Interferon Production and Host Resistance to Type II Avian (Marek's) Leukosis Virus (JM Strain)

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Interferon production in both susceptible S- and resistant K-line chickens infected with type II leukosis virus of JM strain and turkey herpesvirus was studied. The resistant line of chickens produced higher levels of interferon than did the susceptible with JM virus infection during the experimental period. When both susceptible S- and resistant K-line chicks were vaccinated with turkey herpesvirus, the interferon production was quantitatively similar in the two lines.

In 1962, Sevoian and Chamberlain (5) isolated and described an avian leukemogenic virus (JM strain) which was characterized by its capacity to induce a lymphoid leukosis. They found a varied level of neoplastic response to JM virus in different genetic lines of chickens. The highest and lowest leukotic response was in susceptible (S)- and resistant (K)-line chickens, respectively, which were selected from White Leghorn chickens on the basis of their neoplastic response to naturally occurring leukosis (1, 2).

JM strain is considered to be a prototype of type II leukosis viruses causing infection in field flocks of up to 100% and mortality of approximately 15%. Recently, a nonpathogenic turkey herpesvirus (HVT) antigenically related to JM strain was reported and is now being used effectively as a vaccine experimentally and commercially to delay or prevent the development of tumors in chickens; however, the mechanisms of tumor depression or prevention are not known (7).

Resistance to type II leukosis (Marek's disease) has been suggested to be genetically controlled, but by mechanisms operating at different genetic levels. However, the basis for this resistance is not clear at the present time. This study was initiated to investigate a mechanism of host resistance. The results suggest that interferon production by the host at the time of initial infection by JM virus may be one determinant of the outcome of this host-parasite relationship.

MATERIALS AND METHODS

Virus. The JM strain of leukosis virus (28th passage) was used throughout this study. Stock virus was prepared from infected monolayers (10^6 cells per 60-mm petri dish) of duck embryo fibroblast (DEF) as previously described (3). Five days after infection, the cultures with cytopathic effects were harvested by means of a rubber policeman. The infected cells were centrifuged at 400 × g to remove large particles and cell debris and then were frozen and stored in liquid nitrogen. This preparation constituted the stock virus.

For comparison of interferon production, a nonpathogenic HVT (FC-126, 38th passage), a vaccine strain antigenically related to JM strain, was also propagated in DEF monolayers. When a cytopathic effect was evident, the cultures were collected and centrifuged at 400 × g; the supernatant material was used as stock virus.

Virus assays. Monolayers of DEF were grown for 2 days at 37°C in petri dishes (60 mm, Falcon Plastic Co.). After growth medium was removed, 0.1 ml of stock JM virus or HVT was allowed to adsorb for 1 hr at 37°C. The cells were then overlaid with 5 ml of culture medium. The medium was a mixture of medium 199 (Flow Laboratories, Rockville, Md.) supplemented with 10% Trypsine phosphate broth, 5% fetal bovine serum, penicillin (100 units/ml), dihydrostreptomycin (100 μg/ml), and amphotericin B (0.25 μg/ml).

After incubations were carried out at 37°C; the petri dishes were kept under 10% CO₂ tension. Plaques were counted as measured by production of foci of ballooning, round cells or syncytia of various sizes. JM virus titered at 200,000 plaque-forming units (PFU) per ml, whereas HVT titered at 28,000 PFU per ml.

Chicks. Forty-five S-line and 45 K-line chicks (1 day old) were used in this study. Twenty S-line and 20 K-line chicks were each inoculated with 1,000 PFU of JM virus, and equal numbers of each line were each inoculated with 1,000 PFU of HVT. Five birds of each line were kept in isolated modified Horsfall units as controls.

Preparation of interferon. The blood of the experimental chicks was collected by venous puncture, and the plasma was harvested. The plasma was dialyzed for 48 hr at 4°C against approximately 100 volumes of isotonic 0.05 M KCl-HCl (pH 2) solution and then brought to neutrality by dialyzing against phosphate-
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buffered saline (pH 7.2) for 24 hr. The fluid then was centrifuged at 100,000 × g for 2 hr in a Spinco model L centrifuge. This crude preparation contained no infectious virus cell culture or hemagglutinins against sheep red blood cells. Control plasma was treated as the same procedure.

Characterization of interferon. The crude preparation of a viral inhibitor being assayed in these experiments was characterized as interferon by the following criteria: (i) acid stability at pH 2, (ii) stability at 56°C for 60 min, (iii) extracellular inactivation of virus, (iv) dialyzability, (v) species specificity, (vi) sensitivity to proteolytic enzymes (trypsin), and (vii) sedimentation studies.

Assay of interferon. The plaque reduction method described by Wagner (6) was used. Because interferon levels of chicken embryo plasma were being measured, chick embryo fibroblasts (CEF; Spafas, Norwich, Conn.) were prepared in the same manner as described above for DEF and then used for assay. When CEF monolayers were formed, usually in 48 hr, the culture medium was removed and 0.5 ml of twofold dilutions of interferon was added to each of three plates. After 2 hr of absorption, each plate received 4.5 ml of culture medium and was incubated overnight at 37°C. At 24 hr after the interferon treatment, the fluid was removed, and each plate was inoculated with 0.1 ml of HVT (100 PFU) as the indicator virus for interferon assay. After adsorption at 37°C for 1 hr, excess viral fluids were aspirated and the cultures were overlaid with growth medium. Uninoculated CEF controls were also included.

HVT plaques were usually scored on the third day. Interferon titers were assayed as the reciprocal of the dilution that induced 50% inhibition of plaque formation as compared with the numbers of plaques in positive control plates.

RESULTS

Interferon production induced by JM virus. Studies were carried out to determine the level of interferon induced by JM virus in vivo. Susceptible and resistant strains of chicks were each inoculated intraperitoneally with 1,000 PFU of JM virus (28th passages in DEF). Plasma was collected from virus-infected and uninfected control birds at 4 hr and at days 1–7 after inoculation. The plasma was dialyzed as described above. The fluid was then centrifuged at 100,000 × g, and the precipitate was removed. These crude preparations were assayed for interferon activity as described above.

The results averaged from 20 S-line and 20 K-line chickens indicated that the resistant chicks produced higher levels of interferon than did susceptible chicks. The greatest amounts of interferon were consistently present at 48 hr post-infection (Fig. 1). Interferon from plasma of the noninoculated chicks was not detected during the experimental period.

Interferon production induced by HVT. The HVT assays for infecting and sampling were similar to those described for growth of JM virus. Figure 2 shows the results obtained when 20 S-line and 20 K-line plasma samples from HVT-infected chickens and five samples from each respective noninfected control were assayed. Under the experimental conditions employed, quantitative levels of interferon pro-
duction in S- and K-line chickens infected with HVT were similar. The maximal interferon production was observed at 48 hr after inoculation. Interferon levels in plasma from noninoculated chickens were not detected.

Properties of the chicken interferon. Experiments were performed to determine whether the plasma inhibitor possessed the properties of typical interferon. The crude interferon preparations were subjected to the following tests.

Dialyzability and acid stability. Crude interferon prepared in chick plasma was dialyzed as indicated above. The activity was not destroyed, indicating the plasma interferon was nondialyzable at pH 2.0.

Enzymatic sensitivity. The activity of plasma inhibitor was reduced when treated with 0.25% trypsin solution, suggesting a polypeptide nature and sensitivity to proteolytic enzymes.

Heat stability. Incubation at 56°C for 1 hr did not inactivate the plasma inhibitor.

Extracellular inactivation of virus. When 100 PFU of HVT was incubated with plasma interferon or control noninoculated plasma for 2 hr at 37°C, the amount of infectious HVT recovered from two suspensions was the same. Thus, the interferon preparation did not directly affect the virus.

Species specificity. The activity of chicken plasma interferon was reduced when the interferon was assayed on the DEF tissue culture as compared with that on CEF. The plasma interferon was species-specific.

Sedimentation studies. When the plasma inhibitor was centrifuged at 150,000 × g for 2 hr in a Spinco model L centrifuge, the plasma inhibitor remained in the supernatant material. A summary of these results is presented in Table 1.

**DISCUSSION**

Experiments reported here emphasize the relationship between genetically selected strains of chickens susceptible and resistant to type II leukosis and interferon production. The K- and S-line have been selected for many years on the basis of their neoplastic response to naturally occurring leukosis infections (2). Though high natural incidence of infection occurs in both lines, tumors and mortality are as high as 98% in the S-line and as low as 2% in K-lines (1). However, the basis for this resistance is not clear at the present time.

Results of this study indicated that the K-line produced higher levels of circulating interferon than the S-line when both were infected with equal doses of JM virus, a prototype of the most serious disease of poultry. This suggests that the resistance of the K-line chicken to type II leukosis may be due in part to higher levels of interferon production.

HVT stimulated similar amounts of interferon in both K- and S-line chickens. HVT is currently used commercially to "vaccinate" millions of chickens; its protectiveness is better in susceptible lines than in resistant lines as judged by the development of the disease (M. Sevoian, unpublished data). The levels of interferon production induced by HVT in S-line chickens was higher than that induced by JM virus. It is indicated, but not certain, that the higher levels of interferon production by HVT virus in S-lines play a more important role in host resistance than does JM virus infecting the same line of chickens.

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