CONDITIONS OF REPRODUCTION OF AVIAN MYELOBLASTOSIS VIRUS AS A SOURCE OF REVERTASE

N. A. Graevskaya, A. F. Sito, and A. L. Dumina

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The conditions for reproduction of avian myeloblastosis virus, as the main source for isolation of RNA-dependent DNA polymerase, were studied in connection with the "revertase" project. Eight Soviet lines of hens were used. Day-old chicks received an intracardiac or intraperitoneal injection of 0.1 ml of the virus. The titer of the virus was determined with reference to its ATPase activity. Of the strains studied, highest susceptibility to myeloblastosis (up to 75%) and highest titers (over 10^{11}) were observed in lines from the En'ya cross.

KEY WORDS: avian myeloblastosis virus; hens; susceptibility.

Any oncornavirus accumulating in sufficiently high titer during culture can be used in principle as the source of the enzyme "reverse transcriptase" (revertase) [11]. From this point of view, avian myeloblastosis virus, which accumulates in the plasma of infected chickens in high titers (up to 10^{12} virus particles/ml), has great advantages.

The work of Beard et al. [1, 5] showed that the susceptibility of birds to the disease depends on several factors, the chief of which is their genetic constitution. These workers succeeded in selecting an inbred line of hens highly sensitive to lymphomatosis, susceptibility to which reaches 100%.

To obtain large quantities of avian myeloblastosis virus required for isolation of the enzyme, these data could not be utilized, for no corresponding lines of hens were avilable and the susceptibility of Soviet lines to the virus had not hitherto been studied.

The object of this investigation was to study the susceptibility of Soviet lines of hens to myeloblastosis virus and the factors affecting accumulation of the virus in the plasma of infected chickens.

EXPERIMENTAL METHOD

Avian myeloblastosis virus strain BAI-A, obtained from Professor Bonin (West Germany), was used. Eight lines of chickens of the White Leghorn breed were used as possible producers of the virus: cross 288 of lines C and B; En'ya cross of lines C, D, and H; Ivan cross of line E; Katman cross of line 63; interlinear hybrids. Day-old chicks received an intracardiac or intraperitoneal injection of 0.1 ml of virus-containing plasma. Starting from the 10th day after infection, blood films were examined daily and when the number of myeloblasts reached 60% the chicks were totally exsanguinated. The plasma was separated from the cells by centrifugation and stored in liquid nitrogen.

Myeloblastosis virus particles contain the enzyme ATPase, and the activity of the enzyme is directly proportional to the number of virus particles [4, 8, 10]. A shift of the pH of the substrate–indicator solution (ATP + indicators) to the acid side upon addition of virus to it, through the hydrolysis of ATP with the formation of phosphoric acid [6], served as the indicator of ATPase activity. The titer of the virus was determined spectrophotometrically [2, 3]. On the addition of virus to the substrate–indicator solution with

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TABLE 1. Results of Comparative Analysis of Incidence of Myeloblastosis in Chicks of Various Lines and Mean Titers of Virus in Plasma of Affected Chicks

Chicks		Number of chicks studied	Percentage of chicks with disease	Mean time chicks remained in experiment (in days)	Number of samples tested for ATPase activity	Log of geometric mean titer of virus
cross	line					
Katman	63	696	46,0	19,2	234	10,72±0,05
288	B	228	60,0	18,7	75	10,84±0,086
288	C	516	46,1	20,4	191	11,054±0,04
En' ya	H	134	66,4	23,6	45	10,89±0,096
En' ya	D	150	75,3	21,7	66	10,84±0,06
En' ya	C	42	66,4	18,6	10	11,03±0,126
Ivan	E	50	62,5	14,6	15	10,63±0,1
Interlinear hybrids	18×B63	106	43,3	18,1	34	10,91±0,12

TABLE 2. Incidence of Disease and Titer of Virus in Chicks of Different Lines Correlated with Length of Time Chicks Remained in the Experiment

Chicks		Length of stay in experiment (in days)					
		10—17		18—24		25—31	
cross	line	percentage of chicks developing disease	log of geometric mean titer of virus	percentage of chicks developing disease	log of geometric mean titer of virus	percentage of chicks developing disease	log of geometric mean titer of virus
Katman	63	54,3±3	10,83±0,064	18,8±2	10,59±0,081	26,8±2	10,61±0,1
288	B	64,2±4	10,82±0,098	17,5±3	11,3±0,21	18,1±3	10,53±0,19
288	C	47,6±3,2	11,17±0,06	50,0±3,2	10,86±0,06	2,9±1,1	11,10±0,08
En' ya	D	56,2±4,5	11,09±0,11	37,5±4	10,82±0,075	6,2±2	10,58±0,16
En' ya	C	55,5±9,5	11,15±0,17	22,2±7,6	10,75±0,39	22,2±7,6	10,94±0,098
Ivan	E	86,7±6,2	10,71±0,104	13,3±6,2	10,12±0,048	0±3	—
Interlinear hybrid	18×B63	57,8±7,3	10,97±0,23	28,9±6	10,92±0,11	13,2±5	—

phenol red a change in optical density of the solution takes place at the wavelength 550 nm (SF-4A spectrophotometer, 1-cm cuvette). The quantity of enzyme in the virus changing the optical density of the solution from 0.350 to 0.300 during incubation for 1000 sec was taken as the unit of activity. This is equivalent to $4.57 \cdot 10^7$ virus particles in a volume of 50 μl [2]. The titer of virus (i.e., the number of physical particles) was determined by comparison with a standard. A purified [9] preparation of the virus with the number of particles counted electromicroscopically was used as the standard.

Analysis of the purified avian myeloblastosis virus in a sucrose density gradient (15-60%) showed that the virus is distributed in a narrow zone with bouyant density characteristic of oncornaviruses of the C-type, namely 1.16 g/ml. In addition, the peak of optical activity coincided with the peak of ATPase activity.

EXPERIMENTAL RESULTS

Data including the basic factors determining the production of maximal quantities of virus with high titer, required for isolation of the enzyme, are summarized in Table 1.

The incidence of the disease among chicks of line C, cross 288, line 63, and the interlinear hybrids did not exceed 46%, and these chicks are not promising as producers of virus-containing plasma. An incidence of up to 60% was obtained in the line B chicks. The highest incidence of the disease was found in chicks of the En'ya cross. The incidence among chicks of line D of this cross reached 75%. The incidence among chicks infected by the intracardiac route was higher than in those infected intraperitoneally (50.6 and 38% respectively, irrespective of the line).

The titer of the virus obtained is no less important than the incidence of the disease. The highest titers for ATPase activity were found in chicks of line 288-C and En'ya C. However, the difference between maximal geometric mean titer (cross 288 of line C) and the minimal (line E) did not exceed 0.42 log unit. The length of time the chicks remained in the experiment and its correlation with the virus titer in the plasma are of very great importance. The time that the chick remained in the experiment is the sum of the in-

cubation period plus the duration of the clinically manifest disease (from the moment of appearance of the first myeloblast in the peripheral blood until exsanguination). It varied from 12 h to 5-6 days and depended directly on the duration of the incubation period: the shorter that period, the shorter the duration of the clinically manifest disease. The sooner the chick was exsanguinated, the less plasma could be otabined from it; moreover, according to data in the literature [1], the shorter the incubation period the higher the titer of virus. The results showing correlation between the length of time the chickens remained in the experiment and the virus titers in the plasma are given in Table 2.

The results in Table 2 show that most of the chicks developed the disease, irrespective of their line, and were exsanguinated between the 10th and 17th day after infection. Chicks of line C, cross 288, in which the incidence reached a maximum on the 18th-24th day, were the exception. The highest titers of virus also were observed in chicks exsanguinated between the 10th and 17th days. For chicks of line 63, D, E, and line C of cross 288 the differences in titers obtained between the 10th and 17th day and at later times were not statistically significant, in full agreement with the results obtained by other workers [1]. In the chicks of line B maximal titers were found between the 18th and 24th days, but only 17.5% of the chicks had developed the disease at these times, so that the value of this line as a donor of plasma was considerably reduced.

If parameters such as incidence of the disease, the mean length of time the chicks remained in the experiment, and the titer of virus-containing plasma obtained were chosen as the criteria of evaluation, preference among the lines studied must be given to those of the En'ya cross, for the chicks of this cross were the best producers of avian myeloblastosis virus. Investigations of more susceptible lines of hens to myeloblastosis virus are continuing.

The virus was isolated from the plasma of the affected chicks at the Institute of Molecular Biology and Genetics, Academy of Sciences of the Ukrainian SSR (V. Kavsan and A. Ryndich) and at the Institute of Molecular Biology, Academy of Sciences of the USSR (L. Frolova), and a highly purified active enzyme was obtained from it.

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