An Inherited Murine Serum Trace Component Related to Mammary Cancer[1] (35500)

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An interesting protein occurs in minute amounts in mouse serum (1, 2). The concentration of this serum protein is so low that it could not be detected as a separate component by electrophoretic procedures (disk electrophoresis), although it has been ascertained to migrate as an α-globulin. Only immunologic techniques are capable of demonstrating its presence. This serum protein exhibits characteristic occurence patterns (3).

It is absent in all male mice and manifests itself in the serum of most *breeding* female mice. Its presence in *virgin* female mice is markedly strain-dependent and parallels the prevalence of spontaneous mammary tumors in at least 11 mouse strains. Because of this circumstantial relationship to the spontaneous mammary tumor (SMT) in mice and because of its antigenic properties, when injected into rabbits, this trace serum protein has been termed SMT-antigen. There are no indications that it is a viral antigen or that it is related to the mammary tumor virus.

The formation of SMT-antigen has been shown to be governed by a pituitary hormone (4). The latter is not identical with any of the known pituitary factors including prolactin. It was assumed that this pituitary hormone does not normally occur in male mice, and that it is also absent in newborn and in virgin female mice of those strains which are not susceptible to mammary tumors. In female animals of these strains, the pituitary hormone and, consequently, the serum trace protein make their earliest appearance during the first pregnancy and continue to be present thereafter. In female mice which are susceptible to spontaneous mammary tumors, however, the new pituitary hormone and the serum trace protein are present at birth.

When SMT-antigen was determined in the serum of virgin females of commercially available F_1 hybrid mice (CAF_1/J[3] derived from A/J males and BALB/cJ females), 14 out of the 120 animals tested, or 11.7%, were found to exhibit a positive reaction.[4] Since the CAF_1/J hybrids are derived from females of an inbred strain in which virgin females are consistently free of serum SMT-antigen (3, 4), it was suspected that the occurrence of this serum protein in virgin female CAF_1/J hybrids originates through genetic transmittal by the males, *i.e.*, through A/J mice, a strain in which 37% of the virgin females are known to be carriers of SMT-antigen (3).

Further studies have shown that the presence or absence of SMT-antigen in the serum of *virgin* mice is a genetically determined characteristic, transmitted by either sex, as described in the present report.

Methods. Two inbred strains of mice were used for crossbreeding. These were C3H/HeJ and BALB/cJ mice.[3] Previous studies (3) had shown that 90% of the virgin female C3H/HeJ and none of the BALB/cJ virgin females exhibited positive serum SMT-antigen reactions. Reciprocal crosses were made between these two strains of mice and F_1 mice were produced. These F_1 mice were then crossed to F_1 littermates to produce F_2 offspring, and to C3H/HeJ and BALB/cJ inbred mice to produce backcross offspring. Two females were mated with each male. Every female was first tested for the serum SMT-antigen before being mated. Mice were

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[4] Unpublished data.

TABLE I. Serum SMT-Antigen in the Offspring of Reciprocal Crosses from Two Inbred Lines of Mice.

Line no.	Parents used for crosses				Offspring		
	Males	Females	Serum SMT-antigen of mother[a]	Total no. of breeding females	Types of generation	Total no. of litters	Serum SMT-antigen in virgin female offspring[b]
1	C3H/He	BALB/c	—	17	F_1	29	59/72 (82)
2	BALB/c	C3H/He	+	4	F_1	7	16/19 (84)
3	BALB/c	C3H/He	—	1	F_1	2	4/6 (67)
4	F_1[c]	F_1[c]	+	20	F_2	60	96/200 (48)
5	C3H/He	F_1[c]	+	4		8	20/29 (69)
6	C3H/He	F_1[c]	—	2		2	0/3 (0)[d]
7	BALB/c	F_1[c]	+	4		8	5/14 (36)
8	BALB/c	F_1[c]	—	3		4	3/10 (30)
9	F_1[c]	C3H/He	+	6		8	16/19 (84)
10	F_1[c]	BALB/c	—	9		10	7/23 (30)
11	C3H/He	C3H/He	—[e]	—[e]		—[e]	110/124[f] (89)
12	BALB/c	BALB/c	—[e]	—[e]		—[e]	0/147[f] (0)

[a] Before mating.

[b] Number of animals with a positive reaction over total number of female offspring tested; percentage of animals with positive reaction is given in parentheses.

[c] (C3H/He ♂ × BALB/c ♀) F_1; see line 1.

[d] Not significant.

[e] Information not available; the offspring tested was purchased from The Jackson Laboratory, Bar Harbor, Maine.

[f] Data from previous studies (3, 4).

weaned between 21 and 25 days after birth and, at the same time, males were separated from females.

Bleeding. All females to be used for breeding purposes, all surviving female offspring, and some of the male progeny were bled by heart puncture, as previously described (4). Tests for SMT-antigen were initiated within 24 hr after bleeding, performed in the offspring between the ages of 5 and 8 weeks.

SMT-antigen determinations. The presence or absence of serum SMT-antigen was determined in individual mice by means of a specific antiserum against the murine protein, prepared in rabbits, as previously described (2–5). Quantities of 0.01 ml of undiluted, or of twofold and fourfold diluted, fresh mouse serum were used for the immunodiffusion tests, performed according to Ouchterlony (6, 7), as specified previously (4). An animal was considered to exhibit a positive reaction when a precipitation line could be detected at either one or more of these three serum dilutions. When all three mouse serum dilutions failed to produce a positive reaction within 72 hr, the animal was considered free of SMT-antigen. In some animals, the test was repeated on a new serum sample, the blood for which was drawn no earlier than 2 months after the first bleeding. With this interval between the two bleedings, no discrepancies were found between the results of the two serum samples from any one mouse.

Foster nursing of newborn inbred mice from C3H/HeJ parents by BALB/cJ mothers and of newborn inbred BALB/cJ mice by C3H/HeJ mothers was carried out as follows. The natural mothers were removed from their litters within hours after birth and before the first feeding of the newborn mice, as evidenced by the absence of milk in their stomach (the presence of milk in the stomach of newborn mice is visible through stomach lining and skin). About half of the bedding directly underneath the newborn mice was taken off with a scoop and replaced by bedding from the corresponding site of a lit-

ter from the other strain of mice. Finally, the corresponding mothers were exchanged. All manipulations were performed without touching the newborn animals. Acceptance of the exchanged offspring by the foster mothers was not universal. When there was a difference of more than a few hours in the ages of the two litters, the mothers of which were interchanged, only the progeny of the younger litter was used for the study. The fosternursed animals were weaned and their SMT-antigen was determined as described above.

Results. The results of the breeding experiments are shown in Table I. Although only females exhibit the serum SMT-antigen, both reciprocal crosses produced serum-positive females with nearly equal frequencies (lines 1 and 2). It is thus evident that both males and females of the C3H/HeJ strain carry the hereditary factor for the serum trace protein (to occur in virgin females), and that this hereditary factor is dominant.

Since the reciprocal crosses produced nearly equal frequencies of females with serum SMT-antigen, any possibility of a cytoplasmic factor may be ruled out. Evidently, the trait is not sex-linked because the F_1 mice used for backcrosses originated exclusively from SMT-antigen-negative mothers (BALB/cJ), and because every type of backcross to BALB/cJ parents produced certain proportions of SMT-antigen-positive females.

The effect of the gene is not expressed in males, and representative samples (10 animals each) of males from F_1 hybrids (C3H/He ♂ × BALB/c ♀) and F_2 hybrids were found, as expected, to be all negative.

In virgin females, the expression of the effect of the gene is not complete, either in the inbred strain (3) (see also line 11) or in the F_1 offspring (lines 1, 2, and 3). The penetrance is, thus, incomplete and was estimated from the data in lines 1, 2, 3, and 11 to be approximately 86%. The percentage of serum SMT-antigen-positive females, produced by serum-positive F_1 hybrid females was approximately the same as that produced by serum-negative hybrid females, when they were mated to BALB/cJ males (lines 7 and 8).

Since reciprocal crosses and the phenotypes of the F_1 females showed no effect upon the frequencies of the SMT-antigen-positive females among the offspring, the data on progenies of backcrosses involving the same inbred strains were pooled in Table II. Expected frequencies of the SMT-antigen-positive and negative females, based on the hypothesis of a simple dominance with 86% penetrance, were then computed as shown in Table II.[5] The observed data exhibit a significant deficiency of SMT-antigen-positive females in the F_2 generation (first line, Table II). If it is assumed that there is a pair of modifier genes that prevents the expression of the major SMT-antigen genes when present in a homozygous recessive condition and that these modifier genes by themselves have no effect upon the presence or absence of the SMT-antigen, the experimental data fit nearly perfectly the theoretical values, as shown in Table II. These modifiers, then, must be assumed to have been introduced through the SMT-antigen-negative strain, BALB/cJ. Accordingly, a symbol, Smt, is proposed for the

[5] The expected frequencies were calculated in accordance with the fundamental laws of Mendelian inheritance by use of the formulas $E_p = N \times A \times B \times P$ and $E_n = N - E_p$ where E_p and E_n are the expected numbers of positive and negative animals, respectively; N is the total number of animals investigated; A is the expected proportion of positives and negatives if the trait were a simple dominant condition ($A = 75\%$ or 0.75 when both parents are F_1-hybrids from an SMT-antigen-positive and an SMT-antigen-negative inbred strain, *e.g.*, first line of Table II; $A = 50\%$ or 0.50 when one parent is such an F_1-hybrid and the other parent is from an SMT-antigen-negative inbred strain, *e.g.*, BALB/c, line 2 of Table II; $A = 100\%$ or 1 when one parent is an F_1 hybrid and the other parent is from an SMT-antigen-positive strain, *e.g.*, C3H/He, line 3 of Table II); B is a factor introduced to take into account the effect of modifier genes; this factor B is numerically equal to A assuming that the modifiers act like dominant genes ($B = A$), except where the existence of the modifier genes was not taken into consideration, in which case $B = 1$, *e.g.*, in the columns of Table II headed "Expected if the trait were a simple dominant condition with 86% penetrance"; P is the penetrance estimated to be 86% or 0.86 according to the data in lines 1, 2, 3, and 11 of Table I.

TABLE II. Expected Distribution of SMT-Antigen-Positive and -Negative F_2 Hybrid and Backcross Offspring under Two Different Hypotheses.

	SMT-antigen in virgin female offspring[a]							
	Observed		Expected if the trait were a simple dominant condition with 86% penetrance			Expected if a pair of modifiers is involved with no change in other assumptions		
Crosses	Pos.	Neg.	Pos.	Neg.	χ^2	Pos.	Neg.	χ^2
F_1[b] × F_1[b]	96[c]	104[c]	129	71	23.8[d]	96.8	103.2	0.013[e]
BALB/c × F_1[b]	15[f]	32[f]	20.2	26.8	1.93[g]	10.1	36.9	2.46[g]
C3H/He × F_1[b]	36[h]	12[h]	41.3	6.7	3.95[i]	41.3	6.7	3.95[i]

[a] Number of mice with positive or negative serum reactions, see also footnote 5 in text.

[b] (C3H/HeJ ♂ × BALB/cJ ♀) F_1.

[c] Values from line 4 of Table I (F_2 generation).

[d] Expected and observed values are not compatible at the 1% level of significance.

[e] Expected and observed values are almost the same.

[f] Values combined from lines 7, 8, and 10 of Table I.

[g] Expected and observed values are compatible at the 5% level of significance or better; Yates correction term was applied.

[h] Values combined from lines 5 and 9 of Table I.

[i] Expected and observed values are compatible at slightly less than the 5% level of significance; Yates correction term was applied.

major SMT-antigen gene. Under this hypothesis the genotypes of the C3H/HeJ and BALB/cJ mice may be written as SmtSmt MM and smtsmt mm, respectively, if M and m are used to indicate the modifiers. However, it must be pointed out that the concept of a single pair of modifier genes is tentative.

That the transmittal of serum SMT-antigen in virgin females is actually due to a genetic and not to an infectious factor was documented by the following experiment. When five BALB/cJ female mice were first mated to C3H/HeJ males, 20 out of 24 of the resulting female F_1 hybrid offspring from a total of eight litters were serum-positive. If the males would have passed on an infectious agent to their breeding females, subsequent mating of the latter with BALB/cJ males should produce serum-positive female offspring. This was not the case, however, and all of the 18 inbred female offspring, obtained subsequently in a total of six litters from BALB/cJ sires were devoid of serum SMT-antigen.

In addition, transmittal of serum SMT-antigen to the progeny is unrelated to the milk-transmitted mammary tumor virus. This was demonstrated by the finding that foster nursing of newborn BALB/cJ mice by C3H/HeJ mothers did not induce the serum trace protein in the nurslings (see Table III). Conversely, foster nursing of C3H/HeJ newborn mice by BALB/cJ mothers did not prevent the appearance of SMT-antigen in the animals of this strain.

There was no relationship between the presence or absence of SMT-antigen in serum of virgin females and coat color genes, c and

TABLE III. Effect of Foster Nursing on Serum SMT-Antigen in Female Offspring.

True parents			
♂	♀	Foster mother	Serum SMT-antigen in female offspring[a]
BALB/c	BALB/c	C3H/He	0/16[b]
C3H/He	C3H/He	BALB/c	6/6[c]

[a] Number of mice with a positive reaction over total number of female offspring tested.

[b] In a total of six different litters from six different breeding pairs.

[c] In two different litters from two different breeding pairs.

b, segregating in the F_2 generation.

Discussion. There can be no doubt that the occurrence of serum SMT-antigen in virgin females is due to dominant autosomal genes which are carried by both males and females; their effect is not expressed, however, in the males. Serum-negative females, derived from serum-positive ancestry, may also convey the trace protein to their offspring.

In the interpretation of the data, it was assumed that the penetrance of the Smt gene is not complete. It is obvious, however, that a possibility of variable expressivity of the gene cannot be ruled out. Especially, since the protein is present in the serum in minute quantities, the presence of even lower quantities might simply escape detection by the available technique.

It should be emphasized again that the findings reported here pertain only to the occurrence of SMT-antigen in *virgin* female animals, and that the appearance of this trace protein in nearly all breeding female mice must be attributed to an entirely unrelated mechanism which is presumably not subject to genetic limitations.

Because of the close relationship between serum SMT-antigen and a certain pituitary hormone (4), it appears possible that the genetic conditions, assumed above to exist for this serum protein, actually pertain to the new pituitary hormone.

Earlier studies (3) have established an incidental relationship between the occurrence of serum SMT-antigen in virgin female mice and the tendency of these animals to develop spontaneous mammary tumors. It is a well-known fact that four main causative agents are responsible for the formation of this type of tumor in mice, namely hormonal, genetic, viral, and environmental factors, as summarized by Mühlbock (8). It appears quite likely that the genetic control of the occurrence of serum SMT-antigen in virgin female mice or of the new pituitary hormone is related to, or identical with, the genetic factor presumably involved in the causation of the murine tumor. The existence in mice of a genetic factor was most recently reported (9) which is passed equally well by either parent, is prominently involved in mammary tumorigenesis and is of influence on tumor incidence.

Summary. SMT-Antigen, a mouse serum trace protein, occurring in virgin female animals which are susceptible to mammary cancer, was found to be transmitted by a dominant autosomal gene. A symbol, Smt, is proposed for this gene. The expression of the gene is limited to females. Its penetrance is not complete and is estimated to be approximately 86%. It was necessary to assume the presence of a pair of modifier genes, preventing the expression of the effect of the major SMT-antigen genes when present in a recessive homozygous condition. These modifier genes are assumed to have no effect by themselves upon the presence or absence of the SMT-antigen. This serum trace component, or the pituitary hormone involved in its formation, is likely to represent the genetic factor involved in the formation of spontaneous mammary tumors in mice.

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