Responses of Isolator-Derived Japanese Quail and Quail Cell Cultures to Selected Animal Viruses

WENDALL M. FARROW,* MARTIN W. SCHMITT, AND VINCENT GROUPE

Life Sciences Research Laboratories, Life Sciences, Inc., St. Petersburg, Florida 33710

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Thirteen oncogenic and necrotizing animal viruses were assayed in Life Sciences, Inc. (LSI)-specific pathogen-free Japanese quail and LSI-specific pathogen-free chicken embryo cell cultures. Nine viruses produced similar titers in the quail and chicken cell systems, whereas four viruses showed significantly higher titers in chicken. Young Japanese quail and chickens were inoculated with five selected avian viruses and maintained in stainless-steel isolators. Comparable responses were noted in quail and chickens injected with Newcastle disease virus and avian leukosis virus, but quail were significantly more resistant than chickens to fowl pox virus, laryngotracheitis virus, and Marek's disease herpesvirus. Although no overt symptoms of disease were observed in Japanese quail inoculated with most avian viruses, neutralizing antibody or virus was detected, indicating presence of an inapparent infection. In one experiment, neutralizing antibody was detected in a comparable number of quail and chickens after inoculation with avian leukosis virus. Avian leukosis virus viremia was observed at 12 and 70 days postinoculation, with the COFAL (complement fixation for avian leukosis) titers similar for quail and chickens. Most quail infected with Marek's disease herpesvirus produced neutralizing antibody within 70 days but showed no classical symptoms of Marek's disease even when held for 5 months. In contrast, all chickens inoculated with Marek's disease herpesvirus died within 20 days. The utility of quail embryo cell cultures in the preparation of vaccines and biological reagents is discussed.

Domesticated Japanese quail (*Coturnix coturnix japonica*, Temminck and Schlegel) are useful in biological research because of their small size, early maturity, and high egg productivity (14, 21). *Coturnix* produce fertile eggs as early as 35 days and maintain a relatively high fertility rate (70 to 80%) for a period of approximately 7 months. Eggs, which are usually speckled, hatch in 16 to 17 days, and adult birds average 95 and 125 g for males and females, respectively. Within a barrier environment, Japanese quail live up to 5 years, which is equivalent to the longevity of chickens.

Previous studies (12, 15, 25-27) have demonstrated the varied responses of conventional *Coturnix* to inoculation with certain avian tumor viruses, whereas quail embryonated eggs (10, 17) were found to support the growth of several viruses. Additionally, Japanese quail cell cultures have been utilized in studies on the leukosis-sarcoma group of viruses (8, 9, 25, 29, 30, 32) and certain herpes-type viruses (2, 13, 16). Quail embryo cells were essentially resistant to subgroup B virus (31) and showed a reduced susceptibility to subgroup C viruses (6).

Since 1967 we have developed and maintained a rigidly monitored (7), specific pathogen-free (SPF), outbred flock of Japanese quail. The flock, derived from an isolator environment, is free of exogenous avian leukosis virus(es) (ALV), Marek's disease herpesvirus (MDHV), and other common avian viral and bacterial pathogens.

Definitive (germfree, SPF) *Coturnix* have been used for studies (1, 18, 21) only on selected Rous sarcoma viruses [RSV, RSV(O)]. Therefore, it was important to extend studies to determine the response of SPF quail and quail cell cultures to common avian viruses and to certain mammalian viruses. ALV and MDHV were included because of the importance of establishing a source of cells free of ALV and MDHV.

MATERIALS AND METHODS

Viruses. CELO virus, fowl pox virus (FPV), herpes simplex virus, and vesicular stomatitis virus at high passage levels in chicken eggs were passaged an additional two to three times in chicken embryo secondary cells (CEF). Influenza (WSN-125) virus and the Roakin strain of Newcastle disease

virus (NDV) were prepared from infected allantoic fluids after seven passages in chicken embryonated eggs. Laryngotracheitis virus (LTV) was passaged four times on chicken embryo chorioallantoic membranes. All of the above viruses were obtained from J. W. Frankel, Life Sciences, Inc., St. Petersburg, Fla. The Beaudette strain of infectious bronchitis virus (IBV) was obtained from C. H. Cunningham, Michigan State University. The virus was propagated in chicken eggs and then passaged many times in chicken kidney cells (CK) (4); three additional passages were made in CK in this laboratory. The MDHV (GA isolate) was supplied by C. Eidson, University of Georgia, and the MDHV inoculum was derived from skin and feather tips of resistance-inducing factor, Life Sciences, Inc. (LSI)-SPF chickens. ALV-42 was received as a low-passaged (two to three times) CEF preparation from R. M. Dougherty, State University of New York at Syracuse. The Rous sarcoma pseudotypes, RSV(RAV-1) and RSV(RAV-6), were supplied by P. Meyers, Mayo Medical School at Rochester, and viral stocks were prepared at the second passage in CEF. Preparations of the Bryan high titer RSV, derived from a chicken tumor, and RSV(RAV-50) were obtained from the Resources and Logistics Segment, Virus Cancer Program, the National Cancer Institute, and passaged two times in CEF.

Cell cultures. Quail embryo secondary cells (QEF) and CEF were prepared from 5- to 6-day-old primary fibroblasts derived from 7- to 9-day-old embryos. Cells were trypsinized and seeded in 60-mm plastic petri dishes at a concentration of 4.0×10^5 cells/plate. Growth medium was Eagle minimum essential medium (MEM) prepared with Earle salts, supplemented with double-strength vitamins and glutamine, 10% heat-inactivated fetal calf serum (FCS), 10% tryptose phosphate broth, and antibiotics. Cultures of QEF and CEF were incubated at 37 C, and cell monolayers were confluent within 48 h. Quail primary cells (QK) and CK, prepared from 1-day-old birds and seeded at 8.0×10^5 cells/plate in MEM, formed confluent monolayers in 72 h.

To determine viral infectivity titers, confluent cell monolayers were inoculated with 0.5-ml volumes of virus suspension and absorbed for 1 h at 37 C. The overlay medium (MEM) containing 5% FCS and 0.4% agar (Difco) was added, and infected cell cultures were observed for cytopathic effect (CPE) during a period of 9 days. For titrating RSV and RSV pseudotypes, the virus suspension was inoculated into the growth medium, an agar overlay was added at 18 h, and two additional overlays were added 3 days apart. Polybrene (Aldrich Chemical Co., Milwaukee) was added to the diluent at a concentration of 10 μ g/ml to enhance the absorption of RSV(RAV-6) and RSV(RAV-50). MDHV was absorbed for 30 min at 37 C in MEM containing 0.05% Versene and 2% FCS, and fresh medium without Versene was added at 24 h. When the medium was changed 18 h later, 0.4% agar (Difco) was included and FCS was reduced to 2%. Foci were recorded 7 days later.

Neutralization tests. Quail and chicken sera were inactivated at 56 C for 30 min and diluted 1:20

in MEM. The serum dilution was mixed with an equal volume of virus suspension and incubated for 30 min at room temperature prior to inoculation of CK or CEF. The virus control contained diluent in a volume equivalent to the virus suspension. After inoculation, the mixture was absorbed for 30 min at 37 C, an overlay was added, and the number of foci was counted 8 days later. A positive neutralizing antibody (NA) response was recorded when 90% or greater reduction in focus-forming units or plaque-forming units was observed.

Complement fixation for avian leukosis (COFAL) test. The complement fixation test was performed using the microtiter technique (24). The antiserum used to detect COFAL antigen (23) was obtained from hamsters inoculated with the Schmidt-Ruppin strain of RSV. Whole blood collected from fasted birds was diluted 1:5 in cold saline (0.85%) and dispensed in 0.5-ml volumes to plastic petri dishes seeded with CEF. MEM supplemented with 10% FCS was added, and cells were split every 5 to 7 days during a period of 3 weeks. Cell cultures were frozen and thawed twice prior to testing for COFAL antigen. Group-specific antigen was also determined in quail embryo livers derived from 14-day-old embryonated eggs (5).

Birds. The LSI-SPF *Coturnix* used in this study were derived from a closed outbred flock maintained at the Germfree Life Research Center, Tampa, Fla. Fertile eggs obtained from this flock were surface disinfected and hatched in a sterile Reyniers stainless-steel isolator (19). Newborn quail were reared for approximately 30 days in the isolator environment and then transferred aseptically to barrier-sustained cubicles (20) for expansion of small breeder flocks.

LSI-SPF White Leghorn chickens were derived from a flock developed at the University of Connecticut by R. Luginbuhl. Selected, fertile eggs collected from trap-nested chickens over a period of several months were surface disinfected and hatched in the same manner as described for quail eggs. The chickens were reared to 2 weeks of age and later developed as a pedigreed flock in a primary barrier system.

Monitoring of both SPF quail and chicken flocks for avian pathogens followed established procedures (11). Serum neutralization and hemagglutination-inhibition tests were used to detect antibodies to ALV, CELO virus, FPV, IBV, LTV, MDHV, and NDV. Slide agglutination tests were performed for *Mycoplasma gallisepticum*, *M. synoviae*, and *Salmonella pullorum*. No detectable avian pathogens were observed in these monitoring tests. Additionally, COFAL (23) and resistance-inducing factor (22) tests were repeatedly negative.

RESULTS

Infectivity titers in cell culture. The titers of 13 oncogenic and necrotizing animal viruses in Japanese quail and chicken cell cultures are shown in Table 1. The infectivity titer of RSV (Bryan) was 1 log lower in QEF than in CEF, and a similar response was observed with

RSV(RAV-6). With the pseudotypes RSV(RAV-1) and RSV(RAV-50), there was no significant difference in titers in the quail and chicken cell systems. The titer of MDHV in QK and CK was comparable, although the time required to obtain distinct foci was 2 days longer in the quail system. Two other avian viruses, FPV and NDV, gave slightly higher titers in QEF. CPE was detected within 72 h in QEF and CEF infected with FPV and 24 h after inoculation with NDV. CELO virus, IBV, LTV, and MDHV were assayed in kidney cells since CPE was more distinct than in embryo cell culture. The titers of CELO virus and LTV were significantly lower in QK. CPE was detected within 48 h in CK inoculated with CELO virus, IBV, LTV, and MDHV, whereas in QK the viruses showed no CPE earlier than 72 h. The infectivity titers of herpes simplex virus and vesicular

stomatitis virus were identical in QEF and CEF, with cell cultures showing CPE within 24 h and a maximum effect by 48 h. The virus titer of WSN was 4 logs lower in QEF than in CEF. Maximum CPE was observed within 48 h in CEF and by 96 h in QEF.

Response of LSI-SPF Japanese quail inoculated with selected avian viruses. LSI-SPF *Coturnix* and chickens, hatched and maintained in an isolator environment, were inoculated simultaneously at 3 days of age with the avian viruses shown in Table 2 and observed for morbidity and mortality for a period of 10 weeks. Quail inoculated with FPV or LTV showed no overt symptoms characteristic of disease, whereas all infected chickens developed typical responses 4 to 7 days postinoculation, showing a variable mortality rate of 27 to 60%. The average day of death was 10 for chickens inoculated with FPV and 12 for those injected with LTV. Liver and spleen extracts were prepared from FPV- and LTV-inoculated quail and chickens held for 10 weeks. When 10% suspensions of these extracts were injected onto the chorioallantoic membrane of 9-day-old embryonated chicken eggs, numerous pocks typical of FPV and LTV were detected after 6 days of incubation. The response of *Coturnix* and chickens inoculated with NDV was similar although some difference was observed in time of death; the disease progressed rapidly in chickens and all injected birds died within 48 h, whereas quail survived up to 96 h. Quail and chickens inoculated with MDHV showed marked variations in response. None of the characteristic symptoms of Marek's disease was detected in *Coturnix* during a period of 10 weeks, nor were classical visceral and neural tumors found in tissues of birds. However, NA was detectable in serum samples of five randomly selected MDHV-inoculated quail at 10 weeks. The response in LSI-SPF chickens was

TABLE 1. Virus titers in cell cultures derived from LSI-SPF quail and from chickens

Virus	Cell culture type	Log infectivity titer (FFU or PFU/ml) ^a	
		Quail	Chicken
RSV (Bryan)	Embryo	5.5	6.5
RSV (RAV-1)	Embryo	5.0	4.5
RSV (RAV-6)	Embryo	3.5	4.5
RSV (RAV-50)	Embryo	2.5	2.5
FPV	Embryo	4.5	3.5
NDV	Embryo	9.0	8.5
Herpes simplex	Embryo	8.0	8.0
Vesicular stomatitis	Embryo	8.0	8.0
Influenza (WSN)	Embryo	3.0	7.0
MDHV (GA)	Kidney	3.5	4.0
CELO	Kidney	4.0	9.0
IBV	Kidney	2.0	6.0
LTV	Kidney	4.5	6.0

^a FFU, Focus-forming units; PFU, plaque-forming units.

TABLE 2. Responses of LSI-SPF Japanese quail and chickens inoculated with various avian viruses

Inoculation			Cumulative responses ^a			
Virus	Dose (FFU or PFU) ^b	Route	Quail		Chickens	
			Symptoms ^c	Mortality ^c	Symptoms ^c	Mortality ^c
FPV	10	Comb scarification	0	0	100	27
LTV	10	Intratracheal	0	0	100	60
NDV (Roakin)	10	Intraperitoneal	100	87	100	100
MDHV (GA)	10	Intracerebral	0	0	100	100
ALV	10	Intraperitoneal	0	0	0	0

^a Groups of 15 birds held for 70 days.

^b FFU, Focus-forming units; PFU, Plaque-forming units.

^c Percentage.

similar to that seen earlier (7). The mortality rate was 100%, and all chickens died 12 to 18 days after injection with MDHV. No gross symptoms of infection were detected in quail and chickens inoculated with ALV. In contrast, kidney, liver, and spleen homogenates derived from ALV-inoculated quail and chickens did show COFAL titers of 1:4 to 1:8 after passage in CEF for 18 days.

Antibody response of LSI-SPF Japanese quail and chickens inoculated with ALV or MDHV. In one experiment, 3-day-old *Coturnix* and chickens, hatched and maintained in an isolator barrier, were inoculated intraperitoneally with 100 mean tissue culture infective doses of ALV or with approximately 200 focus-forming units of MDHV. Ten weeks after inoculation serum samples were collected from each bird and NA was determined. NA to ALV was detected in 12 of 15 quail and in a slightly higher number (14/15) of chickens (Table 3). The NA response in MDHV-inoculated LSI-SPF quail and chickens could not be compared since all chickens died 12 to 20 days after infection. In one group of 15 MDHV-inoculated quail, 11 showed detectable NA 10 weeks after injection. Similarly, 6 of 8 birds held for 5 months contained MDHV antibody. Quail dem-

onstrating a positive antibody response showed no gross symptoms of Marek's disease when necropsied after an extended holding period of 5 months.

ALV-viremia and COFAL antigen responses in LSI-SPF quail and chickens. Whole blood samples were collected from ALV-inoculated *Coturnix* and chickens at 12 and 70 days. The blood was inoculated in CEF, and the cells and fluids were tested for presence of COFAL antigen at 21 days, using the microtiter complement fixation test. Viremia was detected in quail and chickens at 12 days and was persistent in birds bled at 70 days (Table 4). The range of COFAL titers was similar in quail and chickens at 12 and 70 days postinoculation. The titers of COFAL antigen were relatively low at 12 days but increased significantly during the course of the experiment. The number of quail responding to inoculation with ALV was somewhat less than chickens.

Beginning with the egg laying period at approximately 45 days, eggs were randomly collected from ALV-inoculated *Coturnix* and tested for presence of vertically transmitted COFAL antigen. Eggs were incubated 7 to 9 days, and QEF cell cultures were prepared. After three passages at 7-day intervals COFAL antigen titers were determined. Titers of 1:4 to 1:8 were obtained in approximately 40% of 30 quail eggs. Similar COFAL antigen titers were also demonstrated in extracted single quail embryo livers derived from 13- to 14-day-old embryonated eggs.

DISCUSSION

Although the titers of a few viruses were lower in quail embryo cell culture than in quail embryonated eggs (17), most of the viruses produced equivalent titers in the two systems. Additionally, the titers of a number of viruses in LSI-SPF Japanese quail cell cultures were quite similar to those obtained in the LSI-SPF

TABLE 3. Detection of ALV-antibody in serum from isolator-held Japanese quail and chickens 10 weeks after inoculation with ALV

Host	Inoculum	No. with antibody/total ^a
Quail	ALV ^b	12/15
Quail	Diluent	0/15
Chickens	ALV	14/15
Chickens	Diluent	0/15

^a 1/20 serum dilution effecting a 90% or greater reduction with 10² focus-forming units of RSV (RAV-1).

^b 100 mean tissue culture-infective doses per bird.

TABLE 4. ALV-viremia and COFAL antigen response in LSI-SPF quail and chickens after intraperitoneal inoculation with ALV

Host	Inoculum	ALV-viremia ^a		Reciprocal of COFAL titer			
		12 days	70 days	Range		Geometric mean	
				12 days	70 days	12 days	70 days
Quail	ALV ^b	12/15	12/15	4-16	16-64	7	43
Quail	Diluent	0/15	0/15	<2	<2	<2	<2
Chicken	ALV	14/15	15/15	2-8	16-64	5	38
Chicken	Diluent	0/15	0/15	<2	<2	<2	<2

^a COFAL tests; fractions denote number of birds positive/number inoculated.

^b Approximately 100 mean tissue culture infective doses per bird.

chicken system. However, notable exceptions were CELO virus, IBV, and WSN virus, which produced substantially lower titers in quail cells. The passage history of these viruses in the chicken system may account for the marked differences observed. In a study (3) with IBV, some degree of adaptation to growth in chicken eggs was found essential before the virus was adapted to tissue culture, suggesting that this procedure may be necessary to achieve comparable titers with CELO virus, IBV, and WSN virus in quail and chicken cell cultures.

The absence of overt symptoms of illness and mortality in LSI-SPF *Coturnix* inoculated with FPV, LTV, and MDHV indicated greater resistance to these avian pathogens than was observed with the LSI-SPF chickens. The response was not altered in quail held for an extended period after inoculation, whereas LSI-SPF chickens responded to the viruses within a normal incubation time (4 to 18 days). The holding time for ALV-inoculated quail and chickens was probably insufficient as indicated by the absence of illness and gross lesions in chickens. In this experiment, detectable antibody or virus was found in serum samples or organs (kidney, liver, spleen) of surviving SPF quail and chickens inoculated with ALV, FPV, LTV, and MDHV, indicating that inapparent infection did occur. Only with NDV did SPF Japanese quail and chickens show comparable susceptibility (100%) and mortality (87, 100%). It is conceivable that increasing the dose of virus and using virus preparations derived from the quail system might alter the response of SPF quail to some of the viruses.

In one experiment, where the dose level of ALV was increased, LSI-SPF *Coturnix* and chickens still did not show overt symptoms or gross tumors during a period of 10 weeks. However, NA was detected in 80% of inoculated quail, which was similar to the response observed in chickens (93%). Moreover, the SPF Japanese quail and chickens were similar in the number of birds showing viremia and in the titers of COFAL antigen detected at 12 and 70 days postinoculation. In SPF quail inoculated with a higher dose of MDHV, the antibody response was similar to that of quail injected with ALV. Similar results were also noted in the lack of tumor development and absence of mortality.

The utility of SPF Japanese quail and cell cultures in virus research is demonstrated by their responses to a variety of oncogenic and nononcogenic viruses. Vaccine viruses could conceivably be produced in quail cell cultures, in which endogenous oncornaviruses have not been identified. Also, a variety of diagnostic

viral and serum reagents might also be prepared in quail and quail cell cultures.

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