ABSTRACT

Antigen and tumor incidences in BALB/c and C57BL mice after living as weanlings for 5 weeks in cages with mouse mammary tumor virus-infected females were compared with control BALB/c and C57BL mice living in the same laboratory. All mice were bred continuously, and third-lactation milks were tested for mouse mammary tumor virus antigen by Ouchterlony microimmunodiffusion test. Mammary tumor incidences in the cagemates were not significantly different from those in the controls, although the antigen incidences were significantly greater. However, phosphate-buffered salt solution (0.02 M phosphate, pH 7.4; 0.15 M NaCl; and 0.1% bovine serum albumin) and sham-inoculated mice also had elevated antigen incidences. Repeat tests of milks at the fourth or fifth lactations indicated that more than 50% of those positive at the third became negative at later lactations.

INTRODUCTION

The question of whether or not breast cancer in mice is a communicable disease has already received considerable attention. Borrel (5, 6), who investigated a mouse breeding establishment, believed that cage contact was an important factor in the high incidence of mammary tumors observed in some cages but not in others. Parasite vectors were postulated by Borrel, as well as by others (8-10, 12). Fleas have been strongly implicated as a possible transmission vector for MuMTV (17). Blair et al. (3) and Blair and Lane (2) have presented evidence for airborne transmission of mouse-spleen cell-sensitizing factor(s).

During the past 5 years, we have carried out experiments on possible cagemate transmission of factors associated with MuMTV infection, the results of which are reported here.

MATERIALS AND METHODS

Mice. High-mammary-tumor females of strains RIII, GR, C3H, and BALB/cfC3H that had cast 2 litters and whose milks were positive for MuMTV were used as virus carriers. C57BL weanling females which are highly susceptible to RIII and GR viruses (8, 13, 14) and BALB/c weanling females which are highly susceptible to the C3H virus carried in C3H and the BALB/cfC3H subline (13, 14) were used as experimental recipients for possible cross-infections.

Experimental Method. RIII or GR females that had cast and nursed 2 litters and then had been isolated for 4 weeks were placed in a 7 x 10-inch stainless steel cages with weanling C57BL females 3 to 5 weeks of age. After 5 weeks the C57BL females were transferred to cages with C57BL males and force bred [newborn litters sacrificed (1, 4)]. After the third litter their milks were tested for the presence of MuMTV antigen by standard procedures; a microimmunodiffusion method was used (7). In addition all mice were observed for 2 years for mammary tumors. Similarly, BALB/c weanlings were kept in cages with BALB/cfC3H or C3H mothers for a period of 5 weeks and then bred and tested in the same manner. Previously, it was shown that age 2 to 10 weeks is the most susceptible age of C57BL mice for RIII-MuMTV infection (14).

The control mice consisted mainly of the foundation stocks of the C57BL and BALB/c maintained in our laboratory and various experimental animals listed in Table 2. All mice, except the foundation stocks, were in the same room. Some of the mice of the foundation stocks were not force bred because litters were saved whenever mice were needed for experiments. However, the data in Table 1, "Antigen Incidence, Control" columns, include tests on many mice (about one-third of the total) that were force bred. It appears that force-breeding C57BL or BALB/c mice did not cause the antigen secretion.

RESULTS

The results are summarized in Tables 1 and 2. The first 2 experiments (Table 1, Lines 1 and 2), started in 1973, indicated an elevated MuMTV antigen incidence in third-lactation milks of C57BL and BALB/c cagemates of RIII mice over that found for the controls (see Table 2). The mammary tumor incidences, however, were not significantly different from the controls.

In 1975 these experiments were repeated and expanded to include B57BL weanlings living for 5 weeks with GR multiparous females and BALB/c weanlings living with C3H multiparous females. In every case the incidence of MuMTV antigen expression in the experimental animals was more than 10 times greater than that in the controls, although the incidence of mammary tumors was not significantly higher and the tumours always occurred very late in life.

In the 1973 experiments the tumor incidence in control and experimental BALB/c mice (Table 1, Line 2) was greater than in the 1975 experiments (Table 1, Lines 5 and 6). This difference occurred because 2 substrains of BALB/c were used. In 1973 we had 2 sublines of BALB/c, both derived...
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Table 1

Table 2

Summation data on MuMTV antigen incidences in milks of C57BL and BALB/c mice after various treatments

<table>
<thead>
<tr>
<th>Description of mice</th>
<th>No. of experiments</th>
<th>3rd lactation</th>
<th>4th or 5th lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL Foundation stock</td>
<td>13</td>
<td>4/333 (1.2)</td>
<td>3/128 (2.3)</td>
</tr>
<tr>
<td>PBS-inoculated</td>
<td>2</td>
<td>17/145 (11.7)</td>
<td>0/25 (0)</td>
</tr>
<tr>
<td>Sham-inoculated (needle only)</td>
<td>3</td>
<td>5/31 (16.1)</td>
<td>0/25 (0)</td>
</tr>
<tr>
<td>Cagemates (from Table 1)</td>
<td>3</td>
<td>27/131 (20.6)</td>
<td>8/98 (8.2)</td>
</tr>
</tbody>
</table>

| BALB/c Foundation stock | 13                 | 2/103 (1.9)   | 8/92 (8.7)          |
| PBS-inoculated         | 9                  | 21/105 (20%)  | 1/49 (2.1)          |
| Experimental b         | 4                  | 4/9/49 (18.4) | 1/49 (2.1)          |
| Cagemates (from Table 1) | 3         | 34/127 (26.7) | 11/100 (11)         |

a Numbers in parentheses, percentage.

b Inoculated with C57BL skim milk (at dilutions of 10^{-1}, 10^{-3}, and 10^{-5}).

From the breeding pair we received from the Cancer Research Genetic Laboratory, Berkeley, Calif., in 1965. The subline that constituted most of our mice in 1973 had a mammary tumor incidence of 27% at a mean tumor age of 20.2 months (13). However, third-lactation milks always tested negative for MuMTV antigen and electron microscopic examination of tumors was negative for B particles. Since we wanted to maintain our BALB/c stock as a low-tumor strain, we expanded the subline that appeared to be tumor-free, and this subline was used for the other 2 experiments which were started in 1975 (data reported in Table 1, Lines 5 and 6). The number of dead controls in the foundation stock of this subline is not yet very large, but the tumor incidence in the stock is very much lower than the subline that has now been discontinued. The expression of MuMTV antigen in the foundation stock of both sublines has always been very low (1 to 2%).

Pertinent to the data of Table 1 are extensive unpublished data on antigen and tumor incidences in C57BL and BALB/c mice inoculated i.p. with PBS, which is the fluid used as a diluent for milk in infectivity titrations. In the period 1973 to 1976, in 29 tests involving 145 C57BL and 105 BALB/c mice, 0.1 ml PBS inoculated i.p. at 3 to 5 weeks of age resulted in 11.7 and 20% expression, respectively, of MuMTV antigen in third-lactation milks (Table 2).

In 2 experiments i.p. insertion of a sterile needle with no inoculum into C57BL weanlings resulted in a MuMTV antigen incidence in third-lactation milks of 2 of 16 (12.5%) and 3 of 15 (20%), giving a total of 5 of 31 (16.1%) as shown in Table 2. All of these positive readings turned negative at later lactations, and no tumors developed by 24 months of age. Five positive milks were tested by Dr. A. Vaidya of this laboratory for the presence of hybridizable MuMTV RNA, and none was found.

Additionally, 49 BALB/c mice were inoculated with foundation stock C57BL milk, which is believed to be MuMTV-free. These inoculations resulted in an antigen incidence of 8.4% in third- and 2.1% in fourth-lactation milks (Table 2).
Since the cagemate experiments were completed, a radioimmune assay for MuMTV glycoprotein with a molecular weight of 52,000, a glycoprotein of the virion coat, was developed (19) and detection of this antigen in milks was compared with our standard immunodiffusion assay. Twelve samples of third-lactation milk from PBS-inoculated C57BL mice that were found to be positive by immunodiffusion were tested by radioimmune assay and found to be strongly positive. Many (>160) milks, both positive and negative by immunodiffusion, have been checked by radioimmune assay and agreement between the 2 types of measurements was excellent. Therefore, we believe that the high MuMTV antigen incidences in experimental cagemates were not spurious but represent a somewhat ephemeral expression of MuMTV antigen.

The cagemate experimental mice reported in Table 1 more closely resemble the foundation stock mice in that they were not given injections; however, the incidence of MuMTV antigen expression in third-lactation milks was higher, although probably not significantly higher, than in any of the other groups reported in Table 2.

DISCUSSION

Our conclusion from these experiments is that cagemate exposure of young mice to MuMTV-infected multiparous females did not affect the mammary carcinoma incidence in the exposed animals; however, there was an increase in the incidence of viral antigen secretion in their third-lactation milks.

Correlation between antigen expression and tumor incidence in C57BL and BALB/c mice when they were inoculated i.p. with virus-containing milk has usually been good (7, 13, 15), although in the C57BL mice the antigen incidence has always been somewhat higher than the tumor incidence (7, 13, 14).

Blair et al. (3) found that spleen cells from BALB/c females over 14 weeks of age were reactive against primary mammary tumor cells of the syngeneic BALB/cfC3H subline in a microcytotoxicity assay. Later Blair and Lane (2) reported that, in contrast to the laboratory-raised mice, spleen cells of the BALB/c mice raised in isolation away from the laboratory were not reactive. Lopez et al. (11) reported cellular immunity to MuMTV-specific antigen at a certain age in mice of their BALB/c colony.

There is the possibility that the antigen expression does not represent a viral infection but results from MuMTV exposure or from some kind of stress (18) or other factor(s) that turns on certain genes of the endogenous MuMTV genome that exists in all cells of the species Mus musculus (16, 20); this causes some synthesis of MuMTV antigen but not of MuMTV RNA. This antigen synthesis is unstable and in later life usually disappears. Varmus et al. (20) showed that lactating mammary gland tissues of C57BL and BALB/c contained a number of MuMTV RNA genome equivalents per cell. These do not normally result in the secretion of virus or the development of mammary tumors, but it is conceivable that under certain conditions a viral component could be synthesized.

The results of these experiments were not originally anticipated, but now we recognize that it is necessary to try to elucidate the factors that arouse MuMTV antigen secretion in C57BL and BALB/c mice housed with a different strain. The arousal may not depend on the fact that the cagemates are carriers of MuMTV but on their strangeness as detected possibly through scent.

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REFERENCES

Observations on the Question of Horizontal Transmission of Mouse Mammary Tumor Virus

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