SUPPRESSIVE VERSUS AUGMENTING EFFECT OF THE SAME PRETREATMENT REGIMEN IN TWO MURINE TUMOR SYSTEMS WITH DISTINCT EFFECTOR MECHANISMS*1

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The effect of presensitization with X-irradiated tumor cells on the development of host’s immune resistance against the tumor-associated transplantation antigens (TATA) was investigated in two syngeneic tumor systems with distinct effector mechanisms.

When X5563 plasmacytoma, to which immune resistance was mediated exclusively by killer T lymphocytes, was intravenously inoculated into syngeneic C3H/He mice with lower number after 7000 R X-irradiation, the mice failed to exhibit any protective immunity against the subsequent challenge with viable tumor cells. Moreover, these mice lost their capability to develop any immune resistance even after an appropriate immunization procedure. The immunodepression induced by such a pretreatment regimen was specific for X5563 tumor. While no suppressor cell activity was detected in the above pretreated mice, serum factor(s) from these mice was virtually responsible for this suppression. When the serum factor mediating this tumor-specific suppression was fractionated on the Sephadex G-200 column, the suppressive activity was found in albumin-corresponding fraction, free of any immunoglobulin component.

In contrast, in MM102 mammary tumor system, in which immune resistance is solely mediated by tumor-specific antibody, the pretreatment with X-irradiated MM102 cells augmented the induction of anti-tumor immunity.

These results indicate that while tumor antigens given in the form of X-irradiated tumor cells suppress the induction of killer T cell-mediated immunity in one system, the same presensitization regimen of tumor antigens augments the antibody-mediated immunity in another system, thus giving a divergent effect on the distinct effector mechanisms of syngeneic tumor immunity.

Various hypotheses have been presented with respect to the escape mechanism of tumors from host’s immune surveillance. The most important and interesting mechanism is that specifically suppressive potentials or factors may be generated in tumor-bearing host. According to one view, the specific suppressive factor may be either solubilized form of tumor antigens, anti-tumor antibody or their complex. According to the other view, the suppressive potentials may be the suppressor cells themselves or factors derived from suppressor cells. While in the past decade these inhibiting activities of suppressive potentials have been analyzed at the implementation phase of effector cells, it seems to be important to analyze further the effect of these immunosuppressive factors on the induction phase of effector system.

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*1 This constitutes Part I of a series entitled “Suppressive Effect of X-irradiated Tumor Cell Presensitization on the Induction of Syngeneic Tumor Immunity.”

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We reported previously that in the lethal murine tumor X5563 plasmacytoma system, the protective T cell population, as measured by the in vivo tumor neutralization test, could be developed around 7 days after the surgical resection of intradermally (id) implanted tumor. In contrast, in the tumor-bearing hosts whose tumors had not been removed, the development of such T cell activity was significantly suppressed. Interestingly, such T-cells suppression in tumor-bearing hosts could also be reproduced by the additional intravenous treatment with X-irradiated tumor cells after immunizing procedure of tumor resection. These results suggest that the presence of inadequate form of tumor antigens may fail in developing sufficient effective immunity to reject the tumor even though the host was potentially capable of being primed with tumor-associated transplantation antigens.

In the present study, we addressed ourselves to the question of how the precursors of effector cell population would be affected by the preexistence of tumor antigens at the primary induction phase, and whether such a phenomenon can be generalized to any kind of syngeneic tumor system. To do this, unprimed hosts were pretreated with intravenous (iv) inoculation of smaller number of X-irradiated tumor cells as a model system of small quantity of tumor antigens in the host, and the subsequent induction of immune resistance was analyzed. In this study, we utilized X5563 plasmacytoma and MM102 mammary tumor of C3H/He origin. The immune resistance mechanisms in these two tumor systems have been firmly established to be mediated exclusively by the immune T lymphocytes and tumor-reactive antibody, respectively.

It will be shown that the same tumor antigen-presensitization regimen does not always manifest the same outcome on the induction of tumor-specific immune resistance, but rather brings about divergent influences on the distinct effector mechanisms of syngeneic tumor immunity.

**Materials and Methods**

**Mice** C3H/He and DBA/2 mice of both sexes were used at 7~9 weeks of age.

**Tumors** X5563 plasmacytoma and MM102 mammary tumor, both derived from C3H/He strain and maintained by serial intraperitoneal passages into syngeneic C3H/He mice in the ascitic form, were utilized.

**Procedure for Induction of Effective Tumor-specific Immunity** C3H/He mice were intradermally (id) inoculated with 10⁶ of viable X5563 or MM102 tumor cells, followed by surgical resection of the tumor 7 days later.

**Pretreatment of C3H/He Mice with X-irradiated Tumor Cells** C3H/He mice were intravenously (iv) inoculated with 10³ to 10⁶ of 7000 R X-irradiated X5563 or MM102 tumor cells, 3 times at 4-day intervals.

**Procedure for Induction of Allogeneic Transplantation Immunity** C3H/He mice were intraperitoneally (ip) immunized with 10⁸ of DBA/2 spleen cells at 1 week intervals: Spleen cells were submitted to cytotoxicity assay 1 week after the last immunization, utilizing P815 mastocytoma cells (DBA/2 origin) and MOPC-70A plasmacytoma cells (BALB/c origin) as targets, respectively.

**In vivo Tumor Neutralization Test** The spleen from non-immunized or tumor-immunized mice were removed aseptically and single cell suspensions were prepared. One million viable X5563 tumor cells were mixed with 10⁸ normal or immune spleen cells and injected ip into normal syngeneic mice. Intraperitoneal tumor growth was measured by quantitation of myeloma protein in sera of tumor-inoculated mice with rabbit anti-idiotypic antibody, as described previously.

**In vitro Cytotoxicity Test** Cytotoxicity was determined by the method described previously. Briefly, tumor cells were radiolabeled with ³H-uridine. After the interaction of effector and target cells was allowed to proceed for 16 hr, the radioactivity remaining in the viable target cells was measured using a liquid scintillation counter. Cytotoxicity index (percentage specific killing) was calculated as follows:

\[
\text{Specific killing (\%)} = \left(1 - \frac{\text{cpm in the immune well}}{\text{cpm in the normal well}}\right) \times 100,
\]

where the normal or immune well denotes the viable target cells remaining after incubation with normal or immune spleen cells.
Antibody Response to Thymus-independent Antigen

ε-Amino-dinitrophenyl-lysine-substituted dextran (DNP-dextran) was prepared using a modification of the method of Fielder et al., as described by Haba et al. Two hundred micrograms of DNP-dextran was injected iv. Five days after injection, anti-DNP plaque-forming cells (PFC) in the spleen were enumerated by a modification of the hemolytic plaque technique, using trinitrophenyl-substituted sheep erythrocytes as indicator cells.

Fractionation of Serum from Mice Pretreated with X-irradiated tumor Cells on Sephadex G-200 Column

Four milliliters of serum was submitted to Sephadex G-200 column, and eluted with borate buffered saline (pH 8.6). Fractions of 5 ml each were collected.

RESULTS

Suppression of Acquisition of T Cell-mediated Immune Resistance against X5563 Plasmacytoma in Syngeneic Mice Pretreated with X-irradiated X5563 Tumor Cells

In X5563 plasmacytoma--C3H/He mouse combination, tumor-specific resistance could be induced by the id implantation of viable tumor cells, followed by the surgical resection of tumors (immunization procedure) and this immunity was demonstrated to be mediated exclusively by immune killer T lymphocytes. Effect of the pretreatment with X-irradiated tumor cells on the induction of effective immune resistance was investigated. The result in Fig. 1 shows that mice inoculated iv with 10⁶ of 7000 R X-irradiated tumor cells 3 times at 4-day intervals could not exhibit any resistance against subsequent challenge with 10⁶ viable tumor cells even after the above immunization procedure. Specificity of the inhibitory effect of the pretreatment was evaluated by determining the effect of the same pretreatment on immune responses against various antigens other than tumor-associated transplantation antigen (TATA) of X5563 tumor. The results in Table I demonstrated that this pretreatment did not affect the induction of immune resistance against MM102 mammary tumor despite positive inhibitory effect in the X5563 tumor system. Furthermore, as shown in Table II, the pretreatment did not exhibit any non-specific inhibition in B cell- and T cell-immune responses against thymus-independent antigen, DNP-dextran, or allo-antigens.

In our previous report, although T cell-mediated immunity involved in this tumor system can be measured by in vivo tumor neutralization test as well as the in vitro cytotoxicity test, the former assay was much more closely correlated with in vivo protective immunity. Thus, the effect of the pretreatment was also investigated by measuring the in vivo tumor-neutralizing activity mediated by spleen cells. The spleen cells (10⁶) from either unprimed mice (Group A), from mice which received the immunization procedure (Group B), or from mice which received the im-
Table I. Specificity of the Inhibitory Effect of Pretreatment on the Induction of Immune Resistance against Syngeneic Tumors

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Pretreatment(^a)</th>
<th>Immunization procedure(^b)</th>
<th>Tumor challenge(^c)</th>
<th>Incidence of resistance(^c) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>PC-implanted→Ope.</td>
<td>PC</td>
<td>6/7 (85.7)</td>
</tr>
<tr>
<td></td>
<td>10^8 irradiated PC (× 4)</td>
<td>PC-implanted→Ope.</td>
<td>PC</td>
<td>2/7 (28.6)</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>MM-implanted→Ope.</td>
<td>MM</td>
<td>7/7 (100.0)</td>
</tr>
<tr>
<td></td>
<td>10^8 irradiated PC (× 4)</td>
<td>MM-implanted→Ope.</td>
<td>MM</td>
<td>6/7 (85.7)</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>PC-implanted→Ope.</td>
<td>PC</td>
<td>4/6 (66.7)</td>
</tr>
<tr>
<td></td>
<td>10^8 irradiated PC (× 3)</td>
<td>PC-implanted→Ope.</td>
<td>PC</td>
<td>0/7 (0.0)</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>MM-implanted→Ope.</td>
<td>MM</td>
<td>5/6 (83.3)</td>
</tr>
<tr>
<td></td>
<td>10^8 irradiated PC (× 3)</td>
<td>MM-implanted→Ope.</td>
<td>MM</td>
<td>4/5 (80.0)</td>
</tr>
</tbody>
</table>

\(^a\) Pretreatment was made by the iv inoculation of 10^8 or 10^9 X-irradiated X5563 tumor cells (PC) 4 or 3 times.
\(^b\) The pretreated and non-pretreated mice were inoculated id with 10^6 of PC or MM102 tumor cells (MM) 4 days after the last inoculation of X-irradiated cells, followed by surgical resection of the tumor 7 days thereafter.
\(^c\) Mice were challenged id with 10^8 of indicated tumor cells 7 days after tumor resection.
\(^d\) The value represents the number of surviving mice out of total number of mice 4 weeks after the tumor challenge.

Table II. Specificity of the Inhibitory Effect of Pretreatment

<table>
<thead>
<tr>
<th>Group</th>
<th>B-cell response against DNP-dextran(^b)</th>
<th>Killer T-cell response against allogeneic (H-2(^d)) cells(^c) (% specific killing)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-DNP PFC/spleen</td>
<td>Exp. 1</td>
</tr>
<tr>
<td>Normal</td>
<td>69765 (1.18)</td>
<td>39.9</td>
</tr>
<tr>
<td>Pretreated(^a)</td>
<td>146717 (1.05)</td>
<td>34.9</td>
</tr>
</tbody>
</table>

\(^a\) The same pretreatment as in Fig. 1.
\(^b\) Normal or pretreated mice were immunized iv with 200 μg of thymus-independent antigen, DNP-dextran, 5 days after the last pretreatment. Five days thereafter, direct anti-DNP PFC were enumerated in the spleen, and expressed as the geometric mean of PFC in each group of 4 animals. The value in parentheses represents the standard error.
\(^c\) Both groups of mice were immunized ip with 10^6 of DBA/2 spleen cells twice at 1-week intervals. Spleen cells were submitted to cytotoxicity assay 1 week after the last immunization. Cytotoxicity assay was performed at an effector-to-target cell ratio of 100:1, using P815 mastocytoma cells (DBA/2 origin) and MOPC-70A plasmacytoma cells (BALB/c origin) as target cells in Exp. 1 and Exp. 2, respectively.

Table III. Lack of Tumor-neutralizing Activity of Spleen Cells from Pretreated Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment of donor mice of spleen cells used in tumor-neutralization test(^a)</th>
<th>Tumor growth(^b)</th>
<th>Mean survival time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immunization procedure</td>
<td>(myeloma protein in sera, μg/ml)</td>
<td>(days±SE)</td>
</tr>
<tr>
<td>A</td>
<td>−</td>
<td>10137 (1.36)</td>
<td>12.3±0.48</td>
</tr>
<tr>
<td>B</td>
<td>−</td>
<td>835 (1.63)</td>
<td>23.5±1.55</td>
</tr>
<tr>
<td>C</td>
<td>+</td>
<td>12712 (1.14)</td>
<td>11.5±0.65</td>
</tr>
</tbody>
</table>

\(^a\) Either normal mice (Group A), mice inoculated id with viable X5563 tumor cells followed by tumor resection (Group B), or mice pretreated with 10^8 of X-irradiated X5563 cells 3 times before the above immunization procedure (Group C) were sacrificed 7 days after the tumor resection. Spleen cells from these animals were mixed together with 10^6 of X5563 cells and inoculated ip into syngeneic normal mice.
\(^b\) Tumor growth was measured on day 9 by the quantitation of myeloma protein in sera and expressed as the geometric mean of 4 to 5 animals per group. The value in parentheses represents the standard error.
munization procedure after the pretreatment (Group C) were mixed with $10^6$ of X5563 tumor cells and inoculated ip into non-X-irradiated syngeneic normal mice. Intraperitoneal tumor growth was measured by the quantitation of myeloma protein in sera. As shown in Table III, any significant tumor-neutralizing activity could not be detected in the mice of Group C.

Thus, it can be concluded from the above results that such a pretreatment rendered the mice unable to develop any tumor-specific immune resistance and that this unsuccessful protection against tumor was further substantiated by the lack of tumor-neutralizing activities in the spleen cells from these animals. In the following work, the nature of the suppressive potentials responsible for this inhibition was investigated.

**Analysis of Suppressive Potentials in Mice Pretreated with X-irradiated Tumor Cells**

The involvement of suppressor cells or serum factors in the above inhibition was tested. First, examination was made to see whether the spleen cells from pretreated mice affect tumor neutralization mediated by immune spleen cells. As evident from the results in Table IV, when immune spleen cells were mixed with spleen cells from pretreated mice, the latter cell type did not induce any inhibitory effect on the tumor-neutralizing activity afforded by the former cell type. Thus, the iv presensitization of unprimed mice with X-irradiated tumor cells before immunization procedure resulted in inhibition of development of effector cell population. On the other hand, when the same treatment was made on the immune mice,

### Table IV. Failure to Detect the Suppressor Activity in the Spleen Cells from Pretreated Mice (at Effector Phase)

<table>
<thead>
<tr>
<th>Group</th>
<th>Spleen cell type used in tumor-neutralization test</th>
<th>Tumor growth$^a$ (myeloma protein in sera, µg/ml)</th>
<th>Mean survival time (days ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>$10^6$ normal</td>
<td>8094 (1.08)</td>
<td>10.2 ± 0.37</td>
</tr>
<tr>
<td>B</td>
<td>$5 \times 10^6$ normal + $5 \times 10^6$ immune$^a$</td>
<td>&lt;312</td>
<td>&gt;20</td>
</tr>
<tr>
<td>C</td>
<td>$5 \times 10^6$ immune$^a$ + $5 \times 10^6$ pretreated$^b$</td>
<td>&lt;312</td>
<td>&gt;20</td>
</tr>
<tr>
<td>D</td>
<td>$5 \times 10^6$ normal + $5 \times 10^6$ pretreated$^b$</td>
<td>7108 (1.12)</td>
<td>10.5 ± 0.29</td>
</tr>
</tbody>
</table>

$^a$ Mice which manifested complete resistance against ip challenge of $10^6$ viable X5563 cells after the immunization procedure.

$^b$ Mice which received 3 times iv inoculations with $10^4$ X-irradiated X5563 cells.

$^c$ Tumor growth was measured on day 9.

### Table V. Failure to Detect the Suppressor Activity in Lymphoid Cells from Pretreated Mice (at Induction Phase)

<table>
<thead>
<tr>
<th>Group</th>
<th>Mice</th>
<th>Immunization procedure</th>
<th>Incidence of resistance$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal</td>
<td>-</td>
<td>0/6 (0.0)</td>
</tr>
<tr>
<td>B</td>
<td>Normal</td>
<td>+</td>
<td>5/6 (83.3)</td>
</tr>
<tr>
<td>C</td>
<td>Pretreated$^a$</td>
<td>+</td>
<td>1/6 (16.7)</td>
</tr>
<tr>
<td>D</td>
<td>Transferred with spleen cells from pretreated mice$^c$</td>
<td>+</td>
<td>7/7 (100.0)</td>
</tr>
<tr>
<td>E</td>
<td>Transferred with thymus and bone marrow cells from pretreated mice$^c$</td>
<td>+</td>
<td>6/6 (100.0)</td>
</tr>
</tbody>
</table>

$^a$ The same pretreatment as in Table IV.

$^b$ Spleen cells ($10^8$) from pretreated mice were transferred iv one day before the immunization procedure.

$^c$ Thymus cells ($5 \times 10^8$) and $2 \times 10^8$ bone marrow cells from pretreated mice were transferred.

$^d$ No. of survivor/No. of challenged mice (%)
which already manifested complete resistance against tumor challenge after the immunization procedure, no significant inhibition could be induced against the tumor-neutralizing activity of the spleen cells from those immune animals (data not listed). These results, taken together with the results in Table IV, may imply that such a pretreatment affects primarily the developmental stage of effector cell population. Therefore, further experiments were designed to analyze the effect of lymphoid cells or serum factors on the induction phase of tumor-specific immunity.

Either normal mice (Group B), mice pretreated with $10^6$ irradiated tumor cells 3 times (Group C), or mice transplanted iv with $10^8$ spleen cells (Group D) or $5 \times 10^7$ of thymocytes and $2 \times 10^7$ bone marrow cells (Group E) from pretreated mice were implanted id with $10^6$ tumor cells, followed by the tumor resection 7 days thereafter (Table V). These mice and unprimed mice (Group A) were then challenged id with $10^6$ viable tumor cells 7 days after tumor challenge. The results shown in Table V indicate that the lymphoid cells from pretreated animals did not exhibit any suppressive activity in the induction of immune resistance.

In contrast to the failure of detecting the suppressor cell activity in lymphoid cells from the pretreated animals on the induction as well as the implementation stage of tumor immunity, serum from these pretreated animals exhibited quite a significant suppressive effect on the development of effector cell population (Table VI). Normal C3H/He mice were inoculated iv with 1 ml of serum from pretreated animals (Group C) or normal serum (Group B), and one day later, both groups of mice received the immunization procedure. Then the spleen cells from these two groups of mice and normal mice (Group A) were submitted to the in vivo tumor neutralization test. As evident from the results shown in Table VI, the development of effector cell population was eventually suppressed in the mice of Group C.

It is, thus, concluded that in this tumor system, the development of immune cell population is inhibited by serum factors but not by suppressor cells.

**Evidence for Non-association of Antitumor Antibody with Tumor-specific Suppressive Factor in the Sera from Pretreated Mice** In order to investigate whether any antibody, either in the free form or complex form with tumor antigen, is responsible for this suppression, the inhibiting activities of various fractions from pretreated sera through the Sephadex G-200 column were determined. Fig. 2 shows the gel filtration pattern of the sera from pretreated mice on Sephadex G-200 column. These three peaks will be denoted as IgM, IgG, and al-
DIVERGENT EFFECT OF TUMOR-ANTIGEN PRESENSITIZATION

bumin-corresponding fractions. Because of the possibility that IgG bound to TATA would shift from the peak of IgG to the left, both fractions of IgM and IgG were pooled together. Each fraction corresponding to alb-

Fig. 2. Gel filtration profile of the serum from pretreated mice on Sephadex G-200 column

bumin or IgM plus IgG was concentrated to 4 ml, equivalent to the original volume. Normal C3H/He mice were inoculated iv with 1 ml of each concentrated fraction or sera from normal or pretreated mice, followed by the immunization procedure. The development of *in vivo* tumor-neutralizing activity in the spleen cells from these animals is illustrated in Fig. 3. As evident from these results, the whole sera from pretreated mice again exhibited a significant suppressive effect on the development of effector cell population, and activity comparable to the whole sera was observed in the albumin-corresponding fraction. On the other hand, the IgM plus IgG-corresponding fractions failed to show any inhibitory activity. These results strongly suggest that humoral antibody may play no crucial role in the suppression of immune induction.

Elicitation of Antitumor Effect by the Same Pretreatment in MM102 Mammary Tumor System In order to interpret the above phenomenon as a general mecha-

Fig. 3. Inhibitory effect of albumin- or IgM plus IgG-corresponding fraction on the development of effector cell population

Normal C3H/He mice were treated iv with indicated fractions from Fig. 2. One day later, each group of mice received the immunization procedure. Seven days after the tumor resection, the tumor neutralization test was performed. Tumor growth was determined on day 15. The growth of $10^6$ X5563 tumor cells inoculated with normal unprimed spleen cells was 9873 ± 1.31 μg/ml.
nism of immunodepression in syngeneic tumor immunity, it seemed important to investigate whether the same pretreatment regimen of X-irradiated tumor cells would affect the induction of immune resistance in other syngeneic system in which anti-tumor antibody can be induced. As such a tumor model, MM102 mammary tumor derived from C3H/He mice was utilized and influence of pretreatment with X-irradiated MM102 tumor cells in the development of immune resistance was analyzed by the same protocol as for the X5563 tumor system in Fig. 1. These results are shown in Fig. 4. Similar to the observation in X5563 plasmacytoma system, the immunization procedure resulted in a significant protection against the challenge of $10^6$ MM102 tumor cells. However, in contrast to the X5563 tumor system, it was revealed that the pretreatment with X-irradiated MM102 tumor cells did not exhibit any inhibitory effect, but rather augmented the acquisition of immune resistance upon the immunization procedure. This protecting effect by the pretreatment was also observed when MM102 tumor cells were directly challenged into mice after the pretreatment without any immunization procedure. As shown by Exp. 1 in Fig. 5, the growth of MM102 tumors implanted into mice after pretreatment with

Fig. 4. Augmenting effect of pretreatment with X-irradiated MM102 tumor cells on the induction of immune resistance against MM102 tumor

Experimental protocol and legend are the same as in Fig. 1.

Fig. 5. Inhibitory effect of pretreatment with X-irradiated MM102 cells on the growth of $10^6$ MM102 cells

Normal C3H/He mice were inoculated iv with $10^6$, $10^5$, $10^4$ or $10^3$ of 7000 R X-irradiated MM102 cells 3 times at 4 day intervals. Four days after the final inoculation, each group of mice and non-pretreated mice were implanted id with $10^6$ viable MM102 cells. Tumor growth was measured 7 days after the tumor implantation.

<table>
<thead>
<tr>
<th>Exp</th>
<th>Dose of X-irradiated No. tumor cells in pretreatment</th>
<th>Tumor growth after id implantation of $10^6$ MM102 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$10^5 \times 3$</td>
<td><img src="image1.png" alt="Graph" /></td>
</tr>
<tr>
<td>2</td>
<td>$10^5 \times 3$</td>
<td><img src="image2.png" alt="Graph" /></td>
</tr>
</tbody>
</table>

- - - - - - - geometric mean and standard error.
10^6 X-irradiated MM102 cells was significantly suppressed compared with that in the non-pretreated control mice. Further investigation was made to see whether any conditions such as pretreatment with much smaller number of X-irradiated cells would induce tumor-enhancement. As evident from the results of Exp. 2 in Fig. 5, while the pretreatment with 10^3 to 10^6 X-irradiated cells was found to induce a slightly less protection than that with 10^6, it never brought about any tumor enhancement. Thus, from the results in Figs. 4 and 5, it can be concluded that in MM102 mammary tumor system, in which immune resistance is solely mediated by tumor-specific antibody, pretreatment with X-irradiated MM102 cells eventually augmented the induction of anti-tumor immunity without any enhancement of tumor growth.

**DISCUSSION**

In the present study, the effect of pretreatment with relatively small number of X-irradiated tumor cells on the subsequent induction of immune resistance against tumor was studied in two syngeneic tumor systems in which quite distinct immune resistance mechanisms have been demonstrated.9,10,28)

In X5563 tumor system, to which immune resistance was mediated exclusively by tumor-specific killer T lymphocytes, free form of tumor antigen in circulation released from pretreated X-irradiated tumor cells appears to suppress subsequent effective induction of tumor-specific immunity. In contrast, in MM102 mammary tumor system, to which immune resistance is solely mediated by tumor-reactive antibody, the same pretreatment regimen of X-irradiated MM102 cells augmented the induction of anti-tumor immunity without any enhancement of tumor growth.

Various studies concerning the effect of pretreatment with X-irradiated tumor tissue or soluble tumor antigen on the development of immune resistance against the homologous tumor have been reported. Some observed the tumor-enhancing effect,1,4,14,21,27) whereas the others demonstrated positive induction of tumor-resistance by such a pretreatment.16,17,19) It has been difficult to explain altogether the above mutually controversial phenomena. However, at least two explanations were entertained by Rao et al.21): (a) These discrepancies may result from the immunogenic activity and configurational changes of TATA in soluble tumor antigen preparations, and (b) the dose, route, and time schedule used in the presensitization may also influence the response of host's immune apparatus. In our system, X-irradiated tumor cells were used as the source of tumor antigens, and the same pretreatment regimen was employed in two syngeneic tumor systems. Therefore, in addition to the above two explanations, we may also be able to raise another possibility that the various outcome of pretreatment with tumor antigens may be ascribed, in part, to the difference in the host's effector systems responding to each respective tumor system. While this possibility remains to be confirmed by further experimentations, it seems important to know how such a divergent effect of pretreatment with tumor antigens on subsequent induction of immunity is induced in various tumor systems in a certain syngeneic mice, and how the host immune mechanisms would be affected by such a pretreatment regimen.

With respect to the suppressive potentials affecting the development of immune resistance against tumors, there are at least two possible explanations. Firstly, suppressor cells are activated in the pretreated animals, and those cells or factors derived from them suppress effector cell population. Such a suppressor cell activity was reported by Kölisch et al.15) In the X5563 tumor system, however, such suppressor cell activity was not found.
in the pretreated mice both at implementation and induction phases of immune T-cell activity. Another possible explanation is that humoral factors may be involved in the suppression of development of immune resistance by such a pretreatment. In fact, serum factors were found to be responsible for suppression in the X5563 tumor system (Table VI). In contrast to the nonspecific suppressive factors observed in some tumor-bearing systems, this suppressive activity was specific for X5563 tumor system, and this may suggest that those humoral factors could possibly be either free tumor antigens, anti-tumor antibody, or their complex.

It has been suggested that anti-tumor antibody is responsible for the enhanced growth of the tumor challenged after pretreatment with X-irradiated tumor tissue. It has also been recognized that the serum-blocking factors from the tumor-bearing hosts can inhibit the in vitro reactivity of effector cells in the implementation phase of immunity. Although these factors have been demonstrated to be the complex of tumor antigens and antibody, Nepom et al. reported recently that even if these factors are present in the form of antigen–antibody complex in the tumor-bearing hosts, the critical component for the effector cell blockade may be the part of tumor antigens. This was demonstrated by separating the complex and testing the effect of each component on the blocking of effector cell activity. As a matter of fact, in X5563 tumor system, when the serum from pretreated animals responsible for the inhibition of acquisition of immune resistance was fractionated on Sephadex G-200 column, the most potent inhibiting activity was found in the albumin-corresponding fraction, indicating no involvement of antibody in this inhibition. Thus, taking their results and ours collectively, it may be concluded that tumor antigens present in the serum exhibit a deleterious effect on the induction and implementation of T cell-mediated immunity without absolute requirement of association with anti-tumor antibody.

As a possible mechanism of the divergent effect of presensitization of tumor antigens on subsequent effective induction of tumor immunity in those two different tumor systems, the following postulation may be feasible: As we demonstrated in this study, the effective tumor-specific immunity could be induced in both tumor systems by the id inoculation of viable tumor cells followed by surgical resection of the tumor. Especially in the X5563 plasmacytoma system, as we demonstrated previously, TATA on the X5563 cells induce preferential generation of killer T-cell activity without any helper T-cell activity. In contrast, the TATA on MM102 tumor cells exclusively induce helper T-cell activity without any killer T-cell generation. Therefore, the divergent outcome of tumor antigen presensitization in these two different tumor systems may be ascribed, in part, to the inherent sensitivity differences of these respective T-cell populations to the antigens. This feasible postulation is indeed very important when one tries to establish a new immunotherapy model of these tumor systems. Hence, the easily inhibitable induction of tumor immunity in the X5563 system by pretreatment with tumor antigen may be due to the lack of helper T-cell activity which otherwise may be responsible for augmenting killer T-cell generation and eventually preventing tolerance induction of killer T-lymphocyte system. In this context, therefore, if the hosts were presensitized with tumor cells modified by additional antigenic determinants with which the helper T cell could react, the suppressive activity of pretreated tumor antigen will not function and definite augmentation of induction of protective immunity can be predicted. In fact, when C3H/He mice were sensitized with mitomycin-C-treated or X-irradiated tumor cells after modification with additional antigenic determinants (trinitrophenyl, TNP) under the condition in
which TNP-reactive helper T cells were generated and capable of reacting with TNP on the surface of tumor cells, definite augmentation of in vitro cytotoxic and in vivo protective immunity against the native X5563 tumor was observed (Hamaoka et al., in preparation). Thus, these results indicated that in this X5563 tumor system a smaller number of X-irradiated tumor cells per se may not be the effective immunogens but rather tolerogens to the killer T-cell population and therefore the other cell type such as helper T-lymphocytes which amplify killer T-cell generation may be required in the case of attenuated cell immunization. Thus, in the X5563 tumor system, the inappropriate presentation of TATA resulted in a failure to induce effective tumor-specific immunity. The cellular mechanism of this negative effector T-cell responses induced by inadequate form of TATA presentation is further under investigation.

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