Cellular Immunity to Tumor-associated Antigens of Transplantable Mammary Tumors of C3H/HeN Mice

Aldo Tagliabue,1 Ronald B. Herberman, Larry O. Arthur, and James L. McCoy

Laboratory of Immunodiagnosis, National Cancer Institute, NIH, Bethesda, Maryland 20014 [A. T., R. B. H., J. L. M.], and Viral Oncology Programs, National Cancer Institute, Frederick Cancer Research Center, Frederick, Maryland 21701 [L. O. A.]

ABSTRACT

Cellular immunity to tumor-associated antigens of C3H/HeN-MTV+ (hereafter called C3H+) and C3H/HeN-MTV− (hereafter called C3H−) mice bearing syngeneic transplantable mammary tumors was assessed by the production of migration inhibition factor in response to mouse mammary tumor virus (MTV)-related antigens or 3 M KCl tumor extracts, by the use of an indirect agarose microdroplet migration inhibition assay. C3H+ mice bearing a transplantable syngeneic mammary tumor (MAT-2) were generally unable to produce migration inhibition factor in response to MTV, whereas a strong immune response in those mice was elicited by a 3 M KCl extract of MAT-2 tumor. C3H−-tumor-bearing mice were reactive to MTV as well as to 3 M KCl extract. The latter reactivity was found to be specific since these mice failed to react against similarly prepared 3 M KCl extracts of various normal or chemically induced tumor tissues. Two other spontaneously arising mammary tumors (MAT-3 and MAT-4) of C3H+ mice appeared to share an antigen with MAT-2. Furthermore, a 3 M KCl extract of C3H+ embryos, with no detectable MTV antigens, was found to stimulate migration inhibition reactivity in C3H+ and C3H− mice bearing MAT-2 tumor, which suggests that tumor growth induces reactivity against non-virus-related tumor-associated antigens, which are common to embryo cells. Induction of migration inhibition factor production by MAT-2 tumor-associated antigens or MTV appeared to be dependent on T-cells, since reactivity was eliminated by pretreatment of immune spleen cells with anti-Thy plus complement.

INTRODUCTION

The concept that new antigens are expressed by tumor cells is now widely accepted. These antigens have been defined as TAA,2 and it has been shown that, in general, chemically induced tumors express individually distinct TAA (23), whereas tumors induced by oncogenic viruses have common TAA (9). However, in vivo (29) and in vitro (12, 26) studies suggested that tumors induced by MTV may express either common TAA or individually distinct TAA. The latter antigens do not seem to be directly related to MTV.

1 On leave from Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy. To whom requests for reprints should be addressed, at Laboratory of Immunodiagnosis, National Cancer Institute, NIH, Building 10, Room 8B07, Bethesda, Md. 20014.

2 The abbreviations used are: TAA, tumor-associated antigens; MTV, mouse mammary tumor virus; MI, migration inhibition; MIF, migration inhibition factor; FBS, fetal bovine serum; PEC, peritoneal exudate cells; gp52, a glycoprotein with a molecular weight of 52,000.

Received March 2, 1978; accepted September 25, 1978.

MATERIALS AND METHODS

Animals. Female BALB/c, C57BL/6, C3H+, and C3H− mice were obtained from the Frederick Cancer Research Center Animal Colony, Frederick, Md. The C3H− colony was freed of milk-transmitted MTV-S by cesarean derivation and foster nursing on C57BL/6 mice that are reported not to be infected with MTV (22). These mice, however, should be considered still to be infected by the MTV-L variant that is reported to be present after foster nursing. The C3H+ mice were obtained by reincubation with purified C3H milk-derived MTV. The spontaneous tumor incidence in C3H− and C3H+ mice was 5 and 100%, respectively (C. Hansen, personal communication).

Tumors. Three mammary tumors pathologically defined as adenocarcinomas, MAT-2, MAT-3, and MAT-4, which arose spontaneously in C3H+ exbreeder mice, were used in this study. The tumors were maintained by s.c. injection of trocar fragments, and in this way no differences in tumor growth were observed between C3H+ and C3H− mice. MAT-2 was used between the third and the sixth transplant.
generations, whereas MAT-3 and MAT-4 were used at the first transplant generation. ADJ-PC5 plasmacytoma was maintained by s.c. transplantation of cell suspension into syngeneic BALB/c mice. MCA-1063, a 3-methylcholanthrene tumor induced in C3H+ mice (3), was maintained by s.c. transplantation of cell suspension in syngeneic immunodepressed C3H+ mice. Tumor weights were determined as previously described (27).

**Antigens.** MTV was obtained from Frederick Cancer Research Center Viral Resources Laboratory, Frederick Cancer Research Center. The MTV was prepared from the MmsMT/C, mammary tumor cell culture, as previously described (8).

KCl extracts (3 M) of fresh normal organs, tumors, and C3H/HeN embryos obtained on Day 14 of gestation were prepared as previously described (20). Concentration and dialysis of the 3 M KCl extracts were performed with an Amicon Multi Micro Ultrafiltration System (Lexington, Mass.) by the use of a UM-10 membrane. The concentrated extract was passed through a 0.2-μm Millipore filter, checked for sterility with thioglycolate media, and stored in aliquots at −70° prior to use.

The doses of all antigens used were experimentally determined to be optimal for induction of MIF in spleen cells of tumor-bearing mice without concomitant toxic effects or induction of MIF by spleen cells of normal mice.

**Indirect Agarose Microdroplet MIF Assay.** We used the indirect agarose microdroplet assay as previously described (27). In vitro synthesis of MIF by spleen cells from individual mice was carried out as follows. Spleen cells (5 × 10⁶) from individual mice were suspended in 1 ml of Roswell Park Memorial Institute Medium 1640 containing 1% FBS and then cultured with or without (medium control) antigen for 4 hr at 37° in a humidified 5% CO₂ incubator. The cells were then centrifuged at 150 × g for 10 min, and the supernatants were discarded to remove most of the antigen. The cells were then resuspended at 5 × 10⁶/ml with fresh medium containing 1% FBS and placed in a 37° humidified 5% CO₂ incubator. After 48 hr, the culture supernatants were collected, supplemented with an additional 9% FBS, and stored at −70° until tested for MIF activity. Indirect MIF assay was carried out in the following manner. Normal C57BL/6 mouse PEC were induced by i.p. inoculation with 3 ml of cold Hanks' balanced salt solution with 10% FBS and 100 units of heparin per ml. PEC from 5 to 10 mice were pooled and washed 3 times at 200 × g for 10 min. The PEC were then centrifuged at 200 × g for 5 min, and the supernatant was removed. The cell pellet was incubated briefly at 37° in a water bath and resuspended by gentle agitation to a concentration of 4 × 10⁶ PEC per ml in 0.2% agarose (Marine Colloids, Rockland, Maine). A 2-μl droplet of 0.2% agarose cell suspension containing 8 × 10⁶ PEC per droplet was placed in the center of each well of Costar plastic culture plates (No. 3596; Costar, Cambridge, Mass.) with a micro-dispenser (Drummond Scientific Co., Broomall, Pa.). Each droplet was allowed to solidify at room temperature for 5 min, and then 0.1 ml of supernatant from antigen-stimulated or control cultures was added to each well. The plates were incubated for 24 hr at 37° in a humidified 5% CO₂ incubator. The migration area images were projected onto paper and drawn. The areas of the agarose droplet and of the outer cell migration for each well were quantitated by planimetry. The net area of migration of the cells was then obtained by subtracting the inner agarose area from the outer cell area. A migration index (MIX) was calculated by the formula:

\[
MIX = \frac{\text{Av. migration area of 4 replicates of supernatants from cultures with antigen}}{\text{Av. migration area of 4 replicates of supernatants from medium control cultures}}
\]

**Treatment of Effector Cells with Anti-Thy 1.2 Antibody.** Anti-Thy 1.2 antibody was kindly donated by Dr. H. Holden, National Cancer Institute, NIH, Bethesda, Md. The method of preparation and the specificity of this antibody have been previously described (13). In this study, we followed the procedure described by Holden et al. (14). Briefly, effector cells were incubated for 30 min at room temperature with a final dilution of 1:2 of antiserum. The cells were washed once, resuspended in undiluted fresh rabbit serum, previously screened and found to lack nonspecific cytotoxic effects, as a source of complement, and incubated at 37° for 45 min. In all experiments, a complement control was performed in which the first incubation was in medium alone and the second was with complement. In addition, controls of anti-Thy 1.2 antibody absorbed with normal C3H brain homogenate were performed.

**gp52 Determination Assay.** Competition assays for MTV gp52 were performed with the use of gp52 purified by DEAE-cellulose, followed by Sephadex G-100 chromatography as previously described (1). The purified protein was iodinated using the chloramine-T method described by Greenwood and Hunter (10), and free 125I was separated from bound 125I by sequential chromatography on Sephadex G-25 and G-100 columns. The iodinated protein gave a single radioactive peak at M.W. 52,000 when analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The competition assay was initiated by incubating limiting dilutions of rabbit anti-MTV sera (1:10,000) with competitor once, resuspended in undiluted fresh rabbit serum, previously screened and found to lack nonspecific cytotoxic effects, as a source of complement, and incubated at 37° overnight at 4°. Staphylococcus aureus (Cowen I strain), prepared according to the procedure of Kessler (16), was added to each tube to facilitate precipitation of the antigen-antibody complexes. Incubation proceeded for 15 min at room temperature; pellets were collected at 1500 × g for 30 min. Radioactivity remaining in the pellet was determined with a Searle gamma counter.

**Statistical Analysis.** The average migration index minus 2 S.D. from several controls of normal C3H+ and C3H− mouse spleen cells in the presence of the different antigens was determined to be 0.85 to 0.87 for the various antigens used. To be conservative, only values ≥0.85 were considered to be positive. Statistical differences among groups of
individual MI's were analyzed by the Mann-Whitney non-parametric U test. The results in Charts 1 and 3 were obtained by pooling the data of different individual experiments. Individual mice or pools of 6 to 8 mice were used in this study.

RESULTS

We have previously shown, with the indirect macrophage MI test, that normal C3H+ and C3H− mice younger than 14 weeks of age do not exhibit a detectable natural immune response to MTV-associated antigens (27). However, when a spontaneous mammary tumor (MAT-2) was transplanted into C3H+ and C3H− mice younger than 14 weeks of age, most C3H− mice became reactive to MTV or its major glycoprotein, gp52, and this immunity was influenced by the magnitude of tumor burden. In contrast, tumor-bearing C3H+ mice generally failed to produce MIF in response to MTV or gp52.

In the present study, we tested the ability of both C3H+ and C3H− mice younger than 14 weeks of age bearing transplanted MAT-2 tumor to react in MI to a 3 M KCl extract of MAT-2 tumor (Chart 1). We had determined earlier from dose-response studies with this antigen that 25 μg/ml was the optimal dose for inducing MIF and that this dose did not induce MIF or toxicity with normal mouse spleen cells (Chart 1). A representative experiment showing reactivity with different doses of 3 M KCl extracts of MAT-2 tumor is presented in Table 1. Tumor-bearing mice of both C3H substrains reacted to this dose of the 3 M KCl extract of MAT-2 tumor. Tumor burden seemed to influence the degree of MI reactivity. A total of 4 of 9 (44%) C3H+ mice and

---

**Table 1**

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Dose (μg/ml)</th>
<th>Normal C3H+</th>
<th>Normal C3H−</th>
<th>Tumor-bearing C3H+</th>
<th>Tumor-bearing C3H−</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTV</td>
<td>10.0</td>
<td>0.87</td>
<td>0.97</td>
<td>0.86</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.91</td>
<td>1.08</td>
<td>0.93</td>
<td>0.80b</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>1.03</td>
<td>1.00</td>
<td>1.07</td>
<td>0.88</td>
</tr>
<tr>
<td>3 M KCl extract of MAT-2</td>
<td>250.0</td>
<td>1.03</td>
<td>1.04</td>
<td>1.07</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td>25.0</td>
<td>0.96</td>
<td>1.09</td>
<td>0.79b</td>
<td>0.80b</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>0.96</td>
<td>0.93</td>
<td>0.85b</td>
<td>0.83b</td>
</tr>
<tr>
<td>3 M KCl extract of MCA-1063</td>
<td>200.0</td>
<td>1.06</td>
<td>1.00</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>1.05</td>
<td>0.97</td>
<td>1.00</td>
<td>1.08</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>1.00</td>
<td>0.98</td>
<td>1.04</td>
<td>0.98</td>
</tr>
<tr>
<td>3 M KCl extract of spleen of</td>
<td>50.0</td>
<td>0.88</td>
<td>0.93</td>
<td>0.98</td>
<td>1.02</td>
</tr>
<tr>
<td>C3H− mice</td>
<td>10.0</td>
<td>0.95</td>
<td>0.99</td>
<td>1.16</td>
<td>0.95</td>
</tr>
<tr>
<td>3 M KCl extract of liver of</td>
<td>50.0</td>
<td>0.97</td>
<td>0.96</td>
<td>1.20</td>
<td>0.97</td>
</tr>
<tr>
<td>C3H− mice</td>
<td>10.0</td>
<td>0.89</td>
<td>0.93</td>
<td>1.01</td>
<td>0.96</td>
</tr>
<tr>
<td>3 M KCl extract of kidney of</td>
<td>200.0</td>
<td>0.97</td>
<td>1.03</td>
<td>1.14</td>
<td>0.91</td>
</tr>
<tr>
<td>C3H− mice</td>
<td>50.0</td>
<td>0.96</td>
<td>NDc</td>
<td>1.08</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>1.07</td>
<td>NDc</td>
<td>1.07</td>
<td>0.99</td>
</tr>
<tr>
<td>3 M KCl extract of mammary</td>
<td>200.0</td>
<td>1.13</td>
<td>1.04</td>
<td>1.24</td>
<td>1.10</td>
</tr>
<tr>
<td>gland of C3H− mice</td>
<td>50.0</td>
<td>1.03</td>
<td>0.98</td>
<td>1.03</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>1.06</td>
<td>1.09</td>
<td>1.09</td>
<td>1.02</td>
</tr>
<tr>
<td>3 M KCl extract of embryo of</td>
<td>200.0</td>
<td>1.04</td>
<td>1.07</td>
<td>0.97</td>
<td>0.87</td>
</tr>
<tr>
<td>C3H− mice</td>
<td>50.0</td>
<td>1.04</td>
<td>1.09</td>
<td>0.81b</td>
<td>0.81b</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>1.10</td>
<td>1.02</td>
<td>0.85b</td>
<td>0.85b</td>
</tr>
</tbody>
</table>

*Animals were inoculated s.c. 19 days previously with trocar fragments of MAT-2 tumor.

*Significant level of MI.

*ND, not done.
4 of 11 (36%) C3H— mice were positive on Day 12, when the tumors were small (0.05 to 0.2 g). On Day 19, when the tumors were of medium size (0.5 to 1.0 g), 21 of 24 (87%) and 21 of 25 (84%) C3H+ and C3H— mice, respectively, were reactive. No significant difference, as assessed by the Mann-Whitney nonparametric test, was seen between C3H+ and C3H— mice with small or medium-sized tumors, whereas the values obtained on Day 26, with tumors weighing 2.0 g, were found to be significantly different (p < 0.05). A total of 7 of 10 (70%) of the C3H— mice and 0 of 9 (0%) of the C3H+ mice were able to produce MIF in response to the 3 m KCl extract of MAT-2. No MI reactivity was found in either group of C3H mice on Day 33, when the tumors were more than 4 g.

The specificity of this reactivity was next investigated by means of a 3 m KCl extract of a mineral oil-induced plasmacytoma (ADJ-PC5). The results reported in Chart 2 show that C3H+ and C3H— mice bearing MAT-2 tumor weighing 1 g failed to produce MIF in response to 3 m KCl extract of ADJ-PC5, whereas all these particular C3H mice were reactive to the 3 m KCl extract of MAT-2 tumor (data not shown). Moreover, the ADJ-PC5 extract was able to induce reactivity in BALB/c mice bearing ADJ-PC5, whereas no MIF production was evident in these tumor-bearing mice when the 3 m KCl extract of MAT-2 tumor was used as antigen in MI assay (Chart 2).

A series of 3 m KCl extracts of normal tissues and of the methylcholanthrene-induced MCA-1063 tumor were prepared to assess further the specificity of the MI reactivity. The data presented in Table 1 show that 3 m KCl extracts of normal spleen, liver, kidney, and mammary gland (the last obtained from 14-day pregnant mice) of normal C3H— mice were not capable of inducing any reactivity with spleen cells from C3H— and C3H+ mice bearing MAT-2 tumors even though the same spleen cells were reactive with the 3 m KCl extract of the MAT-2 tumor. Moreover, the 3 m KCl extract of MCA-1063 was negative in the same experiment (Table 1).

We next tested the ability of C3H+ and C3H— mice bearing 2 other mammary tumors (MAT-3 and MAT-4) to react in MI to MTV or to the 3 m KCl extract of MAT-2. MAT-3 and MAT-4 were used during the first transplant generation, and the tumor-bearing mice were tested 19 days after tumor implantation, at a time when both tumors were approximately 0.5 to 1 g in weight. The results are shown in Table 2. C3H+ mice bearing MAT-4 tumor gave results similar to the C3H+ mice bearing MAT-2 tumor (0 of 5 positive reactors to MTV and 4 of 5 to 3 m KCl extract of MAT-2 tumor in both the cases), whereas most of the C3H+ mice bearing MAT-3 were unreactive to either antigen.

Similar results were obtained between the C3H— mice bearing MAT-2 or MAT-3, with most reacting to both MTV and 3 m KCl extract of MAT-2. By contrast, only 2 of 5 C3H—
mice bearing MAT-4 tumor were reactive to MTV and 4 of 5 mice of the same group were reactive to 3 M KCl extract of MAT-2.

We were interested in determining whether a 3 M KCl extract of C3H embryos might also contain an antigen detectable in MI with spleen cells from animals bearing MAT-2 tumors. A 3 M KCl extract of 14-day-old embryos from a pregnant C3H+ mouse was prepared. The results are presented in Chart 3. It was determined from dose-response studies that a concentration of 40 μg of this extract per ml did not produce MIF with spleen cells from normal mice, and this antigen concentration was used with spleen cells from tumor bearers. Both C3H+ and C3H− mice bearing MAT-2 tumors were able to produce MIF in response to this embryonic extract at times when the spleen cell donors were bearing small- (0.05 to 0.2 g) or medium- (0.5 to 1.0 g) sized tumors. Reactivity was still maintained in C3H− mice when the tumors were approximately 2 g in weight, but it was no longer evident in C3H+ mice at this time.

To examine the possible relationship between the antigens in MAT-2 tumor cells and in embryos, MI reactivity of C3H+ and C3H− tumor-bearing mice was evaluated simultaneously with the 3 M KCI extracts of MAT-2 tumor and of embryos (Table 4). Analysis of the correlation coefficients revealed $r = 0.6111$ for the C3H+ mice and $r = 0.4341$ for the C3H− mice. These correlations in reactivity to the 3 M KCl extract of MAT-2 and of the embryos were significant ($p < 0.01$ for C3H+ mice and $p < 0.05$ for C3H− mice).

Since the 3 M KCl extract of the embryos was obtained from a C3H+ mouse harboring MTV, we wanted to see if MTV-associated antigens could account for the reactivity to this extract. This extract, along with the extract of MAT-2, was tested in a competitive inhibition radioimmunoassay for the major glycoprotein (gp52) of MTV (Table 3). No gp52 was detected in the preparation of the embryos, whereas a small amount of gp52 was present in the 3 M KCl extract of the MAT-2 tumor. When a 3 M KCl extract of embryos of C3H− mice was prepared and tested simultaneously with extracts of normal adult tissues, there was no reactivity with spleen cells of normal or tumor-bearing mice (Table 1). A positive reaction was seen in both the C3H+ and C3H− mice bearing MAT-2 tumors.

![Chart 3](image-url)

**Chart 3.** MI reactivity of normal or MAT-2 tumor-bearing C3H+ (●) and C3H− (○) mice, using a 3 M KCI extract of C3H+ embryos (40 μg/ml) as antigen.

![Chart 4](image-url)

**Chart 4.** MI reactivity of C3H+ and C3H− mice bearing MAT-2 tumors weighing 0.05 to 0.2 (●), 0.5 to 1.0 (○), or >2.0 (△) g, tested simultaneously with 3 M KCI extract of MAT-2 (25 μg/ml) and 3 M KCI extract of C3H+ embryos (40 μg/ml).

**Table 3.** Concentration of gp52 in the antigen extracts used in MI assay as assessed by radioimmunocompetition assays.

<table>
<thead>
<tr>
<th>Antigen preparation used for competition</th>
<th>Protein (ng/ml) used in the MI assay</th>
<th>gp52 (ng/ml) used in the MI assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTV</td>
<td>1,000</td>
<td>150,000</td>
</tr>
<tr>
<td>3 M KCl extract of MAT-2</td>
<td>25,000</td>
<td>432</td>
</tr>
<tr>
<td>3 M KCl extract of C3H+ embryos</td>
<td>40,000</td>
<td>&lt;40</td>
</tr>
</tbody>
</table>

**Table 4.** Effect of treatment with anti-Thy 1.2 antibody plus complement on MIF production of spleen cells from MAT-2 tumor-bearing C3H+ and C3H− mice in response to MTV or the 3 M KCI extract of MAT-2

<table>
<thead>
<tr>
<th>Antigen used for triggering MIF</th>
<th>3 M KCl MAT-2 tumor extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTV</td>
<td></td>
</tr>
<tr>
<td>Normal C3H+</td>
<td>0.98</td>
</tr>
<tr>
<td>Tumor-bearing C3H+</td>
<td>0.98</td>
</tr>
<tr>
<td>Normal C3H−</td>
<td>1.13</td>
</tr>
<tr>
<td>Tumor-bearing C3H−</td>
<td>0.79</td>
</tr>
</tbody>
</table>

**a** The animals were inoculated s.c. 19 days previously with trocar fragments of MAT-2 tumors.

**b** Significant level of MI.
mice bearing MAT-2 tumors with the 3 M KCl extracts of C3H— embryos.

To characterize partially the nature of the lymphoid cells required for MIF production, spleen cells from a pool of normal C3H+ and C3H— mice, or from mice bearing transplantable MAT-2 tumors (19 days after transplantation), were treated with anti-Thy 1.2 antibody plus complement (Table 4). This treatment abolished the ability of spleen cells to produce MIF when exposed in vitro to either MTV or the 3 M KCl extract of MAT-2 tumor. No decrease in M1 reactivity was observed when the spleen cells were treated with complement alone or with C3H brain-absorbed anti-Thy 1.2 plus complement.

**DISCUSSION**

We have demonstrated that C3H mice bearing transplantable MAT-2 tumors produce a factor, presumably MIF, in response to MTV or a crude 3 M KCl extract of MAT-2 tumor. As in other mouse tumor systems (17), production of MIF appeared to be dependent on T-cells, since anti-Thy 1.2 sera plus complement abrogated the response. Specificity of this reactivity was indicated by the lack of activity of MAT-2 tumor bearers against an antigenic, similarly prepared 3 M KCl extract of a chemically induced plasmacytoma, ADJ-PC5. Further, 3 M KCl extracts of normal spleen, liver, kidney, and mammary gland of C3H— mice and of a 3-methylicholanthrene-induced tumor of C3H+ mice were not able to induce M1 reactivity in either of the C3H substrains bearing MAT-2 tumors. Two other mammary tumors (MAT-3 and MAT-4) of C3H+ mice appeared to share antigens with MAT-2, since animals bearing the tumors reacted to the extract of MAT-2. C3H+ mice bearing MAT-2 or MAT-4 tumors reacted with the 3 M KCl extract of MAT-2 tumor, and C3H— mice bearing MAT-2, MAT-3, or MAT-4 tumors reacted to the extract of MAT-2. None of these mammary tumors, however, was able to induce reactivity in C3H+ mice in response to MTV. An unexpected result was the lack of reactivity of C3H+ mice bearing MAT-3 tumor against the 3 M KCl extract of MAT-2 and the low reactivity (2 of 5 positive) of C3H— mice bearing MAT-4 tumor against MTV. One possible explanation for these results is that an eclipse in immunity induced by mammary tumors, as reported in this and in other studies (7, 27), could occur earlier with MAT-3 and MAT-4 tumors in comparison with the MAT-2 tumor. However, MIF production by spleen cells of mice bearing MAT-3 or MAT-4 tumors, when the MAT-2 extract was used, strongly suggests antigenic cross-reactivity among these 3 mammary tumors. We cannot totally rule out the possibility that this antigenic cross-reactivity was entirely or partially due to MTV or its major glycoprotein, gp52. However, evidence would support the hypothesis that the cross-reactivity is due to nonviral TAA, since the MAT-2 extract used to test cross-reactivity with spleen cells from MAT-3 and MAT-4 tumor-bearing mice contained only minute quantities of gp52 as detected by radioimmunoprecipitation assays. Moreover, in determining the optimal dose of gp52 to induce MIF production in C3H— mice bearing MAT-2 tumor, we found that 10.8 ng of gp52 per ml, i.e., the concentration of gp52 contained in the dose of 3 M KCl extract of MAT-2 tumor used in the M1 assay, did not induce reactivity in those mice (A. Tagliabue and J. L. McCoy, unpublished observation). Also, both C3H+ and C3H— tumor-bearing mice reacted to the MAT-2 extract, whereas C3H+ tumor-bearing mice rarely reacted to gp52 or MTV (27). Some investigations have shown that there is a sharing of antigens of some spontaneously arising mouse mammary tumors (12, 26). Although this has usually been assumed to be entirely due to MTV antigens, our present results suggest that other common antigens may also be present. The finding that 3 M KCl extracts of C3H+ and C3H— mouse embryos could efficiently induce production of MIF in spleen cells from both C3H+ and C3H— mice might suggest that one of the tumor antigens being recognized by this in vitro technique could be embryo associated. Since no gp52 could be detected by radioimmunoassay in the C3H+ embryo extract, the antigenic activity of the embryonic preparation was presumably not MTV related or at least was a virion antigen other than gp52. The known vertical transmission of MTV through the milk of C3H rather than through the placenta is consistent with these data. The significant correlation in reactivity against the 3 M KCl extracts of MAT-2 and of the C3H+ embryo in simultaneous M1 tests suggests that similar antigenic determinants were being recognized on the tumor and embryo cells. Blair et al. (4) reported the failure to induce transplantation resistance to mouse mammary tumors by immunization with embryonic tissue. These differences in results are compatible with previous findings that embryonic antigens can induce detectable immunity but usually do not induce resistance against tumor growth (2, 28). The results of this study and of our previous (27) as well as other studies (12, 26) indicate that an array of different antigens (some common and some possibly distinct) may elicit cellular immune responses in the mammary tumor-bearing host.

The M1 reactivity against the 3 M KCl extract of MAT-2 in the 2 substrains of animals was influenced by the magnitude of tumor burden. Reactivity was readily observed in both C3H+ and C3H— hosts, while tumors ranged in size from 0.05 to 1.0 g. As the tumor burden approached 2 g, the C3H+ animals became refractory in their ability to produce MIF in response to this antigen extract, whereas C3H— mice were still reactive. Both substrains were unreactive to the extract when the tumor load was greater than 4 g. We have similarly demonstrated an effect of tumor burden on the reactivity of MAT-2 tumor-bearing C3H mice against MTV and gp52 and have shown that C3H— mice react more vigorously to MTV and gp52 antigens than do C3H+ mice (27). This more rapid loss of reactivity of C3H+ than that of C3H— mice may partially explain the more rapid growth of MAT-2 tumor in C3H+ mice that we previously reported (27).

The mechanism(s) responsible for this loss of M1 reactivity with increased tumor burden is not yet known. It has been shown previously that the highest titers of antibody against MTV occur in mice bearing palpable primary tumors (15), and that sera from BALB/cfC3H mice can block in vitro cellular cytotoxicity against mammary tumors (6, 11). Cremer and Bentvelzen (7) have also reported the transient production of adherent suppressor cells during mouse mammary tumor development. The question of whether these or other factors are influencing the development of
immunological eclipse in mice with large tumor burdens is being investigated.

ACKNOWLEDGMENTS
We wish to thank Dr. David H. Lavrin for his helpful suggestions.

REFERENCES

Cellular Immunity to Tumor-associated Antigens of Transplantable Mammary Tumors of C3H/HeN Mice


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/39/1/35

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pub@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.