Proceedings of the National Academy of Sciences
Vol. 67, No. 1, pp. 377–384, September 1970

Genetic Transmission of Viruses That Incite Mammary Tumor in Mice

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Presented at Symposium on Virus Genome Effects in Normal and Cancer Cells, April 27, 1970

Abstract. Electron microscopy, immunofluorescence, and bioassay demonstrated the presence of a mammary tumor inciting virus in untreated mice of three different inbred strains, and in irradiated or urethan-treated mice of two other mouse strains, indicating the ubiquitous nature of this group of viruses. In general these viruses are transmitted vertically by the gametes of the mouse strain in which they naturally occur. The virus is present in every cell, although often in an incomplete form. If a mammary tumor inciting virus is introduced into a different mouse strain, only milkborne transmission will take place, after which the virus is found in a limited number of tissues.

It has been speculated that mammary tumor inciting viruses are transmitted as genetic factors of the host strain to which they belong. There is some evidence that a repressor, produced by a regulator gene, controls the rate of release of such a genetically transferred virus. Repression can be abrogated by a carcinogenic treatment. The repressor would also cause resistance to a superinfecting mammary tumor inciting virus by interference with its replication.

The development of mouse mammary tumors usually results from the interaction between hormones, a suitable genetic constitution and viral agents.[1–4] The intention of this paper is to discuss only the interrelation between viral and genetic factors with particular reference to the possible genetic transmission of mouse mammary tumor inciting viruses.

It has been firmly established that the virion of the mouse mammary tumor virus (MTV) is the so-called B-type particle[5,6] as described by Bernhard.[7] We have no evidence that C-type particles,[7] associated with many neoplastic diseases in various animal species, can also induce mammary tumors. With the C particle viruses, MTV constitutes the group of the oncogenic RNA viruses (oncorna viruses).[8]

Distribution of MTV in the Mouse after Postnatal Infection. After infection MTV not only replicates in its target, the mammary gland, but in other organs as well[9,10] (see Table 1). In male mice the virus is found in accessory sex organs like the epididymis,[11] in which B particles are produced. We have no single indication that MTV induces tumors in these organs. B particles have

TABLE 1. *Distribution of MTV-S and expression of the various virus functions in postnatally infected mice.*

	Infectivity	MTV antigens	Intracytoplasmic A particles	B (virion)	Tumor
Erythrocytes	+	−	−	−	−
Lymphoid tissues	+	+	(+)	−	−
Epididymis	+	+	+	+	−
Mammary gland	+	+	+	+	+

also been found in chemically induced brain[12] and pulmonary tumors,[13] but it is not clear whether these particles represent MTV and whether they are etiologically involved in the genesis of these tumors.

We could confirm claims that an infectious form of the virus occurs in erythrocytes,[14] but the majority of blood-borne infectivity seems to be associated with leukocytes.[15] Infectivity has also been reported for bone marrow cells[16] and cellfree extracts from lymphomas of MTV-infected animals[17] which suggests that the hemopoietic stem cell is the primary site of MTV multiplication.

Electron microscopy did not reveal the presence of B particles in erythrocytes[14] or in spleen cells.[18] In some lymphoid cells, especially aged or neoplastic ones, intracytoplasmic A type particles are seen[19−21] which are thought to be the precursory form of the B particle.[21,22]

Immunofluorescence studies with polyvalent, highly absorbed rabbit anti-MTV sera, demonstrated virus specific antigens in the mammary gland, epididymis, spleen, and other hemopoietic tissues of infected mice,[18,23] but not in erythrocytes, liver, or brain.[23] In summary (see Table 1), it may be concluded that after infection MTV is present in several organs and that the epigenetic status of the cell strongly influences the different functions of the MTV genome.

Vertical Transmission of MTV-Strains. The mammary tumor virus is split up into several strains, which differ in virulence, tumor histology, and antigenic properties[24−29] (see Table 2). Host range does not play an important role in discriminating the various virus strains[30] as it does in mouse leukemia.[31]

TABLE 2. *Vertical transmission of strains of the mammary tumor virus (MTV).*

MTV strain	Characterization	Reference mouse strain	Modes of vertical transmission* Milk	Ovum	Sperm
S	Standard, virulent	C3H	+++	−	(+)
P	Induces plaques	GR	+++	+++	+++
L	Low oncogenic	C3Hf	(+)	++	++
O	Very low oncogenic	BALB/c	−	++	++
X	Radiation-induced	020	−	+	+
Y	Radiation-induced	C57BL	(+)	+	+

* − indicates no transmission, (+) incidental transmission, + always transmission, but no spontaneous manifestation, + + always transmission but not always manifestation, + + + always transmission and manifestation.

MTV-S: The standard strain (MTV-S) as discovered by Bittner and associates,[32,33] is vertically transmitted in its "natural" host, the C3H strain, by the milk[32] and occasionally by the sperm.[25,34,35] Eggborne transmission has never been observed.[36]

MTV-P: This virus strain, which induces typical lesions called plaques,[37] occurs in the GR strain[38] in which it is vertically transmitted not only via the milk but also by the sperm[25,30,38] and ovum.[39] If this virus is introduced into other mouse strains, it will be transmitted to the offspring by the milk only.[25,30] The transmission via the gametes in the GR strain is confined to MTV-P: MTV-S will not even be transmitted by the milk of GR females.[26] Male and eggborne transmission of MTV-P in the GR strain are controlled by a single dominant Mendelian factor.[24-26,30] It could be excluded that this gene causes male transmission by releasing large amounts of virus into the semen.[25,30] The two possible functions of this gene can be (α) causation of such an extreme susceptibility to MTV-P that the very small amounts of virus, contaminating the gametes, are still sufficient for a complete infection, or (β) the transfer of MTV-P as a genetical factor of the host. Objections against the first postulate are (1) susceptibility would be confined to MTV-P only, whereas alleles of this gene determine this for both MTV-S and MTV-P;[26,30] (2) when B particles are isolated from GR strain mammary tumors according to the technique of Calafat and Hageman[40] and the yields are estimated, smaller amounts seem to be present as compared with some other mouse strains,[41] the number of particles in a tumor being positively associated with susceptibility[42]; (3) some rabbit anti-sera to MTV detect a virus-specific antigen in GR livers and brains, while mice postnatally infected with MTV-P did not contain this antigen in the same organs.[23] Also infectivity has been found in cellfree extracts of these GR-strain organs.[43] It seems that MTV-P is present in every cell of GR mice although it needs not to function always normally: in the liver cells the ordinary MTV-antigens as found in the spleen are not present.[23]

MTV-L: The low-oncogenic variant of MTV (MTV-L) is transmitted by the germ cells in the C3Hf mouse strain.[44] Mature virions are found in C3Hf milk,[6] but seldomly milkborne infections have been observed.[44] The transmission of MTV-L by C3Hf gametes can certainly not be explained on the basis of a susceptibility gene, because after introduction of MTV-L into the more susceptible BALB/c strain,[26] only milkborne infection is found.[45] The C3Hf genome seems to be concerned with only the transmission of MTV-L. It fails to support such mode of transmission of MTV-S and MTV-P.[26] In crosses between C3Hf and resistant strains like O20 or C57BL no B particles are found in mammary tumors arising after strong hormonal stimulation.[4,25] These particles were found, however, in several tumors which arose in the F2 generation of these crosses.[26] This indicates that genes from the O20 and C57BL strains suppress the manifestation of MTV-L in the F1 hybrids with C3Hf.

MTV-O: The BALB/c strain has been said to be MTV-free,[44] but we recently found B particles in our BALB/cAnDeA line and also got immunological evidence for the presence of MTV in the germfree BALB/c colony of the Radiobiological Institute TNO. B particles have also been observed in mammary tumors arising in the BALB/cCrgl subline.[46] This overlooked MTV-strain (MTV-O) probably has an even lesser oncogenic potential than MTV-L. Its presence could not be demonstrated in BALB/c milk[28] which points to the gametes as vectors of transmission. It is important to note that MTV-L, -S,

and -P are only transmitted by the milk, if introduced into the BALB/c strain.[26,45] This demonstrates again the specificity of the host genome for transmission via the gametes of the virusstrain, which "naturally" occurs in a mouse strain.

MTV-X: We have examined electron microscopically many O20 strain mammary tumors, which were induced by extreme hormonal stimulation: no B particles were found in any of them.[47] Tumors induced in this strain by X-irradiation followed by the addition of urethan in the drinking water, contained a virulent MTV.[47] This virus is called MTV-X,[26,27] because it has been induced by X-irradiation and relatively little is known about its biological and antigenic properties.

It seems very likely that the same genes, which repress the manifestation of MTV-L in (C3Hf xO20)F1, also repress the manifestation of MTV-X in the O20 strain itself.

MTV-Y: The C57BL strain, which is highly resistant to the development of mammary tumors[4] and does not show the presence of an MTV, gets a relatively high tumor incidence after irradiation followed by an extreme hormonal stimulation.[48] There is some evidence that an acellular factor, presumably a virus, which induces mammary tumors becomes activated by irradiation.[48]

Rabbit anti-MTV sera do not react in the indirect immunofluorescence test with C57BL spleens and other hemopoietic tissues.[18,23] Radiation-induced C57BL lymphomas produce a weak but significant fluorescence, while lymphomas induced by X-rays followed by the addition of urethan to the drinking water give a very strong reaction with anti-MTV sera.[49]

MTV-specificity is proven by that AKR-strain lymphomas, which spontaneously developed under influence of the Gross leukemia virus, give no reaction with anti-MTV sera in the indirect immunofluorescence test. The C57BL strain MTV is tentatively called MTV-Y. However, electron microscopy failed to detect MTV virions in urethan-induced C57BL mammary tumors, but the finding of such particles in a chemically induced C57BL brain tumor[15] indicates the possibility that they are produced after carcinogenic treatments.

Ubiquity of MTV. Our results suggest that every mouse strain would carry an MTV, in other words that MTV would be ubiquitous. If this virus cannot be demonstrated in untreated animals, irradiation- or urethan-treatment makes it detectable. In most cases, these MTVs are vertically transmitted via the gametes in the mouse strain in which they naturally occur. If these viruses are introduced into other mouse strains, only milkborne transmission will take place. In two instances (MTV-L and MTV-P) we could exclude the possibility that susceptibility genes would be responsible for the gamete-borne transmission: host genes were directly concerned with this mode of transfer of the virus. The very intimate relationship between host genome and virus strain with regard to transmission,[26,30], suggests strongly that MTV-strains are transmitted as genetical factors of the mouse strain they belong to. The results with the O20 and C57BL strains demonstrate that other host factors control the release and subsequent manifestation of genetically transferred MTV.

Host Factors in Mammary Carcinogenesis. Much genetic work has been done

on host factors in the origin of mouse mammary tumors despite the complexity of the problem. The use of highly inbred mouse strains, having a different genetic origin, has been very helpful in this respect. In Table 3 are reported mammary tumor incidences for several inbred mouse strains in force-bred females, which received (α) no further treatment, or (β) 0.5 μg purified MTV-S[43] intraperitoneally at 4 weeks of age, or (γ) 0.05% urethan continuously in their drinking water beginning at the age of 2 months.

TABLE 3. *Parallels in susceptibility to "spontaneous," virus- and urethan-induced carcinogenesis of the mammary gland in mice.*

Mouse strain	Country of origin	Breeder	MTV unmasked	Mammary tumor incidences*: Spontaneous at 2 years	MTV-S at 1 year	Urethan at 1 year
GR	Switzerland	Grumbach	Yes	36 (100)	27 (100)	18 (100)
C3Hf	U.S.A.	Strong	Yes	30 (37)	21 (52)	32 (78)
BALB/c	U.S.A.	Bagg	Yes	20 (30)	50 (82)	30 (53)
MAS	Switzerland	Maier	No	55 (11)	23 (17)	22 (32)
O20	The Netherlands	Korteweg	No	27 (0)	38 (0)	14 (0)
TS	Germany	Schäfer	No	29 (0)	23 (0)	31 (10)
C57BL	U.S.A.	Little	No	32 (0)	45 (0)	24 (0)

* In parentheses tumor incidences; preceding parentheses the number of animals used.

Except for the GR all these strains are regarded as "low-mammary-cancer strains" because they do not get tumors before one year of age. This can be due either to the low oncogenic activity of the MTV strains they harbor or to the repression of a virulent MTV. Obviously the moderate tumor incidences at two years of age in the C3Hf and BALB/c strains are due to free MTV. Just as in the O20 and C57BL strains, we could not detect electronmicroscopically or immunologically the presence of MTV in MAS and TS mice. As can be observed in Table 3 there is a remarkable correlation in susceptibility to each mode of mammary tumor induction. This correlation is also found if other doses or modes of application of urethan or virus are used.[50] Only if very high doses of urethan are used strain differences are obscured because several tumors will then also develop in O20 and C57BL mice.[51] The observed correlation cannot be due to chance events, especially if the great genetic differences between the strains are taken into account. It signifies that the same system, which controls the degree of susceptibility to one mode of tumorigenesis, also determines the rate of tumor development after another carcinogenic treatment. It has been clearly established that this system operates at the level of the mammary gland itself.[52–54] The resistance of the C57BL strain to mammary tumor-induction by MTV-S is genetic in nature.[55,56] From the results presented in Table 4 the same can be concluded for the O20 strain as compared to the susceptible BALB/c strain. We may therefore conclude, that host genes control susceptibility to mammary carcinogenesis, irrespective of the mode of induction.

In the case of "spontaneous" tumorigenesis, the differences between the mouse strains can be explained to a great extent by host gene control of the release of MTV. Since these genes would also operate in susceptibility to viral carcinogenesis, it has to be postulated that the same gene-product, which pre-

TABLE 4. *Genetics of the difference in susceptibility to MTV-S between the O20 and BALB/c mouse strain.*

Strain or hybrid	Mammary tumor incidences at 1 yr with MTV-S dilutions*		
	10^{-3}	10^{-4}	10^{-5}
O20	38 (0)	16 (0)	10 (0)
BALB/c	30 (83)	50 (82)	27 (56)
(O20 × BALB/c)F1	45 (78)	25 (52)	38 (11)
O20 × (O20 × BALB/c)F1	69 (45)	96 (32)	19 (0)
BALB/c × (O20 × BALB/c)F1	29 (75)	60 (52)	18 (44)
(O20 × BALB/c)F2	135 (73)	158 (65)	51 (51)

* The stock inoculum is 0.5 mg of wet weight of purified virus. In parentheses tumor incidences, preceding parentheses the number of animals used.

vents the release of MTV in O20, C57BL, TS, and MAS mice, also causes resistance to superinfecting virus. In the O20 and C57BL strains it could be demonstrated that resistance to MTV-S is associated with a poor replication of virus,[57–59] which would suggest that the repressing substance interferes with the replication of superinfecting MTV. In a Mendelian analysis we acquired evidence that the strong resistance of O20 and C57BL and the moderate resistance of C3Hf to MTV-S as compared with the BALB/c strain is controlled by genes, which are located in the same linkage group as the GR-strain gene determining the transmission of MTV-P,[26,30] suggesting once more that similar genetic mechanisms are operating in the release of MTV and resistance to superinfection.

The correlation in susceptibility to spontaneous and urethan-carcinogenesis can also be explained on a virologic basis. The activation of an MTV by urethan as observed in the O20 and C57BL mouse strains can be due to abrogation of the repressing system, which prevents the release of virus. The strain differences in susceptibility to urethan-carcinogenesis could reflect the variation in the ease with which the repressing system can be abrogated. It cannot be excluded, however, that interferon-depression[60] and immune impairment[61] by urethan also play a role.

Provirus Theory. As the most economical explanation for all our data on transmission we repeatedly advocated the hypothesis that a DNA copy of the viral RNA is integrated into the host genome.[24,26,30] Since the MTV genome seems to consist of three RNA molecules,[62,63] it seems feasible that three separate "proviral chromosomes" are part of the host genome. Transcription of the provirus(es) would be controlled by a regulator gene, which produces a repressor that also can interfere with the replication of superinfecting viruses. In strains like C3Hf and BALB/c the regulator gene would be mutated in such a way that suboptimal amounts of repressor are produced, which causes the spontaneous release of their own MTV and susceptibility to superinfecting MTV's. In hybrids with strains like O20 and C57BL, which would have a wildtype regulator gene, sufficient repressor is released to prevent the manifestation of MTV-L and -O. These hybrids are also much less susceptible to superinfecting MTV-S (Table 5).

O20 or C57BL strain genes cannot repress in hybrids with GR the release of

TABLE 5. *Susceptibility of some inbred mouse strains and their F1-hybrids to MTV-S.*

Strain or hybrid	Mammary tumor incidences at 1 year with MTS-V dilutions* 10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
BALB/c	10 (90)	30 (83)	50 (82)	27 (56)	22 (45)
C3Hf	20 (80)	16 (50)	21 (52)	13 (23)	31 (0)
O20	18 (28)	38 (0)	16 (0)	10 (0)	7 (0)
C57BL	15 (0)	29 (0)	17 (0)	N.T.	N.T.
(C3Hf × BALB/c)F1	14 (86)	25 (64)	15 (93)	13 (62)	10 (20)
(O20 × BALB/c)F1	20 (60)	45 (78)	25 (52)	38 (11)	43 (0)
(C57BL × BALB/c)F1	10 (50)	32 (56)	37 (35)	11 (18)	19 (0)
(C3Hf × O20)F1	31 (48)	24 (54)	25 (4)	36 (0)	22 (0)
(C57BL × C3Hf)F1	13 (69)	20 (60)	19 (11)	36 (3)	15 (0)

* The stock inoculum is 0.5 mg of wet weight of purified virus. In parentheses tumor incidences, preceding parentheses the number of animals used. N.T. = not tested.

MTV-P. Since the release seems not to be affected by the presence of a repressor, we assumed the GR to have a mutation in the corresponding operator gene. This corresponds with our observation, that in GR mice MTV-S fails to replicate sufficiently that it could be detected in our test system,[29] indicating the presence of the repressing factor, in the GR mouse strain.

This provirus theory has to be tested by DNA-RNA hybridization experiments, which are still very difficult in the MTV field. Also linkage studies on the fine structure of the provirus can be very helpful. We have some preliminary observations that the virulence-marker of MTV-X, being present in the O20 strain can recombine with the C3Hf-strain regulatory gene, which allows the release of the avirulent MTV-L, resulting in the spontaneous release of a virulent MTV.

For the acquisition of the new data presented in this paper the valuable technical assistance is acknowledged of Jan Brinkhof, Rochelle van Essen-Griffin, Dolf Aarssen, Annemarie Amsen, Jaap Arends, Toos Schrijvers-Zonjee, Margreet Luning, Liedeke Kleine, Henk Lobbrecht, and Harriette Eppenga.

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