Role of Inflammatory Neutrophils in Antitumor Effects Induced by Intraperitoneal Administration of Corynebacterium parvum in Mice

Alan K. Lichtenstein, Jonathan Berek, Janet Kahle, and Jacob Zigelboim

Department of Medicine, Wadsworth Veterans Administration Hospital, Los Angeles, California 90073; and Departments of Gynecological Oncology (J. B.) and Microbiology and Immunology (J. Z.), UCLA Medical Center, Los Angeles, California 90024

ABSTRACT

We studied the role of inflammatory neutrophils in the antitumor effects that follow i.p. injection of Corynebacterium parvum (1400 μg) into C3HeB/FeJ mice challenged with the murine ovarian teratocarcinoma. Peritoneal neutrophils, obtained from mice 6 hr after injection of C. parvum, exerted significant antitumor effects when injected admixed with murine ovarian teratocarcinoma cells into the peritoneal cavities of normal mice. Treatment of recipient mice with whole-body irradiation or repeated injections of silica prevented the antitumor effects, indicating that neutrophils were activating a second effector mechanism in recipient mice. Peritoneal cells obtained at 24 or 72 hr or at 7 or 11 days following C. parvum injection were considerably less effective in activation of this effector mechanism. Heat-killed C. parvum (6 hr)-induced neutrophils activated antitumor responses, but thioglycolate-induced cells were without effect. Antitumor responses in mice receiving peritoneal neutrophils were not due to simple transfer of C. parvum organisms in the inocula. These results indicate that inflammatory neutrophils, elicited into the peritoneal cavity by injection of C. parvum, play an important role in the activation of subsequent antitumor effects.

INTRODUCTION

Many BRMs with antitumor properties in mice are microbial products (1) that induce acute inflammatory responses at the site of injection (9). PMNs are the initial host cells infiltrating these sites. It is well accepted that these cells, by virtue of their inherent bactericidal properties, play an important role in the clearance of infectious organisms (5, 19). In addition, PMNs activate a second antibacterial mechanism through their release of chemotactic factors that induce the influx of bactericidal mononuclear cells (18).

In contrast to the above, the role of PMNs in tumor rejection is not well established. However, several studies have documented that PMNs, if appropriately stimulated, have tumoricidal properties (4, 6, 10, 15). During an investigation of BRM-induced tumor rejection in a murine ovarian cancer model, we also documented that inflammatory PMNs could directly lyse tumor cells (13). Tumor-lytic PMNs were detected in the PC between 6 and 72 hr following i.p. administration of Corynebacterium parvum, and correlated temporally with the onset of in vivo tumor cytology (13). In addition, ovarian cancer cells could not be detected in peritoneal fluids 48 hr after treatment (13), suggesting that tumor rejection was rapidly accomplished by PMNs.

We have continued our investigation by using a bioassay as a more objective test to determine when tumor rejection was completed. We anticipated that mice treated with transfer of peritoneal cells from C. parvum-injected donors would not demonstrate tumor outgrowth if transfer occurred 48 hr after treatment. In contrast, the results of the bioassay and other experiments, reported herein, indicate that: (a) tumor rejection is not completed until 7 to 10 days following treatment; and (b) in addition to direct tumor lysis, inflammatory PMNs activate a second distinct effector mechanism which is required for tumor rejection. Thus, the antitumor effects of PMNs in this model closely resemble their antibacterial effects. They both involve a direct interaction with the target (tumor or bacterial cell), followed by an activation of a second distinct effector mechanism.

MATERIALS AND METHODS

Mice. Female C3HeB/FeJ mice were obtained at 6 to 8 weeks of age, and rested in our vivarium for at least 1 week prior to initiation of an experiment. Mice were obtained from The Jackson Laboratory (Bar Harbor, ME).

Tumor. The MOT (7), a gift from Dr. R. Bast, is maintained by weekly i.p. passage in C3HeB/FeJ mice. Tumor growth remains confined to the PC in this model. In addition, the tumor is susceptible to regional immunotherapy with C. parvum. Injection (i.p.) of C. parvum, 1400 μg, 24 hr after challenge with 10⁶ MOT cells cures 70 to 95% of mice (2, 11, 20).

Tumor Challenge. Mice are given injections i.p. of 10³ viable tumor cells in 0.2 ml PBS (Grand Island Biological Co., Grand Island, NY), adjusted to pH 7 with NaOH, and receive i.p. treatment with 0.2 ml of PBS, thioglycolate (4%), or C. parvum (strain CN6134, Burroughs Wellcome Co., Research Triangle Park, NC).

Peritoneal Lavage and Tumor Cell Enumeration. At varying intervals after injection, mice were sacrificed and their PCs were lavaged by rapid injection of 10 ml of cold PBS. Peritoneal fluid was withdrawn and reinjected 2 more times. After a washing, cells were counted, and cytospin preparations were made. After staining with Wright-Giemsa, the percentage of tumor cells was determined in at least 300 cell differentials. Total tumor cell burden was calculated by multiplying total peritoneal cell yield by percentage of tumor cells. Tumor cell burden per mouse is presented as mean ± S.D. of 5 to 6 mice per time point.

Bioassay. MOT cells (1 × 10⁶) were injected i.p. into C3HeB/FeJ mice. C. parvum (1400 μg), PBS, or thioglycolate (4%) was injected i.p. 24 hr later. At various times after treatment, mice were sacrificed and peritoneal cells (host plus tumor) were obtained by lavage. Peritoneal cells were centrifuged once (1200 rpm for 4 min), resuspended in 0.4 ml PBS, and reinjected into naive mice. Each naive recipient mouse received...
Neutrophil-mediated Antitumor Effects

the total peritoneal cell contents of one donor mouse. The development of ascites and mortality was assessed in transfer-treated mice. In preliminary experiments, we performed the bioassay by transferring peritoneal cell contents from untreated mice 4 hr after i.p. tumor challenge. Recipient mice of peritoneal cells obtained 4 hr after injection of 10^5 tumor cells demonstrated progressive tumor growth, while those receiving cells from mice challenged with 5 × 10^6 did not. This suggests that when recipient mice of the bioassay do not experience tumor growth, the tumor burden in the treated donor mice had been decreased below 10^5 tumor cells.

Winn Assay. MOT cells were obtained from a passaged mouse, washed, and resuspended to a final concentration of 5 × 10^5 cells/ml. PEC, obtained at varying times after i.p. C. parvum injection (1400 μg), were washed and resuspended to the desired concentration. MOT cells (0.2 ml) and PEC (0.2 ml) were then mixed together immediately before injecting (0.4 ml) into naive mice i.p. Spleen cells from C. parvum-injected mice were used as controls. They were obtained by expression of the spleen through wire mesh, followed by 3 washes. Cells from at least 2 spleens were pooled, counted, resuspended to desired concentration, admixed with tumor cells (0.2 ml of effectors with 0.2 ml of tumor cells), and injected i.p. into naive mice. The development of ascites and mortality was assessed in transfer-treated naive mice.

WBI. WBI (250 or 500 rads) was delivered by an X-ray generator (250 kV, 12 ma, 0.3 mm Cu and 2 mm Al) at a rate of 100 rads/min. WBI was administered to recipient mice of the Winn assay 24 hr prior to i.p. injection.

Silica Treatment. Silica crystals (Sigma Chemical Co., St. Louis, MO; 5 μm in diameter) were resuspended (40 mg/ml) in PBS, pH 7.4. Ultrasonic vibration was used to completely disperse the crystals. One ml of the suspension was injected i.p. on Days 2, 3, and 5 after transfer of the Winn assay inocula.

Percoll Gradient Fractionation of PEC. Percoll fractionation of PEC was performed by a modification of the procedure of Hamburger et al. (8). Briefly, 70% percoll was prepared by mixing 27 ml of Percoll (Pharmacia Fine Chemicals, Piscataway, NJ) with 3 ml of 10× RPMI and 12.85 ml of 1× RPMI. The solution was adjusted to pH 7.0 with NaOH, and to an osmolality of 285 with 10× RPMI. The self-generating gradient was formed by mixing 1 ml of peritoneal cells (1.5 × 10^7/ml in RPMI and 10% fetal calf serum) with 8 ml of 70% Percoll in 10-ml high-speed centrifuge tubes. Tubes were spun at 60,000 × g for 20 min at 25° in a Beckman high-speed centrifuge, using a Ti-50 fixed-angle rotor. The PMN-enriched, macrophage-depleted fraction was found to be band corresponding to a density range of 1.075 to 1.090, as determined by density-marker ultracentrifugation using a Ti-50 fixed-angle rotor. The PMN-enriched, macrophage-depleted fraction was found in a band corresponding to a density range of 1.075 to 1.090, as determined by density-marker ultracentrifugation using a Ti-50 fixed-angle rotor.

Heat Killing of PEC. Peritoneal cells were killed by incubation in a 56° water bath for 45 min. After treatment, viability was 0%, as judged by trypan blue exclusion.

Labeling of C. parvum and Quantification of C. parvum in PEC. C. parvum was labeled with 125I by the technique of Hamburger et al. (8), previously described (17). Briefly, 5 ml of organisms were washed 3 times in 0.9% NaCl solution and were resuspended in PBS. 125I (2 mCi) ([radiochemical Centre, Amersham, England) was added, followed immediately by 0.4 ml chlorine-T (5 mg/ml). After 5 min, 0.4 ml sodium bisulfite (25 mg/ml) was added, followed by 0.4 ml sodium iodide (10 mg/ml). The suspension was washed once in 0.9% NaCl solution and dialyzed for 5 days against daily changes of 2 liters of 0.9% NaCl solution at 4°. Suspensions were stored at 4°. Less than 1% of 125I was released after incubating labeled C. parvum for 24 hr at 37°. Fourteen hundred μg of 125I-labeled C. parvum (430,000 cpm) were injected i.p. into C3H/HeJ mice. Six hr later, mice were sacrificed and peritoneal cells were obtained by lavage. Cells were washed 3 times and then counted in a γ counter. Less than 1% of total cpm was present in the supernatant of the last wash. The amount of 125I contained in 6- hr PEC was 24.6 ± 3% (S.D.) of injected cpm (for 6 mice). The total number of cells retrieved 6 hr after i.p. injection of C. parvum (1400 μg) was 5.2 ± 0.6 (×10^6 cells/mouse). Thus, 9.5% of the initially injected dose of C. parvum was contained in 2 × 10^4 host PEC.

Statistics. Statistically significant differences between survival experiments were determined by the Gehan modification of the generalized Wilcoxon method. The t test was used for all other p value determinations.

RESULTS

Tumor Rejection Completed by 7 to 10 Days following i.p. Injection of C. parvum. Our initial studies documented that in vivo tumor cytolysis begins between 6 and 24 hr following i.p. injection of C. parvum (13). In addition, by 48 hr following treatment, tumor cells are not detected by microscopic examination of peritoneal fluids (13). This early time period (6 to 48 hr posttreatment) coincided with a rapid influx of tumor-lytic PMNs into the PC (13). Collectively, the data suggested that tumor rejection was completed by PMNs within 48 hr of C. parvum administration. To confirm this notion, a bioassay (Table 1) was used. At varying intervals after treatment with PBS, C. parvum, or thiglycolate, tumor-challenged mice were sacrificed and their peritoneal cells (tumor and host) were transferred directly into the PC of normal recipient mice. A separate group of mice challenged with 10^5 tumor cells and treated with 1400 μg of C. parvum was observed as a treated-control group (Table 1, Group A). Mice transferred with peritoneal cells from PBS- or thiglycolate-injected donors all succumbed to progressive tumor growth within 24 days (data not shown). When peritoneal cell transfer occurred 3 days after treatment with C. parvum, tumor outgrowth occurred in all mice (Table 1). In contrast, only 41% of progressive tumor growth.

Table 1

<table>
<thead>
<tr>
<th>Time of transfer after C. parvum injection</th>
<th>Survival of mice dying of tumor growth*</th>
<th>No. alive at 78 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Treated-control group, cells transfused</td>
<td>26 days (1 mouse)</td>
<td>11</td>
</tr>
<tr>
<td>B. 6 hr</td>
<td>34.4 ± 3.6°</td>
<td>9</td>
</tr>
<tr>
<td>C. 3 days</td>
<td>11</td>
<td>32 ± 6.2</td>
</tr>
<tr>
<td>D. 7 days</td>
<td>12</td>
<td>38.7 ± 4.1</td>
</tr>
<tr>
<td>E. 10 days</td>
<td>12</td>
<td>36.38 (2 mice)</td>
</tr>
</tbody>
</table>

* Data are presented as survival in days following transfer of recipient mice dying of progressive tumor growth.

Twelve mice were challenged with 10^6 tumor cells, treated with C. parvum i.p., 1400 μg 24 hr later, and observed for tumor outgrowth.

° Mean ± S.D.

* Survival significantly different than control (Line A); p < 0.05.
of mice treated with transfer of cells obtained 7 days after treatment exhibited progressive tumor growth. If transfer occurred 10 days after treatment, survival of recipient mice was comparable to the treated-control group. Thus, tumor rejection is not completed until 7 to 10 days after treatment. Since tumorlytic peritoneal PMNs are only detected between 6 and 72 hr after C. parvum injection, a second distinct antitumor mechanism must be activated in treated mice to complete the process of tumor rejection.

PEC Obtained 6 Hours after Treatment and Activation of a Second Antitumor Response. In addition to the above conclusion, the bioassay (Table 1) demonstrated that recipients of peritoneal cells, obtained 6 hr after C. parvum injection (Group B), demonstrated survival comparable to the treated-control group. Since viable tumor cells must have been present in these transferred inocula, the results suggested that donor PEC interacted with host cells of recipient mice to complete tumor rejection some time after transfer. This phenomenon was further studied by a Winn assay. As shown in Table 2, PEC obtained 6 hr after C. parvum injection exert an antitumor effect when injected admixed with fresh tumor cells into the PCs of normal mice. At an E:T of 200:1, tumor outgrowth is completely prevented in 53% of mice. At lower E:T, the anti-tumor effect is diminished. In addition, equivalent numbers of spleen cells, obtained from the same C. parvum-injected mice, have no effect.

In the next experiment, recipient mice were either treated with WBI prior to transfer, or were repeatedly given injections of silica beginning on Day 2 after transfer. When 6-hr PEC were mixed with tumor cells and transferred into mice pretreated with WBI, antitumor effects were abrogated (Chart 1). Tumor protection was absent at 500 rads and was significantly decreased at 250 rads. Although mice receiving 500 rads and tumor cells (with or without effectors) demonstrated significant early radiation toxicity (hunched posture, weight loss), tumor growth was documented in all mice, either by the development of ascites, or by microscopic identification of >10⁶ MOT cells within the PC immediately prior to death. Early death in some irradiated, tumor-challenged mice not receiving effector cells appeared to be due to the combination of radiation toxicity and tumor growth.

Repeated administration of silica to recipient mice beginning on the second day after injection of the Winn assay inoculum also prevented antitumor effects (data not shown). Silica injections had no effect on the survival of mice receiving tumor cells alone. The results of these 2 experiments confirm that 6-hr peritoneal cells prevent tumor growth by activating a second

<table>
<thead>
<tr>
<th>Host cells transferred</th>
<th>E:T</th>
<th>n</th>
<th>Survival of mice dying of tumor growth</th>
<th>No. alive at 75 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor cells alone, 10⁶</td>
<td>12</td>
<td>12</td>
<td>24 ± 2²</td>
<td>0</td>
</tr>
<tr>
<td>Peritoneal cells</td>
<td>200:1</td>
<td>15</td>
<td>40.2 ± 6.6</td>
<td>9²</td>
</tr>
<tr>
<td>Peritoneal cells</td>
<td>100:1</td>
<td>15</td>
<td>38 ± 4.4</td>
<td>3²</td>
</tr>
<tr>
<td>Peritoneal cells</td>
<td>10:1</td>
<td>15</td>
<td>34.6 ± 7.3</td>
<td>1²</td>
</tr>
<tr>
<td>Spleen cells</td>
<td>200:1</td>
<td>8</td>
<td>28 ± 2.2</td>
<td>0</td>
</tr>
<tr>
<td>Spleen cells</td>
<td>100:1</td>
<td>8</td>
<td>25.4 ± 2.3</td>
<td>0</td>
</tr>
</tbody>
</table>

a Survival in days following injection for mice dying of tumor outgrowth.

b MOT cells (10⁶) injected alone i.p.

c Mean ± S.D.

d Survival significantly different than that for tumor-injected control mice (top line); p < 0.05.

**Chart 1.** Abrogation of neutrophil-mediated antitumor effects by prior WBI of recipient mice. Peritoneal effector cells (2.5 x 10⁶) obtained 6 hr after i.p. injection of C. parvum (1400 μg) were mixed with 10⁶ MOT cells (E:T = 250:1) and were injected into the PCs of normal or WBI-treated mice. Survival curves of mice receiving tumor alone, 250-rad irradiated mice receiving tumor alone, 500-rad-irradiated mice receiving tumor alone, normal mice receiving PEC and tumor, 250-rad-irradiated mice receiving PEC and tumor, and 500-rad-irradiated mice receiving PEC and tumor. Twelve mice were used per group in one experiment. Survival of irradiated mice (250 and 500 rads) receiving PEC and tumor is significantly (p < 0.05) decreased compared to survival of normal mice receiving PEC and tumor.

**Chart 2.** Tumor cytoreduction in PCs of mice receiving PEC-tumor cell Winn assay inoculum. Tumor cell counts in peritoneal fluids retrieved from normal (△) or preirradiated (○) mice at varying time intervals after i.p. injection of the Winn assay inocula (2.5 x 10⁶ PEC + 10⁶ MOT cells). Data presented as MOT cells/mouse. Points, mean of 6 mice/time point; bars, range. Zero time is actually 4 hr after inoculation of Winn assay inocula.
Neutrophil-mediated Antitumor Effects

Morphological examination of peritoneal fluids of recipient mice indicate that this second antitumor mechanism begins between 24 and 72 hr following transfer (Chart 2), and continues beyond 72 hr after injection of 6-hr PEC.

PEC Obtained 6 Hours after *C. parvum* Treatment and Effectiveness in Activation of Antitumor Mechanism in Recipient Mice. Chart 3 demonstrates that peritoneal cells obtained 6 hr after *C. parvum* injection were the most effective at transferring tumor protection in the Winn assay. Peritoneal cells obtained at 24 or 72 hr or at 7 days following *C. parvum* injections exerted only modest antitumor effects. Cells obtained 11 days after *C. parvum* (Chart 3), at 6 hr, and at 1, 3, 7, and 11 days after thioglycolate injection (data not shown) had no effect on tumor growth.

Neutrophil Transfer and Tumor Protection in the Winn Assay. The proportion of neutrophils (PMNs) in peritoneal fluids peaks at 6 hr after *C. parvum* injection (92%), is maintained at 24 hr (75%), and then rapidly decreases (42% at 48 hr; 27% at 72 hr; 12% at Day 7; and 8% at Day 11). The proportion of PMNs correlated with the ability of PEC to prevent tumor outgrowth in the Winn assay (Chart 3), suggesting that these were the critical cells that activated antitumor effects in recipient mice. To confirm or refute this notion, a continuous Percoll gradient was used to enrich peritoneal cell populations for PMNs. As shown in Table 3, PMN-enriched (and macrophage depleted) host cells effectively transferred protection in the Winn assay.

The PMN-enriched inoculum contained a small number of peritoneal lymphocytes (Table 3). Since stimulated T-lymphocytes are capable of recruiting other effector cells, it was possible that they were instrumental in activating a second effector mechanism. To address this question, 6-hr *C. parvum*-induced PEC were treated with monoclonal anti-TH antibodies and complement prior to transfer to recipient mice. Table 3 demonstrates that T-lymphocyte depletion did not affect the ability of 6-hr PEC to adoptively transfer tumor protection.

Heat-killed PMNs and Effective Transfer of Tumor Protection. To investigate the neutrophil requirements for effective transfer of tumor protection, 6-hr *C. parvum*-induced PMNs were killed by incubation at 56°C for 45 min prior to transfer in the Winn assay (using an E:T of 250:1). Survival of mice receiving heat-killed PMNs (15 of 17 mice alive 75 days after tumor challenge) was comparable to those receiving viable cells (12 of 17 mice alive at 75 days).

PMN-mediated Tumor Protection and Simple Transfer of *C. parvum* Organisms. Since heat-killed PMNs were effective in transferring tumor protection, it was possible that simple transfer of *C. parvum* organisms in the Winn assay inocula induced tumor rejection in recipient mice. Utilizing 125I bound to *C. parvum* organisms, we determined that 10% of the originally injected dose of bacteria was being transferred in 2 × 10⁶ 6-hr PEC (in E:T of 200:1). Table 4 demonstrates that injection of this dose of free organisms (140 μg) was ineffective in preventing tumor outgrowth, while the same number of organisms in the 6-hr PMN inoculum is effective. These results confirm our previous studies (2), where antitumor effects were not detected with injection of *C. parvum* doses below 350 μg. Thus, the detected antitumor response is above and beyond what would be expected from simple transfer of an equivalent number of organisms.

---

**Table 3**

**Demonstration of ability of PMN-enriched and T-lymphocyte-depleted peritoneal cell populations to transfer antitumor protection in Winn assay**

<table>
<thead>
<tr>
<th>Source of effector</th>
<th>Lympohocytes</th>
<th>Macrophages</th>
<th>Treatment with anti-TH and complement</th>
<th>No. of transferred mice alive at 75 days²</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Control, 10⁶ tumor cells alone</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/8</td>
</tr>
<tr>
<td>B. 6-hr PEC, unfraccionated</td>
<td>88</td>
<td>5</td>
<td>7</td>
<td>68³</td>
</tr>
<tr>
<td>C. 6-hr PEC, Fraction 4 (PMN enriched)</td>
<td>95</td>
<td>4</td>
<td>1</td>
<td>5,9⁴</td>
</tr>
<tr>
<td>D. 6-hr PEC, unfraccionated</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>7/8</td>
</tr>
</tbody>
</table>

*Data are presented as number of mice alive at 75 days after injection per number of mice given injections. Mice in Group A all died within 32 days of tumor inoculation. Survival times of mice dying of tumor outgrowth in Groups B, C, and D were comparable and ranged from 36 to 50 days. *(Significantly better survival; p < 0.05 compared to control (Group A)).

---

**November 1984**

5121
Although we have not been able to detect tumoricidal macrophages, we have identified macrophages cytostatic to MOT cells (10⁴) injected i.p., immediately followed by either (B) i.p. injection of C. parvum, 1400 μg, (C) i.p. injection of C. parvum, 140 μg, or (D) 2 x 10⁶ PEC obtained from donor mice 6 hr following i.p. injection of C. parvum, 1400 μg.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Dying of tumor growth</th>
<th>No. alive at 75 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Tumor alone, 10⁴</td>
<td>7</td>
<td>29.5 ± 3.6⁰</td>
<td>0</td>
</tr>
<tr>
<td>B. Tumor + C. parvum (1400 μg)</td>
<td>8</td>
<td>34, 36 (2 mice)</td>
<td>0⁶</td>
</tr>
<tr>
<td>C. Tumor + C. parvum (140 μg)</td>
<td>8</td>
<td>32.4 ± 0.7</td>
<td>0</td>
</tr>
<tr>
<td>D. Tumor + 6-hr PEC (10⁶ + 2 x 10⁷)</td>
<td>8</td>
<td>34.4 ± 4.1</td>
<td>5⁸</td>
</tr>
</tbody>
</table>

⁰ Mean ± S.D.
⁶ Survival significantly better than Control Group A; p < 0.05.

**DISCUSSION**

The results of this study emphasize the critical role played by inflammatory PMNs in the process of tumor rejection induced by regional immunotherapy with BRMs. In this particular animal model, PMNs have 2 distinct antitumor effects: (a) they rapidly lyse tumor cells within the PC beginning between 6 and 24 hr following treatment; and (b) they activate a subsequent effector mechanism that completes tumor rejection by 7 to 10 days after treatment.

Although the proportion of PMNs in peritoneal fluids obtained 24 hr after C. parvum treatment is similar to that of 6-hr PECs, the former were considerably less effective in transferring tumor protection (Chart 3). The major morphological difference between these populations is that PMNs obtained at 6 hr are engorged with bacterial organisms (>30/cell in >75% of PMNs), while those present at 24 hr contain many fewer organisms (1 to 5/cell in <50% of cells). In addition, nonviable C. parvum-induced PMNs activate significant antitumor responses, but 6-hr thiglycollate-induced PMNs are without effect. These data suggested that tumor rejection in recipient mice was simply due to the transfer of a sufficient number of bacterial organisms, either free or cell associated, in the Winn assay inocula. However, Table 4 demonstrates that antitumor effects are much greater than would be predicted from the amount of corynebacteria present in these inocula. This indicates that although organisms and PMNs must be present in the Winn assay inocula, they interact synergistically to stimulate effector mechanisms in recipient mice.

Most of the results of this study are consistent with the hypothesis that PMN-activated secondary antitumor effects are mediated by macrophages. (a) They were not detected if recipient mice were pretreated with WBI or repeated injections of silica. By preventing entry of blood-borne monocytes into inflammatory exudates (WBI; Ref. 16) and exerting nonspecific toxic effects on mononuclear phagocytes (silica; Ref. 12), these manipulations prevent the activation of peritoneal macrophages. (b) Chapes and Haskell (3) have demonstrated that PMNs elicited by i.p. injection of C. parvum and transferred into the PC of normal mice, activate tumoricidal macrophages. In their study, macrophage activation was equally achieved by injection of nonviable 6-hr PMNs, but PMNs obtained 48 hr after C. parvum injection were ineffective. These conditions are quite similar to those of PMN-mediated antitumor effects in the Winn assay. Although we have not been able to detect tumoricidal macrophages, we have identified macrophages cytostatic to MOT targets in the PCs of mice given injections of 6-hr C. parvum-elicited PMNs. In contrast, injection of thiglycollate-elicited PMNs, or PMNs obtained 24 or 48 hr after C. parvum injection, are much less effective in causing activation of cytostatic factors.

The data do not exclude the possibility that B- or T-lymphocyte responses play a role in the secondary antitumor effects of recipient mice. We consider this unlikely, however, since tumor cytolysis was significant by 72 hr following peritoneal cell transfer (Chart 2), which is too rapid for the development of a primary immune response.

Our initial study documented that PMN-mediated tumor lysis significantly reduces the tumor burden with an absence of detectable MOT cells in peritoneal fluids by 48 hr following treatment. The results presented in this report, however, clearly indicate that the early PMN tumoricidal effect is insufficient by itself to fully prevent tumor outgrowth. It is unclear whether the second antitumor response is sufficient by itself to complete tumor rejection, or whether it must work in tandem with the initial response. We suspect that this depends upon the magnitude of tumor challenge. In our protocol, where C. parvum is injected 24 hr after tumor challenge, the second antitumor response would not begin until approximately 72 hr following tumor challenge. During this time, the initial tumor burden of 10⁶ cells would undergo 3 to 4 doublings (14), if it was unencumbered by the initial PMN-mediated tumor cytolyis. The second effector mechanism might not be capable of coping with this degree of tumor load. Challenges with lower tumor cell numbers, however, might be readily rejected by either of the mechanisms acting independently.

The mechanism by which inflammatory PMNs activate antitumor mechanisms in mice treated by transfer is unknown. One possibility is that their release of chemotactic factors induces a marked influx of mononuclear cells into the PC which are then susceptible to activation by the small number of C. parvum organisms released by PMNs. In this model, the small amount of C. parvum is ineffective when injected i.p. by itself, because it cannot elicit sufficient neutrophilic exudation. An alternative hypothesis is that PMNs actually process organisms intracellularly, such that they attain a heightened stimulatory capacity when subsequently presented to secondary effectors. These theories are currently under investigation.

**REFERENCES**

10. Jong, E. C., and Klebanoff, S. J. Eosinophil mediated mammalian tumor cell...
Neutrophil-mediated Antitumor Effects


Announcements

MEETING OF THE RADIATION RESEARCH SOCIETY

The annual meeting of the Radiation Research Society will be held at the State University of Iowa, Iowa City, on June 22—24, 1953. The Society will be the guest of the University, and all meetings will be held on the campus. The program will consist of: (1) Two symposia, one on “The Effects of Radiation on Aqueous Solutions,” which includes the following speakers: E. S. G. Barron, Edwin J. Hart, Warren Garrison, J. L. Magee, and A. O. Allen. The second is “Physical Measurements for Radiobiology” and companion talks by Ugo Fano, Burton J. Moyer, G. Failla, L. D. Marinelli, and Payne S. Harris. (2) On Monday night, June 22, a lecture by Dr. L. W. Alvarez on meson physics has been tentatively scheduled. On Tuesday night, June 23, Dr. L. H. Gray of the Hammersmith Hospital, London, will speak on a topic to be announced. Dr. Gray’s lecture is sponsored by the Iowa Branch of the American Cancer Society. Those desiring to report original research in radiation effects, or interested in attending or desiring additional information, please contact the Secretary of the Society, Dr. A. Edelmann, Biology Department, Brookhaven National Laboratory, Upton, L.I., New York.

ERRATUM

The following correction should be made in the article by Beck and Valentine, “The Aerobic Carbohydrate Metabolism of Leukocytes in Health and Leukemia. I. Glycolysis and Respiration,” November, 1952, page 891; substitute for the last paragraph:

The data in Table 3 permit several interesting calculations. If one compares the amount of glucose actually disappearing with the sum of the amount equivalent to lactic acid produced plus that equivalent to O₂ consumption, it is seen that the amount of glucose “cleavage products” exceeds the amount of glucose utilized by 12 per cent in N and 27 per cent in CML and is exceeded by the glucose utilized by 16 per cent in CLL. If the assumption is made that, in this respect, the myeloid and lymphoid cells of leukemia are similar to those of normal blood, it may be that the computed normal figure represents a summation of the myeloid (M) and lymphoid (L) cells that make up the normal leukocyte population. Thus, if M = +0.27 and L = −0.16 and the normal differential is 65 per cent M and 35 per cent L, then

\[ 0.65(0.27) + 0.35(-0.16) = 0.12 \]

a figure identical to the observed +0.12 for normal leukocytes.
Role of Inflammatory Neutrophils in Antitumor Effects Induced by Intraperitoneal Administration of Corynebacterium parvum in Mice


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/44/11/5118

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 pubs@aacr.org.

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