Complement-Fixing Antigens in Tissue Cultures of Avian Leucosis Viruses

Abstract. *Avian sarcoma and leucosis virus preparations grown in chick embryo tissue cultures contained complement-fixing antigens, which were demonstrated with the use of antiserums from hamsters carrying sarcomas induced by the Schmidt-Ruppin strain of avian sarcoma virus. The complement-fixing antigens shared by three different strains of avian leucosis and one strain of avian sarcoma viruses were largely "soluble" (having failed to sediment when centrifuged at 59,364g for 70 minutes). Sedimented particles, presumably virus particles, contained the common sarcoma-leucosis antigen as well.*

It has been reported (*1, 2*) that sarcomas induced in chickens and hamsters by the Schmidt-Ruppin (S-R) strain of avian sarcoma virus contained antigens which reacted in complement-fixation (CF) tests with serums from hamsters bearing the S-R tumors, but not with the serums from hamsters carrying tumors induced by adenovirus types 12 and 18 and SV-40 virus. Subsequently we noted that these antigens contained a component which was not sedimented at 26,384*g* for 1 hour (*2*), a sedimentation force regarded as adequate for sedimentation of Rous sarcoma virus or myxoviruses (*3, 4*). We also noted that other avian sarcomas, the Bryan strain of Rous sarcoma virus (RSV) (*2*) and a wild sarcoma found in a natural outbreak (*2, 5*) contained a similar antigen.

We found that RIF-free (*6*) secondary chick embryo tissue cultures infected with various members of the leucosis complex contained a soluble complement-fixing antigen similar to that produced by the Bryan and S-R strains of avian sarcoma. These viruses (Table 1) were inoculated into secondary chick embryo tissue cultures after preliminary growth for 24 hours in 60-mm Falcon petri dishes under 5 percent CO_2 (*7*); the medium, which was changed every 4 days, consisted of Baluda's medium (*8*) containing 10 percent tryptose phosphate broth and 7 percent inactivated newborn calf serum. On the 14th day after inoculation, cells and fluid were collected and frozen at −70°C. After thawing, the cell suspension was centrifuged at 2000 rev/min for 20 minutes in an International refrigerated centrifuge, after which the supernatant was again centrifuged at 30,000 rev/min (59,364*g*) for 70 minutes in a No. 40 head of a Spinco high-speed centrifuge. The upper half of the supernatant was drawn off, then the lower half, without disturbing the pellet. The pellet was then suspended in Eagle's growth medium to one-tenth the volume of the original supernatant. The uncentrifuged original virus preparation and the three portions obtained after centrifugation were then titrated as antigens in the complement-fixation test with 8 to 16 units of hamster serum antibody as determined in tests with a standard antigen from the hamster tumor produced by the S-R-strain (*1*). The results (Table 1) show that the original complement-fixing antigens were retained in all of the fractions except in the pellets, where they were reduced at least tenfold.

Table 1. Antigen titers of fractions of chick embryo tissue culture (CETC) cell suspension when tested against hamster antiserum (8 to 16 units) to Schmidt-Ruppin virus.

CETC infected viruses	CF titer of antigens (reciprocal of dilution)			
	Starting material	Supernatant		Pellet 1/10 volume
		Upper half	Lower half	
Sarcoma virus				
S-R strain*	4	4	4	<2
Leucosis complex				
Erythroblastosis†	64	64	64 (partial)	64
RPL 12†	32	16	16	32
RIF†	64	64	64	32
Uninfected CETC				
	<2	<2	<2	<2

* S-R strain of avian sarcoma obtained from C. G. Ahlstrom. † Obtained from American Type Culture Collection (*6*).

The solubility of these newly demonstrated complement-fixing antigens in avian leucosis virus preparations considered together with other properties of the avian tumor viruses, namely, size, mode of replication at the cell membrane, and RNA content (*9, 10*) suggest that these viruses may have *generic* relationship to the myxoviruses. However, the sedimentable and soluble antigens of chicken and hamster tumors induced by avian sarcoma strains were found uniformly nonreactive when tested with various specific guinea pig serums used in previous studies of myxovirus relationships in this laboratory (*4, 10*). Likewise, serums from hamsters bearing tumors induced by S-R virus which showed high-titered reactions with antigens of leucosis virus were uniformly negative when tested against the standard myxovirus antigens (*4, 10*). It is important to note that the cross reaction of complement-fixing antibody between the S-R hamster antiserum and the prototype leucosis viruses which are closely related to the Bryan strain of Rous (*11*) is not reflected in the neutralization test, where the S-R and Bryan strains appear to be distinct (*12*). The higher titers shown by the heterologous leucosis strains may be due to the higher infectivity titers which these strains achieve in tissue culture of chick embryo cells as compared to the S-R strain (*12*).

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References and Notes

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6. The erythroblastosis strain "R" was supplied to the American Type Culture Collection by Dr. J. W. Beard, Duke University; the RPL 12 strain of avian lymphomatosis was supplied by Dr. B. Burmester; and the Rous Interfering Factor (RIF), a strain of avian lymphomatosis, was supplied by Kimber Farms.
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10. Antiserums and antigens included standard reagents for influenzas A, B, and C; parainfluenzas 1, 2, 3, and 4; mumps, Newcastle, and SV 5 viruses; the preparations contained both soluble and viral antigens. Measles and respiratory syncytical virus antiserums were also included.
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