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Enhancement and Interference in Chickens Inoculated with Marek's Disease Herpesvirus and Oncornaviruses[1] (39082)

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(Introduced by Vincent Groupé)

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Previous studies (1) showed that the course of Marek's disease (MD), a lymphoproliferative disease of chickens, was influenced by the presence of an avian leukosis virus (ALV). Enhanced mortality and gross tumor development were observed following contact-exposure of specific pathogen-free (SPF) chickens to others inoculated with Marek's disease herpesvirus (MDHV) and Rous-associated virus type 2 (RAV-2), an ALV of subgroup B, compared to those exposed to either virus alone. RAV-2 also altered the response of cell cultures to MDHV (2). Pre-infection of chicken embryo fibroblast (CEF) cultures with RAV-2 rendered the cells resistant to MDHV focus-formation. This interference phenomenon was accompanied by enhanced ALV complement-fixing antigen (COFAL) titers.

This report presents evidence that other subgroups of ALV and reticuloendotheliosis virus (REV), an avian oncornavirus antigenically unrelated to ALV (3), also affected the responses of chickens to MDHV.

Materials and methods. Viruses. Three cell-free MDHV preparations (Figs. 1A, 1B & C, 1D) were extracted (4) from the feather follicle epithelium (FFE) of isolator-held LSI-SPF (from Life Sciences, Inc., SPF line) or SPAFAS (embryonated eggs, COFAL negative from MD-free chickens, obtained from SPAFAS, Inc., Norwich, Connecticut) chickens at 4 weeks of age. The chickens had been inoculated intraabdominally (IA) at 3 days of age with approximately 100 MDHV focus-forming units (FFU) of a duck embryo fibroblast (DEF) suspension infected with a Georgia isolate (supplied by Dr. C. S. Eidson, University of Georgia, Athens, Georgia) of MDHV (5). Infectious ALV was not detected in the FFE extracts or the DEF suspension by examination of inoculated, passaged CEF cultures. COFAL (6), resistance-inducing factor (RIF) (7), and 60–70s RNA (8) assays were employed. Tests (9) for the presence of other avian viruses and microbial agents in the inocula were uniformly negative.

RAV-1, RAV-2, RAV-7, and RAV-50 (ALV subgroups A, B, C & D, respectively) and corresponding Rous sarcoma viruses were supplied (by Dr. E. H. Bernstein, University Laboratories, Highland Park, New Jersey) as fluids from infected CEF cultures. REV (supplied by Dr. H. Bose, University of Texas, Austin, Texas) was concentrated by ultracentrifugation after cultivation in SPAFAS CEF cultures.

Cell cultures and virus assays. CEF cultures (10) were prepared from 10-day-old LSI-SPF embryos and chicken kidney (CK) cell cultures (11) were prepared from strictly quarantined 10-day-old chickens. MDHV infectivity titers were determined (12) in CK cell cultures. MD antigen was demonstrated by agar gel precipitin (AGP) tests (13) with 10% extracts of the skin and FFE in SPGA stabilizer (14) and chicken anti-MDHV serum. ALV infectivity titers ($TCID_{50}$) were assayed by the RIF procedure.

Chickens. The derivation and characteristics of the isolator-derived, barrier-sustained LSI-SPF line of chickens that was used in all experiments have been described (1). Infectious ALV, MDHV, and other avian viruses or adventitious microbial agents have not been demonstrated in this line of chick-

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ens. The C/O phenotype is predominant, although the C/B phenotype is occasionally detected. The chickens were hatched in a barrier-sustained environment and transferred in groups of 20–40 to isolators at 3 days of age. On the following day, the groups were inoculated IA with 0.2 ml of MDHV (100 FFU), RAV (10^5TCID$_{50}$), or a mixture of MDHV and RAV. REV was administered at a 1/10 dilution in a comparable study. SPGA stabilizer with antibiotics was used as a diluent for the viruses and also to inoculate control groups. All chickens were observed daily for symptomatology and mortality. An independent proportions formula was used in the statistical analysis of differences in mortality rates.

Results. In a preliminary experiment, concurrent inoculation of MDHV and RAV-2 in LSI-SPF chickens resulted in 33% mortality by 6 weeks. No deaths occurred in the RAV-2 inoculated group and only 5% mortality among chickens that received MDHV. Fig. 1 illustrates similar enhancement of mortality rates in all groups of chickens inoculated with mixtures of MDHV and other oncornaviruses, compared to the respective groups inoculated with each virus alone. There were no deaths among the controls inoculated with diluent.

Chickens inoculated with MDHV and RAV-1 showed 33% cumulative mortality at 6 weeks, whereas the mortality among chickens inocuated with each of these viruses alone was 6% or less (Fig. 1A). In similar experiments, MDHV and RAV-7 or MDHV and RAV-50 mixtures produced mortalities of 83% and 75%, respectively (Figs. 1B and C). Lower rates were observed among groups inoculated with MDHV (44%), RAV-7 (5%), or RAV-50 (25%). By 16 days post inoculation 78% of the chickens that received a mixture of MDHV and REV (Fig. 1D) were dead, and the remaining six birds were moribund. In view of the early, high mortality in this group, as well as 52% mortality in the REV group, this experiment was terminated at 2½ weeks. In the experiments with RAV-1, RAV-2, RAV-7, or REV, there were significant differences ($P < 0.05$) between the terminal cumulative mortality of the chickens inoculated with virus mixtures and the

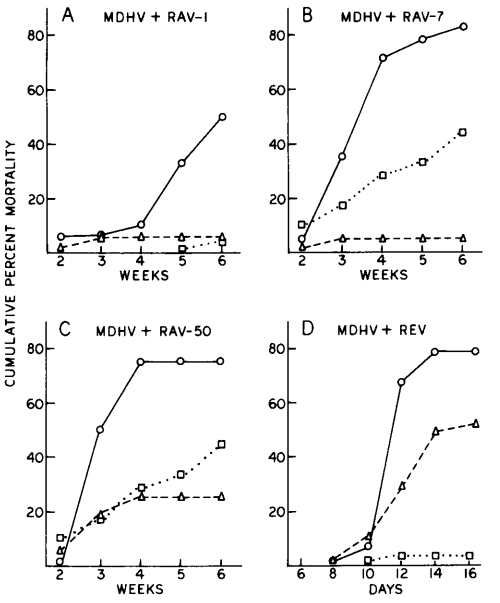

FIG. 1. Cumulative mortality among groups of 20–40, isolator-held LSI-SPF chickens inoculated IA at 4 days of age with MDHV preparations and oncornaviruses. □ · · · · · □ MDV (100 FFU); Δ---Δ RAV (10^5 TCID$_{50}$) or REV; ○——○ MDHV + RAV or MDHV + REV. No mortality occurred in diluent treated control groups.

mortality of the respective groups inoculated with the individual viruses.

Accompanying the enhanced mortality, an increased incidence of neurological symptoms was observed in the chickens inoculated with mixtures of MDHV and RAV-1 or MDHV and RAV-2. These symptoms included ataxia, paresis with unilateral wing droop, and paralysis.

Tests to detect the presence of MD antigen were performed with FFE extracts from chickens inoculated with MDHV alone or MDHV and RAV-1. ALV interfered with MDHV replication as evidenced by the frequency and the time to detection of MD antigen (Fig. 2). At 3 weeks after inoculation, none of the FFE extracts from representative chickens inoculated with the MDHV and RAV-1 combination showed the presence of MD antigen, whereas 60% of those that received only MDHV were positive. After 4 weeks, MD antigen was detected in 50% of the chickens inoculated with both viruses and was demonstrated in all extracts from

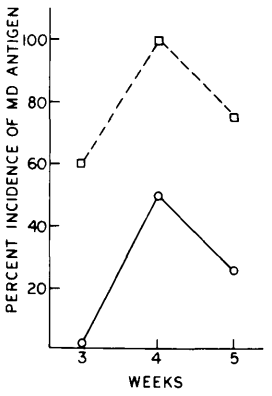

FIG. 2. MD antigen (AGP test) in the FFE extracts from isolator-held LSI-SPF chickens ionculated IA at 4 days of age with MDHV (100 FFU) + RAV-1 (10^5 $TCID_{50}$) O——O, or MDHV □---□. Each point represents the percent incidence of MD antigen detected in four to five extracts.

the MDHV group. By 5 weeks the frequency of MD antigen detection among chickens inoculated with MDHV and RAV-1 or MDHV alone was 25% and 75%, respectively. A summary of these data shows that, during the 3–5 week monitoring period, MD antigen was detected in 23% of the FFE extracts from chickens inoculated with MDHV and RAV-1. Over the same period, MD antigen was present in 79% of the chickens that received MDHV alone.

Discussion. These experiments show that, in addition to RAV-2 (1), other avian oncornaviruses affected the responses of LSI-SPF chickens to MDHV. Viral enhancement has been demonstrated by nucleic acid hybridization techniques (15). Significantly higher levels of ALV-specific mRNA were detected in tissues from conventional chickens exposed to MDHV alone or MDHV and RAV-2 than in corresponding tissues from chickens exposed to RAV-2 or from untreated controls.

Our data indicate that ALV (RAV-1) interfered with MD antigen production in the FFE. This observation is in agreement with the results of *in vitro* studies in which oncornaviruses interfered with the replication of herpesviruses. MDHV focus formation was inhibited in CEF cultures preinfected with RAV-2 (2) and herpes simplex virus was restricted in cells transformed by a variety of oncornaviruses (16).

MDHV isolates have been shown to exhibit marked differences in pathogenicity for chickens (17). Under field conditions, MDHV is transmitted by chicken dander (18), and it was shown that enveloped, infectious virions are produced in the FFE (4). Electron microscopic examination of the FFE from chickens inoculated with both an ALV and MDHV revealed the coexistence of particles resembling oncornaviruses and herpesviruses (19). Our observations suggest that oncornaviruses that may be present in the inoculum could influence host response to various isolates of MDHV.

Summary. Enhancement of mortality rates and symptomatology was observed in isolator-held LSI-SPF chickens concurrently inoculated with MDHV and avian oncornaviruses (RAV-1, RAV-2, RAV-7, RAV-50, or REV). Interference with MD antigen production also was demonstrated in extracts of the feather follicle epithelium from chickens inoculated with both MDHV and RAV-1.

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