Two Loci Controlling Genetic Cellular Resistance to Avian Leukosis-Sarcoma Viruses

LYMAN B. CRITTENDEN, HOWARD A. STONE, RICHARD H. REAMER, AND WILLIAM OKAZAKI

Regional Poultry Research Laboratory, U. S. Department of Agriculture, East Lansing, Michigan 48823

Received for publication 20 April 1967

Female chickens known to be heterozygous for resistance to subgroups A and B of the avian leukosis-sarcoma viruses were mated to males known to be homozygously resistant to both. The progeny were assayed both on the chorioallantoic membrane (CAM) and in tissue culture for resistance to representative viruses of the A, B, and tentatively defined C subgroups. Segregation ratios of resistance to A and B subgroup viruses agreed with the previously suggested hypothesis of single-autosomal-recessive genes controlling resistance to each subgroup. Mixed infection on the CAM and replicate plate infection in tissue culture with subgroup A and B viruses showed that resistance to the A and B subgroups was inherited independently. Assays with viruses tentatively classified as subgroup C indicated that they were largely composed of a mixture of subgroup A and B viruses or of particles possessing the host range specificity of both. However, virus stocks of the subgroup C category, as well as some stocks classified as subgroup B, produced small numbers of pocks or foci on individuals known to be resistant to subgroup A and B viruses. It is suggested that these Rous sarcoma virus stocks carry between 1 and 10% of a true subgroup C virus.

The avian leukosis-sarcoma viruses may be classified into at least three subgroups (A, B, and C), based on properties of the virus coat. These properties are *host range*, *interference patterns*, and *antigenic type* (6, 14, 15; Vogt et al., Symposium on Subviral Carcinogenesis, Nagoya, Japan, *in press*).

A single-autosomal-recessive gene controls specific cellular resistance to the *in vitro* and *in vivo* growth of subgroup A viruses (1-3, 8). Similarly, a second single-autosomal-recessive gene controls resistance to subgroup B viruses (10, 11). It is clear that resistance to these viral subgroups is independent at the phenotypic level, because all four combinations of resistance or susceptibility are found (14). However, no studies have utilized critical matings which would reveal whether these genes are linked or independent. Preliminary data from this laboratory suggest that these genes are, in fact, independently inherited (R. H. Reamer, *unpublished data*). The present study was designed specifically to test this hypothesis, by inoculation of back-cross progeny from matings designed to segregate in a one-to-one ratio of resistance to susceptibility to both subgroups A and B with Rous sarcoma viruses (RSV) representative of these subgroups.

The same progeny were also inoculated with

viruses tentatively classified as subgroup C in an attempt to identify resistance to this subgroup.

MATERIALS AND METHODS

Phenotypic and genotypic nomenclature. Vogt and Ishizaki (14) suggested a phenotypic nomenclature based on the terminology for host bacterium resistance used by bacteriophage workers. We suggested (3) genotypic terminology, using *Rs* and *rs* for gene symbols representing an abbreviation for RSV. Since it is now known that these genes control resistance to leukosis viruses as well as to RSV, and since it is suspected that at least two loci are involved, we have adopted the terminology presented in Table 1. We call these loci the "tumor virus a" (*tva*) and the "tumor virus b" (*tvb*) loci, specifying susceptibility patterns to the avian tumor virus subgroups A and B, respectively. The superscripts *r* and *s* stand for resistance and susceptibility, respectively, and multiple alleles may be designated by numbered superscripts.

Virus stocks. Table 2 gives the virus stocks used, their subgroup classification, and their abbreviations.

The Bryan high-titer pseudotypes BH-RSV(RAV-1) and BH-RSV(RAV-2) were obtained from P. K. Vogt. These stocks were originally produced by activation of nonvirus-producing transformed (NP) cells with RAV-1 or RAV-2 which had been "cloned" by limit dilution (14). The BH-RSV (RAV-1) seed virus was propagated once by passage in the pectoral muscle of line 151 isolated chickens (7); BH-RSV(RAV-2) was

TABLE 1. Genotypic and phenotypic nomenclature for genetic cellular resistance to the avian leukosis-sarcoma viruses controlled by the *tva* and *tvb* loci

Genotype	Phenotype	Susceptibility (S) or resistance (R) to avian tumor virus subgroup	
		A	B
<i>a^s a^s b^s b^s</i>	<i>C/O</i>	S	S
<i>a^s a^r b^s b^s</i>	<i>C/O</i>	S	S
<i>a^r a^r b^s b^s</i>	<i>C/A</i>	R	S
<i>a^s a^s b^s b^r</i>	<i>C/O</i>	S	S
<i>a^s a^r b^s b^r</i>	<i>C/O</i>	S	S
<i>a^r a^r b^s b^r</i>	<i>C/A</i>	R	S
<i>a^s a^s b^r b^r</i>	<i>C/B</i>	S	R
<i>a^s a^r b^r b^r</i>	<i>C/B</i>	S	R
<i>a^r a^r b^r b^r</i>	<i>C/A, B</i>	R	R

propagated in line 7, known to be resistant to subgroup A viruses. The tumor minces were suspended in 0.15 M potassium citrate with 1 mg of hyaluronidase per 100 ml, homogenized in a Waring Blendor, and clarified by centrifugation at 2,000 × *g* in an International refrigerated centrifuge.

The Schmidt-Ruppin stocks, SR-RSV-1 and SR-RSV-2, were prepared from heterogeneous material obtained from Padman Sarma (12). These stocks were selected for A and B subgroup specificity by growing them for five consecutive passages of supernatant fluid in C/B and C/A cells, respectively. A second preparation of SR-RSV-2 was obtained from P. K. Vogt. This was passaged on C/A cells and classified as predominantly subgroup B. These stocks were propagated in line 151 or line 7 chickens for one passage as indicated above.

Since it was apparent, at least in tissue culture, that the resistance to B viruses could be overcome rather easily, the relative titers of several of the viruses were determined in C/O and C/A, B embryos and in tissue culture. Tenfold dilutions of the virus preparations were inoculated into the cells or embryos of line 6 and 7. The logarithm (base 10) of the titers in line 6 (C/O), line 7 (C/A, B), and the difference between them are given for each virus in Table 3. Substantial differences of at least three logs were observed in both assay systems on inoculation with both subgroup A viruses.

The subgroup B viruses showed a different picture. Large differences in titer were observed on inoculation with BH-RSV (RAV-2). However, the differences in titer in both systems were substantially lower when two different sources of SR-RSV-2 were used. Therefore, the unexpected susceptibility to these viruses of embryos carrying the *b^r* allele in the homozygous state must be attributed to the virus stocks used and not to the assay method. For this reason, difficulty was experienced in identifying the C/B and C/A, B phenotypes unequivocally when SR-RSV-2 was used.

The BS-RSV was prepared as described by Purchase and Okazaki (10). The HA-RSV preparation has been described by Crittenden and Okazaki (1). Both of these stocks have demonstrated their ability to differentiate among phenotypes on chorioallantoic membrane (CAM) assay (1).

Matings. Inbred lines 6 and 7, and their F₁ and back-cross matings, were used in this study. Extensive data have shown that line 7 is homozygously resistant (*a^r a^r*), line 6 is homozygously susceptible (*a^s a^s*), and the back-cross progeny of an F₁ × line 7 mating segregates in a ratio of one resistant to one susceptible in response to inoculation with BS-RSV, which is predominantly subgroup A (1, 3, 14). Table 4 presents the susceptibility of these lines upon inoculation with HA-RSV and PR-RSV. It is clear from these data and other unpublished data (Crittenden) that line 6 is homozygously susceptible to HA-RSV (*b^s b^s*), whereas line 7 is segregating for resistance to this subgroup B virus. However, subline 2 of line 7 appears to be homozygously resistant (*b^r b^r*) to this subgroup. This same segregation pattern is seen with PR-RSV, suggesting a possible relationship with subgroup B. Thirty-nine F₁ females from reciprocal cross matings of lines 6 and 7 were available for study. Since the subline origin of the line 7 parent was unknown, these dams could have been double heterozygotes (*a^s a^r, b^s b^r*) or homozygously susceptible to subgroup B while heterozygous for susceptibility to subgroup A (*a^s a^r, b^s b^s*). These females were mated to males of subline 2 of line 7, known to be *a^r a^r, b^r b^r*. A preliminary test of these matings by inoculation on the CAM with BH-RSV (RAV-2) revealed 24 which produced at least two resistant progeny. These 24 matings were assumed to be the double back-cross or test-cross matings appropriate for one test of independent segregation. That is, if these genes are independent, the two parental (C/O, C/A, B) and the two recombi-

TABLE 2. Stocks of virus used and their subgroup identity according to Vogt and Ishizaki (14)

Stock	Subgroup		
	A	B	C or unknown
Bryan high-titer RSV pseudotypes	BH-RSV (RAV-1)	BH-RSV (RAV-2)	
Schmidt-Ruppin RSV	SR-RSV-1	SR-RSV-2	
Bryan Standard RSV	BS-RSV		
Harris RSV		HA-RSV	
Carr-Zilber RSV			CZ-RSV
Prague RSV			PR-RSV

TABLE 3. Differences in titers ($\log_{10}$) of pairs of subgroup A and B virus preparations in line 6 (C/O) and line 7 (C/A,B) embryos when assayed in tissue culture and on the CAM

Assay	Subgroup A		Subgroup B	
	BH-RSV(RAV-1)	SR-RSV-1	BH-RSV(RAV-2)	SR-RSV-2
<i>Tissue culture assay</i>				
Line 6 (C/O) titer	6.62	6.00	6.11 ^a 6.00	6.23 4.88 6.30
Line 7 (C/A,B) titer	≤1.00	≤2.00	≤2.00 2.00	4.04 3.26 3.54
Difference	≥5.62	≥4.00	≥4.11 4.00	2.19 1.62 2.76
<i>CAM assay</i>				
Line 6 (C/O) titer	6.58	5.93	5.62	5.80 4.11 ^b
Line 7 (C/A,B) titer	3.20	1.70	2.36	4.91 2.95
Difference	3.38	4.23	3.26	0.89 1.16

^a More than one entry means that the titration was repeated.
^b The second entry represents virus obtained from P. K. Vogt and propagated by us.

TABLE 4. Tabulation of approximate pock counts on the embryonic clorioallantoic membranes of lines 6 and 7 and their cross after inoculation of HA-RSV and PR-RSV

Line	Sub-line	Inoculum	Pock count ^a					
			0	1-20	21-50	51-100	100+	Total
6	All	HA-RSV	0	0	2	11	18	31
6 × 7	All	HA-RSV	0	0	1	26	33	60
7	1	HA-RSV	23	4	9	25	23	84
	2	HA-RSV	79	5	0	0	0	84
	3	HA-RSV	3	1	35	82	95	216
	4	HA-RSV	25	0	1	7	1	34
6	All	PR-RSV	1	0	4	21	100	126
7	1	PR-RSV	3	2	0	1	4	10
	2	PR-RSV	11	8	0	0	0	19
	3	PR-RSV	2	0	10	5	11	28
	4	PR-RSV	4	0	0	0	0	0

^a Estimates over 20.

nant (C/A, C/B) phenotypes should occur in equal frequency.

Assay procedures. The CAM and tissue culture assay procedures were essentially those previously described (3) and follow the procedures given by Groupé et al. (4) and Temin and Rubin (13).

RESULTS

CAM assays. Twenty-two test-cross matings were used for the CAM assay experiments. The

FIG. 1. Distribution of pock counts on the CAM of test-cross embryos inoculated with two dilutions of BH-RSV(RAV-1). CAM numbers were grouped for tabulation in successive five-pock categories. Data represent one of three settings of eggs. Pocks became confluent when more than 300 were present; therefore, all membranes showing 300 or more pocks were tabulated as 300+.

eggs from these matings were incubated in 10 consecutive biweekly settings. In each setting, a group of line 6 (C/O) and line 7 (C/A,B) eggs were set as known susceptible and resistant controls, and these behaved as expected. Four different inocula were used in concentrations which would give an average of at least 100 pocks per

TABLE 5. Segregation ratios of test-cross embryos susceptible and resistant to four inocula of Rous sarcoma virus

Virus	Test-cross mating ♂ × (♂ × ♀)	Sex of embryo	No. susceptible	No. resistant
BH-RSV (RAV-1)	7 × (7 × 6)	♂	33	36
	7 × (7 × 6)	♀	46	38
	7 × (6 × 7)	♂	43	46
	7 × (6 × 7)	♀	51	46
	Total		173	166
BH-RSV (RAV-2)	7 × (7 × 6)	♂	45	65
	7 × (7 × 6)	♀	65	53
	7 × (6 × 7)	♂	52	63
	7 × (6 × 7)	♀	51	59
	Total		213	240
BH-RSV (RAV-1) mixed with BH-RSV (RAV-2)	7 × (7 × 6)	♂	37	11
	7 × (7 × 6)	♀	34	7
	7 × (6 × 7)	♂	37	14
	7 × (6 × 7)	♀	42	19
	Total		150	51
PR-RSV	7 × (7 × 6)	♂	50	9
	7 × (7 × 6)	♀	33	11
	7 × (6 × 7)	♂	50	16
	7 × (6 × 7)	♀	46	13
	Total		179	49

membrane. The sex of each embryo was determined when the pocks were counted.

Representative distributions of pock counts for embryos inoculated with BH-RSV(RAV-1) are given in Fig. 1. The 10⁻³ dilution was used to provide most of the segregation data collected in three settings. Any embryo inoculated with this dilution showing four or fewer pocks was considered resistant. It is clear that the distributions are bimodal and that it was not difficult to differentiate between resistant and susceptible embryos. The segregation data are given in Table 5. These data are grouped by sex of the embryo and the reciprocal mating status of the dam in order to evaluate any possible influence of sex linkage. The fact that chi-square tests showed no significant heterogeneity of segregation ratios among the four mating type-sex groups indicated that this locus is not on the sex chromosome. The ratio of 173 susceptible to 166 resistant embryos agrees very well with the 1:1 ratio expected for subgroup A viruses, assuming that susceptibility is controlled by a single-autosomal-dominant gene.

Similar results with BH-RSV(RAV-2) were obtained (Fig. 2 and Table 5). Although the segregation ratios are not as close to 1:1 as in the case of BH-RSV(RAV-1), they still agree

FIG. 2. Distribution of pock counts on the CAM of test-cross embryos inoculated with two dilutions of BH-RSV(RAV-2).

with the single-autosomal-gene hypothesis for the control of resistance to subgroup B viruses.

The next inoculum used was a mixture of BH-RSV(RAV-1) and BH-RSV (RAV-2) in such concentrations that each virus alone should have produced an average count of at least 100 pocks. If the *tva* and *tvb* genes were at the same locus or closely linked, one would expect only the parental C/O and C/A,B phenotypes in equal frequency. However, if *tva* and *tvb* were located at unlinked loci, the recombinant C/A and C/B phenotypes would be expected in frequency equal to the parental C/O and C/A,B phenotypes. This would give a 3:1 ratio of susceptible and partially susceptible (C/O, C/A, and C/B) to resistant (C/A,B) phenotypes. This method of testing for linkage in the RSV system was first used by Payne and Biggs (9). Again, the segregation ratios were found to be homogeneous (Table 5). The very close fit to a 3:1 ratio of susceptible to resistant embryos shows that these two single-gene systems are not at closely linked loci. Figure 3 gives the distribution of pock counts for one of

FIG. 3. Distribution of pock counts on the CAM of test-cross embryos inoculated with a mixture of BH-RSV(RAV-1) and BH-RSV(RAV-2). Data represent one of two settings of eggs.

TABLE 6. Median and range of focus counts on control line 6 and line 7 cells inoculated with six strains of RSV in 14 trials

Line	Phenotype	Determination	Subgroup A		Subgroup B		Subgroup C	
			SR-RSV-1	BS-RSV-1	SR-RSV-2	HA-RSV	PR-RSV	CZ-RSV
6	C/O	Median	2,180	3,000+ ^a	2,380	3,000+	3,000+	3,000+
		Range	520-3,000+	600-3,000+	740-3,000+	1,110-3,000+	1,100-3,000+	1,520-3,000+
7	C/A,B	Median	0	0	0	0	0	0
		Range	0-0	0-0	0-450	0-120	0-40	0-360

^a Pocks became confluent when counts were more than 3,000 per 60-ml plastic dish.

the two settings inoculated with the mixture. It is clear that the distribution is bimodal, but no clear distinction can be made between the C/O and selectively resistant (C/A and C/B) phenotypes.

Vogt and Ishizaki (15) suggested that PR-RSV belongs to a third or C subgroup. Since the C/A,B subline of line 7 and line 6 (C/O) were found to be resistant and susceptible to these viruses, these same matings were appropriate for studying segregation ratios with this virus stock. Again, segregation ratios were found to be homogeneous, and a 3:1 ratio of susceptible to resistant embryos was observed (Table 5). This suggests that susceptibility to this virus stock is controlled by two independent loci, perhaps *tva* and *tvb*.

Tissue culture assay. Secondary cell cultures of a single embryo may be inoculated with different virus stocks in separate plates to estimate the embryo phenotype. This is an obvious advantage when more than one virus subgroup is involved.

The test-cross embryos from four F₁ dams were used for tissue culture assay. Primary cell cultures were made each week from 10 test-cross embryos, one line 6 (C/O) embryo, and one line 7 (C/A,B) embryo. Replicate secondary cultures were inoculated in 60-mm plastic plates with each of six virus preparations in concentrations which would give an average of at least 1,000 foci per plate. Two plates from each embryo were held as uninoculated controls. The median and range of focus counts on line 6 and 7 control plates for the 14 trials are given in Table 6. In each case, the subgroup A viruses produced high focus counts on the line 6 (C/O) and no foci on the line 7 (C/A,B) cell sheets. However, foci were noticed on some of the C/A,B cell sheets when inoculated with subgroup B and C viruses. The significance of these foci will be considered in the Discussion. It was arbitrarily decided to call embryos with 10% or less of the line 6 control count resistant for the purpose of classifying a culture.

Table 7 gives the phenotypic classification of

TABLE 7. Phenotypes of test-cross embryos, based on susceptibility to SR-RSV-1 and SR-RSV-2 only, with 10% of the line 6 focus counts as the criterion of resistance

Dam	C/O	C/A	C/B	C/A,B	Total
18	15	5	3	9	32
22	8	10	7	8	33
25	4	10	5	10	29
36	13	8	7	5	33
Total	40	33	22	32	127

127 test-cross embryos based on the susceptibility of embryo tissue cultures to SR-RSV-1 and SR-RSV-2 (stocks produced at this laboratory). Inoculation with SR-RSV-1 gave a ratio of 62 susceptible (C/O and C/B) to 65 resistant (C/A and C/A,B), an excellent fit to the expected 1:1 segregation at the *tva* locus. For SR-RSV-2, the ratio was 73 susceptible (C/O and C/A) to 54 resistant (C/B and C/A,B). This ratio is not significantly different from 1:1. The ratio of 72 parental (C/O, C/A,B) to 55 recombinant (C/A, C/B) phenotypes, while showing an excess of parental phenotypes, is not significantly different from the 1:1 ratio expected for independent segregation.

To investigate the possibility that genes independent of the *tva* and *tvb* loci control a cell surface property specific for the proposed subgroup C viruses, a composite tabulation of the cellular phenotypes from all six inocula was made. Sufficient information was available for classification of 122 embryos. Again, the criterion used to define resistance was a plating efficiency of 10% or less compared with the line 6 controls. The involvement of three independent loci in the genetic cellular resistance seen in our material would lead to the appearance of eight cellular phenotypes in equal frequencies. However, only four phenotypes occurred in appreciable frequency (Table 8). The phenotypes for susceptibility and resistance to the subgroup A and B viruses segregated approximately 1:1 and were independent as

TABLE 8. *Cell phenotypes of test-cross embryos determined by use of 10% of the line 6 focus counts as the criterion of resistance for all six virus stocks*

Dam	C/O	C/A	C/B	C/C	C/A,B	C/A,C	C/B,C	C/A,B,C	Total
18	15	4	3	1	0	0	0	8	31
22	8	10	7	0	0	1	2	4	32
25	4	10	5	0	1	0	0	9	29
36	12	5	5	0	1	1	1	5	30
Total	39	29	20	1	2	2	3	26	122

before. However, the response to the proposed subgroup C viruses gave a segregation ratio of 90 susceptible to 31 resistant, a 3:1 ratio, as was found on the CAM assay with PR-RSV. Furthermore, subgroup C susceptibility is clearly dependent on both the *tva* and *tvb* loci. That is, the cells susceptible to either A or B subgroups (or both) are usually susceptible to subgroup C. The 3:1 ratio observed is a consequence of this dependence.

As pointed out in the previous section, it was sometimes difficult to identify the cellular phenotypes with certainty. We believe that the cases in which presumptive subgroup C susceptibility or resistance appears to be independent of the phenotypes controlled by the *tva* and *tvb* loci represent errors of classification.

DISCUSSION

Both the CAM and tissue culture assay systems confirm that two single-autosomal-dominant genes control susceptibility to subgroup A and B viruses, respectively. There is no evidence of sex linkage because the segregation of progeny of F₁ dams produced by reciprocal matings of lines 6 and 7 was 1:1. Also, male and female progeny each gave 1:1 ratios of susceptibility to resistance on CAM assay. Results of both methods of assay indicate that the genes controlling susceptibility to subgroup A and B viruses are inherited independently, showing that the *tva* and *tvb* loci are not closely linked.

CZ-RSV and PR-RSV have been tentatively placed in subgroup C (15). The present data indicate that the ability of these viruses to infect cells is influenced by both the *tva* and *tvb* loci. This suggests that these virus preparations are approximately equal mixtures of subgroup A and B viruses or that single particles possess protein coat properties complementary to cell surface sites for attachment or entry of both subgroup A and B viruses. The latter could occur either by genetic recombination of the subgroup A and B controlling elements so that they occur in one particle, or by production of protein envelopes with both specificities by dual infection with subgroup A and B viruses, that is, phenotypic mixing. In-

vestigations of cloned virus stocks are necessary to differentiate among these several alternatives. Recent studies by Vogt (Virology, *in press*) show that phenotypic mixing among two subgroups, A and B, does occur in the avian tumor viruses.

A small proportion of embryos appeared to have phenotypes for susceptibility to subgroup C viruses independent of the *tva* and *tvb* loci. It is unlikely that these are due to close linkage of a *tvc* locus to the other two loci, for two reasons. First, a segregation of three susceptible embryos to one resistant embryo is observed after inoculation with these viruses, whereas a 1:1 ratio would be expected if a single *tvc* locus controlled resistance. Second, if a third locus were closely linked to the *tva* and *tvb* loci, these also should show evidence of linkage with each other. They do not. It is most likely that the exceptional phenotypes occurred through misclassification, but the possibility of their existence should not be ignored. It is clear, then, that at least the major portions of the CZ-RSV and PR-RSV stocks are probably not of a third subgroup, C, at least by host range criteria.

The successful infection of C/A,B cells and embryos, particularly with subgroup B viruses, raises the question of the degree of resistance controlled by these loci. At least two alternatives to true infection of C/A,B cells with subgroup A or B viruses exist. One is that some viable cells are present in the inoculum and produce foci or pocks through transplantation and virus production. This is possible because the inocula were centrifuged but not filtered or subjected to ultrasonic vibration. However, each inoculum did go through at least two cycles of freezing and thawing. It seems unlikely that the presence of viable cells is the full explanation, because the methods of preparation of the two SR-RSV-2 stocks were very similar to the methods used for the stock showing larger differences in titer between the C/O and C/A,B phenotypes (Table 3). The fact that difference in titer found with the relatively pure stock of BH-RSV(RAV-2) was much greater than in the case of SR-RSV-2 suggests that particularly the SR-RSV, PR-RSV, and CZ-RSV stocks may contain 1 to 10% of a true subgroup C

virus which does grow in C/A,B cells. Observations by Vogt, Ishizaki, and Duff (Proceedings of the Symposium on Subviral Carcinogenesis, Nagoya, Japan, *in press*) confirm the presence of a large proportion of subgroup A and B viruses in the PR-RSV and CZ-RSV stocks. Furthermore, they found evidence for a small proportion of a virus plating on C/A,B cells in these stocks. The fact that RAV-50 was isolated from SR-RSV (5) and has been shown to be largely subgroup C suggests that our stocks of SR-RSV-2 could have been mixed with virus of this subgroup. Therefore, it seems most likely that viral mixtures explain the unexpected susceptibility of C/B embryos and cells.

LITERATURE CITED

1. CRITTENDEN, L. B., AND W. OKAZAKI. 1965. Genetic influence of the Rs locus on susceptibility to avian tumor viruses. I. Neoplasms induced by RPL12 and three strains of Rous sarcoma virus. *J. Natl. Cancer Inst.* **35**:857-863.
2. CRITTENDEN, L. B., AND W. OKAZAKI. 1966. Genetic influence of the Rs locus on susceptibility to avian tumor viruses. II. Rous sarcoma virus antibody production after strain RPL12 virus inoculation. *J. Natl. Cancer Inst.* **36**:299-303.
3. CRITTENDEN, L. B., W. OKAZAKI, AND R. H. REAMER. 1964. Genetic control of responses to Rous sarcoma and strain RPL12 viruses in the cells, embryos, and chickens of two inbred lines. *Natl. Cancer Inst. Monograph* **17**:161-177.
4. GROUPÉ, V., V. C. DUNKEL, AND R. A. MANAKER. 1957. Improved pock counting method for the titration of Rous sarcoma virus in embryonated eggs. *J. Bacteriol.* **74**:409-410.
5. HANAFUSA, H., AND T. HANAFUSA. 1966. Determining factor in the capacity of Rous sarcoma virus to produce tumors in mammals. *Proc. Natl. Acad. Sci. U. S.* **55**:532-538.
6. ISHIZAKI, R., AND P. K. VOGT. 1966. Immunological relationships among envelope antigens of avian tumor viruses. *Virology* **30**:375-387.
7. OKAZAKI, W. 1964. Discussion. *Natl. Cancer Inst. Monograph* **17**:504-507.
8. PAYNE, L. N., AND P. M. BIGGS. 1964. Differences between highly inbred lines of chickens in the response to Rous sarcoma virus of the chorioallantoic membrane and of embryonic cells in tissue culture. *Virology* **24**:610-616.
9. PAYNE, L. N., AND P. M. BIGGS. 1966. Genetic basis of cellular susceptibility to the Schmidt-Ruppin and Harris strains of Rous sarcoma virus. *Virology* **29**:190-198.
10. PURCHASE, H. G., AND W. OKAZAKI. 1964. Morphology of foci produced by standard preparations of Rous sarcoma virus. *J. Natl. Cancer Inst.* **32**:579-589.
11. RUBIN, H. 1965. Genetic control of cellular susceptibility to pseudotypes of Rous sarcoma virus. *Virology* **26**:270-276.
12. SARMA, P. S., R. J. HUEBNER, AND D. ARMSTRONG. 1964. A simplified tissue culture tube neutralization test for Rous sarcoma virus antibodies. *Proc. Soc. Exptl. Biol. Med.* **115**:481-486.
13. TEMIN, H. M., AND H. RUBIN. 1958. Characteristics of an assay of Rous sarcoma virus and Rous sarcoma cells in tissue culture. *Virology* **6**:669-688.
14. VOGT, P. K., AND R. ISHIZAKI. 1965. Reciprocal patterns of genetic resistance to avian tumor viruses in two lines of chickens. *Virology* **26**:664-672.
15. VOGT, P. K., AND R. ISHIZAKI. 1966. Patterns of virus interference in the avian leukosis and sarcoma complex. *Virology* **30**:368-374.