Detection of Agglutinins in Chickens Infected with JM Leukosis Virus

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Hemagglutinins for sheep red blood cells were detected in sera from JM virus-infected single-comb White Leghorn (susceptible S line) chickens. The agglutinins were not sedimented at 97,000 × g and were not affected by freezing and thawing, possibly indicating a soluble hemagglutinating factor. Agglutination titers were read in a relatively short period, after 4 hr of incubation at 4 C. The usefulness of this test for quick and low-cost screening of a large number of samples is indicated.

The JM-type leukemia infections in poultry is common and widespread. The general consensus is that type II leukemia comprises the single most significant loss within and without the avian leukemia complex. Type II infections are highly contagious by direct and indirect contact (1). Chickens exposed parenterally or naturally to JM virus showed signs of paralysis within 4 to 6 weeks (4, 5). Tumors of the gonads and dorsal root ganglia with ensuing mortality in many affected birds were also noted.

A serological procedure for the detection of agglutinins in chickens with JM leukemia virus was investigated in this study. Both formalized and fresh sheep red blood cells were agglutinated by fresh plasma from JM virus-infected chickens which showed signs of infection such as paralysis, tumors of the gonads, or dorsal root ganglia (or combinations of these signs).

MATERIALS AND METHODS

Experimental animals. Single-comb White Leghorn (susceptible S line) chickens were inoculated intraperitoneally with JM-infected whole blood from birds of the same strain showing all signs of infection: paralysis and tumors of the gonads and dorsal root ganglia.

Inocula. Whole blood was collected from infected paralyzed single-comb White Leghorn chickens with sterile citrated equipment. Infected tissue culture duck embryo fibroblast cells were lysed by three cycles of freezing and thawing in dry ice and 70% ethyl alcohol.

Titrations. All titrations were done by using the microtiter technique of Sever et al. (3). The microtiter equipment was purchased from Cooke Engineering Co., Alexandria, Va. Phosphate-buffered saline (PBS), pH 6.4, with 1% crystalline bovine serum albumin (BSA), purchased from Sigma Chemical Co., St. Louis, Mo., was used as a diluent. A 50-μliter amount of the diluent was administered into each of the wells of disposable plastic U plates. Twofold dilutions of experimental or control chicken plasma were applied with 50-μlitter dilution sticks. A 50-μlitter amount of 0.125% (v/v) fresh sheep red blood cells or 0.5% (v/v) formalinized red blood cells was added to each well. Titers were read after 4 hr of incubation at 4 C.

RESULTS

Plasma from 42 paralyzed, JM virus-inoculated chickens was found to contain agglutinins to formalinized and fresh sheep red blood cells. Reciprocal titers as high as 256 were detected for the formalized cells and 64 for the fresh sheep red blood cells. Agglutinins were not detected in plasma from control, isolated, and unexposed chickens. Centrifugation at 40,000 rev/min in a type 50 rotor of a model L Beckman preparative ultracentrifuge for 2 hr did not seem to affect the titer of the supernatant plasma, possibly indicating a soluble hemagglutinating factor. Twice freezing and thawing in dry ice and 70% ethyl alcohol did not lower the titer (Table 1).

DISCUSSION

The JM-type II leukemia infections in poultry are highly contagious by direct and indirect contact (1) or by the airborne route (6). Ovarian transmission of the virus to the offspring is possible. The signs of infection are a useful aid to diagnosis. However, earlier and quicker diagnosis is desirable. The Ouchterlony double-diffusion method is effective, but undiluted serum and 4 to 7 days are required to obtain results. Fluorescein-labeled antibody procedures require the preparation of a fluorescein isothiocyanate-labeled antiserum for the direct method; with the sandwich technique, there is the hazard of nonspecificity of results. Hemagglutination can be used for wide-scale screening investigations.
Table 1. Agglutination of sheep red blood cells exposed to plasma from JM virus-infected 4-week-old chickens

| No. of chickens tested | Sheep red cell treatment | Titer (logs) | Range of titer | Source of plasma |
|------------------------|--------------------------|--------------|----------------|------------------|
|                        |                          |              | Plasma centrifuged at 40,000 rev/min (logs) | Twice frozen and thawed plasma (logs) |
| 42                     | Fresh                    | 3-6          | 3-5            | 3-6              | JM virus-infected 4-week-old chickens |
| 42                     | Formalin treated         | 3-8          | 3-8            | 3-8              | JM virus-infected 4-week-old chickens |
| 25                     | Fresh                    | 0            | 0              | 0                | Control unexposed chickens           |
| 25                     | Formalin treated         | 0            | 0              | 0                | Control unexposed chickens           |

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