Tumor Immunity Induced by Preimmunization With BALB/c Mouse Myeloma Protein 1, 2

Peter M. Freedman, Johnna R. Autry, Sei Tokuda, and Ralph C. Williams, Jr. 3, 4

SUMMARY—Prior sc immunization of BALB/c mice with 1 mg isolated M component of MOPC-11 mouse myeloma resulted in significant relative immunity to subsequent sc or ip challenge with 10^4 living cells from the same plasmacytoma. However, challenges of 10^6 and 10^10 tumor cells overcame immune status engendered by preimmunization with M component. Despite evidence for the specificity of the immunity induced by one isolated M component as opposed to another, no clear cytotoxic antibody, cell-mediated tumor-cell lysis, or predominance of either humoral or cell-mediated immune mechanisms were demonstrated. These findings were compatible with a relatively slight tumor-specific antigenicity of M components expressed on tumor surfaces, compared with the tumor specificity of other tumor-related, cell-surface antigens.—J Natl Cancer Inst 56: 735–740, 1976.

Preimmunization with intact or modified M components from specific plasmacytomas results in prevention or significant retardation of syngeneic plasma cell tumors in BALB/c mice (1–3). Anti-idiotypic antibody has been demonstrated by various techniques in mice immunized with isolated mouse myeloma proteins (1–3); however, the presence of such antibody has not appeared to correlate with suppression or enhancement of tumor growth (3). Kolb et al. (6), using plasmacytoma cells treated with sodium iodoacetate, immunized BALB/c mice and demonstrated subsequent hemagglutinating antibodies against tumor extracts. Using the same method of immunization, Lespineats (7) demonstrated that titers of IgG2 antitumor antibody generated in immunized mice did not correlate with subsequent development of tumor in mice challenged with plasmacytoma cells.

Immunity to mouse plasmacytomas has been produced by injection of viable tumor into one limb and subsequent removal of the tumor-bearing limb (8, 9). The findings of Rouse et al. (8) appeared to support the concept that tumor resistance was a cell-mediated process dependent on T cells. These findings were corroborated by Wagner and Röllinghoff (10), who sensitized cortisone-resistant thymocytes in vitro to syngeneic plasmacytoma cells and induced specific lymphocyte-mediated tumor lysis in a 51Cr-release assay. It has not been clear whether immunity to plasmacytomas induced by preimmunization with M components functions through direct cell-mediated T-cell killing, antibody-mediated lymphocyte toxicity, or T-cell sensitization to idiotypes on plasma cells.

The principal aim of the present study was to confirm previous observations that preimmunization with the myeloma tumor cell product—M component—could confer immunity to subsequent challenge with whole tumor cells and, if possible, to examine the role of both humoral and cellular immunity in such tumor resistance.

MATERIALS AND METHODS

Mice.—BALB/c mice 7–12 weeks old were obtained from the Charles River Breeding Laboratories (North Wilmington, Mass.) or the Laboratory Supply Company (Indianapolis, Ind.). Recipients of in vivo transfers of adherent peritoneal exudate cells (PEC), tumor cells, and sera were males; females were used in all other experiments.

Preparation of M component.—Ascitic fluid from MOPC-11 (IgG2) tumor-bearing animals was separated on G-200 Sephadex columns in phosphate-buffered saline (PBS), pH 7.4. The second (7S) peak was concentrated and subjected to starch-block electrophoresis in barbital buffer (pH 8.6) at 30°C for 24 hours. Mopane myeloma M component was eluted from starch-block fractions by displacement with PBS (pH 7.4) and examined for purity with simple immunodiffusion in agarose, with goat antiserum against mouse whole serum, IgA, and IgG2 (Meloy Laboratories, Springfield, Va.). Isolated M components were then dialyzed against PBS (pH 7.4) and adjusted to a concentration of 2 mg/ml. Isolated M components showed only one line when tested in immunoelectrophoresis against antibody to mouse whole serum. In addition, a much more specific test of purity was achieved in that isolated mouse M components, when 1 mg emulsified in complete Freund's adjuvant (CFA) was used for sc immunization of rabbits, produced antiserum showing only one line in Ouchterlony analysis with isolated M component, whereas serum from a mouse with myeloma, or ascitic fluid.

Immunization schedule.—The procedure outlined by Lynch et al. (9) was used. Each mouse received 1 mg isolated M component in three weekly injections. The first two doses were given in CFA and the last dose was given in incomplete Freund's adjuvant (IFA). The injections consisted of a total volume of 0.32 ml/dose, half of which was adjuvant; injections were given in multiple subcutaneous locations and into the footpads. The animals were inoculated with tumor 7–14 days after the last M-component injection. The end point for tumor growth from sc injection of tumor was the time at which tumor nodules reached 2 cm in largest diameter. When ip injections were used, mice were scored positive for tumor growth when the abdomen was distended with ascitic fluid. Sera from all animals challenged with tumor were studied at weekly intervals by cellulose acetate electrophoresis for presence of M components. In general, this method provided the earliest indication of tumor growth.

Tumor inoculum.—MOPC-11 plasmacytoma, MOPC-315 (IgG), and MOPC-6A (IgA) were provided by Dr. Matthew Scharff (Albert Einstein Medical Center, New York, N.Y.); MOPC-70A was provided by the Salk Institute, San Diego, California. Tumor cells from sc implants were minced in RPMI-1640 and passed through fine mesh gauze. Tumor cells from ascitic fluid were collected after centrifugation at 200 × g for 10 minutes. Various numbers (10^3–10^7) were injected sc or ip as cell...
suspensions in RPMI-1640 (Gibco, Grand Island, N.Y.), after centrifugations through a Ficoll–Hypaque gradient (9.0 g Ficoll, Pharmacia, Uppsala, Sweden; 30 ml of 50% wt/vol sodium Hypaque, Winthrop–Sears, Inc., New York, N.Y.; and 115 ml distilled H2O) to remove dead cells, polymorphonuclear leukocytes, and erythrocytes. Such cells showed a viability of 98% as measured by trypan blue dye exclusion. Tumor cells were washed twice in RPMI-1640 before use. P-815 mastocytoma (DBA/2) cells were used in some experiments for coupling M components to cell surfaces.

Cell-mediated cytotoxicity assay.—We used the radioactive 51Cr-release assay as described by Canty and Wunderlich (11) and modified by Goldberg et al. (12). Spleen cells from normal or immune mice were incubated at 37°C in 10% CO2 with radioactive target cells for 5½–6 hours; 51Cr-labeled MOPC-11 M component-tagged mastocytoma cells were then used. The latter target was prepared by use of 0.5 mg 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride/ml as the coupling agent to link M component to the 51Cr-labeled mastocytoma (13, 14). Spleen cell to target cell ratios varied from 100:1 to 1,000:1.

Antibody titers of immune sera.—Hemaggulinating antibodies were measured by use of MOPC-11 M component coupled to mouse erythrocytes with chromic chloride (1). Following the coupling procedure, 0.025 ml indicator cells was added to microtiter plates containing serial dilutions of pooled sera from either normal, tumor-bearing, or immune animals (0.025 ml/well). The plates were placed on a shaker for 10 minutes and then allowed to sediment before hemagglutination was recorded. Precipitating antibody was tested by immunodiffusion with serum samples against various concentrations of MOPC-11 M component. We examined cytotoxic antibody to mastocytoma cells using suspensions of freshly prepared tumor cells and 0.05 ml rabbit complement, by the procedures of Gorer and O’Gorman (16), as modified by Boyse et al. (17).

In vivo cell transfers.—Spleen cells from immune or normal mice were minced in RPMI-1640; the supernatant was removed and passed through a fine mesh gauze. Viability at this step was 80–85% as determined by trypan blue exclusion. Spleen cell suspensions were washed twice in RPMI-1640 and injected sc or ip after the cell count had been corrected for the 15–20% dead cells. Spleen cells were injected at the same time as tumor cells; in some instances immune or normal sera were also injected according to individual protocols.

Adherent PEC preparation.—Immune or normal mice were inoculated ip with 0.3 ml Marcol mineral oil, and PEC were harvested 72 hours later with repeated washes totaling 10 ml RPMI-1640 containing 1 U heparin/ml (18). The PEC were washed twice with media, layered over a 100×15-mm glass petri dish, and incubated at 37°C with 10% CO2 for 2 hours with occasional shaking. The nonadherent cells were decanted. The adherent cells were removed with a rubber policeman and counted; their viability was determined by trypan blue exclusion. Peroxidase staining (19) of the adherent cell population plus morphologic differences under light microscopy facilitated calculation of the percentage of macrophages, lymphocytes, and polymorphonuclear cells in these PEC preparations. Adherent PEC were injected ip with tumor cells and in some instances with immune or normal mouse serum.

RESULTS

After preimmunization with M component, sera of most mice showed low titers of hemagglutinating antibody (1:8–1:16), with specificity for M component detected only in the sera of immunized mice. No antiidiotype antibody was found in animals bearing transferred tumor or in normal mice. Specificity for M-11 idiotype was confirmed by inhibition of agglutination.

Normal nonimmune BALB/c mice challenged sc with various doses of viable MOPC-11 tumor cells developed tumor nodules at decreasing rates as the tumor dose was lowered (text-fig. 1). With doses of 104 and 105 tumor cells, the time until tumor developed within groups was increased; this was not so at larger dose levels. BALB/c mice inoculated ip with different numbers of tumor cells are also represented in text-figure 1. Again, a decreasing rate of tumor growth was recorded as the tumor inoculum was lowered; however, it was not as great as with the sc tumor cell challenge.

BALB/c mice were immunized with three doses of purified MOPC-11 M component and challenged sc with various tumor cell doses 7–14 days after the last M-component injection. Preimmunization with M component clearly conferred significant immunity to a challenge dose of 1×104 tumor cells, since all animals studied (9/9) showed no detectable tumor for observation periods up to 50 days, whereas all 10 animals not preimmun-
nized with M component developed tumor within 50 days after inoculation. All immune animals were later challenged repeatedly with tumor at 14-day intervals. The 14-day interval was chosen because at that time control nonimmunized animals receiving identical tumor doses had developed MOPC-11 M component in their sera, as determined by cellulose acetate electrophoresis. Therefore, animals showing either no M component on cellulose acetate electrophoresis of sera or having no grossly palpable tumor 14 days after their tumor doses were repeatedly rechallenged.

The results of rechallenging immune mice sc with various doses of MOPC-11 tumor cells are shown in table 1. Delay in tumor growth to 2 cm was apparent in immune animals receiving one injection of $10^6$ or $10^5$ tumor cells. Analysis of variance plus an interaction term procedure indicated a significant difference ($P<0.001$) between immune and nonimmune animals at these doses of challenge tumor cells. The most clear-cut results were recorded when $10^4$ tumor cells were injected into immune and nonimmune recipients. Four of 13 immune animals receiving three repeated challenges developed 2-cm tumors after 50 days; however, 1 animal showed no tumor growth up to 120 days, and 8 immune animals developed tumors that never reached 2 cm over 120 days' observation. Of these 8, 5 showed gradual regression in tumor size beginning at 45 days, and 2 showed complete disappearance of palpable tumor. All animals with residual tumor nodules (2-10 mm) were still alive after 120 days. By contrast, 10 of 10 nonimmune animals receiving the same ($10^4$-cell) inoculum regime had 2-cm tumors at 40 days or less, and all eventually died of tumor by 55 days. Two animals given $1 \times 10^4$ MOPC-11 tumor cells resisted five such challenges, and at 70 days after their last dose (126 days after M-component immunization) showed no signs of gross tumor and had normal serum cellulose acetate electrophoresis patterns. However, when the size of tumor cell inoculum was increased to $10^7$ tumor cells, relative immunity induced by M-component preimmunization could be overcome (table 1).

The specificity of the immune response to MOPC-11 preimmunization is demonstrated in table 2. Animals were immunized with normal saline in CFA and IFA instead of being immunized with MOPC-11 component; the same dose schedule and the same challenge doses of MOPC-11 tumor were used. Other groups were immu-

### Table 1.—Resistance of immunized and nonimmunized mice to sc MOPC-11 challenge(s)

<table>
<thead>
<tr>
<th>Immune status</th>
<th>Number of MOPC-11 tumor cells</th>
<th>Number of mice in each group</th>
<th>Number of sc doses given every 14 days</th>
<th>Significance</th>
<th>Time, in days, until tumor nodule = 2 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune</td>
<td>$10^5$</td>
<td>6</td>
<td>1</td>
<td></td>
<td>12, 15, 15, 16, 18, 13 (av, 14.8)</td>
</tr>
<tr>
<td>Immune</td>
<td>$10^6$</td>
<td>8</td>
<td>1</td>
<td>$P&lt;0.001$</td>
<td>[18, 24, 31, 30, 45, 49, 49, 54 (av, 37)]</td>
</tr>
<tr>
<td>Nonimmune</td>
<td>$10^4$</td>
<td>8</td>
<td>1</td>
<td></td>
<td>[15, 16, 17, 16, 16, 22, 30, 30 (av, 20)]</td>
</tr>
<tr>
<td>Immune</td>
<td>$10^5$</td>
<td>8</td>
<td>1</td>
<td>$P&lt;0.001$</td>
<td>[28, 30, 43, 29, 45, 49, 56, 64 (av, 41)]</td>
</tr>
<tr>
<td>Nonimmune</td>
<td>$10^4$</td>
<td>8</td>
<td>1</td>
<td></td>
<td>[18, 18, 15, 19, 19, 21, 31 (av, 20)]</td>
</tr>
<tr>
<td>Immune</td>
<td>$5 \times 10^4$</td>
<td>2</td>
<td>1</td>
<td></td>
<td>No growth, no growth</td>
</tr>
<tr>
<td>Nonimmune</td>
<td>$5 \times 10^4$</td>
<td>3</td>
<td>3</td>
<td></td>
<td>36, no growth, no growth</td>
</tr>
<tr>
<td>Immune</td>
<td>$5 \times 10^4$</td>
<td>3</td>
<td>3</td>
<td></td>
<td>16, 16, 16, 22, no growth</td>
</tr>
<tr>
<td>Nonimmune</td>
<td>$10^4$</td>
<td>5</td>
<td>1</td>
<td>$P&lt;0.006$</td>
<td>[30, 55, 57, 54, no growth] b</td>
</tr>
<tr>
<td>Nonimmune</td>
<td>$10^4$</td>
<td>13</td>
<td>3</td>
<td></td>
<td>65, no growth, no growth, no growth</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>4</td>
<td></td>
<td>[26, 29, 31, 37, 40, 21, 24, 28, 40 c (av, 30)]</td>
</tr>
</tbody>
</table>

* No growth refers to no visible tumor after 60 days’ observation.

b Eight animals developed tumors that never reached 2 cm; 5 of the 8 showed regression in size of tumors beginning at 45 days; all 8 were still alive with tumor nodules (2-10 mm) after 120 days.

### Table 2.—Specificity of M-component immunization

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>Tumor challenge</th>
<th>Dose</th>
<th>Number of mice with tumor (2 cm)/No. tested</th>
<th>Time, in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (nonimmune)</td>
<td>MOPC-70A</td>
<td>$1 \times 10^4$</td>
<td>3/3</td>
<td>20, 21, 30</td>
</tr>
<tr>
<td>MOPC-11 M component—CFA+IFA</td>
<td>MOPC-70A</td>
<td>$1 \times 10^4$</td>
<td>3/3</td>
<td>18, 19, 28</td>
</tr>
<tr>
<td>None (nonimmune)</td>
<td>MOPC-11</td>
<td>$1 \times 10^4$</td>
<td>3/3</td>
<td>17, 17, 17 b</td>
</tr>
<tr>
<td>Normal saline—CFA+IFA</td>
<td>MOPC-11</td>
<td>$1 \times 10^4$</td>
<td>3/3</td>
<td>15, 17, 18</td>
</tr>
<tr>
<td>MOPC-11 M component—CFA+IFA</td>
<td>MOPC-11</td>
<td>$1 \times 10^4$</td>
<td>3/3</td>
<td>28, 30, 43 b</td>
</tr>
</tbody>
</table>

* Time until tumor diameter reached 2 cm.

b $P<0.025$
nized with MOPC-11 component and then challenged with a different IgG1 plasmacytoma (MOPC-70A). The results show tumor resistance only in the group immunized with MOPC-11 component and challenged by MOPC-11 tumor, which indicated that tumor protection was specific and dependent on M-component immunization. Furthermore, cross-challenge experiments were performed to extend these data. Groups of 5 mice each were preimmunized with MOPC-11, MOPC-315 (IgG), and MOPC-6A (IgA) and then challenged by sc implantation with each respective tumor. Specificity of relative protection correlated with the immunizing M component (table 3). We used a one-way analysis of variance to test the hypothesis that the means of all groups were equal. Each pair of means was then tested by the Newman-Keuls procedure, which takes into account all pairs of means rather than single pairs. Significant protection appeared to be present for all three M components tested ($P < 0.02$–0.001).

Attempts were next made to define the mechanism of tumor resistance in the BALB/c mice immunized with M component; $1 \times 10^7$ spleen cells from immune mice were collected 8 and 10 days after the first tumor challenge and cultured with $1 \times 10^6$ or $1 \times 10^4$ radioactive $^{31}$Cr-tagged MOPC-11 tumor cells in a $^{31}$Cr-release assay. In multiple experiments, however, no increased specific lysis was noted in comparison with results for normal mouse spleen cells. MOPC-11 M component was also linked to radioactive $^{31}$Cr-tagged mastocytoma cells, with carbodiimide as coupling agent. Using spleen cells obtained from immune mice 5, 7, 8, and 10 days post tumor challenge and varying the ratio of immune spleen cells to target cells from 50:1 to 200:1, we recorded no specific increase in target cell lysis compared with findings for normal, unimmunized spleen cells.

In addition, multiple attempts to detect direct cytotoxic antibodies by use of fresh suspensions of MOPC-11 tumor cells as targets and sera of several mice to both immune and normal groups initially showed only a positive electrophoresis and no visible sign of tumor. These animals did not develop tumor during observation for 100 days; at 100 days their serum electrophoretic patterns had reverted to normal. Thus an ip tumor cell dose of $1 \times 10^6$ plus either $1 \times 10^2$ immune or normal spleen cells allowed the immune system of some animals to reject a subclinical growth of tumor, evident initially only by transient appearance of M component in the sera.

Experiments were done with $1 \times 10^7$ spleen cells from immune animals, with a smaller dose ($1 \times 10^5$) of tumor cells given ip, and with $1 \times 10^5$ normal mouse spleen cells. At this dose of tumor cells, the time range within groups was greater prior to development of tumor in both the control and noncontrol groups. However, even with a 10,000:1 ratio of immune spleen cells to tumor cells, resistance to tumor was not increased among the adoptive, transferred immune mice.

All mice in this experiment either developed tumor or had sera positive for M component by cellulose acetate electrophoresis at or before 28 days. Sera of several mice from both immune and normal groups initially showed only a positive electrophoresis and no visible sign of tumor. These animals did not develop tumor during observation for 100 days; at 100 days their serum electrophoretic patterns had reverted to normal. Thus an ip tumor cell dose of $1 \times 10^6$ plus either $1 \times 10^2$ immune or normal spleen cells allowed the immune system of some animals to reject a subclinical growth of tumor, evident initially only by transient appearance of M component in the sera.

Using a different method of inducing immunity to plasmacytoma, Rollinghoff et al. (9) showed significant tumor resistance to doses of $1 \times 10^5$ tumor cells and protection with in vivo immune spleen cell transfers of recipients previously irradiated with 450 rads. Therefore, we tried a similar approach using normal recipient BALB/c mice irradiated with 400 rads and challenged with $1 \times 10^5$ or $1 \times 10^4$ spleen cells from immune or normal animals, plus $1 \times 10^5$ MOPC-11 tumor cells ip. No significant protection from tumor growth was recorded for the irradiated recipients.

<table>
<thead>
<tr>
<th>Immunizing M component a</th>
<th>Days survival in transplanted tumor strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MOPC-11 (IgG)</td>
</tr>
<tr>
<td></td>
<td>MOPC-6A (IgA)</td>
</tr>
<tr>
<td></td>
<td>MOPC-315 (IgG)</td>
</tr>
<tr>
<td>MOPC-11 (G) b</td>
<td>48, 63, 65, 67, 70+</td>
</tr>
<tr>
<td>MOPC-6A (A) c</td>
<td>17, 19, 20, 23, 24</td>
</tr>
<tr>
<td>MOPC-315 (G) d</td>
<td>23, 28, 30, 32, 32</td>
</tr>
</tbody>
</table>

* For each immunizing M component, a one-way analysis of variance was based on allowing equation: Days survival = overall mean + effect of transplanted tumor strain + random deviation.

† MOPC-11 preimmunization: $P < 0.001$ vs. challenge by MOPC-6A and MOPC-315.

‡ MOPC-6A preimmunization: $P < 0.001$ vs. challenge by MOPC-11 or MOPC-315.

§ MOPC-315 preimmunization: $P < 0.005$ vs. challenge by MOPC-6A, and $P < 0.025$ vs. challenge by MOPC-11.

![Table 3.—Survival post tumor transplantation in BALB/c mice immunized with specific M component protein](image-url)

![Table 4.—Cell transfers (ip) with immune spleen cells and MOPC-11 tumor cells](image-url)
To examine the possibility that the mechanism of tumor resistance was mediated largely by a cell type other than lymphocytes, we harvested PEC from immune animals (10 days post tumor challenge) and removed the adherent cells; 5 × 10^6 adherent PEC (50% of which were macrophages as determined by microscopic morphologic criteria and by uptake of peroxidase stain) were given ip with 1 × 10^6 MOPC-11 tumor cells alone or with 0.1 ml serum from immune or normal animals. Control groups consisted of adherent PEC from normal mice (58% of the PEC were macrophages), with similar numbers of tumor cells and immune or normal sera. The results indicated no significant tumor resistance over that shown by controls. Of interest was the finding that all 4 donors of immune PEC, after initiation of ascites by Marcol mineral oil, subsequently lost their immune status and developed tumor.

**DISCUSSION**

The clear evidence for immunity to mouse myeloma after preimmunization with specific M component, obtained in this study, confirmed previous reports (4, 5). The rate of MOPC-11 tumor formation in BALB/c mice depended both on the number of tumor cells injected and the route of injection; ip inoculations of tumor cells showed the greatest tumor-stimulating potential and sharpest end point, best seen in the ranges of 10^3 and 10^4 tumor cells. Mice immunized with MOPC-11 component could resist numerous sc challenge injections of 10^4 MOPC-11 tumor cells. Resistance to tumor challenge in immunized mice was less prominent with dose levels of 10^5 and 10^6 tumor cells. Similar results indicating low-dose tumor resistance have been reported by others (3, 4). Immunization with M component from one plasmacytoma did not protect against tumor formation from cells of another plasmacytoma. Protection appeared to depend on the M component's developing specific anti-idiotypic immunity to the specific M component involved. Tumor resistance induced by prior in vivo inoculations of intact plasmacytoma cells has resulted in a broader spectrum of immunity to other plasmacytomas (9), without so-called idiotypic restriction. The concept that plasmacytoma cell-surface M components or the secreted cell product may function like a tumor-specific antigen may be relevant to other conditions in which tumor cells have also been shown to bear restricted monoclonal determinants (20, 21).

Using intact or modified plasmacytoma cells to induce tumor immunity, several groups of workers (8, 9, 22, 23) demonstrated cellular recognition of tumor antigens in immune spleens. Specifically, Rouse et al. (8) showed that the cell principally responsible for protection in immune animals was a T lymphocyte present in PEC, spleen, and thoracic duct, and that macrophages have little or no function in immune protection in this system. In vivo transfers of 1 × 10^6 immune spleen cells have been able to prevent tumor challenges of 1 × 10^6 tumor cells in sublethrally irradiated recipients (9). Immune, cortisone-resistant thymocytes sensitized in vitro to plasmacytoma cells show significant lysis of tumor cells in 51Cr-release assays (10). Lymphocytes sensitized in vitro to plasmacytomas can confer tumor resistance in adoptive transfers with tumor cells (21).

Our attempts to demonstrate cell-mediated cytotoxicity in spleen cells from immune mice, with 51Cr-labeled MOPC-11 tumor cells or MOPC-11 component-tagged 51Cr-labeled mastocytoma cells as targets, were unsuccessful.

The difference between our results and those of others may be due in part to the antigens used in preparing the immune animals. The intact plasmacytoma cell carries many antigens (25–29). Whereas the isolated M component differs only with respect to the idiotypic determinant. Our data suggest that tumor immunity induced by an M component is considerably weaker than that conferred by preimmunization with the intact plasmacytoma cell.

The present studies confirm significant immunity engendered by prior immunization with isolated M components from mice; however, clear-cut predominance of either humoral or cellular immune mechanisms could not be demonstrated. Plasmacytoma M components probably do not represent strong, tumor-specific, antigenic systems as compared with other antigens represented on the same tumor cell surfaces. From the studies reported here, it seems unlikely that M components alone represent a major system participating in induction of tumor immunity in the mouse plasmacytoma model.

**REFERENCES**

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