Infection of Resistant Avian Cells by Subgroup B Rous Sarcoma Virus

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Received for publication 12 April 1976

Chicken fibroblasts derived from the H & N flock, which have been characterized as resistant to subgroup B avian oncornaviruses in focus assays, can be infected in suspension shortly after trypsinization by subgroup B sarcoma and leukosis viruses. Once cells are plated, resistance to infection reappears rapidly. C/BE cell suspensions obtained by treatment with EDTA instead of trypsin are not as sensitive to infection. Late interference established by preinfection with subgroup B leukosis viruses is not overcome by trypsinization. In addition to C/BE H & N chicken cells, C/ABE RPRL line 7 cells can also be infected by subgroup B viruses shortly after trypsinization; however, none of the cell types can be made sensitive to subgroup E infection. These results are discussed in relation to current information on the genetic control of resistance to avian oncornaviruses.

Cellular susceptibility or resistance to subgroups A through E avian RNA tumor viruses is controlled in chickens by a series of genetic loci, *tva*, *tub*, *tvc*, and *tve*. Susceptibility at the *tvc* locus is dominant to resistance; heterozygotes *tux*^r *tux*^s are susceptible to the virus in question. This has suggested that the *tvc* locus codes for a surface receptor molecule (for review see reference 21). Some evidence indicates that, in at least some inbred chicken lines, the *tub* locus controls the existence of a receptor for both subgroups B and E viruses (3, 5). Other genes then modify susceptibility to subgroup E viruses so that a chicken can be C/BE or C/E but never C/B (5, 14). The genetically controlled resistance to at least two subgroups, A and B, has been shown to be at a step subsequent to adsorption; either at penetration or uncoating (1, 15, 18). Resistance to infection may not be absolute under certain conditions. C/A cells can be infected with Rous sarcoma virus-Rous-associated virus type 1 [RSV(RAV-1)] (subgroup A) if the C/A cells are co-cultivated with irradiated cells producing the virus (1).

Resistance to sarcoma virus infection can also occur if cells are preinfected with a non-focus-forming virus (RAV or avian leukosis virus [ALV]) of the same subgroup, a phenomenon which has been called interference (16, 17, 24). Two types of interference have been described (19). The first is early interference in which cells are infected with an ALV at a multiplicity of infection (MOI) much greater than

1. It has been suggested that in this type of interference initially added viral particles, either infectious or noninfectious, could block receptor sites and prevent adsorption of the superinfecting sarcoma virus (20). In the second type, late interference, cells are infected at an initial low MOI and interference is established by viral replication after several cell passages. In this case, interference could be established by viral particles produced internally, which then block outer receptor sites as they are released from the cells in a manner analogous to early interference (19).

During the course of studies on Sendai virus-mediated fusion of various subgroups of RSV into resistant cells, it was found that efficient infection of C/BE cells by subgroup B viruses could occur if cells were infected in suspension shortly after trypsinization. This report describes the conditions necessary for the infection of genetically resistant cells by subgroup B viruses. Data are also presented on the infection of C/E cells by RSV-B in which interference to B sarcoma viruses has been established by preinfection with an ALV of subgroup B or D.

MATERIALS AND METHODS

Viruses and cells. C/BE and C/E chicken embryo fibroblasts were obtained from embryos of non-inbred chickens supplied by H & N Inc., Redmond, Wash. All C/BE and C/E cells used were chicken helper factor (*chf*) negative (7, 25). C/ABE cells were from line 7 chickens maintained at H & N and

occasionally backcrossed to chickens from the main H & N flock. The line 100 C/BE embryo used was obtained from L. Crittenden. Japanese quail embryonated eggs were obtained from Life Sciences, Inc., St. Petersburg, Fla. A list of the viruses used is presented in Table 1.

Tissue culture. Conditions for cultivation of cells, focus assays, and agar colony assays have been previously described (9, 23, 26). All cultures were grown on Hams F10 (GIBCO) supplemented with 5% calf serum and 10% tryptose phosphate broth (GM) and maintained at 37°C. Cells were infected in suspension by the procedure for Sendai virus-mediated fusion (8; M. Linial and P. E. Neiman, *Virology*, in press) with or without the addition of Sendai virus (about 2,000 hemagglutination units). Briefly, cells were trypsinized using 0.05% trypsin (Difco, 1:300) in Tris buffer (23) and counted, and 3×10^6 cells were pelleted by centrifugation. Cells were suspended in 0.5 ml of GM. Virus (0.5 ml) was then added with or without the addition of 0.05 ml of 0.05% DEAE-dextran. Cell suspensions were incubated at 4°C for 20 to 30 min with shaking every 10 min and then at 37°C for 20 to 30 min. In later experiments the incubation at 4°C was omitted and cells were incubated at 37°C for 30 min. After incubation, cells were plated in 60-mm dishes containing 4 ml of medium and passaged when confluent. Infections in monolayers were carried out in the presence of 0.0025% DEAE-dextran, added at the time cells were initially plated.

For direct enumeration of infected cells using the infectious center technique, C/E or C/BE wells were infected in suspension for 30 min at 37°C. Dilutions of infected cells were then plated and allowed to settle for 30 min. The medium was removed, and sufficient chicken anti-subgroup B neutralizing antiserum was added to inactivate all free virus (after incubation for 30 min at 37°C). The antiserum was then removed, cells were washed extensively, and C/E indicator cells were added. Plates were overlaid the next day, and foci were counted in 7 days.

In experiments in which EDTA was used instead of trypsin to remove cells from monolayers, the following procedure was used. Cell monolayers in 100-mm dishes were washed twice with Tris buffer (24),

and 5 ml of EDTA buffer (containing, per liter, Na-EDTA, 0.2 g; NaCl, 8 g; KCl, 0.2 g; Na₂HPO₄, 1.15 g; KH₂PO₄, 0.2 g; glucose, 0.2 g; pH 7.3; reference 12) was added. Monolayers were incubated at 37°C for about 5 min until the cells were free of the dish surface. Cell suspensions were pipetted to disperse clumps, counted, centrifuged, and suspended in growth medium as in trypsinization experiments. This method does not produce good single cell suspensions of chicken embryo fibroblasts and yields many large clumps of cells. Cell viability after EDTA treatment is poor compared to that after trypsinization.

RESULTS

Replication of subgroup B viruses in C/BE cells. Initial experiments were performed with a laboratory stock of RSV-PR-B (PR-B) that had not been recently cloned. It was found that even without beta-propiolactone-inactivated Sendai virus to induce cell-virus fusion, efficient infection of C/BE cells in suspension could be obtained. Table 2 describes three separate

TABLE 2. Infection of C/BE cells by subgroup B viruses in the presence or absence of Sendai virus^a

Virus	MOI	Sendai	Measure of Infection		
			% Cells forming agar colonies	% Infected cells	IU/ml ^b
Expt 1 ^c					
PR-B-3	0.3	+	>1		
PR-B-3	0.3	—	0.01		
None		+	0		
Expt 2 ^d					
PR-B-3	0.01	+		55	
PR-B-3	0.01	—		2	
None		+		0	
Expt 3 ^e					
RAV-6	ND ^f	+			10 ⁵ –10 ⁶
RAV-6	ND ^f	—			10 ⁴ –10 ⁵

^a C/BE cells were infected with PR-B and RAV-6 in suspension as described in Materials and Methods. Sendai virus was obtained from Connaught Lab. Ltd., Ontario, Canada, and used at the optimal dilution for fusion of replication-defective Bryan high titer RSV [BH-RSV(–)] and chicken cells.

^b Interference inducing units (IU) using end-point dilution assay on C/E cells.

^c After 6 days of culture, infected cells were trypsinized, and equivalent cell numbers were plated in soft agar. Agar colonies were counted after 8 days.

^d Cells infected and passaged twice were trypsinized, and portions containing equivalent numbers of cells of each sample were plated on monolayers of C/E cells for infectious centers. After 1 day, cultures were overlaid with agar, and foci were counted 6 days later.

^e Cells were infected in suspension, and supernatant fluids were harvested after two passages.

^f ND, Not determined (but the same both with and without Sendai).

TABLE 1. Viruses used

Virus	Sub-group	Origin
PR-B	B	Lab stock
PR-B-3	B	Clone of PR-B
PR-B-3b, c	B	Subclones of PR-B-3
RSV(RAV-2)	B	H. Hanafusa via W. Mason
PR:r2:B	B	Recombinant of PR-A and RAV-2 P. Vogt via W. Mason
PR-B wt	B	W. Mason
RAV-6	B	W. Mason
PR-A	A	Lab stock
PR-C	C	Lab stock
RAV-50	D	W. Mason
PR-E-21, 53	E	Linial and Neiman (in press)

experiments comparing B virus infection of C/BE cells in suspension with or without the addition of inactivated Sendai virus to enhance fusion events. In both cases, freshly trypsinized cells were infected in suspension as described above. PR-B-3 was a newly cloned stock, grown only in *chf*⁻ cells. Experiments 1 and 2 were performed using the same C/BE embryo; experiment 3 utilized cells from a different embryo. Both cultures were scored as resistant to B virus in focus assays, and therefore they were presumably homozygous resistant at the *tub* locus. Both embryos tested were susceptible to subgroup B virus infection immediately after trypsinization. In each case, significant replication of freshly cloned PR-B or RAV-6 was obtained after one or more cell transfers, compared to cells infected in the presence of Sendai virus (from 1 to 10%). These results suggested that C/BE cells were not completely resistant to infection by subgroup B viruses under certain conditions of infection. The PR-B-3 virus was not capable of focus induction in monolayers of C/BE cells in a routine focus assay. Nevertheless the virus was recloned, and B subgroup viruses were obtained from other laboratories (see Table 1) to test for infection of C/BE cells.

The number of C/E or C/BE cells initially infected by PR-B or PR-C (in experiment 3A) were compared in three different sets of embryos by infecting cells in suspension and plating immediately (without cell passage) into soft

agar (Table 3). Colony formation in soft agar is a direct measure of viral transformation. It can be seen that there was a large variation in the number of C/BE cells that were infected by PR-B in the three experiments, even after taking into account the differences in absolute efficiency in each assay, determined by the formation of agar colonies by the control C/E cells. In experiment 1A, the C/BE cells were about 17% as sensitive to infection as the C/E cells; in experiment 2A, they were 0.5% as sensitive. In experiment 3A, the ability of C/BE and C/E cells to be infected by PR-B and PR-C was determined. Twice as many C/E cells were transformed by PR-C as compared to PR-B; about 200 times as many C/BE cells were transformed by PR-C. Correcting for the difference in susceptibility of the control C/E cells to PR-C and PR-B, it was calculated that the initial number of C/BE cells infected by PR-B was 1.7% that of the initial number infected by PR-C.

Direct enumeration of initially infected cells using an infectious center technique is also shown in Table 3. The overall efficiency of this measure of infection was low, but comparable to that of agar colony formation. Using infectious centers, the infection of C/BE cells was calculated to be 18 and 34% of that of C/E cells in two experiments using cells from different C/E and C/BE embryos, comparable to the highest efficiency obtained in experiment 1A.

Although it was unlikely that the lab stocks

TABLE 3. Comparison of infection in suspension of C/E and C/BE cells by PR-B

Expt	Cell	Virus	MOI	% Cells forming agar colonies ^a	% Cells forming infectious centers ^b	(C/BE)/(C/E) ^c
1A	C/E	PR-B	0.05	2		0.17
	C/BE	PR-B	0.05	0.33		
2A	C/E	PR-B	0.01	0.13		0.005
	C/BE	PR-B	0.01	0.0006		
3A	C/E	PR-B	0.05	0.58		0.07
	C/BE	PR-B	0.05	0.04		
	C/E	PR-C	0.05	1.1		4.1
	C/BE	PR-C	0.05	4.5		
1B	C/E	PR-B	0.25		0.44	0.18
	C/BE	PR-B	0.25		0.068	
2B	C/E	PR-B	0.25		2.6	0.34
	C/BE	PR-B	0.25		0.88	

^a Cells were infected by PR-B or PR-C in suspension at 37°C for 30 min, and aliquots were plated directly into soft agar. Colonies were counted after 8 days. The percentage of cells forming agar colonies was determined from the maximal number that could have been infected at the given MOI.

^b Assayed as described in Materials and Methods. The percentage of cells forming infectious centers was calculated assuming 100% efficiency of infection in suspension.

^c Ratio of number of C/BE cells to C/E cells forming agar colonies or infectious centers.

employed were contaminated with other subgroups of virus, since the stocks did not yield foci when assayed on C/BE cells (the ratio of focus formation on C/E cells as compared to that on C/BE cells was greater than 10^6) and since they were in some cases twice newly cloned, viruses obtained from other sources were compared to PR-B-3c. The results are shown in Table 4. In all cases, efficient infection of C/BE cells was obtained. The resultant titers were about 1% of the usual titer (10^6 to 2×10^6 focus-forming units/ml) obtained when B sarcoma viruses were grown in C/E cells. The virus produced after infection of the C/B cells was unable to transform C/BE cells in focus assays and was therefore still subgroup B. It was found that the addition of DEAE-dextran was unnecessary for infection in suspension; equivalent titers of virus were produced with and without DEAE-dextran in all cases except that of RSV(RAV-2) (data not shown). The data in Table 4 demonstrate that cells from the same embryo as those infected in suspension became relatively resistant to infection once the cells had formed monolayers. None of the cultures infected as monolayers exhibited any apparent transformation after four cell passages, although a small number of transformed cells might have been expected.

To determine how soon after plating cells became resistant to infection, the experiment described in Table 5 was performed. Cells were infected in suspension (zero-time culture) or at 20 or 40 min after plating the cells in complete growth medium on 60-mm dishes. Cells had settled, but not flattened, at 20 min after seed-

ing. Cells did not flatten in the presence of the DEAE-dextran until about 40 to 60 min after plating. When viral yields were titrated, it was found that cells, infected as soon as 20 min after plating in dishes, showed about a 100-fold decrease in resulting viral titers compared to the same cells infected in suspension. These yields were determined after three cell passages and therefore represented an augmentation of the initial infection. As was seen in Table 4, at 4 h after plating C/BE cells were completely resistant to PR-B infection.

Requirement for trypsin in acquisition of susceptibility. Although fibroblasts are generally removed from monolayers by trypsinization, it is possible to use a EDTA solution in which the presence of the chelating agent disperses the cell monolayer. Such a procedure was performed to ascertain whether the presence of a proteolytic enzyme was necessary to render C/BE cells susceptible to PR-B. The use of EDTA instead of trypsin decreased the B subgroup susceptibility of C/BE cells about 1,000-fold (Table 6). The resultant viral titer of 2×10^2 focus-forming units/ml obtained was equivalent to that obtained after infection of monolayer cultures (Table 4). In contrast, C/BE

TABLE 4. Infection of C/BE cells by various subgroup B sarcoma viruses

Infection	Virus	Virus released from C/BE cells titered on: ^a	
		C/E	C/BE
In suspension ^b	PR-B-3c	1.5×10^4	<10
	PR-B wt	6×10^3	<10
	PR:r2:B 1b-1	1.1×10^4	<10
	RSV(RAV-2)	7×10^3	<10
In monolayers ^c	PR-B-3c	10^2	<2
	PR-B wt	<2	<2
	PR:r2:B	3×10^1	<2
	RSV(RAV-2)	<2	<2

^a FFU/ml of the fourth passage after infection. All infected C/BE cultures were positive for transformation.

^b Cells were infected in suspension as described in Materials and Methods.

^c Cultures of C/BE cells were trypsinized, and 3×10^6 cells were plated on a 60-mm dish in the presence of DEAE-dextran. Four hours later, after monolayers had formed, cultures were infected with the same input FFU as cells in suspension.

TABLE 5. Time course of infection of C/BE cells by PR-B wt after trypsinization

Time (min) ^a	Titer ^b	Transformation ^c
0	$>3 \times 10^5$	+
20	7×10^3	—
40	1×10^3	—

^a Cells were trypsinized, and 3×10^6 cells were infected in suspension (zero-time sample), or 3×10^6 cells were plated on 60-mm dishes containing 5 ml of GM containing 0.0025% DEAE-dextran. PR-B wt virus was added at the indicated times after plating.

^b FFU/ml at third passage plated on C/E cells. No foci were formed by 0.1 ml of any sample when plated on C/BE cells.

^c At the fourth passage.

TABLE 6. Infection in suspension of C/BE cells dispersed from monolayers by EDTA treatment or trypsin

Virus	Treatment	Titer ^a	Transformation ^b
PR-B wt	Trypsin	$>3 \times 10^5$	+
PR-B wt	EDTA	2×10^2	—
PR-C	Trypsin	3.8×10^6	+
PR-C	EDTA	3.0×10^6	+

^a FFU/ml of PR-B or PR-C titrated on C/E cells after two cell passages. No foci were obtained if 0.1 ml of either PR-B supernatant was plated on C/BE cells.

^b At the fourth passage.

cells collected by EDTA treatment were as sensitive to infection by PR-C as those obtained by trypsinization. In addition, C/E cells were equally infected by PR-B after EDTA or trypsin treatment (data not shown).

Trypsinization of virus particles. To test whether trypsinization had any effect on the PR-B itself, 5 ml of a PR-B wt transformed cell supernatant was pelleted at 40,000 rpm for 40 min and resuspended in 5 ml of phosphate-buffered saline or growth medium without serum. Portions of virus were either incubated with trypsin (final concentration, 0.05%) for 2 min at room temperature (the conditions under which cell trypsinization is carried out) or left untreated. Virus was then plated on C/E and C/BE cells. Treatment with trypsin was found to inactivate the PR-B 3- to 4-fold when assayed on C/E cells. However, after trypsinization no foci were produced when a 0.1-ml portion was plated on C/BE cells, indicating that trypsin could not change the subgroup specificity of the virus.

Subgroup B infection of C/BE cells preinfected with B or D leukosis virus. Late interference with PR-B was established in C/E cells by infection with RAV-6(B) or RAV-50(D). Both B and D RAVs interfere with subgroup B sarcoma virus infection (6). Since interference may be caused by blocked receptor sites, it was of interest to determine whether trypsinization could alleviate interference, as was seen with neutralizing antiserum treatment (19). Table 7 shows the results of infecting C/E cells in suspension in which complete leukosis virus induced interference to subgroup B had been established. C/E cells preinfected with RAV-6 or

RAV-50 were completely resistant to either PR-B or RSV(RAV-2) virus replication and induced cellular transformation under the same conditions in which C/BE cells were susceptible.

Infection of quail and line 7 cells in suspension. To test the generality of the subgroup B infection after trypsinization, avian cells other than H & N C/BE were tested. Japanese quail cells are always resistant to subgroup B in focus assays. It was found that this resistance could not be overcome by infection in suspension (data not shown). Subgroup B resistant ring-necked pheasant cells were also not susceptible to infection in suspension.

RPRL line 7 chickens maintained at H & N can be obtained with the C/ABE phenotype. This was an opportunity to test a different resistant chicken line and also to see whether resistance to subgroup A and E could be overcome by the method described here. The results are shown in Table 8. PR-B could infect line 7 C/ABE cells as efficiently as H & N C/BE cells. PR-A could replicate in the C/ABE cells to a titer which was less than 1% that of PR-B in the same cells or less than 0.1% the titer of PR-A in permissive C/BE cells. Transformation of C/ABE cells by PR-A occurred very slowly; after four cell passages the PR-B-infected C/ABE cultures were confluent transformed, whereas only a few foci of transformation were apparent in the PR-A-infected plates. Further study of PR-A infection was not done. The replication of PR-B RPRL line 100 C/BE cells (from an embryo obtained from L. Crittenden) was also examined. Preliminary results indicated that PR-B could replicate in these cells after infection in suspension to a somewhat lesser extent than in H & N C/BE cells. Because the

TABLE 7. Subgroup B virus infection of C/E cells preinfected with RAV-6 or RAV-50^a

Preinfected with:	Challenge sarcoma virus ^b	Sarcoma virus titer ^c	Transformation ^d
None	PR-B wt	>10 ⁵	++++
RAV-6	RSV(RAV-2)	3 × 10 ¹	—
RAV-6	PR-B wt	<5	—
RAV-6	PR-A	>10 ⁵	++++
RAV-50	PR-B wt	5–10	—

^a C/E cells were infected with RAV-6 or RAV-50 or left uninfected and passaged three times, at which time the infected cells showed complete interference with PR-B in focus assays. They were then infected in suspension as described in Materials and Methods.

^b All viruses were at an MOI of 0.1 to 0.5.

^c FFU/ml at fourth passage after infection, titered on C/E cells.

^d Morphology of cells at the second passage after sarcoma virus infection. ++++ indicates complete transformation.

TABLE 8. Infection of H & N C/BE and line 7 C/ABE cells by PR-A, PR-B and PR-E

Cell	Virus ^a	Titered on: ^b		
		C/BE	Pheasant	C/E
Line 7				
C/ABE	PR-A	1.2 × 10 ³		10 ³
C/ABE	PR-B	<5		6 × 10 ⁵
C/ABE	PR-E	<5	<5	<5
H & N				
C/BE	PR-A	1.8 × 10 ^{6c}		1.8 × 10 ^{6c}
C/BE	PR-B	<5		1.8 × 10 ⁵
C/BE	PR-E	<5	<5	<10
C/BE	None	<5		<10

^a All cells were infected at an initial MOI of 0.1 to 0.5.

^b FFU/ml in supernatants at fourth passage after infection, assayed on various cell types.

^c Titered at second passage.

cells used were not in good condition, infection was difficult to quantitate. In contrast, infection of C/ABE or C/BE cells by PR-E was not seen. No virus able to form foci on E-susceptible pheasant cells was recovered, and no transformation by subgroup E was observed. This result with PR-E has been obtained with over 20 separate embryos of C/E and C/BE phenotypes (Linial and Neiman, in press; these studies).

DISCUSSION

From the results presented here, it would appear that cellular resistance to subgroup B in at least two groups of white leghorn chickens, RPRL line 7 and the H & N flock, is not absolute. Cells derived from embryos that have been stored as resistant to subgroups B and E in focus assays can be infected by various Prague and Bryan strain RSV-B if they are infected in suspension immediately after trypsinization. These cells were homozygous resistant at the *tub* locus, since heterozygotes (*tub^s tub^r*) would be sensitive to B virus (4, 5, 13). Since susceptibility is dominant to resistance, it has been suggested that a specific cellular substance present in susceptible cells is necessary for viral infection (1, 15). However, the data presented here suggests that resistant cells can be made susceptible by treatment with the proteolytic enzyme trypsin. Although the effect of other proteolytic enzymes has not been determined, it has been shown that cells dispersed from monolayers by EDTA cannot be infected (less than 1% of the virus produced by trypsin treatment is recovered). Trypsinization of subgroup B virus does not allow the virus to infect C/BE monolayers.

There are two possible explanations for these results. The first possibility is that the *tub^s* and *tub^r* loci code for different "receptor" molecules; the *tub^r* molecule being in an inaccessible or masked state for subgroup B virus. Treatment with trypsin unmasks the cellular molecules and allows viral penetration and/or uncoating. This effect is transient, and the masking substance is quickly regenerated (Table 5). Since sensitivity is dominant, the cell surface of *tub^r tub^s* heterozygotes would be a mosaic of masked and active molecules, but enough active receptors would be present for the cells to be infected. The second hypothesis is that all chicken cells contain a receptor molecule that must be cleaved by a cellular protease to be functional. The *tub^s* allele would code for this enzyme, and the unmasking activity would be present in *tub^s tub^s* and *tub^s tub^r* cells but not in homozygous resistant cells. The action of this cellular protease is mimicked to some extent by trypsin

in vitro. Further biological and biochemical experiments will be necessary to differentiate between these hypotheses.

Experiments by Steck and Rubin (19, 20) have suggested that late interference involves blockage by interfering viruses of sites on the cell surface that are necessary for penetration or uncoating of the superinfecting virus. The superinfecting virus can absorb to the cells but does not become insensitive to antibody neutralization. It had been suggested (1) that the mechanisms of both genetic resistance and viral-induced interference involve a blockage in uncoating or penetration. The data reported here show that resistance caused by interference cannot be overcome by trypsinization, unlike genetically controlled resistance. Although trypsin has an inactivating effect on suspensions of virus, if the assumption that viral particles are the agents blocking receptors in the cells exhibiting interference is correct, trypsinization cannot release the blocking particles and allow superinfection. This is similar to the results of Steck and Rubin (20), who found that mild heat treatment of RAV-1 destroyed viral infectivity but left viral interference capacity exact. They suggested that minor alterations in viral coat protein could remove infectivity but not ability to interfere with other viruses of the same subgroup.

Although the genetic control of resistance to subgroup B and E has not been studied in H & N chickens, it has been analyzed in some inbred lines: Reaseheath W (10), Reaseheath C and I (11, 14), and RPRL lines 6, 7, 15, and 100 (3, 5). In all these lines there is a relationship between susceptibility to E and B; all E-susceptible chickens are also B susceptible, but all B-susceptible chickens are not E susceptible (e.g., there is no C/B phenotype). This nonreciprocal relationship is also seen in interference patterns; subgroup B leukosis virus interferes with subgroup E focus formation (22), but subgroup E virus does not interfere with subgroup B (8). It has been reported both that the *tub* locus controls resistance to subgroup E as well (2, 3, 5) and that there are two separate loci, *tve* and *tub*, involved in cellular susceptibility (10). The results reported here indicate a fundamental difference in cellular susceptibility to subgroups B and E; resistance to subgroup B can be overcome by treatment of cells with trypsin; resistance to subgroup E cannot. These results suggest that at least the molecular sites involved in uncoating and/or penetration of the two viral subgroups are different. It would be most attractive to postulate that two different receptor-like molecules for subgroups B and E exist in H & N and line 7 chickens. However,

the possibility cannot be excluded that one gene in these chickens controls a single receptor with two different conformational states for subgroup B and E.

ACKNOWLEDGMENTS

Dick Raymond of H & N generally supplied fertile chicken eggs of specified phenotypes. I thank Paul Smith, Nancy Hopt, and Sue Brown for aid with these experiments, Paul Neiman and Bill Mason for critical comments, and the latter for supplying many of the viruses used.

This work was supported by Public Health Service grants CA 20068, CA 15704, CA 18029, and CA 18282 from the National Cancer Institute and an American Cancer Society Institutional Research Grant.

LITERATURE CITED

- Crittenden, L. B. 1968. Observations on the nature of a genetic cellular resistance to avian tumor viruses. *J. Natl. Cancer Inst.* 41:145-153.
- Crittenden, L. B. 1975. Two levels of genetic resistance to lymphoid leukosis. *Avian Dis.* 19:281-292.
- Crittenden, L. B., and J. V. Motta. 1975. The role of the *tub* locus in genetic resistance to RSV(RAV-O). *Virology* 67:327-334.
- Crittenden, L. B., E. J. Smith, R. A. Weiss, and P. S. Sarma. 1974. Host gene control of endogenous avian leukosis virus production. *Virology* 57:128-138.
- Crittenden, L. B., E. J. Wendel, and J. V. Motta. 1973. Interaction of genes controlling resistance to RSV(RAV-O). *Virology* 52:373-384.
- Duff, R., and P. K. Vogt. 1969. Characteristics of two new avian virus subgroups. *Virology* 39:18-30.
- Halpern, M. S., D. P. Bolognesi, R. R. Friis, and W. S. Mason. 1975. Expression of the major viral glycoprotein of avian tumor virus in cells of chf(+) chicken embryos. *J. Virol.* 15:1131-1140.
- Hanafusa, T., H. Hanafusa, and T. Miyamoto. 1970. Recovery of a new virus from apparently normal chick cells by infection with avian tumor viruses. *Proc. Natl. Acad. Sci. U.S.A.* 67:1797-1803.
- Linial, M., and W. S. Mason. 1973. Characterization of two conditional early mutants of Rous sarcoma virus. *Virology* 53:258-273.
- Pani, P. K. 1974. Studies in genetic resistance to RSV(RAV-O). A third type of genetic resistance typified by the W line of fowl and further support for the two loci genetic model. *J. Gen. Virol.* 23:33-40.
- Pani, P. K., and L. N. Payne. 1973. Further evidence for two loci which control susceptibility of fowl to RSV(RAV-O). *J. Gen. Virol.* 19:235-244.
- Paul, J. 1972. Cell and tissue culture. The Williams and Wilkins Co., Baltimore.
- Payne, L. N., and P. M. Biggs. 1970. Genetic resistance of fowl to MH2 reticuloendothelioma virus. *J. Gen. Virol.* 7:177-185.
- Payne, L. N., P. K. Pani, and R. A. Weiss. 1971. A dominant epistatic gene which inhibits cellular susceptibility to RSV(RAV-O). *J. Gen. Virol.* 13:455-462.
- Piraino, F. 1967. The mechanism of genetic resistance of chick embryo cells to infection by Rous sarcoma virus-Bryan strain (BS-RSV). *Virology* 32:700-707.
- Rubin, H. 1960. A virus in chick embryos which induces resistance *in vitro* to infection with Rous sarcoma virus. *Proc. Natl. Acad. Sci. U.S.A.* 46:1105-1118.
- Rubin, H. 1961. The nature of virus-induced cellular resistance to Rous sarcoma virus. *Virology* 13:200-206.
- Rubin, H. 1965. Genetic control of cellular susceptibility to pseudotypes of Rous sarcoma virus. *Virology* 26:270-276.
- Steck, F. T., and H. Rubin. 1966. The mechanism of interference between an avian leukosis virus and Rous sarcoma virus. II. Early steps of infection by RSV of cells under conditions of interference. *Virology* 29:642-653.
- Steck, F. T., and H. Rubin. 1966. The mechanism of interference between an avian leukosis virus and Rous sarcoma virus. I. Establishment of interference. *Virology* 29:628-641.
- Tooze, J., ed. 1973. The molecular biology of tumor viruses, chapter 12. Cold Spring Harbor Laboratory, New York.
- Vogt, P. K. 1967. A virus released by "nonproducing" Rous sarcoma cells. *Proc. Natl. Acad. Sci. U.S.A.* 58:801-808.
- Vogt, P. K. 1969. Focus assay of Rous sarcoma virus, p. 198-211. In K. Habel and N. P. Salzman (ed.), *Fundamental techniques in virology*. Academic Press Inc., New York.
- Vogt, P. K., and R. Ishizaki. 1966. Patterns of viral interference in the avian leukosis and sarcoma complex. *Virology* 30:368-374.
- Weiss, R. A., R. R. Friis, E. Katz, and P. K. Vogt. 1971. Induction of avian tumor viruses in normal cells by physical and chemical carcinogens. *Virology* 46:920-938.
- Wyke, J. A., and M. Linial. 1973. Temperature sensitive avian sarcoma viruses: a physiological comparison of twenty mutants. *Virology* 53:152-161.