

Structural Studies of Avian Myeloblastosis Virus: Absence of Leukemogenic Activity of the Core Component (38255)

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The subject of the biological activity *in vivo* of the core component of avian oncornaviruses has received little attention, in contrast to the intense investigation of their biochemical and morphological characteristics (for review, see 2). Initial attempts to demonstrate an infectious activity in avian cores were unsuccessful (1). In contrast, a more recent report mentions that core preparations were infectious for chick embryo fibroblasts (3). Neither report evaluated oncogenesis of avian core preparations in neonatal chicks. Consequently, our laboratory has examined the leukemogenic effects in susceptible chicks of intraperitoneal injections of core preparations of avian myeloblastosis virus (AMV) that had been well characterized by biochemical and morphological methods.

Materials and Methods. Reported methods of treatment of Ficoll-purified AMV (6) with the nonionic surfactant, Sterox SL (7), using improved conditions for isolation (8), were followed to obtain preparations of the core component with a morphological homogeneity and a polypeptide composition as previously described (9). A comparison of the leukemogenic activity of preparations of AMV virions and AMV cores was made by injection of several concentrations of these two types of particles into the peritoneal cavity of 1-day-old chicks of a susceptible genetic strain (SPAFAS, Gettysburg, PA). After

1 week the various groups of birds were evaluated for the development of myeloblastosis by frequent peripheral blood smears. Chicks which developed myeloblastosis were exsanguinated and an autopsy was performed. The period of observation for all groups of chicks was 7 weeks. At that time all chicks were exsanguinated, and an autopsy was also performed. In the groups of chicks injected with AMV core material, the possibility of viral infection without noticeable pathologic effect was evaluation by purification of the serum of each chick following exsanguination, and electron microscopic examination by the thin section technique of the pellet from the region of buoyant density of 1.16 g/ml.

Results. Table I compares the leukemogenic activity of AMV virions and AMV cores which were isolated either by means of isopycnic centrifugation or velocity centrifugation (8). Evaluation of the latter, more rapid method of core isolation was considered important because the prolonged technique of isopycnic centrifugation might remove biologic activity of AMV cores. However, with either method of isolation, it is evident from Table I that the AMV core component, at concentrations of between 50 μ g and 330 μ g of viral protein injected per chick, was not capable of inducing myeloblastosis in a total of 40 neonatal chicks. In contrast, intraperitoneal injection of AMV virions caused an incidence of leukemia of approximately 55% for virions obtained by velocity centrifugation and 70% for virions obtained following isopycnic centrifugation. The incidence of leukemia in the several groups of AMV

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TABLE I. Comparative Leukemogenesis of AMV Virions and the AMV Core Component.

Method of isolation	Type of particle	Amount of protein injected	Chicks injected	Chicks leukemic	Total injected	Total leukemic	% incidence leukemia	
Isopycnic centrifugation ^a	Virion	$\mu\text{g}/\text{chick}$						
		100 μg	18	12				
		50 μg	17	13	55	39	70.9	
		25 μg	20	14				
	Core component	$\mu\text{g}/\text{chick}$						
		100 μg	5	0				
		50 μg	10	0	15	0	0	
Velocity centrifugation ^b	Virion	$\mu\text{g}/\text{chick}$						
		330 μg	5	4				
		115 μg	10	8				
		60 μg	10	7	45	25	55.6	
		30 μg	10	5				
		3 μg	10	1				
	Core component	$\mu\text{g}/\text{chick}$						
		330 μg	5	0				
		115 μg	10	0	25	0	0	
		50 μg	10	0				

^a Isopycnic centrifugation in linear 25–65% w/w sucrose gradients was for 18–22 hr in the Beckman L2 65B centrifuge using the SW 27 rotor with the 2.5×8.89 cm tubes. The light scattering bands of either AMV virions or AMV cores were collected from the gradients with the ISCO Density Gradient Fractionator as previously described (8). The light scattering bands at a buoyant density of 1.16 g/ml (AMV virions) and buoyant density of 1.27 g/ml (AMV cores) was used for direct injection into the peritoneal cavities of neonatal chicks.

^b To obtain AMV cores by velocity centrifugation (8), Sterox SL-treated AMV was placed in a polyallomer SW 56 tube which contained 0.3 ml of 15% w/w sucrose over 1.0 ml of 48% w/w sucrose and sedimented for 2 hr at 53,000 rpm into a pellet.

^c Protein determinations were performed according to a modification of the procedure of Lowry *et al.* (5).

Viremic plasma which contained over 10^{12} particles of AMV (BAI strain A) by ATPase assay was obtained from Dr. J. W. Beard of Life Sciences, Inc., through the Virus Cancer Program of the National Cancer Institute. AMV from aliquots of this high-titer viremic plasma were purified by use of Ficoll gradients and rate-zonal centrifugation as previously described (6). Conditions for core isolation (8) included resuspension of this purified AMV in a buffer of 50 mM sodium phosphate and 1 mM ethylenediamine tetraacetic acid to a turbidity of 20% transmission (or approximately 1 mg/ml of viral protein) and quick-frozen in dry ice-acetone in 2 ml aliquots. The core component of AMV was obtained by either isopycnic centrifugation or velocity centrifugation (8); however, in both cases, each 2 ml of this purified AMV was treated for 10 min at 0° with 0.5 ml of a 7.5% v/v concentration of the non-ionic surfactant, Sterox SL (7). Purified AMV was mixed with buffer alone to obtain the samples of AMV virions, and sedimented by either isopycnic centrifugation or velocity centrifugation as described above.

virion-injected chicks did not vary greatly within the tenfold concentration range of about 300 μg to 30 μg of viral protein injected per chick. A control group of 20

chicks which were injected with the buffer solution alone did not develop myeloblastosis. At autopsy, microscopic examination of the visceral and thoracic organs, as well

as sections of bone marrow from the vertebral column and the upper portion of the femur bone revealed the characteristic pathologic features of leukemia (4) in those virion-injected chicks which by peripheral smear were myeloblastic, and the absence of neoplastic changes in the bone marrow and other tissues of the core-injected chickens. In addition, the serum of the core-injected chicks also failed to contain viral particles as evaluated by electron microscopy as described above.

Discussion. This study documents that removal of the outer envelope from the AMV virion also removes the characteristic oncogenic effect of this representative avian RNA tumor virus. A likely explanation is that the core component alone is unable to attach and penetrate the cellular membranes of chick cells, and so fails to initiate the infectious and leukemogenic process in neonatal chicks. In addition, the presence of ribonuclease activity (10) may have degraded the 70 S RNA genome of AMV cores. Experiments are in progress to evaluate the ability of AMV core preparations to immunize against the development of leukemia in chickens prior to injection of AMV virions.

Summary. Preparations of the core component of avian myeloblastosis virus (AMV)

failed to induce myeloblastosis following intraperitoneal injection into neonatal chicks of a susceptible genetic strain.

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