitis virus 3 infection, *Biomed. Pharmacol.* **37:**68–74 (1983).
78. Dupuy, J. M., C. Dupuy, and D. Decarie, Genetically determined resistance to mouse hepatitis virus 3 is expressed in hematopoietic donor cells in radiation chimeras, *J. Immunol.* **133:**1609–1613 (1984).
79. Lamontagne, L., and J. M. Dupuy, Natural resistance of mice to mouse hepatitis virus type 3 infection is expressed in embryonic fibroblast cells, *J. Gen. Virol.* **65:**1165–1171 (1984).
80. Lamontagne, L. and J. M. Dupuy, Persistent infection with mouse hepatitis virus 3 in mouse lymphoid cell lines, *Infect. Immun.* **44:**716–723 (1984).
81. Taguchi, F., N. Hirano, Y. Kiuchi, and K. Fujiwara, Difference in response to mouse hepatitis virus among susceptible mouse strains, *Jpn. J. Microbiol.* **20:**293–302 (1976).
82. Weiser, W., and F. B. Bang, Macrophages genetically resistant to mouse hepatitis virus converted *in vitro* to susceptible macrophages, *J. Exp. Med.* **143:**690–695 (1976).
83. Taylor, C. E., W. Y. Weiser, and F. B. Bang, *In vitro* macrophage manifestation of cortisone-induced decrease in resistance to mouse hepatitis virus, *J. Exp. Med.* **153:**732–737 (1981).
84. Taguchi, F., A. Yamada, and K. Fujiwara, Resistance to highly virulent mouse hepatitis virus acquired by mice after low-virulence infection: Enhanced antiviral activity of macrophages, *Infect. Immun.* **29:**42–49 (1980).
85. Weiser, W. Y., and F. B. Bang, Blocking of *in vitro* and *in vivo* susceptibility to mouse hepatitis virus, *J. Exp. Med.* **146:**1467–1472 (1977).
86. Arnheiter, H., T. Baechi, and O. Haller, Adult mouse hepatocytes in primary monolayer culture express genetic resistance to mouse hepatitis virus type 3, *J. Immunol.* **129:**1275–1281 (1982).