Communication

Immunization of Mice against Murine Mammary Tumor Virus Infection and Mammary Tumor Development

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ABSTRACT

Formalin-inactivated whole murine mammary tumor virus (MuMTV), MuMTV membranes, the acid-soluble component of MuMTV, and purified MuMTV glycoprotein with a molecular weight of 55,000 (gp55; also designated as gp52) were used as vaccines in an attempt to identify the MuMTV antigen(s) that can protect mice from exogenous MuMTV infection and subsequent tumor development. Formalin-inactivated whole MuMTV, MuMTV membranes, and purified MuMTV gp55 were effective immunogens, whereas the acid-soluble component of MuMTV (which consists mainly of MuMTV gp55) failed to protect mice from challenge with live virus. These results suggest that (a) MuMTV gp55 is the major immunizing antigen and (b) its native conformation must be maintained for it to be an effective vaccine.

INTRODUCTION

Mice immunized with syngeneic tumor cells (8, 15, 17, 25) or extracts from normal or neoplastic mammary tissue (7, 26) infected with the MuMTV have acquired resistance to subsequent transplantation of mammary tumors. Immunization of mice with formalin-treated MuMTV also confers partial resistance to challenge with syngeneic mammary tumor cells (1, 24).

Similarly, the immunization of mice with low doses of crude MuMTV extract enriched for the major viral glycoprotein delays the growth of transplanted tumor cells, but large doses of the immunizing antigens accelerate tumor cell growth (6). Although vaccination with inactivated tumor cells (5, 23), tumor cell extracts (13, 16), formalin-inactivated MuMTV (2, 3), or MuMTV extract (6) delayed the onset of tumor development in some strains of mice, it was only marginally effective in preventing tumor development in strains of mice that have a high incidence of spontaneous breast tumors (2, 3, 22). A delayed tumor incidence was also observed in GR mice, which have a high incidence of spontaneous mammary tumors, after they had been immunized with MuMTV derived from C3H mice, which also have a high incidence of breast cancer (24). Recently, and Moore (3) demonstrated that C57BL mice, which normally do not develop spontaneous breast cancer, can be completely protected against exogenous MuMTV infection by a single injection of formalin-inactivated MuMTV. Furthermore, vaccination with formalin-inactivated MuMTV prevents tumor development in mice that have a relatively low incidence of spontaneous tumors (2, 22).

These results suggest that MuMTV vaccines are feasible and can control exogenous infection in animals that do not normally overtly express endogenous virus and that they can also prevent tumor development in strains of mice that have a low virus load. Before a safe and effective vaccine can be obtained from MuMTV, the antigens involved in the immune response must be defined. We have therefore attempted to identify the antigen(s) of the MuMTV, which can immunize mice against MuMTV infection and subsequent mammary tumor development.

MATERIALS AND METHODS

MuMTV was purified from the milk of RIII mice by sucrose density gradient centrifugation as described previously (19). Viral membrane fragments were released from intact virus by treating MuMTV with Tween 80 and ether and were isolated by gradient centrifugation (20). Isolated virus and viral membrane preparations were examined in the electron microscope following negative staining with phosphotungstic acid. Projections consisting of knobs and stalks, a characteristic structural component of the MuMTV surface, were present on the surface of the isolated membrane fragments, which varied in size. Polyacrylamide gel electrophoresis of the membrane preparation revealed that it contained the 2 major viral glycoproteins (MuMTV gp55 and gp34). The preparation also contained small amounts of other glycoproteins (gp90 and gp68), the origin of which is not yet established. The morphology and polypeptide composition of the membrane fragments (rosettes) have been described in detail elsewhere (20). The MuMTV membrane preparation used in this study was also free of viral RNA as measured by UV absorption analysis. The knobs of the viral surface projections were isolated by treating MuMTV with 0.05 n HCl at 4° for 20 min and contained mainly gp55 (20).

To obtain purified MuMTV gp55, we solubilized the virus by incubation at 4° for 1 hr in phosphate buffer (pH 6.8) containing 1% Nonidet P-40, 0.01 M EDTA, and 0.2% (v/v) β-mercaptoethanol. During the incubation period the mixture was subjected to 10 separate sonic extractions, each lasting for 30 sec. Pure MuMTV gp55 was obtained from the resulting crude extract by a combination of DEAE-cellulose
and Sephadex G-200 column chromatography, as described previously (18). All steps in the purification procedure were carried out at 4°C. Quantitation of viral protein was done by the method of Lowry et al. (14).

Purified whole virus to be used for immunization was inactivated by treatment with formalin at a dilution of 1:4000 for 48 hr at room temperature, whereas the subviral components or purified MuMTV gp55 was used without any formalin treatment. Prior to the immunization studies, 10 μg each of purified virus, virus treated with formalin, and viral components without formalin treatment were injected into different groups of 3- to 4-week-old C57BL mice (a strain of mice with an extremely low incidence of spontaneous mammary tumors) to determine whether these preparations were infectious. Aliquots of these materials were kept at -70°C for future use. The inoculated mice were then force-bred, and their milk at the third and fifth parturitions was tested for viral antigen by the microimmunodiffusion technique (4) with a polyvalent rabbit anti-MuMTV (ether-disrupted) serum. The methods of preparation of this serum and the tests for its specificity have been described earlier (21). The inoculated mice were also kept for observation of possible development of mammary tumors for a period of 2 years. Since a long time period is needed for the completion of the infectivity experiments described above (i.e., for the observation of tumor development), we relied on the results of the microimmunodiffusion analysis (see "Results") of the fifth parturition milk of the inoculated mice to initiate the immunization experiments.

None of the mice inoculated with formalin-treated MuMTV, MuMTV rosettes, the acid-soluble component, or MuMTV gp55 were infected with MuMTV, and therefore these preparations were used for immunization studies. All preparations were emulsified with Freund's complete adjuvant (Difco Laboratories, Detroit, Mich.), and various amounts (varying from 0.01 to 10 μg) of each sample were inoculated once i.m. into 3- to 4-week-old C57BL mice. After 3 weeks each mouse was challenged with live virus (10 μg/mouse), and the milk at the third parturition was tested for the presence or absence of MuMTV-specific antigen(s) by microimmunodiffusion. Most of the inoculated mice were kept for observation of tumor development.

RESULTS

Table 1 shows that about 50% of the C57BL mice, each inoculated with 10 μg of purified MuMTV, developed mammary tumors by the time they were 2 years old. MuMTV-specific antigens were detected in the milk of about 50 and 60% of the mice at the third and fifth parturition, respectively. In contrast, mice inoculated with formalin-inactivated MuMTV, a preparation containing MuMTV membranes (MuMTV rosette), the acid-soluble component of MuMTV, and a preparation of purified MuMTV gp55 did not produce any MuMTV antigens in their milk or develop mammary tumors during 2 years of observation.

The effect of immunization of C57BL mice with the same preparations mentioned above on the occurrences of MuMTV expression and tumorigenesis by inoculation of live virus at a concentration of 10 μg/mouse is shown in Table 2. Mice immunized with 1 or 10 μg of formalin-inactivated intact virus were completely protected against challenge of live virus. No MuMTV antigens could be detected in the third parturition milk of these mice, and they remained tumor-free during the 2 years of observation. When mice were vaccinated with 0.1 or 0.01 μg of formalin-inactivated virus and then challenged with live virus, only about 6 and 20% of the mice, respectively, developed mammary tumors. Immunization of C57BL mice with 10 μg of MuMTV membrane preparation and the MuMTV rosette or purified MuMTV gp55 preparation resulted in complete protection against infection by exogenous MuMTV. One μg of the rosette and MuMTV gp55 preparation was also very effective in immunizing the mice. About 20% of the mice

<table>
<thead>
<tr>
<th>Virus and/or viral components inoculated (10 μg/mouse)</th>
<th>MuMTV antigen in the milk of the inoculated micea (no. positive/no. tested)</th>
<th>Tumor incidence during 2 yrb (no. of mice with tumor/no. of mice examined)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact MuMTV</td>
<td>11/19 (50.5)%</td>
<td>9/16 (56.3)%</td>
</tr>
<tr>
<td>Formalin-inactivated MuMTV</td>
<td>0/20 (0)</td>
<td>0/17 (0)</td>
</tr>
<tr>
<td>MuMTV rosette</td>
<td>0/20 (0)</td>
<td>0/19 (0)</td>
</tr>
<tr>
<td>Acid-soluble MuMTV component</td>
<td>0/19 (0)</td>
<td>0/16 (0)</td>
</tr>
<tr>
<td>Purified MuMTV gp55</td>
<td>0/15 (0)</td>
<td>0/12 (0)</td>
</tr>
<tr>
<td>Phosphate-buffered saline (control)</td>
<td>0/20 (0)</td>
<td>0/19 (0)</td>
</tr>
<tr>
<td>Freund's complete adjuvant (control)</td>
<td>0/20 (0)</td>
<td>0/16 (0)</td>
</tr>
</tbody>
</table>

a Three- to 4-week-old female C57BL mice were given i.m. inoculations.

b After inoculation of virus and/or subviral components, all mice were force-bred. Milks were collected from individual mice at the third and fifth parturitions and analyzed for the presence or absence of MuMTV antigen(s) by microimmunodiffusion.

c During this 2-year period, some mice died. Those dying without mammary tumors were not taken into account.

d Numbers in parentheses, percentage.
immunized with 0.1 μg of the rosette preparation per mouse developed mammary tumors. A 10-fold less concentrated preparation of the same material, however, was also equally effective as an immunogen, since only about 28% of the mice challenged with live virus developed mammary tumors. A similar result was obtained when 0.1 μg of purified MuMTV gp55 was used for immunizing each mouse. In contrast, mice immunized with a dose of 0.01 μg of MuMTV gp55 were not at all protected against virus challenge. About 60% of this group of vaccinated mice, an incidence similar to that in the control group, developed mammary tumors. An unexpected result was obtained in those experiments where preparations of MuMTV gp55, made after treating MuMTV with acid, were used for immunizing mice. About 55 to 75% of the mice vaccinated with various amounts (0.01, 0.1, or 10 μg/mouse) of the acid-soluble component produced viral antigen(s) in the milk of the third parturition. Since the detection of viral antigen in mouse milk is a good predictive test for eventual mammary tumor genesis, most of the mice in this group were sacrificed, except those vaccinated with 10 μg of acid-soluble component. As predicted, about 62% of this latter group of mice developed mammary tumors, an incidence similar to the control group. These results suggest that, although the major envelope glycopeptide MuMTV gp55 is the antigen involved in immunizing mice, the native structure of the molecule must be preserved during its purification to obtain an effective vaccine.

DISCUSSION

MuMTV inactivated by formalin treatment is effective in immunizing C57BL mice against exogenous MuMTV infection and subsequent mammary tumor development. However, as shown in this study, for complete protection about 1 μg of purified virus (Table 2) is required. This result agrees very well with those reported by Charney et al. (2), who also used formalin-inactivated purified MuMTV to immunize C57BL mice. However, our results of tumor incidence in mice immunized with 0.1 or 0.01 μg of virus apparently differ from those obtained by Charney et al. (2). For example, in this study only 6 and 20% of the mice immunized with 0.1 and 0.01 μg of virus, respectively, developed mammary tumors, compared to about 65% of the nonvaccinated mice. Using a similar concentration of virus for immunization, Charney et al. (2) observed 50 and 68% tumor incidences, respectively, in the immunized mice, as compared to the 56 to 67% tumor incidence in the control groups. This apparent discrepancy may be due to a number of factors, such as: (a) the integrity of the virus used for immunization (i.e., the amount of the immunizing antigen present in a particular batch of virus preparation) and (b) the differing susceptibility of individual mice to immunization and virus infection (for instance, it is often found that, within a matched group of mice inoculated with the same amount of virus at the same time, some will be infected and produce tumors, while others will remain tumor-free throughout their lifetime).

We have conclusively demonstrated in this study that purified MuMTV gp55, the major envelope glycoprotein of the virus, can be used to immunize MuMTV-free C57BL mice against exogenous MuMTV infection and subsequent development of mammary tumors (Table 2). The protective effect is clearly dose dependent.

For complete protection of mice from infection with MuMTV, we found that between 1 and 10 μg of the purified
MuMTV gp55 vaccine per mouse were required. At lower doses no protection was produced by the vaccine. Our results parallel the findings of Hunsmann et al. (10), who found that protection against MuLV-induced leukemia could be rendered by immunizing the mice with a high dose (about 100 µg/mouse) of purified MuLV gp71, the major MuLV envelope glycoprotein. The number of mice that we used for immunization in each group is rather small, and therefore reliable dose-response relationships cannot be established. However, the data presented in Table 2 are sufficient to make a rough comparison of the potency of the various preparations used in immunizing mice. The amount of MuMTV gp55 necessary to protect all of the vaccinated mice is between 1 and 10 µg/mouse. This dose of vaccine is slightly more than that needed when the MuMTV rosette preparation is used. However, our results indicate that similar protection can be achieved with a 10-fold less amount of formalin-inactivated whole virus. It appears, therefore, that the native conformation of MuMTV gp55 may be an important factor in its immunogenicity. Our failure to immunize mice with HCl-prepared gp55, which was probably denatured, is consistent with this conclusion. Attempts should be made to purify MuMTV gp55 in its native form so that an effective immune response can be elicited with smaller amounts of MuMTV gp55 than have been used in this study.

Using crude MuMTV extract as a vaccine, Creemers et al. (6) found that low doses (1 µg/mouse) of the MuMTV vaccine resulted in delay of the onset of tumor growth, whereas higher doses (10 µg/mouse) resulted in enhancement of the growth of tumors. Since these authors used crude extract of MuMTV instead of purified MuMTV gp55 to immunize mice and subsequently challenged them with tumor cells, not purified virus, the results of this study cannot be directly compared with our results. However, it would be of interest to determine whether mice immunized with substantially higher doses of MuMTV gp55 than have been used in this study would have an increased incidence of tumor development after challenge with live virus.

Protection against the development of mammary tumors or leukemias in laboratory mice by active or passive immunization may depend on various factors, including the genetic makeup of the mice (which controls the expression of viral genetic information, as well as the production of infectious virus) and the host immune response to oncornaviral antigens. Huebner et al. (9) have shown that immunization with type-specific oncornavirus viruses prepared from inactivated MuLV results in suppression of virus expression in strains of mice that have been cross-bred with the highly viremic AKR mouse strain. In contrast, attempts to induce immunity against leukemia induced by the endogenous viruses of AKR and C57BL/6 mice by immunization of these mice with gp71 isolated from Friend leukemia virus were unsuccessful (11, 12), although this component did serve as an effective immunogen for protecting STU mice against subsequent challenge with Friend virus (10). These findings indicate that the method of immunization plays an important role in eliciting immune response in different strains of mice. Similarly, using formalin-inactivated whole MuMTV, Charney et al. (2) have shown that those mice that receive infectious virus through their mother’s milk and have a high incidence of mammary tumors cannot be immunized by vaccination. In contrast, mouse strains with a low mammary tumor incidence infected with gamete-borne virus manifest a high degree of immunity upon immunization, and the incidence of tumors in these mice is reduced. However, those strains of mice that do not express virus or develop spontaneous mammary tumors, when immunized with inactivated whole virus [Charney et al. (2); this report] or MuMTV gp55 (as shown in this study), can be completely protected from exogenous virus infections and subsequent mammary tumor development. It remains, therefore, to determine whether MuMTV gp55 vaccine would inhibit the expression of virus and reduce the incidence of spontaneous mammary tumors in mouse strains with a high and/or low cancer incidence. We are hopeful that the glycoprotein vaccine will be effective in preventing spontaneous tumor development in mice with a low cancer incidence. Thus, the cellular and/or humoral immune responses to MuMTV gp55 should be monitored in the immunized low-cancer strain, as well as in the MuMTV-negative strain, to obtain an insight into the immune mechanisms involved in the prevention of tumor development in the former and MuMTV infection in the latter strain of mice.

ACKNOWLEDGMENTS

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REFERENCES

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