TUMORICIDAL ACTIVITY OF PERITONEAL EXUDATE CELLS FROM RATS TREATED WITH MITOMYCIN C*1

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In spite of the wide use of anticancer drugs in local treatment for malignant pleural and peritoneal effusions, the mode of changes induced in regional antitumor mechanisms involving lymphocytes and macrophages is poorly understood. In recent studies, we demonstrated that intraperitoneal (ip) injection of Nocardia rubra cell wall skeleton resulted in the induction of tumoricidal activity of peritoneal macrophages,1) and also that the activity of the cells was considerably impaired after in vitro exposure to mitomycin C (MMC), but was not affected by the concurrent ip injection of MMC.2) The immunosuppressive effects of anticancer drugs have generally been considered to be comprehensive, but surprisingly, we found a significant cytolitic activity of peritoneal exudate cells (PEC) from rats injected with MMC alone. This communication is a preliminary report on the tumoricidal PEC induced by ip injection of MMC.

Male ACI/N rats used were 9 to 10 weeks old, and weighed approximately 200 g. One and 4 days after a single ip injection of 40 or 200 µg, in vitro cytolytic tests with PEC against 125I-iododeoxyuridine (UdR) labeled syngeneic fibrosarcoma, AMC-60 tumor cells, were performed by the method reported previously.3) Briefly, PEC were harvested by washing twice with Eagle’s minimum essential medium containing heparin and antibiotics, then washed, and resuspended in RPMI-1640 medium supplemented with 10% fetal calf serum (RPMI-FCS). The cytolysis test was performed in a volume of 1 ml of RPMI-FCS containing 1 x 10^6 whole PEC and 1 x 10^4 radiolabeled target cells in a plastic tube (Falcon Plastics, Oxnard, Calif.). When adherent PEC were used as effectors, nonadherent PEC were removed by extensive washings with a jet of the medium after incubation for 2 hr, and radiolabeled target cells suspended in 1 ml of RPMI-FCS were added. After culture for 24 hr at 37° in humidified air and 5% CO2, the radioactivities of the culture supernatant and the cell pellet were counted separately. Cytolytic activity was calculated by means of the formula indicated in Table I.

When rats were injected with 40 µg of MMC, cytolytic activity was not detectable on day 1 but became apparent on day 4 (Table I). The injection of 200 µg MMC, however, resulted in a significant increase in cytolytic activity of the PEC obtained on both days 1 and 4. This may indicate a dependence of the reactivity on time and dose of MMC given. Fractionation by allowing PEC to adhere to the bottom of a plastic tube revealed the adherent PEC to be the cell type responsible for the cytolytic activity. Accordingly, the present results suggested strongly the association of macrophages with the tumoricidal activity of PEC induced by ip injection of MMC. This seems to be in op-
position to the general concept that cancer
drugs are also suppressive for various immune
cells. On the other hand, the present results
seem to be in agreement with the reported
induction of macrophage cytostatic activity
by treatment with adriamycin and cyclo-
phosphamide.3)

Though the mechanism(s) responsible for
the present phenomenon remains obscure,
it may be related to direct action of MMC
on the differentiation of a series of macro-
phages.

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### Table I. Cytolytic Activity of Peritoneal Exudate Cells (PEC) from Rats Injected with Mitomycin C (MMC)

<table>
<thead>
<tr>
<th>Dose of MMC (µg)</th>
<th>PEC harvested on day</th>
<th>No. of rats</th>
<th>Mean PEC yield (\times 10^7)</th>
<th>Cytolytic activity* (%)</th>
<th>Mean ± SE</th>
<th>Adherent PEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>—</td>
<td>4</td>
<td>1.1</td>
<td>2.1 ± 0.4</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>1</td>
<td>4</td>
<td>1.9</td>
<td>2.6 ± 0.7</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3</td>
<td>1.1</td>
<td>16.3 ± 2.2</td>
<td>9.9 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>1</td>
<td>5</td>
<td>1.4</td>
<td>19.3 ± 3.3</td>
<td>16.8 ± 3.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6</td>
<td>1.9</td>
<td>37.7 ± 3.7</td>
<td>28.1 ± 2.2</td>
<td></td>
</tr>
</tbody>
</table>

* Radiolabeled tumor cells \(1 \times 10^4\) cells were cultured with \(1 \times 10^4\) whole PEC and the resulting fraction of adherent PEC for 24 hr, and cytolytic activity was calculated by means of the following formula:

\[
\text{Cytolytic activity (\%)} = \frac{\text{cpm of } ^{125}\text{I of the supernatant}}{\text{cpm of } ^{125}\text{I of the supernatant and of the cell pellet}} \times 100 - \text{spontaneous release}
\]

ND = Not detectable.

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### REFERENCES