MAMMARY TUMORS AND MAMMARY TUMOR VIRUS EXPRESSION IN HYBRID MICE OF STRAINS C57BL AND GR

By W. E. HESTON and W. P. PARKS

(From the Laboratory of Biology and Laboratory of Tumor Virus Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014)

Geneticists in 1933 and 1934 made the original discovery that led to the identification of the murine mammary tumor virus (MMTV) (1, 2).[1] By using reciprocal hybridization of high and low mammary tumor strains of mice, they discovered an extrachromosomal factor or maternal influence in mammary tumorigenesis. Bittner (3) later showed through foster nursing experiments that this factor could be transmitted through the milk and hence it was called the milk agent. Through logical steps this agent was eventually purified and characterized as an RNA-containing virus and shown in its mature form to be a characteristic particle now known as the type B particle or finally the MMTV.

Bittner and Huseby (4) never considered the issue of mammary tumorigenesis as solely viral and persisted with the concept of three sets of interacting factors, the genetic constitution of the host, the hormonal influence, and the milk agent. He carried genetic crosses beyond the F_1, and in the maternal line, F_2, and in first backcrosses observed tumor and nontumor segregation ratios that were in accord with single dominant gene segregation (5).

Subsequently, genetic crosses and foster nursing studies of strains C3H and C57BL carried out by Heston and co-workers in 1945 (6) showed that replication and transmission of virus was under genetic control. Females of the backcross to the susceptible C3H male could transmit the virus more effectively than those of the backcross to the resistant C57BL male. Again the C57BL backcross females showed a 50-50 tumor nontumor segregation ratio but nontumor females could also transmit virus although not as effectively as those that later developed tumors. Successive backcrossing of these C57BL backcross females to C57BL males completely eliminated virus by the third backcross suggesting that the number of genes controlling virus was few and possibly only one (7). However, a subsequent more detailed study of the second backcross populations did not support single gene control (8).

In the 50's and 60's Mühlbock (9) developed strain GR that was unique in that female mice had a very high incidence of mammary tumors which was transmitted by the male as readily as by the female. It was of special interest to us when Bentvelzen in 1968 and 1972 (10, 11) suggested from his data that the GR virus was genetically transmitted as a structural gene, the provirus, controlled by a regulator gene. From tumor nontumor segregation ratios, Bentvelzen concluded that single gene segregation accounted for mammary tumorigenesis. In GR and C57BL crosses carried out by van Nie and co-workers (12) the single gene hypothesis seemed to be supported. However, data from the second backcross appeared to be inadequate particularly in the light of the past observations already discussed. As a further complication in crosses between GR and BALB/c, Nandi

---

[1] *Abbreviation used in this paper:* MMTV, murine mammary tumor virus.

and Helmich (13) observed MMTV segregation ratios in the F_2 and backcross generations that fit a two gene model.

We therefore initiated a study of the genetic transmission of MMTV in which the presence and segregation of MMTV expression in F_1, F_2, and backcross hybrids of the low mammary tumor strain C57BL and the high mammary tumor strain GR was analyzed (14). To test the single gene hypothesis special emphasis was given to the second backcross.

In crosses in which passage of virus was only through the male, MMTV expression in milk samples from early lactations segregated in first backcross females in a 60:40 ratio not significantly different from the expectancy for either single or two gene control as postulated by either Bentvelzen or Nandi and Helmich, respectively (10, 13). Among the second backcross progeny of MMTV-positive first backcross females there were more virus-positive females than among the offspring of virus-negative first backcross females indicating significant segregation of genetic factors influencing virus expression. However, all second backcross families had some virus-positive females and further the families from virus-positive first backcross females had an incidence of positive females above the 50:50 ratio expected with single gene control. Similarly, of 25 second backcross families of first backcross males all but one had MMTV-positive females with no evidence of grouping of families. These results suggested strongly that virus expression in crosses between GR and C57BL mice was regulated by more than a single locus.

In addition to the measure of MMTV expression, females from these crosses have now been observed for appearance of mammary tumors.

In the present report an analysis of the genetic segregation of the tumors in these hybrids and the correlation of tumor frequency with the early measure of expression of virus in milk is reported. These results indicate that MMTV expression and mammary tumorigenesis are highly correlated. Also, analysis of the segregation ratios reveals that genetic control is more complex than can be explained by the single gene hypothesis.

Materials and Methods

A detailed outline of the breeding experiment (see below), the care of the animals, and the measure of MMTV expression was given in the previous publication (14). The C57BL parent strain was the line from our laboratory in which less than 1% of females normally develop mammary gland tumors by 2 yr of age (15). The strain GR parents were derived from the GR strain developed by Mühlbock (9) and received from his laboratories in 1960. This strain has a mammary tumor incidence of approximately 100% males transmit MMTV as readily as do females. It was in strain GR that Bentvelzen et al. (16) described male transmission of MMTV and proposed single dominant gene control for genetically transmitted virus.

The various hybrids produced from these strains are listed in Table I and their designation is given. Our intent was to study only male-transmitted genetic influences to eliminate putative milk-transmitted virus. The first cross between a C57BL mother and a GR father produced the F_1 population which was 100%; positive for virus expression (14); brother sister matings of this F_1 population produced F_2 populations. The first backcross to C57BL populations were done as reciprocal crosses to evaluate the putative milk-transmitted virus influence on virus expression in mammary tumorigenesis. Thus, either an F_1 mother and/or a C57BL mother were employed in matings to produce the two reciprocal groups of first backcross animals designated BC_1. Second backcross populations were derived from 25 male and 25 female BC_1 animals by using C57BL mates. The BC_1 females had been further segregated based on early lactation virus-positive and early lactation virus-negative groups.

After MMTV expression was measured during the first or second lactation, females were retired to holding cages. However the 25 first backcross females were allowed to produce as many second backcross litters as possible before being retired. All females were examined twice weekly

TABLE I

Genetic Crosses Employed in Present Study

	Mother C57BL (Low virus expressor	×	Father GR (High virus expressor)	Offspring Designation
Parental strains Hybrid strains				F_1
1	F_1	×	F_1	F_2
2	C57BL F_1	× ×	F_1 C57BL	First backcross (BC1)
3	C57BL BC1	× ×	BC1 C57BL	Second backcross (BC2)

for the appearance of tumors. After developing tumors or becoming moribund from some other cause the animals were necropsied and all tumors were taken for histologic examination. At the end of 2 yr, near the mean of the natural life span of the hybrid populations, the few remaining females of all groups were sacrificed. The tissues were fixed in Fekete's modification of Tellyecniczyky's solution (70% alcohol, 20 parts; formalin, 2 parts; glacial acetic acid, 1 part), sectioned and stained with hematoxylin and eosin.

Results

Earlier studies by Varmus et al. have shown a high degree of correlation between virus MMTV RNA and protein expression and the propensity for the development of mammary tumors within a given mouse strain (17). Similarly, viral polypeptide expression levels in milk correlate highly with the likelihood of any given female within an inbred strain of developing a mammary tumor (18-20). Until the recent small study by van Nie et al. (21), no study had evaluated the correlation between virus expression and tumorigenesis in various hybrid generations by using high and low mammary tumor incidence mice strains. As shown in Table II, in a very large number of animals the percentage of animals with tumor virus expression parallels the percentage of the various hybrid groups which developed tumors. In subsequent hybrid generations, as increasing genetic information from C57BL is introduced there is a marked decline in both virus expression and tumor frequency, and further, virus expression always exceeds tumor incidence. For example, 88% of the F_2 generation females express virus, but only 73% develop tumors.

The incidence of virus-positive BC-1 females with a C57BL mother was 60%, intermediate between the 50% expectancy for a single gene and the 75% expectancy if either of two genes resulted in virus transmission (14). The incidence of virus-positive first backcross females with an F_1 mother was 88%, considerably higher than in the reciprocal cross. Interestingly, the incidence of tumors in both groups was 41 and 42%. These data and similar results from reciprocal second backcross offspring suggest that the gentically-transmitted virus and/or genetically-transmitted factors are more important in mammary tumorigenesis than is milk-transmitted virus.

A similar lack of maternal influence on virus expression was noted in the second backcross populations. The frequency of virus expression regardless of the first backcross parent was approximately 50%, and the tumor incidence in

TABLE II

Mammary Gland Tumors and MMTV Virus Expression in Females of Strains GR and C57BL and Their Hybrids*, ‡

Strain or hybrid	Designation	Total number	Percent with MMTV§	Percent with tumor
GR	Parental	113	100	96
C57BL (B)	Parental	116	0	3
(B × GR)F₁	F₁	73	100	97
(B × GR)F₂	F₂	100	88	73
B × (B × GR)F₁	1st Backcross-BC₁	103	60	41
(B × GR)F₁ × B	1st Backcross-BC₁	99	88	42
[B × (B × GR)F₁]BC₁ × B	2nd Backcross-BC₂	283	53	14
B × [B × (B × GR)F₁	2nd Backcross-BC₂	580	47	16

* In all crosses the female is listed first.

‡ 1 GR, 1 C57BL, 4 F₂, 3 [B × (B × GR)F₁]BC₁, and 1 [(B × GR)F₁ × B]BC₁ females included in previous publication (1) died in the cages and no records were obtained.

§ Data from previous publication (1).

both groups was approximately 15%. Thus, in both the first and second backcross generations there was no evidence of a maternal influence on tumorigenesis and only in the first backcross generation was there an apparent influence on the frequency of virus expression.

Although the incidence of virus-positive females is significantly greater than would be predicted with a single gene in all crosses, the incidence of mammary tumors in the F_2 and the first backcross groups shown in Table II do not differ significantly from ratios expected with a single dominant gene hypothesis; 73% observed, 75% expected ($\chi^2 = 0.104$; $0.80 > P > 0.70$); 41 and 42% observed, 50% expected ($\chi^2 = 1.7418$; $0.20 > P > 0.10$) ($\chi^2 = 1.1486$; $0.30 > P > 0.20$). However, such an analysis of tumor incidence breaks down upon the analysis of the various second backcross generations as will be shown subsequently.

Correlation between Virus Expression and Tumors in GR and C57BL Strains and Their Hybrids. The quantitative relationship between mammary tumor virus expression and mammary tumorigenesis is not clear from the above data. Thus, a comparison of tumor-positive and virus-positive mice in the hybrid groups is shown in Table III. With but few exceptions, all mammary tumors occurred in females that had been mammary tumor virus positive at either the first or second lactation. The exceptions were three C57BL females which were virus negative and developed mammary tumors. It is possible that the three females listed in Table III may have become infected venerally at later matings with their MTV-positive GR and F_1 cage mates. Other exceptions to the high correlation between virus expression and mammary tumors were one first backcross and six second backcross females that had been classified as virus negative and later developed mammary tumors. These tumors could not be attributed to venereal infection since these females had been mated only to C57BL males, but might be explained by testing errors or tabular mistakes.

TABLE III

Correlation between Virus Expression and Tumors in Females of Strains GR and C57BL and Their Hybrids*‡

Strain or hybrid	MMTV positive				MMTV negative			
	With tumor		Without tumor		With tumor		Without tumor	
	No.	Average age	No.	Average age	No.	Average age	No.	Average age
		mo		mo		mo		mo
GR	109	9	4	13	0	–	0	–
C57BL	0	–	0	–	3	19	113	21
(B × GR)F₁	71	12	2	16	0	–	0	–
(B × GR)F₂	73	14	17	14	0	–	10	20
[B × (B × GR)F₁]BC₁	41	14	21	20	1	21	40	23
[(B × GR)F₁ × B]BC₁	42	14	45	19	0	–	12	19
[B × (B × GR)F₁]BC × B – BC₂	36	16	113	19	4	19	130	20
B × [B × (B × GR)F₁]BC₂	91	16	179	20	3	20	307	21

* In all crosses the female is listed first. For test designations of strain or hybrids, refer to Table I.

‡ 1 GR, 1 C57BL, 4 F₂, 3 [B × (B × GR)F₁]BC₁, and 1 [(B × GR)F₁ × B]BC₁ females included in previous publication (1) died in the cages and no records were obtained.

Additionally, there was a mammary tumor in a virus-negative second backcross female that was probably caused by an active chromophobe adenoma of the pituitary gland that occurred in this animal.

In subsequent backcross generations, the proportion of virus-positive females that developed mammary tumors was much lower. As will be shown subsequently, lower tumor incidence was in part due to lower levels of virus expression. Therefore, the simple presence of virus expression in the milk did not necessarily equal tumor formation. As is also shown in Table III, decreasing virus expression in backcross groups prolonged the latency to tumor formation from 9 mo in the GR strain to approximately 16 mo in the second backcross generations. Thus, the correlation between the proportion of any parental strain or hybrid group which expresses MMTV virus and the percentage of that group that develops tumors is very high and further correlates with tumor latency.

Correlation between Virus Expressor Status and Mammary Tumors in Second Backcross Females. With the high degree of correlation between virus expression and the propensity for mammary tumorigenesis, it was possible to evaluate critically various second backcross generations for tumor and nontumor segregation ratios. Specifically, this analysis is to test the single gene hypothesis for mammary tumorigenesis to test the role of milk-transmitted virus and finally to extend the correlation between virus expression and tumor formation. As shown in sections A and B of Table IV, by selecting families from virus-positive and virus-negative first backcross mothers the frequency of mammary tumorigenesis differed significantly, 35 and 1%, respectively.

Female progeny from virus-positive first backcross mothers (Table IV, section A) demonstrate a clear association between levels of virus expression and mammary tumor propensity. 74% of their female offspring were virus positive and 35% developed tumors. Although the numbers are too low to analyze individual families, summation of the results indicates that approximately 50% of animals with + + status (indicating the presence of MMTV p14 in a 1:400 dilution of milk) develop mammary tumors. Animals with + − status (meaning

MMTV p14 antigen positive at a 1:40 milk dilution but not at the 1:400 dilution) only rarely developed tumors.

Section B of Table IV shows the correlation between virus expression and tumorigenesis in the female offspring of 13 first backcross females classified at early lactations as virus negative. Only one mammary tumor was noted in 170 offspring and only 32% were virus positive. The ability to separate first back-cross mothers so strikingly into two groups clearly indicates that genetic factors are important in the regulation of virus expression and tumorigenesis. More importantly, factors regulating viral expression, regardless of their complexity are also important determinants of mammary tumorigenesis.

As indicated earlier, the larger number of animals available from the progeny of these first backcross females shown in both sections of Table IV enables a more precise statistical analysis of the single gene hypothesis for mammary tumorigenesis. This hypothesis would predict a 50% mammary tumor incidence among the BC_2 female offspring of virus-positive BC_1 mothers. Of 113 BC_2 female offspring of the early positive backcross females, 35 of those with virus developed tumors. However, this incidence does not support the single gene hypothesis since the observed 31% tumor incidence of tumors associated with virus is significantly lower than 50% expectancy; $\chi^2 = 8.01$ $P < 0.01$. Such an analysis of these second backcross females (section A) is complicated by possible milk transmission of virus in addition to the genetic influences transmitted by these first backcross mothers. However, if milk virus transmission was opera-tive one might expect an enhancement of genetically-transmitted virus and actually expect a figure greater than 50% tumor incidence. Given that the observed values were significantly lower than would be predicted by a single gene, antibody influences in milk (22) or negative influences from the C57BL genotype must be postulated to be consistent with a single GR gene hypothesis.

Analysis of the Reciprocal Second Backcross Population with a Male First Backcross Parent. The breeding test of the 25 first backcross males to produce second backcross females has the disadvantage that it was not possible to classify the fathers as virus positive or negative. However, this cross has the advantage that large numbers of female offspring are produced allowing analy-sis of individual second backcross families. As previously reported 24 of the 25 first backcross males produced female progeny that were virus positive (14). As shown in Table V, 15 males produced female offspring that developed mammary tumors. If a single gene were involved we would assume that these 15 BC_1 males had that gene and the incidences of mammary tumors in their families would be distributed about a 50% incidence. In contrast, the tumor incidences range from 5-58% with no evidence of grouping (Table VI). There was a total of 347 offspring in these 15 second backcross families of which 95 animals or 27% of the total had tumors. This incidence is significantly below the 50% expected with a single gene segregation: $\chi^2 = 37.43$; $P < 0.001$. Thus, although the incidence of mammary tumors in the F_2 and first backcross generations do not deviate significantly from that expected for a single gene hypothesis, such an hypothesis is not supported with the data from this second backcross generation. These data strongly support a more complex genetic situation than can be explained as a single mendelian trait.

TABLE IV

Correlation between Virus Expressor Status and Mammary Tumorigenesis in Second Backcross Female Progeny of First Backcross Females Segregated on Basis of Virus Expressor Status

		Virus status of second backcross females					
		+ +		+ −		− −	
A	Early positive first backcross mothers*	With tumor	With-out tu-mor	With tumor	With-out tu-mor	With tumor	With-out tu-mor
	1852	3	3	1	2	2	2
	1853	1	1				2
	1855	2	2		5	1	1
	1856	1	1				1
	1857	5	6		2		1
	1886		2		7		2
	1981	5	1		3		1
	2060	3	4				
	2209	3	6		2		1
	2256	2	2	1	2		
	2261	4	3		2		1
	2262	3	2	1	2	1	2
	Total	32	33	3	27	4	14
		(28)‡	(29)	(3)	(24)	(4)	(12)
B	Early negative first backcross mothers§						
	1854		2		3		9
	1866				3		5
	1867		3		2		1
	1888				3		11
	1940						3
	1978				11		9
	1979				4		18
	1980				2		8
	2114				2		13
	2115				4		14
	2159		3		5		7
	2160	1	1		3		6
	2257				2		12
	Total	1	9	0	44	0	116
		(0.6)	(5)		(26)		(68)

* All first backcross mothers were virus positive in their first or second lactation and are identified in the previous publication. 10 of these 12 mothers developed mammary tumors, the exceptions being 1853 and 2060.

‡ Number in parenthesis represents percent of total.

§ Of 13 virus-negative mothers tested in early lactations, 12 were tumor free at the time of death. One mother, 1979, died and was cannibalized, thus no final record is available.

Comparison of Agouti Gene Segregation and Mammary Tumor Segregation. Evidence that typical mendelian segregation occurred in the GR-C57BL crosses came from studies of the agouti locus through the second backcross generation (Table VI). In the second backcross females there was single gene

TABLE V

Correlation between Virus Expressor Status and Mammary Tumorigenesis in Female Progeny of First Backcross Males

First backcross father	Virus status of second backcross females					
	++		+-		--	
	With tumor	Without tumor	With tumor	Without tumor	With tumor	Without tumor
2158						21
2011				1		21
2057				3		26
2157				3		15
2347				5		21
2287				5		20
2286				6		20
2254		2		6		18
2111		6		8		10
2113		3		8		6
2112		3		14	1	9
2059	1			8	1	18
2208	2	8		5	1	6
2255	4	10		5		7
2058	5	8				11
2056	5	6		6		7
2156	5	5		10		4
2253	6	4				13
2360	9	6		3		8
2207	8	2	1	2		12
1931	7		3	1		14
2358	11	2				12
2110	11	3		4		5
2357	3	1		2		
2809	10	1	1	4		3
Total	87	70	5	109	3	307
	(15)*	(12)	(0.7)	(19)	(0.5)	(53)

* Figure in parenthesis represents percentage of total female progeny from 25 first backcross fathers, a total of 580.

segregation at the agouti locus with no evidence of linkage between presence of virus or mammary tumors and this locus. Both reciprocal second backcross populations had mammary tumor incidences significantly less than the 50% predicted for a single gene in the families where we would assume segregation of this gene. Furthermore, segregation ratios in these individual families showed no evidence of clustering around a 50% mean. The clear-cut single gene segregation of the agouti locus indicated that if mammary tumorigenesis had segregated as a single gene it would have been detected in our crosses.

Distribution of Age and Tumor Death. It has been considered by some authors that there are two kinds of mammary tumors, those that arise relatively early in the life of the animal and are induced by MMTV and those that arise relatively late and are caused by other factors and/or genetically transmitted MMTV (21, 23, 24). The many mammary tumors occurring in these hybrids were tabulated according to tumor age to see if there was evidence for such separation. The distribution presented in the histogram in text Fig. 1 spreads from 8 to

TABLE VI

Comparison of Segregation of Agouti with Segregation of Mammary Tumors in Females of the Second Backcross to C57BL

Cross	Total number families	Number families with agouti	Number animals	Number animals with agouti	Segregation ratio
Agouti					
BC₁ (agouti) × C57BL	13	13	161	74	46:54
BC₁ (nonagouti) × C57BL	12	0	126	0	—
C57BL × BC₁ (agouti)	13	13	303	149	49:51
C57BL × BC₁ (nonagouti)	12	0	283	0	—
	Total no. families	No. families with mammary tumors		No. animals with mammary tumors	
Mammary tumors					
BC₁* × C57BL	12	12	113	35¶	31:69
BC₁‡ × C57BL	13	1	170	1	—
C57BL × BC₁§	15	15	347	95	27:73
C57BL × BC₁‖	10	0	234	0	—

* Females with MMTV expression in early lactations.
‡ Females with *no* MMTV expression in early lactations.
§ Males with female offspring with mammary tumors.
‖ Males with no female offpsring with mammary tumors.
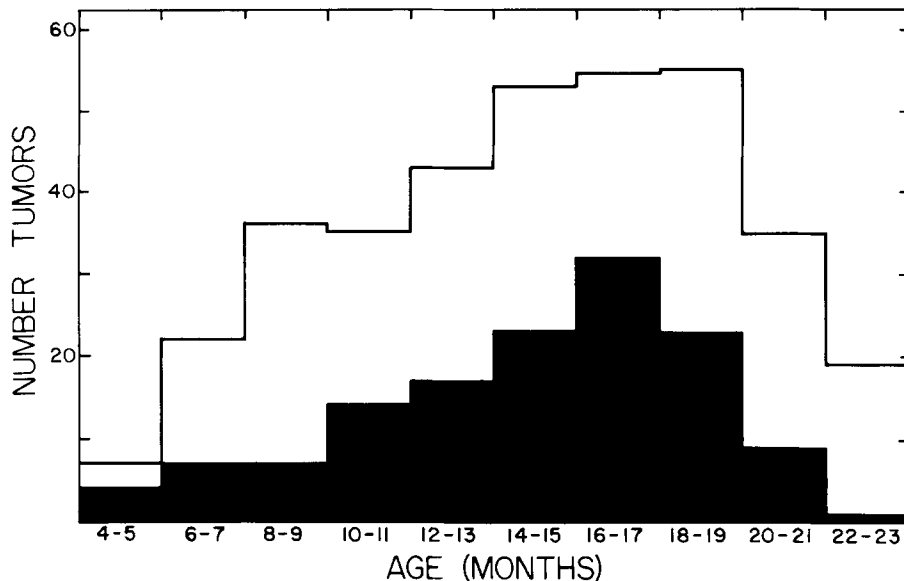¶ Four additional virus-negative females had tumors.

FIG. 1. Mammary tumors of all hybrids distributed according to age of animal when the tumor appeared. Open bars represent all mice and closed bars represent mammary tumors occurring in BC₂ generation females.

25 mo but fails to indicate any grouping according to age at which the tumors appeared. We conclude that early and late are more likely the result of levels of virus expression interacting with a particular genotype.

Further, it should be noted that by 2 yr the age-specific incidence of mammary tumors appears to be declining markedly suggesting that most mammary tumors occurred in this population by 20 mo of age.

Reticular Cell Neoplasms in Second Backcross Females. Earlier studies have shown that type C expression is correlated with reticular cell neoplasms but not with mammary tumorigenesis although no measure of MMTV expression was reported (25). We were interested to determine if mammary tumor virus expression could be directly demonstrated to be independent or dependent of reticular cell tumors in various hybrid groups. An analysis of the second backcross groups for reticular cell neoplasms indicated no correlation between virus expressor status and reticulum cell tumors (Table VII). Thus, the high association between type B virus expression and mammary adenocarcinomas appears to be specific to that class of tumors and not general for all tumor types.

Other Tumors in C57BL, GR, and Their Hybrids. All neoplasms observed in the parent strains and their various hybrids in this study are listed in Table VIII. Most of the mammary tumors were Dunn's types A and B (26). In all cases they were classified depending on whether the cell arrangement was predominantly an adenoid, type A, or predominantly sheets and cords, type B, because most tumors had areas that were of the alternate type. The pale cell carcinoma has been described by van Nie and Dux (27) in the GR strain. The pale cell carcinoma reveals cells that appear to lie in compact sheets but take a very pale stain with hematoxylin and eosin. Thus far we have noted this type of mammary tumor only in the GR strain and hybrids derived therefrom.

It was of interest that adenocanthomas observed in these groups occurred in females whose milk was virus positive. In other studies (28, 29) this type of neoplasm had been noted in strains such as the C3HfB, thought to be free of milk-transmitted virus and especially among mammary tumors induced by chemical carcinogens. Thus adenocanthomas may represent an aberrant or unusual result of the activation of type B virus expression (30). Further studies will be necessary to validate this hypothesis.

After mammary tumors, the next most frequently occurring neoplasm in this study were the reticulum cell neoplasms probably because of the influence of the C57BL strain. This group included both reticulum cell types A and B described by Dunn (30). Other neoplasms in the GR strain included lymphocytic leukemias and lung tumors, but were limited to these two groups probably because of the early death of the GR females from mammary tumors. The list includes in the other groups a number of other tumors, none of which occurred in significant numbers in the population.

Discussion

The initial reports of mammary tumor virus segregation as a single dominant gene in crosses between the GR and C57BL used mammary tumor development as the measure of virus expression (10–12). Previous studies had clearly shown a high degree of correlation between virus expression and mammary tumorigene-

TABLE VII

Reticular Tissue Neoplasms in Second Backcross Female Progeny and Virus Expressor Status

Parental cross	MMTV virus expression			
	Positive		Negative	
	Total number	Number with neoplasms	Total number	Number with neoplasms
[B × (B × GR)F₁] × B	149	14 (9)*	134	30 (22)
B × [B × (B × GR)F₁]	270	41 (15)	310	44 (14)
Total	419	55 (13)	444	74 (17)

* Number in parenthesis represents percentage.

TABLE VIII

Neoplasms in Females of Strains GR and C57BL and Their Hybrids

Neoplasm +	GR	C57BL	(B × GR)F₁	(B × GR)F₂	[B × (B × GR)F₁]BC₁	[(B × GR)F₁ × B]BC₁	[B × (B × GR)F₁]BC × B-BC₂	B × [B × (B × GR)F₁]BC₂
	113* ‡	116	73	100	103	99	283	580
Mammary tumors								
Type A	18	1	25	38	16	24	30	56
Type B	93	2	47	44	31	18	14	43
Pale cell	28		7	6	3	4	1	4
Adenoacanthoma	3	1		1	1	1	2	3
Reticulum cell neoplasm	1	17	2	4	7	4	34	64
Lymphocytic leukemia	7	1		1	4	2	9	14
Lymphoma		1			1		1	7
Plasma cell tumor							1	
Hemangioendothelioma		2		2	4	1	4	14
Lung tumor	8		1	8	3	2	2	1
Hepatoma					1		1	1
Sarcoma								2
Leiomyosarcoma							2	
Osteogenic sarcoma				1				
Chromophobe adenoma							2	1
Adrenal medullary tumor					1			
Tubular adenoma of ovary					1			
Squamous cell carcinoma					1			
Carcinoma of cervix								1
Adenoma of Harderian gland							1	
Carcinoma of pancreas		1						
Papilloma			1	1				

* Number represents females in each group followed.
‡ Since some animals in each group had multiple tumors, the number of neoplasms exceeded the total in some instances

sis either at the level of viral RNA transcription or viral protein expression in inbred strains of mice (17, 23). However, it was important to determine if virus expression and mammary tumorigenesis were closely correlated in hybrid populations to test the validity of the single gene hypothesis for either mammary

tumorigenesis or virus expression. Our previous work indicated that direct immunologic measurements of virus expression did not appear to segregate as a single gene (14). The present study was designed to investigate this point specifically for mammary tumors through the second backcross generation. The analysis of mammary tumorigenesis in the F_1's, F_2's, and first backcross generations yielded results that were consistent with a dominant single gene determinant for mammary tumorigenesis in agreement with the earlier observations of Bentvelzen and van Nie et al. (10–12). However, our concern in initiating these studies, was that statistically, too few second backcross segregants had been analyzed. In our analysis of the second backcross generation the hypothesis for a single gene is not proven. Although we cannot exclude a negative genetic influence contribution from the C57BL genotype, we favor the view that mammary tumorigenesis in these crosses is not a single gene influence but are a threshold expression of multifactorial genetic inheritance from the parental phenotypes. Such inheritance patterns can be expected with threshold or quasi-continuous characters. Several examples of such phenomena in mammalian genetics are known (32–35).

The evidence from this and other studies suggests that the genesis of mammary tumors in such hybrids is an interplay between multiple positive and negative influences. Many of the positive influences appear to affect the expression of mammary tumor virus in early lactations and promote expression of high virus titers. Animals that display this particular phenotype for whatever reasons appear to have a greater potential for the development of mammary tumors. Negative genetic influences that may influence virus expression are largely undefined at the present time and may be exemplified by the regulatory genes present in the C57BL mouse that prevent expression, or the spontaneous 10-fold drop in constitutive virus expression noted in mammary cells in culture (36). Since both the GR and C57BL strains have comparable although not necessarily identical amounts of mammary tumor viral genes in their DNA (37), it would appear that the regulation of the expression of these genes is an important determinant in natural mammary tumorigenesis. The definition of genetic influences that regulate mammary tumor virus expression is an important future concern in the understanding of the regulation of murine carcinomas.

This rather genetic interpretation of mammary tumorigenesis does not adequately deal with what is commonly presumed to be a largely milk-transmitted disease. The milk influence, presumably a milk-transmitted mammary tumor virus into susceptible recipient newborn animals may also play a role in natural disease (38). In this context, the ability to lower or alter tumor incidence by foster nursing (39) suggests that the milk-transmitted virus is relevant although such experiments have not been repeated in the past 20 yr. Certainly the view that cancer is a delicately balanced interplay between positive and negative influences has been altered to a significant degree by geneticist's selection for particular tumor phenotypes. For example, high mammary tumor incidence strains where milk transmission is an important factor might not commonly exist in situations where natural selection is operative. The results of reciprocal cross analysis (Tables I, II, IV and Fig. 1) here suggests that in most natural

situations simple transmission from milk is by no means the only determinant of mammary tumorigenesis. In fact, it would appear that although multiple factors are involved in virus expression it is genetically-transmitted determinants that are primarily responsible for the development of mammary tumors.

There is no natural situation where mammary tumor virus is expressed at high levels and the population is not at risk for some mammary tumors. Nor is there a mouse population that has a mammary tumor incidence of greater than 20% that can be shown to be free of detectable mammary tumor virus expression. Thus, as shown herein there is a high degree of association on the level of individual animals, within inbred strains and at the level of the whole population between virus expression and propensity for the development of mammary tumors. It would appear however that environmental influences may play important roles in determining whether or not an animal expresses high levels of mammary tumor virus. For example, glucocorticoids have been shown to be an important determinant of mammary tumor virus expression in tissue culture (40) and similarly this and other hormones are likely to play important, although as yet largely undefined roles, in natural mammary tumorigenesis.

In view of the clearcut association between virus expression and mammary tumorigenesis it is perhaps surprising that we consider the possibility that mammary tumorigenesis is not the direct effect of a mammary tumor virus gene or genes. However this possibility should be considered in light of recent results from type C virus studies in AKR thymic leukemias (41). The virus currently regarded as the cause of this well-studied disease appears not to be the classic ecotropic AKR virus but the result of recombinational events between the AKR virus and endogenous xenotropic viruses in the AKR mice (41). Similarly in rats, another murine system, recombinational events have been clearly demonstrated which result in altered viral properties and the ability to transform cells in cell culture (42). As yet there is no direct evidence of a viral-encoded transformation gene nor evidence for recombinational events in mammary tumorigenesis. Nevertheless these possibilities are being pursued (24).

Summary

Mammary tumorigenesis in genetic crosses between the high mammary tumor incidence GR and the low incidence C57BL mouse strains is highly correlated with murine mammary tumor virus expression in milk. Although the F_1 and first backcross females had a mammary tumor incidence which was consistent with a single dominant gene segregation, the tumor incidence in the critical second backcross segregants disproved the single gene hypothesis. Genetic factors were clearly involved in regulation of virus expression which in turn correlated with both tumor incidence and tumor latency; these complex phenotypes are however best explained as threshold or quasicontinuous characters. As predicted from this model, the age specific incidence of mammary tumors showed a broad peak at 14–19 mo of age with no evidence of an early or late phase. Hematopoietic tumors showed no correlation with virus expression or mammary tumorigenesis suggesting different etiologies for these tumors.

References

1. Jackson Laboratory Staff. 1933. The existence of nonchromosomal influence in the incidence of mammary tumor in mice. *Science (Wash. D.C.).* 78:465.

2. Korteweg, R. 1934. Proefondervindelijke onderzoekingen aangaande erfelijkheid van Kanker. *Ned. Tijdschr. Geneeskd.* 78:240.

3. Bittner, J. J. 1937. Breast cancer and mother's milk. Relation of nursing to the theory of extra-chromosomal causation of breast cancer in mice – a preliminary report. *J. Hered.* 28:363.

4. Bittner, J. J., and R. A. Huseby. 1946. Relationship of the inbred susceptibility and inherited hormonal influence in the development of mammary cancer in mice. *Cancer Res.* 6:235.

5. Bittner, J. J. 1942. Observations on the genetic susceptibility for the development of mammary cancer in mice. *Cancer Res.* 2:540.

6. Heston, W. E., M. K. Deringer, and H. B. Andervont. 1945. Gene-milk agent relationship in mammary tumor development. *J. Natl. Cancer Inst.* 5:289.

7. Heston, W. E., M. K. Deringer, and T. B. Dunn. 1956. Further studies on the relationship between the genotype and the mammary tumor agent in mice. *J. Natl. Cancer Inst.* 16:1309.

8. Heston, W. E., G. Vlahakis, and M. K. Deringer. 1960. Delayed effect of genetic segregation on the transmission of the mammary tumor agent in mice. *J. Natl. Cancer Inst.* 24:721.

9. Mühlbock, O. 1965. A note on a new inbred mouse strain GR/A. *Eur. J. Cancer.* 1:123.

10. Bentvelzen, P. A. 1968. Genetical control of vertical transmission of the Muhlbock mammary tumor virus in the GR mouse strain. Hollandia, Baarn, Beukenlaan, Amsterdam. 76.

11. Bentvelzen, P. 1972. Hereditary infections with mammary tumor viruses in mice. *In* RNA Viruses and Host Genome in Oncogenesis. P. Emmelot and P. Bentvelzen, editors. North Holland Publishing Company, Amsterdam. 309.

12. Nie, R. van, J. Hilgers, and M. Lenselink. 1972. Genetical analysis of mammary tumor development and mammary tumor virus expression in the GR mouse strain. *In* Recherches Fondamentales sur les Tumeurs Mammaires. Ministère de la Santé Publique, Paris. 21.

13. Nandi, S., and C. Helmich. 1974. Transmission of the mammary tumor virus by the GR mouse strain. II. Genetic studies. *J. Natl. Cancer Inst.* 52:1567.

14. Heston, W. E., B. Smith, and W. P. Parks. 1976. Mouse mammary tumor virus in hybrids from strains C57BL and GR: breeding test of backcross segregants. *J. Exp. Med.* 144:1022.

15. Heston, W. E., G. Vlahakis, and G. H. Smith. 1972. Mammary gland tumors in substrains C57BL/M and C57BL/He Mice. *J. Natl. Cancer Inst.* 49:805.

16. Bentvelzen, P., J. Daams, P. Hageman, and J. Calafat. 1970. Genetic transmission of viruses that incite mammary tumors in mice. *Proc. Natl. Acad. Sci. U.S.A.* 67:377.

17. Varmus, H. E., N. Quintrell, E. Medeiros, J. Bishop, R. Nowinski, and N. Sarkar. 1973. Transcription of mouse mammary tumor virus genes in tissues from high and low tumor incidence strains. *J. Mol. Biol.* 79:663.

18. Blair, P. B. 1969. Detection by immunodiffusion of mouse mammary tumor virus in milk samples and correlation with tumor development. *Cancer Res.* 29: 745.

19. Charney, J., B. Pullinger, and D. H. Moore. 1969. Development of an infectivity assay for mouse mammary tumor virus. *J. Natl. Cancer Inst.* 43:1289.

20. Noon, M. C., R. G. Wolford, and W. P. Parks. 1975. Expression of mouse mammary tumor viral polypeptides in milks and tissues. *J. Immunol.* 115:653.

21. Nie, R. van, A. A. Verstraeten, and J. de Moes. 1977. Genetic transmission of mammary tumor virus by GR mice. *Int. J. Cancer*. 19:383.
22. Furth, J., R. Cole, and M. Boone. 1942. The effect of maternal influences upon spontaneous leukemia of mice. *Cancer Res*. 2:280.
23. Schlom, J., R. Michalides, D. Kufe, R. Hehlmann, S. Spiegelman, P. Bentvelzen, and P. Hageman. 1973. A comparative study of the biologic and molecular basis of murine mammary carcinoma: a model for human breast cancer. *J. Natl. Cancer Inst*. 51:541.
24. Drohan, W., R. Kettmann, D. D. Colcher, and J. Schlom. 1977. Isolation of the mouse mammary tumor virus sequences not transmitted as germinal provirus in C3H and RIII mouse strains. *J. Virol*. 21:986.
25. Peters, R. L., L. Rabstein, G. Spahn, R. Madison, and R. Huebner. 1972. Incidence of spontaneous neoplasms in breeding and retired breeder BALB/c GR mice throughout the natural life span. *Int. J. Cancer*. 10:273.
26. Dunn, T. B. 1959. Morphology of mammary tumor in mice. *In* The Physiopathology of Cancer. F. Homberger and W. H. Fishman, editors. Hoeber-Harper, New York. Chapter 3. 38–84.
27. Nie, R. van, and A. Dux. 1971. Biological and morphological characteristics of mammary tumors in GR mice. *J. Natl. Cancer Inst*. 46:885.
28. Pitelka, D. R., H. A. Bern, S. Nandi, and K. B. DeOme. 1964. On the significance of virus like particles in mammary tissues of C3Hf mice. *J. Natl. Cancer Inst*. 33:867.
29. Kirschbaum, A. 1949. Induction of mammary cancer with methylcholanthrene; histological similarity between carcinogen induced tumors and certain mammary neoplasms occurring spontaneously. *Cancer Res*. 9:93.
30. Dunn, T. B. 1954. Normal and pathological anatomy of the reticular tissue in laboratory mice. *J. Natl. Cancer Inst*. 14:1281.
31. Wright, S. 1934. The results of crosses between inbred strains of guinea pigs, differing in number of digits. *Genetics*. 19:537.
32. Russell, W. L. 1941. Inbred and hybrid animals and their value in research. Misinterpretation of differences between strains. *In* Biology of the Laboratory Mouse. G. D. Snell, editor. Blakiston Division of the McGraw-Hill Book Co., Inc., New York. 346.
33. Gruneberg, H. 1952. Genetical studies on the skeleton of the mouse. IV. Quasicontinuous variations. *J. Genet*. 51:95.
34. Green, E. L. 1951. The genetics of a difference in skeletal type between two inbred strains of mice (BALB/c and C57B). *Genetics*. 36:391.
35. Parks, W. P., E. Hubbell, R. Goldberg, F. O'Neill, and E. Scolnick. 1976. High frequency variation in mammary tumor virus expression in cell culture. *Cell*. 8:87.
36. Varmus, H. E., J. M. Bishop, R. C. Nowinski, and N. H. Sarker, 1972. Mammary tumor virus specific nucleotide sequences in mouse DNA. *Nat. New Biol*. 238:189.
37. Bittner, J. J. 1942. The milk-influence of breast tumors in mice. *Science (Wash. D.C.)*. 1:462.
38. Andervont, H. B. 1949. Studies of the disappearance of the mammary-tumor agent in mice of strains C3H and C1. *J. Natl. Cancer Inst*. 10:201.
39. Parks, W. P., E. M. Scolnick, and E. H. Kozikowski. 1974. Dexamethasone stimulation of murine mammary tumor virus: a possible tissue culture source of virus. *Science (Wash. D.C.)*. 184:158.
40. Hartley, J. W., N. Wolford, L. J. Old, and W. P. Rowe. 1977. A new class of murine leukemia virus associated with development of spontaneous lymphomas. *Proc. Natl. Acad. Sci. U.S.A.* 74:789.
41. Scolnick, E. M., R. J. Goldberg, and W. P. Parks. 1974. A biochemical and genetic analysis of mammalian RNA containing sarcoma viruses. *Cold Spring Harbor Symp. Quant. Biol*. 39:885.