RETROVIRUSES AS VECTORS FOR GERM LINE INSERTION IN THE CHICKEN

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SUMMARY

We are seeking an easy method for inserting beneficial genes into the germ line of poultry. We injected wild type and recombinant avian leukosis viruses (ALV) near the blastoderm of line 0 embryos just before incubation and progeny tested the surviving viremic males for transmitted viral genetic material. A number of positive progeny were identified and their DNAs were analyzed for restriction enzyme fragments that hybidized with ALV cloned DNA. Most of the progeny had simple restriction enzyme patterns unlike the viremic male parent and chicks congenitally-infected by viremic females. These data suggest that viral information has been inserted into the chicken germ line.

INTRODUCTION

Germ line insertion of foreign genes in poultry has lagged behind other animals due to the difficulties of isolating and handling the large, yolky, fertile ova at the optimum stage for microinjection, and of external manipulation and re-insertion of the altered ova into the female oviduct (reviewed in Crittenden and Salter (1985), Wagner (1985) and Freeman and Messer (1985)). Researchers believe that the numerous endogenous viral genes present in domestic chickens (Smith, 1986; Hughes, et al., 1981 and Gudkov, et al., 1981) are a result of retroviral infection of the chicken germ line (Frisby, et al., 1979). We sought, therefore, to use avian retroviruses to reproduce and improve a mechanism of germ line insertion that is presumed to have occurred naturally since the speciation of the chicken. Retroviral infection of early mouse embryos has been used successfully by Jaenisch (1976) and more recently by Van der Putten, et al. (1985) to insert genetic information into the germ line. We report here that retroviral infection of early chicken embryos appears to have inserted viral genetic information into the chicken germ line.

MATERIALS AND METHODS

Viruses, chickens and viral DNA used in this work have been described previously (Salter, et al., 1986). Briefly, we used wild-type ALV, RAV-1, and two recombinant ALVs, 882/-16, RAV-0, a viral vector having mostly exogenous molecular and biological properties except pathogenicity (Hughes, et al., 1986) and RAV-0-A(1), a virus having mostly endogenous molecular and biological properties except subgroup specificity and pathogenicity (Wright and Bennett, 1986 and Salter, et al., unpublished).

We used line 0, a chicken line that lacks endogenous viral sequences (Astrin, et al., 1979), for our studies. DNA sequences used as probes to detect ALV and chicken gene sequences will be described below.

Methods used in this work have been described in detail (Salter, et al., 1986). In brief, viral material was injected near the developing embryo of unincubated fertile eggs, the viremic chicks (designated generation zero (G0)) were identified by dot-blot hybridization and the viremic males were raised to maturity. Viremic males are not known to transmit congenitally (Rubin, et al., 1961; Spencer, et al., 1980). Therefore, their progeny presumably could only receive viral sequences through infection of the germ cells. The viremic male chickens were progeny tested for transmission of viral sequences by mating to specific pathogen free female line 0 chickens. The presence of viral sequences in the progeny (designated generation one (G1)) was determined by dot-blot hybridization. Briefly, a small quantity of blood was boiled in 0.2 M ammonium hydroxide and 2 M NaCl, rapidly cooled, centrifuged, dotted onto nylon filters, baked for 2 hours at 80^{o}C and probed for ALV sequences using nick-translated pRAV-10R, a plasmid DNA containing the complete permuted RAV-1 genome (Sealy, et al., 1983). DNA from the dot-blot positive progeny was further analyzed by restriction enzyme digestion for fragments that hybridize to pRAV-2, a plasmid DNA which contains the complete RAV-2 (subgroup B ALV) genome and cross hybridizes extensively with all ALVs (Smith, et al., 1984).

RESULTS AND DISCUSSION

Progeny testing of viremic males

Table 1 summarizes the transmission results from the progeny testing of the G0 viremic males. Four males from each of the two groups of males viremic with recombinant ALVs transmitted viral information to their G1 progeny; only one RAV-1 viremic male transmitted to his progeny. The frequency of transmission ranged from about 1 % to 11 %. The transmission of viral sequences to the progeny can be explained by one of two routes: 1. The G1 positive progeny were congenitally infected by a transient viremia in the exposed female. 2. The G1 positive progeny obtained the viral sequences via early infection of the germ cells resulting in a chimeric G0 male which then transmitted the proviral sequences genetically to its G1 progeny. We show below that the second route is the most probable.

Restriction enzyme analysis of G1 progeny DNA

DNA of the G1 positive progeny was digested with the restriction enzyme, Sac I, DNA fragments separated by size on agarose gels, transferred to nitrocellulose and probed with ALV plasmid DNA. ALV proviral DNA is cut at least once by Sac I so a single restriction enzyme fragment that hybridized with radiolabelled ALV plasmid DNA would be found representing each site of integration. We have performed Sac I digests on most of the G1 positive progeny and analyzed the restricted DNA for fragments that hybridized with radiolabelled ALV plasmid DNA. We have also analyzed DNA from the viremic G0 male parent and DNA from congenitally-infected progeny of viremic females. Restricted DNA from congenitally-infected progeny and the viremic male parents showed smears

Table 1. Frequency of transmission of ALV genetic information to the G1 progeny of G0 viremic males mated to specific pathogen free line 0 females

Virus	Wingband Number of G0 Parent	G1 Progeny Number	Number Dot-Blot Positive	Frequency (%)
RAV-0-A(1)	U-19725	75	1	1.3
	U-19721	124	10	8.1
	U-19772	134	7	5.2
	U-19770	28	3	10.7
	10 others[1]	454	0	0.0
Total	14	815	21	2.6
882/-16, RAV-0	U-19636	51	1	2.0
	U-19632	65	2	3.1
	U-19658	47	1	2.1
	U-19637	119	1	0.8
	5 others[1]	255	0	0.0
Total	9	537	5	0.9
RAV-1	U-20113	98	2	2.0
	13 others[1]	455	0	0.0
Total	14	553	2	0.4

[1]Number that failed to transmit to any progeny.

of fragments hybidizing to the ALV probe indicating multiple insertions in different cells. The restricted DNA from the G1 positive progeny showed much simplier patterns. Many had a single restriction fragment; some had several fragments (up to five) or single fragments superimposed over a smear. These data are summarized in Table 2. Thus, the restriction enzyme patterns of the G1 progeny DNA were very similar to the restriction enzyme patterns of DNA from chickens harboring endogenous viruses in their germ cells (Crittenden, 1981).

Virological and Antibody Data

We have analyzed the G1 dot-blot positive progeny and G0 female parents for the presence of virus and virus titer. All but one (Table 2, wingband No. U-26754) of the positive G1 progeny were viremic for ALV with titers of 10^4 to greater than 10^7 per ml whole blood. All female G0 parents were negative for ALV. In addition, some of the female parents were negative for ALV antibody indicating that infection had not occurred even with multiple inseminations of viremic semen and, thus, congenital transmission through a female with transient viremia did not occur.

Table 2. Restriction enzyme analysis of dot-blot positive G1 progeny of ALV viremic G0 males that suggest clonal transmission.

Virus and Wingband # of G0 Sire	Wingband # of Dot-Blot Positive G1 Progeny	pRAV-2 positive Sac I Fragments (KB)
RAV-0-A(1)		
U-19772	U-25546	10.9
	U-25547	17.1
	U-25675	14.6
	U-26358	13.4, 10.8, 9.4, 7.5, smear[1]
	U-26661	7.0
	U-26767	9.7, smear
	T-22100	9.7
U-19721	U-25667	>23
	U-26043	12.4
	U-26754[2]	8.1
	U-26710	16.0
	U-26903	>23, 16.1, 12.2, 9.3, smear
	U-26953	11.6, 9.7, 6.5
	U-26907	11.6, smear
	T-22050	9.2, smear
	U-26865	incomplete[3]
	T-22026	incomplete
U-19725	U-26324	6.2
	U-26890	incomplete
	T-22099	17.5, 11.1, 6.2
882/-16, RAV-0		
U-19636	U-26930	incomplete
U-19632	U-26412	12.8, 10.0, 9.0, 7.3, 6.9, smear
	T-22039	9.2, smear
U-19658	U-26603	14.0, 12.0, 10.8, 7.6, smear
U-19637	U-26066	8.5
RAV-1		
U-20113	V-19282	20, 8.4
	V-18903	incomplete

[1] Numerous fragments appearing in background.
[2] Negative for virus; all of the other progeny with positive Sac I fragments were positive for virus.
[3] Analysis not done or needs to be repeated.

CONCLUSIONS

We conclude that each G1 positive progeny represents one or several independent insertions of ALV proviruses into the germ line of the chicken for the following reasons: 1. The G1 dot-blot positive chickens were progeny of G0 viremic males mated to specific pathogen free line 0 females. 2. The G0 female parents were negative for ALV. Some were also negative for ALV antibody indicating that they never were infected by multiple inseminations with semen containing virus and, thus, could never transmit congenitally to their offspring. 3. The restriction enzyme pattern of the G1 positive progeny DNA were quite different from each other and from the viremic male parents and congenitally-infected progeny from viremic females. Some G1 progeny had single fragmemts while other had multiple bands. None of the G1 progeny had restriction enzyme patterns resembling congenital infection from viremic females.

We are in the process of progeny testing each G1 positive male and female by mating with specific pathogen free line 0 males and females. If the G1 positive male parents are germ-line inserted, then the expected 50 % transmission of viral genetic material should be found.

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