AVIAN REOVIRUSES

Yani Drastini

ABSTRACT

The members of the orrereovirus genus are distinguishable into two distinct groups: those of avian origin, and those of mammalian origin (Kawamura and Tsukahara, 1966). Both groups of viruses have a genome consisting of 10 segments of double-stranded RNA (dsRNA), separable into three size classes, which are enclosed within a double protein capsid shell 70 to 80 nm in diameter with a similar protein composition and distribution (Sponholz and Graham, 1976; Schnitzer et al., 1982; Julkunen, 1983). However, avian reoviruses, unlike their mammalian counterparts, exhibit a wide heterogeneity in their neutralizing antigens (Robertson and Wilcox, 1986), possess a different group specific antigen (Pezek et al., 1987), cause no haemagglutination (Glass et al., 1975), have been associated with disease in their natural hosts (Kibenge and Wilcox, 1983), and cause syncytium formation both in infected cell culture and in susceptible chickens (Wilcox and Compaan, 1982; Kibenge and Wilcox, 1983). In addition, avian reoviruses are reported to be heterogeneous in their sensitivities to tropism (Drastini, 1993).

Reovirus Unggas

ABSTRAK

Anggota genus orrereovirus dapat dibedakan dalam dua kelompok: yang berasal dari unggas, dan yang berasal dari mamalia. Kesud kelompok memiliki virus genom yang terdiri atas 10 rantai RNA beragam, dapat didefinisikan menjadi tiga kelompok ukuran, yang berhubungan dengan campak kepulihan protein ganda, dengan diameter 70-80 nm, dengan komposisi dan distribusi protein yang dialing mirlip.

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INTRODUCTION

At least 11 serotypes of avian reovirus, with extensive cross-reactivity among the heterologous types, have been identified among strains isolated in Japan, UK, Germany, and the USA (Wood et al., 1981). Robertson and Wilcox (1986) also found considerable antigenic heterogeneity among Australian avian reovirus isolates, but assigned the 10 selected strains examined to 3 subtypes rather than distinct serotypes. Most avian reovirus serotypes have been associated with a variety of pathologic conditions in chickens worldwide, and some have also been isolated from normal chickens (Robertson and Wilcox, 1986) and diseased chickens infected with other viruses and bacteria (Olson, 1984). These viruses are associated with enteric diarrhea, growth abnormalities, leg deformities and respiratory disease. However, the relationships between the serotypes and specific diseases have not yet been demonstrated. Moreover, avian reoviruses, like the mammalian reoviruses, exhibit marked polymorphism in genomic segment cocricular transduction patterns (genotypes) among isolates of the same serotype as well as among different serotypes (Hidy et al., 1979; Gouveia and Schnitzer, 1982; Loranzo et al., 1992).

Avian reoviruses can be readily grown in the laboratory in primary cultures of cells of avian origin (Gruner et al., 1982; Barta et al., 1984). However, among the established mammalian cell lines tested, only Vero cells have been reported to support replication of certain strains of avian reovirus (Barta et al., 1984; Wilcox et al., 1985; Norweji et al., 1988; Jones and Alarce, 1990). The only report on the nature of avian reovirus replication in Vero cells found the virus to be highly cell-associated, requiring several freeze-thaw cycles to release virus from infected cells (Wilcox et al., 1985).


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The objective of this study is to increase the understanding of the significance of the biological heterogeneity among avian reoviruses.

1. PATHOLOGY OF AVIAN REOVIRUS INFECTIONS IN CHICKENS

1.2. General Introduction

In Canada avian reovirus was first isolated from chickens suffering from chronic respiratory disease (Fahey and Crawley, 1954). This initial isolate was originally termed the Fahey-Crawley (FC) agent, and was later characterized as a member of the reovirus group (Poteé et al., 1967). Subsequently, other isolates of avian reoviruses were made from a variety of clinical conditions in chickens including cecal pasting (Dentmahle and Pomercy, 1966a), non-syncytial viral arthritis (Glass et al., 1973; Jones et al., 1975), hydropericardium (Spradley and Bains, 1974; Jones, 1976), and a running syndrome/malabsorption syndrome (Van der Heide et al., 1981; Page et al., 1982; Robertson et al., 1986). Avian reoviruses have also been isolated from clinical conditions in other avian spp., such as turkeys affected with infectious synovitis (Glass, 1984; Levison et al., 1980), tenosynovitis (Page et al., 1982; Afaaq et al., 1991) and diarrohea and conjunctivitis (McFerran et al., 1976); from ducks exhibiting diarrhoea (Klauchula, 1956; Malkinson et al., 1981); from pigeons with diarrhoea (McFerran et al., 1976); from grey parrots with enteritis (Meulemans et al., 1983); from wedge-tail eagle with tenosynovitis (Apella andax) (Jones and Guneratne, 1984); and quails suffering from enteritis (Ritter et al., 1986). However, the role of avian reoviruses in disease has not been clearly established. In addition to their association with the above disease conditions, avian reoviruses have been isolated from clinically normal chickens (Robertson et al., 1984), and from cell cultures prepared from apparently healthy chicken kidneys (Mustafa Babjev and Spradley, 1971).

To cause disease, a virus must enter a host by specifically adsorbing to target cells, replicate inside these host cells with the resultant viremia disseminating virus within the host in characteristic pathways, causing damage to host tissues (Joahl, 1983). Reoviruses generally infect via the respiratory and enteric tracts of animals, hence the name "RDV" virus (Sabin, 1959), the acronym standing for "respiratory enteric orphan. Initial replication of avian reovirus occurs in the mucosa of the gastrointestinal and respiratory tracts (Menendez et al., 1975; Ellis et al., 1983). In case of the gastrointestinal tract, the virus comes in contact with trypsin, other proteolytic enzymes and bile salts. This interaction may be important in determining the virulence of the virus. If the virus survives within the immune environment, it enters intestinal epithelial cells, causing local inflammation, or enters the systemic circulation (Kaufman, 1983) with resultant disease damage at distant sites.

The pathogenesis of reovirus infection has been studied in detail with mammalian reoviruses. Mammalian reovirus type 1 causes the necrotic barrier via M cells at the peyer's patches (Tyier and Field, 1990). The virus then spreads to the mesenteric lymph nodes and spleen (Kaufman, 1983), or is trapped by hepatic Kupffer cells and is then excreted into the bile (Tyier and Field, 1990). The latter mechanism may also be an important route by which a virus producing systemic disease e-enters the intestinal tract for excretion via faeces (Rubin et al., 1986).

The pathogenesis of avian reovirus infection is similar to that described for mammalian reoviruses. Following oral inoculation of 1-day-old specific pathogen free (SPF) light hybrid chicks with strain R2 of avian reovirus (Jones et al., 1975), the virus was re-isolated from the pancreas, oesophagus, ileum, cecal tonsils and rectum at 24 hours post inoculation (PI) (Kibenge et al., 1985). Maximum virus titres were found in the liver at 3 days PI, and declined by 7 days PI. Viruses could be re-isolated from the heart at 10 days PI and in the hock joint at 14 days PI. Jones et al. (1989) used the same virus strain to report that the virus entered and replicated primarily in the intestinal epithelium and bursa of Fabricius within 12 hours PI, spread to most tissues within 1 to 2 days PI, and finally localized in joint tissues by 4 days PI.

The mortality caused by the virus is higher in 1-day to 1-week-old chickens than in 2 weeks of age or older chickens. Also, the surviving 1-day to 1-week-old chickens frequently develop a persistent virus infection (Toiman, 1987).

Several factors, including virus strain, dose, route of inoculation (Wood and Thorston, 1981; Islam et al., 1988), breed of chicken (Jones and Kibenge, 1984), immune status (Carboni et al., 1975; Wood and Tilston, 1981; Jones and Georganos, 1984a; Teng et al., 1987; Kibenge et al., 1987) and other infections (MacKenzie and Bains, 1977; Kibenge et al., 1982; HILL...
et al., 1989) and non-infectious factors have been shown to influence the course of experimental avian infection in chickens (Table 1).

Table 1. Experimental infection with reoviruses in chickens

<table>
<thead>
<tr>
<th>Experimental Disease</th>
<th>Virus strain</th>
<th>Chickens breed and age used</th>
<th>Route of infection</th>
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<td>1D O</td>
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<td>1D FP</td>
<td>1, 3 W</td>
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</tr>
</tbody>
</table>

Respiratory disease: 1.52 W Egg inoculation | McCormick et al., 1971 |
1.2. Toxoplasmosis/Viral arthritis

Avian toxoplasmosis/viral arthritis occurs worldwide. The disease was first reported in England in 1967 (Dalton and Henry, 1967). It has since been reported in Netherlands (Kramer et al., 1968), Italy (Rossi et al., 1969), France (Montemass et al., 1980), Yugoslavia (Volarovic et al., 1980), U.S.A. (Olsen and Solomon, 1968; Johnson and van der Heide, 1971; Glass et al., 1973; van der Heide et al., 1974), Australia (McKenna and Binder, 1976, 1977; Kibenge et al., 1982), Japan (Kawamura et al., 1976 and 1977), Canada (Eide and DerWalt, 1979), and Egypt (Tattawy et al., 1984).

The term avian toxoplasmosis was originally used to describe an inflammation of the tendon sheaths and tendons caused by Mycoplasma synoviae (Dalton and Henry, 1967), whereas the mycoplasmal disease was referred to as viral arthritis (Olsen, 1973). Currently, both terms are used to describe the mycoplasmal-associated disease (Kibenge and Wilcox, 1983).

Toxoplasmosis or viral arthritis lesions have been found in various degrees in affected birds. The severity of the lesions depends on the breed of chicken (Carbovi et al., 1975; Wood and Thornton, 1981; Jones and Georgiou, 1984a), the age of the chickens at the time of infection (Carbovi et al., 1975; Wood and Thornton, 1981; Jones and Georgiou, 1984a), route of virus inoculation (Wood and Thornton, 1981; Isfan et al., 1985), and presence of secondary bacterial infections (MacKenzie and Rains, 1977; Kibenge et al., 1982; Hill et al., 1989). Among these breeds of chicken examined, the commercial (non-SPF) Ross I broilers were more sensitive to an anthropic retrovirus infection than SPF light-hybrids and commercial (non-SPF) White Leghorn egg layers (Jones and Kibenge, 1984). With regard to the age, the younger chickens were more susceptible to infection (Carbovi et al., 1975; Wood and Thornton, 1981; Jones and Georgiou, 1984a; Tang et al., 1987). Four routes of inoculation, foot-pad, beak-articular, oral, and contact exposure produced toxoplasmosis, with the severe lesions occurring after foot-pad and intra-articular routes (Wood and Thornton, 1981; Isfan et al., 1988). It has been suggested that the toxoplasmosis outbreaks are caused by a primary subclinical viral infection, with Staphylococcus aureus as a secondary infection responsible for the development of clinical signs (MacKenzie and Rains, 1977; Kibenge et al., 1982; Hill et al., 1989). However, bacteria were not present in all clinical outbreaks of toxoplasmosis (Kibenge et al., 1982). The sex of broiler chicks inoculated subcutaneously as 1 day of age with avian mycoplasma strain 81-474 from hock joint had no effect on the mortality rate (Montgomery et al., 1985).
The clinical response to infection following oral inoculation of 1-day-old SPF light-hybrid chicks included depression, prostration (Jones and Georg, 1984a) and anorexia at 2 days PI (Tang et al., 1987), but by 8 days PI the chicks appeared normal. Sometimes no clinical signs were observed until 3 (Kibenge et al., 1985) to 5 weeks PI (Kibenge and Dhillon, 1986). The tenosynovitis lesion was a unilateral swelling on the plantar aspect of the leg, below the hock joint by 3 to 4 weeks PI (Jones and Georg, 1984a), which decreased by 6 weeks PI. Mortality of chicks caused by the virus began 4 days PI (Tang et al., 1987; Al-Aleq and Jones, 1991) and continued until 10 day PI (Kibenge and Dhillon, 1986). Mortality was due mainly to severe hepatitis characterized by congestion, hemorrhage and yellow necrotic areas.

The growth rates observed by measuring the body weight were found to be significantly lower in inoculated chickens than those of the corresponding control group within 5 weeks PI (Kibenge and Dhillon, 1987). However, another experiment in which a different strain of avian reovirus was used showed no significant differences between the weights of infected and the control groups within 2 to 6 weeks PI (Jones and Kibenge, 1984).

The gross lesions in the affected chickens include a tendon swelling below the hock at 3 weeks PI and above the hock by 6 weeks PI. Rupture of digital flexor tendons has been observed at 6 weeks PI, and fusion of the tendons at 9 weeks PI (Jones and Georg, 1984a). A yellowish-brown gelatinous exudate between tendons in the swollen legs, varying degrees of thickening and fusion of tendons, and pitted erosions of the articular cartilage of the hock joints were observed by 12 weeks PI (Jones and Kibenge, 1984; Jones and Georg, 1984a).

1.3. Respiratory disease

Avian reovirus was originally isolated from chickens suffering from respiratory disease (Foley and Crawley, 1954). The virus has also been isolated from laying flocks concurrently infected with infectious bronchitis virus (McFerran et al., 1971), and from geese with a respiratory disease (Conotton and Milkevich-Ku Czantary, 1967). However, respiratory disease has not yet been reproduced by experimental inoculation of chickens with avian reovirus (Table 1). Intranasal inoculation of chicks with a trypanosome sensitive avian reovirus was effective in causing viral arthritis, and therefore it may be that the respiratory route of infection is of greater significance with reoviruses of this type (Al-Aleq and Jones, 1991).

1.4. Enteric disease

Avian reoviruses associated with enteric disease have been isolated from chickens suffering from severe clinical pneumatic (Deinmay and Pomeroy, 1969a), from turkeys with depression, anorexia, 30% mortality (Simmons et al., 1972) or infectious enteritis (Gershovitz and Woolley, 1973), and from quails experiencing severe enteritis (Ritter et al., 1986; Gay et al., 1987).

In experimentally infected chickens, there were neither clinical signs nor microscopic intestinal lesions following oral inoculation of 5-day old SPF chickens (Gay et al., 1986). One of two strains used in that study caused significantly depressed weight gain in virus inoculated chickens compared with the un inoculated controls up to 5 days PI, while the other strains did not affect weight gain. Virus was recovered from faeces by 2 to 8 days PI, and from liver and spleen beginning on day 4 PI (Gay et al., 1986).

1.5. Ranting syndrome

This disease syndrome was initially reported in chickens in the Netherlands (Kouwenhoven et al., 1978a). It was then described in England (Bracewell and Wyeth, 1981), in USA (Page et al., 1982) and Australia (Paas et al., 1982, Roche et al., 1984). The term infectious runtling and runting syndrome were initially used by Bracewell and Wyeth (1981). Other names that have been used to refer to the same condition include infectious perinatalitis, osteoporosis (Kouwenhoven et al., 1978b), hip osteoporosis, beak bone disease, femoral head necrosis, palo bird syndrome (van der Heide et al., 1981), and malabsorption syndrome (Page et al., 1982).

Runtling syndrome in chickens is characterized by stunted growth, elevated feed conversion ratios, poor feathering, leg weakness, decreased weight gain (Kouwenhoven et al., 1978a; Bracewell and Wyeth, 1981; Page et al., 1982), diarrhoea (Vartomeen et al., 1980), femoral head fractures, and osteoporosis (van der Heide et al., 1981).

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Infect ed chickens showed a reduc tion in growth rates ranging from 5 to 20% in 1-week-old chickens, and signs of weaknesses and poor feather development in 2-week-old chickens (Kovacs et al., 1978a). It was also determined that the most prominent lesions were proventriculitis and a catarrhal enteritis. In another observation in chickens, the growth rates were reduced between one and two weeks of age (Bracewell and Wyeth, 1981). The feathering defects and leg weakness were also observed up to 5 weeks of age. One-day-old commercial broiler chickens inoculated orally with a malabsorption-syndrome-suspected-virus exhibited decreased weight gain, elevated feed conversion ratios, feathering defects, lameness, poor pigmentation of the shanks, and poorly digested food in the intestinal tract. Histopathological lesions included proventriculitis, myocarditis, catarrhal enteritis, bursal atrophy and pancreatic atrophy (Page et al., 1982). However, other studies suggested that the rooviruses isolated from affected chickens were not necessarily associated with the syndrome since the isolation rate of roovirus from normal 3-week-old chickens was at least equivalent to that obtained from agamemnon chickens with the running/standing syndrome (Robertson et al., 1984).

1.6. Immunossuppression

Several workers have studied the effect of avian rooviruses on the immune system of their hosts, but there is no report that designates avian roovirus as an immunosuppressive agent. In the bursa of Fabricius of birds infected with a tansy钒ovirus-producing avian roovirus strain WVU 1675, lymphoid cells were decreased as early as 7 days PI (Kerr and Olson, 1969). The bursa of Fabricius was also reported to be atrophied in field cases of roovirus-associated malabsorption (Page et al., 1982). Avian roovirus strain 81-5 isolated from chicken with malabsorption syndrome decreased the bursa of Fabricius and increased the spleen organ-to-body weight ratio up to 3 weeks PI following intravenous inoculation with 10^3.5 PFU/0.1 ml, and altered the spleen weight alone at 1 week PI following the same inoculation with a lower dose of 10^2 PFU/0.1 ml (Montgomery et al., 1983). But this virus strain had no effect when 10^3 PFU/0.1 ml was given orally. Avian roovirus strain WVU 1675 also caused hematological effects (Kerr and Olson, 1969). Poor antigenically similar avian rooviruses were divided into two groups (Kerr and Olson, 1983; Rischart, 1984). One group consisting of two non-pathogenic viruses was found in lymphoid tissues other than thymus, and caused no gross or microscopic changes. The second group of two pathogenic viruses persisted in the bursa of Fabricius and/or thymus over 24 days PI, and caused atrophy of the lymphoid follicles in the bursa of Fabricius and depletion of lymphoid cells in both the bursa of Fabricius and thymus.

Avian rooviruses interfered with the development of immunity to coccidia and Marek's disease virus (Rischart et al., 1983; van der Heide et al., 1983; Ruff et al., 1985), and impaired the T-cell function and the phagocytic ability of circulating monocytes (Montgomery et al., 1986). Chickens dually infected with Eimeria maxima and avian roovirus strain 2035 exhibited synergistic depression of weight gain (Ruff and Rosenberger, 1983). Dual infection with Cryptosporidium baileyi and roovirus strain 2035 resulted in significant weight gain depression over a 21-day period PI. Chickens inoculated with only roovirus strain 2035 showed no effect on weight gain during the same period, and C. baileyi infection significantly decreased weight gain only 8 to 14 days PI (Guy et al., 1986). However, roovirus infection in the dually infected SPF chickens did not modify the microscopic changes caused by C. baileyi (Guy et al., 1988).

1.7. Rooviruses in avian species other than chickens

Avian rooviruses have been isolated from ducks (Kaschula, 1950; Mankin et al., 1981), turkeys (Kawamura et al., 1965; Alfaleq et al., 1980), pigeons (McFerran et al., 1976; Vandevelde et al., 1982), per birds (Meelmann et al. 1983; Panighrahy, 1986), wedge-tail eagle (Jones and Gaveren, 1984), and quails (Ritter et al., 1986). Most of these avian species exhibited enteritis, and some were suffering from tansy钒oviridae/arthritis.

Turkeys inoculated with an attenuated avian roovirus originally isolated from turkey showed that virus distribution in most organs occurred by 3 to 7 days PI (Nersessian et al., 1985). Viruria occurred by 7 days PI. The virus was recovered from tonsils at 3 to 7 days PI and 28 days PI (Nersessian et al., 1985). In addition, turkeys seem to be much more resistant than chickens to the induction of arthritis/vasculitis by rooviruses from other species (Alfaleq and Jones, 1989).

No experimental infections of avian roovirus in other avian species have been reported.
2. CULTIVATION OF AVIAN REOVIRUSES

2.1. Isolation and propagation in primary cell cultures

Primary cell cultures which have been used for the isolation and propagation of avian reoviruses include the whole chicken embryo (CE) (Deshmukh and Pomery, 1964a); chicken embryo lung (CELu) (Peck et al., 1967), fibroblast (CEF) (De et al., 1973), kidney (CEK) (Glass et al., 1973), and liver (CELi) (McForan et al., 1976); chicken kidney (CK) (Kawamura et al., 1965), lung (CL), and teratocarcin cell cultures (Sahu and Olson, 1975); turkey kidney cells (Fujikata et al., 1969); and duck embryo fibroblast cell cultures (Lee et al., 1973). Of these, CELi cell cultures have been reported to be the most sensitive for the cultivation of avian reoviruses (Guerazene et al., 1982; and Barta et al., 1984).

The cytopathic effect (CPE) in cell cultures infected with avian reoviruses is characterized by the formation of multinucleated cells (syncytia) which detach from the monolayers leaving small holes in the cell monolayer (Deshmukh and Pomery, 1964a; Robertson and Wilcox, 1986). In CELi cell cultures, CPE was first detected on the sixth, seventh, and fourth passages of avian reovirus strains Type 24, Type 25 and Type 39, respectively (Deshmukh and Pomery, 1964a). The CPE of these viruses appeared at 5 to 7 days PI, became numerous and larger upon further incubation, until the entire monolayer was destroyed by 9 days PI. However, the maximum virus titers reached in the third passage, which increased to 8.2, 7.2 and 8.4 log10 TCID50, respectively, by the twenty-fifth passage, while the virus propagated in CEF produced a maximum titer of only 6.0 log10 TCID50/ml (Guerazene et al., 1982). In addition to the higher titer of the virus, CELi cell cultures also produced larger plaques than CEF. More blind passages (5 to 7) were required before CPE was observed in virus isolation attempts using CL, CK, and particular cell cultures with avian reovirus strains Type 24, Type 25, Type 39, FC, WWU 1664-2941, WWU 2397, WWU 2986, and WWU 71-92a (Guerazene et al., 1982).

2.2. Adaptation to continuous cell lines

Several mammalian cell lines have been used to grow avian reoviruses, including those of bovine origin [Madin Darby bovine kidney (MDBK), and Georgia bovine kidney (GBK)], canine origin [Madin Darby canine kidney (MDCK)], human origin (Chad C, HBl2 and HeLa), mouse origin (L929), monkey origin (LLC-MK2, and African green monkey kidney (Vero)), feline origin (Grinstead's feline kidney (CFK)), baby hamster kidney (BHK), rabbit kidney (RK), and porcine kidney (PK) (Sahu and Olson, 1975; Barta et al., 1984). Mammalian cell lines have advantages of being convenient for use, and with no risk of contamination with naturally transmitted avian reoviruses in contrast to avian cell cultures.

Avian reovirus strain RAM-1 passed 14 times in CK cells followed by 4 to 5 freeze-thaw cycles produced CPE in Vero cells within 48 h of the first passage, whereas 5 other strains passed 8 to 10 times in CK cells could not be adapted to grow in Vero cells (Wilcox et al., 1983). Another study found that all 22 avian reovirus strains passed several times in avian embryonic cell cultures, produced CPE in Vero cells after 1 to 3 passages (Nwajio et al., 1988). The CPE produced in Vero cells is characterized by focal areas of cell fusion. The virus was so highly cell-associated that it required up to 10 days PI to obtain maximum CPE (Wilcox et al., 1983). In addition, infected cells had to be frozen and thawed at least 4 times before the virus could be passaged successfully. In contrast, in chicken cell cultures, avian reoviruses required only 1 day incubation to produce CPE and one freeze-thaw cycle to release virus from infected cells (Robertson and Wilcox, 1984). In other studies, avian reovirus strains produced CPE in Vero cell monolayers after an incubation period of 4 to 5 days (Barta et al., 1984) or 3 to 6 days (Sahu and Olson, 1975).

Avian reovirus strain WUV 2937 produced CPE after three blind passages on GBE, CEFK, and BHK cells, five passages on RK cells, and ten passages on PK cells. No CPE was produced on rabbit bone marrow (RBM) (Barta et al., 1984). In CELi cell cultures the virus produced CPE on the first passage, 40 h after inoculation. Reovirus strain 51133 penetrated and intoxicated mouse L cells, but there was no viral genome replication or viral progeny formation (Spandolos and Graham, 1976). Subsequent investigations reported that avian reovirus strain 51133 could replicate in mouse L cells at pH 6.4 and 7.2, but not at pH 8.2 (Mallo et al., 1991a). These authors suggested that the pH of the medium barely affected viral transcription, with little or no influence on viral mRNA translation. Vero cells were found to be unsuitable for the isolation of avian reoviruses from field materials, and it was


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3. MOLECULAR STRUCTURE OF AVIAN REOVIRUSES

3.1. Electron microscopy

The morphology structure of avian reovirus has been studied by electron microscopy in chickens (Kawamura et al., 1965; Hirayonmu et al., 1983), turkeys (Simmons et al., 1972; Nenastin et al., 1986), and quails (Ritter et al., 1986).

By a transmission electron microscope, the virus particle from infected chickens showed a double capsid andicosahedral symmetry (Simmons et al., 1972; Hirayonmu et al., 1983). Another observation of a reovirus isolate from the hock joint of a chicken showed that the particle was hexagonal to spherical (Glass et al., 1972). The outer capsid consists of 92 capsomers (Kawamura et al., 1965). In mammalian reoviruses the outer shell is sensitive to digestion by chymotrypsin, but the cores are completely resistant (Shahtin and Sipe, 1966a; Smith et al., 1969). Also, the cores can be disrupted by high concentrations of sodium dodecyl sulfate (Joklik, 1983). The diameter of the avian reovirus particle ranges from 70 to 82 nm (Kawamura et al., 1965), 60 nm (Dutta and Pomeroy, 1967), 58 to 64 nm (Kiddie, 1970), 56 to 61 nm (Deutschkuh et al., 1971), and 62 to 72 nm (Hirayonmu et al., 1983).

3.2. Avian reovirus genome

The avian reovirus genome was initially determined to consist of RNA by using DNA inhibitors (Kawamura et al., 1965; Hirayonmu et al., 1983), and susceptibility to DNase or RNase (Deutschkuh and Pomeroy, 1969; Spandios and Graham, 1976) digestion. The genome was then shown to consist of double-stranded DNA (Glass et al., 1973; Sekiguchi et al., 1968; Spandios and Graham, 1976), and its segments were also separated into three size classes: three large (L1, L2, and L3), three medium (M1, M2, and M3), and four small segments (S1, S2, S3, and S4) in sucrose gradients (Spandios and Graham, 1976), or by analysis of the migration patterns of the viral genome using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Rakite et al., 1990). The approximate molecular weight range of each genomic segment group is 2.5 to 2.7 x 10^6, 1.3 to 1.8 x 10^6, and 0.71 to 1.2 x 10^6 daltons for L1, M, and S segments, respectively (Spandios and Graham, 1976; Gouveia and Schnitzer, 1982; Lomani et al., 1992). The migration patterns of genome segments of avian reoviruses isolated from turkeys and chickens were found to be heterogenous, particularly in the M and S segments, while those of canary and cockatiel isolates were not (Lomani et al., 1992).

3.3. Viral proteins of avian reovirus

Characterization of viral proteins of avian reoviruses has been done (Spandios and Graham, 1976; Schnitzer et al., 1982), but assignment of the specific protein products to individual gene segments has not been reported yet (Roberson and Wilson, 1986). In mammalian reoviruses, each genome segment is transcribed into a mRNA molecule by a DNA-dependent-RNA polymerase within the reovirus core, and is then translated into a single viral coded polypeptide in the cytoplasm of infected cells (Joklik, 1981) (Table 2). The three large genome segments (L1, L2, and L3) encode 3 large proteins (λ1, λ2, and λ3, respectively), the three medium segments (M1, M2, and M3) encode μ1, μ2, and μ3; and a non structural (μNS) protein, respectively, and S1, S2, S3, and S4 segments encode σ1 and σ2, σ3, ε1, and ε2 respectively (Tyler and Fields, 1990).

Similarly to the dsRNA genome segments, the viral proteins from purified avian reovirus particles also resolve into three size classes on SDS-PAGE: 3 large (λA, λB, and λC), 2 medium (μA and μB), and 3 small bands (σA, εA, and εB) with the molecular weights of 115 to 145 K, 70 to 85 K, and 32 to 40, respectively (Spandios and Graham, 1976; Schnitzer et al., 1982) (Table 2). In mammalian reoviruses, the outer capsid consists of three proteins: σ1, σ2, and μC (Tyler and Fields, 1990). The σ1 protein is the primary determinant of reovirus pathogenesis since it functions as the virus attachment protein, as hemagglutinin, and as the antigen for inducing neutralizing antibody production and cell mediated immune responses (Tyler and...
Fields, 1990). The μNS protein encoded by the M3 gene segment functions as a determinant of transcriptase activation following protease treatment in vitro. This protein confers resistance of the outer capsid to protease digestion in vivo, and functions to modulate virulence within a avian type (Tyder and Fields, 1990). Biologic functions of the μNS protein include affinity for dsRNA, zinc metalloproteinase, inhibition of cellular RNA and protein synthesis, establishment of persistent infection, and regulation of viral transcription and translation (Tyder and Fields, 1990).

The mammalian revirus cores contain three major proteins (λ1, λ2 and α2) and two minor proteins (α3 and μβ). The 9, α2 and λ3 were suggested to contain transcriptase activities (Tyder and Fields, 1990).

Besides the viral proteins above, there are three nonstructural proteins (cNS, μNS and c NS). cNS is encoded by the S3 gene segment and functions to condense the mRNP into precursor subviral particles in preparation for dsRNA synthesis (Gomatos et al., 1981). This protein may play other important roles early in revirus replication (Cross and Fields, 1972). The μNS protein encoded by the M3 gene segment is presumed to form part of the viral transcriptase complex (Morgan and Kingsbury, 1980). cNS which is encoded by the S1 gene is thought to play a role in the virus replication cycle (Einst and Shatkus, 1983).

3.4. Physicochemical properties

The physicochemical properties of avian reviruses reported include resistance to trypsin (Kawamura et al., 1965; Alfalio et al., 1991), sodium deoxycholate (Kawamura et al., 1965), zephiran (Deshmukh and Pomeroy, 1969), 2% formaldehyde at 4°C (Meissmann et al., 1982), and the lipid solvents ether and chloroform (Glass et al., 1973; Nenshahin et al., 1986); sensitivity to 7% phenol, 100% ethanol, trichloro isocyanate (Patrick et al., 1976). Enviro D, mercury bromide (Deshmukh and Pomeroy, 1969). The density of virions has been reported to be 1.56 to 1.57 g/ml in cesium chloride gradients (Sandiford and Graham, 1976; Schindler et al., 1982).

The stability of avian reviruses to pH and temperatures vary. Avian revirus strains Type 24, Type 25, and Type 39 isolated from encephalitis in chickens were stable at pH 3.0 for 30 min; (Deshmukh and Pomeroy, 1969). Glass et al. (1973) have reported the revirus isolated from chicken hock joints to be resistant to pH 3.0 and 4.0 for 4 h. The avian revirus strains GM-203 and S1133 isolated from encephalitis were partially sensitive to pH 3.0 and 12.0 for 3 h (Carton et al., 1975). Avian revirus strain BC-5 and BC-7 obtained from turkeys suffering from encephalitis were reported to be stable at pH 3.0 and 7.0 for 3 to 5 h (Glasserowitz et al., 1973), while four other turkey revirus isolates were also stable at pH 5.0 for 4 h (Nenshahin, 1986). The viruses isolated from chickens with encephalitis were stable at room temperature for more than 2 months, at 4°C for more than 3 months (Dutta and Pomeroy, 1967), and at 50°C for only 1 h (Deshmukh and Pomeroy, 1969). The virus recovered from chickens suffering from malabsorption syndrome and encephalitis were stable at 50°C for 1 to 6 h (van der Heide and Kalbac, 1975; Hienranyu, 1983), but other researchers have reported the viruses to be partially inactivated at this temperature within 10 to 30 min (Dutta and Pomeroy 1967; Mustafa-Bubajie et al., 1973). The turkey revirus isolates were stable at 50°C for from 30 min to 6 h (Nenshahin et al., 1985).

It has been reported that avian reviruses isolated from chickens are resistant to trypsin (Kawamura et al., 1965; Jones et al., 1975). The infectivity of an avian revirus strain R1 of chickens (Jones and Gunaratne, 1984) in CSLI cell monolayers was also unaffected after treatment with 0.01% trypsin for 30 min, but that of strain TR1 isolated from a turkey with arthritis was reduced indicating this strain to be trypsin sensitive (Alfalfio and Jones, 1991). Recently, it was also reported that 6 of 14 avian revirus strains from Canada were judged sensitive to trypsin treatment with 2.5% trypsin for 45 min (Draistai, 1992).

4. ANTIGENIC CHARACTERISTICS OF AVIAN REVIRUSES

4.1. Common precipitin antigens

The common antigens among avian reviruses have been detected by a direct fluorescent antibody (FA) (Kawamura and Tsubahara, 1966; Menendez et al., 1975), an indirect FA (Id, 1982), complement fixation (Kawamura and Tsubahara, 1966) and the agar gel precipitin (AGP) test (Kawamura and Tsubahara, 1966; Wood et al., 1980). Up to four precipitin lines, and therefore at least four common precipitin antigens, have been detected in the AGP test (Olson and Weiss, 1972).
14.2. Neutralizing antigen

The serum neutralization test has been used in embyroinjected eggs inoculated via the CAM route (Olsen and Weiss, 1972), in C57 (Kawamura et al., 1965), and in C57B1/c3H cells (Hickoryman et al., 1983). Kawamura et al., (1965) grouped 77 Japanese strains into 5 serotypes. Types 24, 25 and 59 were classified into 2 serotypes, in which Type 59 was antigenically different from the other two (Deodharia and Pomeroy, 1969a; Sahu and Olsen, 1975). The WVU 2937 and FC strains were grouped as one serotype based on one-way serum neutralization tests conducted by CAM inoculation of embryoinjected eggs (Olsen and Weiss, 1972). However, with a cross-neutralization test using a coagulant virus-varying serum plaque reduction test in C57 cell cultures, WVU 2937 was of a different antigenic group from the PC strain (Sahu and Olsen, 1975). A similar observation had been made by Mauro and Waxley (1973) using neutralization kinesics. Later, Sahu et al., (1979) classified strains WVU 2937, Texas, UMI 203 and S1133 as subtypes of the same serotype. Six strains (R1 Uumen 131133, R1 2286, Kleta 122, Kantes, and Kleta 795) have been grouped into 3 serotypes (Wood et al., 1980). Another six strains were also placed into 3 serotypes: S1133, C108 and type 45; type 81-5 and 43A; and type 82-9 (Hickoryman, 1983). Robertson and Wilcox (1984) classified 10 Australian avian reovirus strains into 3 subtypes. Overall, at least 11 serotypes of avian reoviruses have been demonstrated by serum neutralization tests (Wood et al., 1980).

5. CONCLUSIONS

Avian reoviruses, unlike mammalian reoviruses, exhibit a wide heterogeneity in their neutralizing antigens (Robertson and Wilcox, 1986), possess a different group-specific antigen (Petak et al., 1967), cause no haemagglutina-
tion (Glass et al., 1971), have been associated with disease in their natural hosts (Kibenge and Wilcox, 1983), cause syncytium formation both in infected cell culture and in suckling chickens (Wilcox and Compaia, 1982; Kibenge and Wilcox, 1983), and showed a wide variation in the degree of sensitivity to trypsin (Drasti, 1992). Therefore, a knowledge of biological characterization of avian reoviruses is important to increase our understanding of the significance of the biological heterogeneity among avian reoviruses. Furthermore, it might be useful to differentiate the strains of the viruses or to other purposes.

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