Sensitivity of Tumoricidal Function in Macrophages from Different Anatomical Sites of Cancer Patients to Modulation of Arachidonic Acid Metabolism

Donald P. Braun, Mi-Chung Ahn, Jules E. Harris, Elton Chu, Larry Casey, George Wilbanks, and Kalliopi P. Siziopikou

ABSTRACT

The sensitivity of cancer patient macrophages from different anatomical sites to arachidonic acid metabolism was investigated in tumor cell cytotoxicity assays. Alveolar macrophages and peripheral blood monocytes from 13 non-small cell lung cancer patients, peritoneal macrophages and peripheral blood monocytes from 13 ovarian cancer patients, and comparable macrophages from control patients with nonmalignant lung or gynecological diseases were tested. Inhibitors of either the cyclooxygenase pathway or the lipoxygenase pathway together with specific metabolites of each pathway were used to evaluate how these different macrophage populations are regulated by eicosanoids. In addition, metabolic studies were performed to compare directly the arachidonic acid metabolism of macrophages obtained from these different anatomical locations. The results demonstrate that the peripheral blood monocytes from lung cancer and ovarian cancer patients and the peritoneal macrophages from ovarian cancer patients are sensitive to cyclooxygenase inhibition; this was not seen with comparable macrophages from the relevant control patients. Sensitivity to modulation by cyclooxygenase inhibition correlated with increased cyclooxygenase metabolism and with the capacity of prostaglandins to mediate suppression of tumoricidal function in these populations of cancer patient macrophages. In contrast, alveolar macrophages from cancer patients were not sensitive to either cyclooxygenase inhibition or to prostaglandin-mediated suppression. No such differential influences were revealed for the lipoxygenase pathway of arachidonic acid metabolism in any macrophage population tested. Thus, eicosanoids, particularly those of the cyclooxygenase pathway, can be a critical immunoregulatory feature of certain tumor microenvironments.

INTRODUCTION

Macrophages play diverse and contentious conflicting roles in the immune response against neoplasia. Thus, macrophages can contribute to tumor inhibition by exerting cytostatic or cytotoxic responses against tumor cells (1–3) but can also facilitate tumor progression and metastasis (4, 5). In addition, macrophages contribute to the regulation of other cells that are important in tumor immunity including T-cells, NK cells, and lymphokine-activated killer cells (6–9). Not surprisingly, progressive tumor growth has been associated with modification of macrophage cytotoxic and immunoregulatory function in animal systems (10) and in humans (11–13).

One way in which cancer is thought to affect macrophage function is through the alteration of cellular AA metabolism (14, 15). AA is metabolized in most cells including macrophages via the cyclooxygenase and lipoxygenase enzyme systems. The metabolites produced are known collectively as eicosanoids and include PGs and thromboxane from cyclooxygenase metabolism and LTs and HETEs from lipoxxygenase metabolism (16, 17). In patients with cancer, increased levels of eicosanoids in the circulation and in extravascular secretions have been documented (18, 19) and have been implicated in the immunological abnormalities associated with tumor progression (15, 20). The increase in eicosanoids observed in cancer patients has been variably attributed to active secretion by tumor tissues, the products of coagulation and inflammation, and active secretion by immunoregulatory macrophages (20–23). Recently, we demonstrated that the in vitro development of tumoricidal function in peripheral blood monocytes of cancer patients is reduced compared to that of normal peripheral blood monocytes unless the cyclooxygenase inhibitor, indomethacin, was included in the culture (24). We have also observed impairment in the tumoricidal function of alveolar macrophages from lung cancer patients but in that case, indomethacin could not augment cytotoxicity (25). These observations suggested that the impact of AA metabolism on macrophage cytotoxic function might vary with the anatomical site from which the macrophages are obtained.

In the present study, we have investigated the impact of AA metabolism on the development of cytotoxicity in macrophages obtained from 3 different anatomical locations in cancer patients: the peripheral blood; the peritoneal cavity; and the alveolar spaces. We have utilized inhibitors of either the cyclooxygenase pathway or the lipoxygenase pathway together with specific metabolites of each pathway to evaluate how these different macrophage populations are regulated by eicosanoids. We have evaluated whether similar relationships between AA metabolism and tumoricidal function exist in comparable macrophages from patients with nonmalignant diseases. Finally, we have performed metabolic studies to compare directly the AA metabolism of macrophages obtained from these different anatomical locations. The results demonstrate a differential impact of AA metabolism via the cyclooxygenase pathway on the development of tumoricidal function in alveolar macrophages, peritoneal macrophages, and peripheral blood monocytes of cancer patients.

MATERIALS AND METHODS

Patient Populations. Monocytes and macrophages used in this study were obtained during the course of and in conjunction with standard clinical management practices. PBM and AM were obtained from 13 patients with non-small cell lung cancer. The diagnoses for this group were: 5, squamous cell carcinoma; 6, adenocarcinoma; and 2, poorly differentiated large cell carcinomas. At the time of bronchoalveolar lavage, each patient was staged by chest radiograph according to the lung cancer staging system of Stittik and DiSantis (26). By that system, 4 patients had Stage I lesions, 3 patients had Stage II lesions, 4 patients had Stage IIIA lesions, 1 patient had a Stage IIIB lesion, and 1 patient had a Stage IV lesion. PM and PBM were obtained from 13 patients with ovarian cancer by peritoneal aspiration and lavage at the time of initial surgical laparotomy. Patients in this group had a diagnosis of adenocarcinoma, papillary serous adenocarcinoma, or serous cystadencarcinoma of the ovary: 3 patients had Stage I lesions, 4 patients had Stage II lesions, 4 patients had Stage III lesions, and 2 patients had Stage IV lesions. No cancer patient studied in this investigation had received cytotoxic chemotherapy or radiation therapy prior to macrophage collection and testing. For comparative purposes, controls...
for these cancer patient groups consisted of 12 patients with chronic nonmalignant diseases involving the lung (AM and PBM) and 20 patients with infertility (PM and PBM). The diagnoses for the patients with nonmalignant lung diseases were: 3 chronic cough with no pathology; 3 with systemic lupus erythematosus; 2 with sarcoidosis; 3 with hemoptysis; and 1 with a lung abscess. The patients with infertility were found, upon laparoscopy, to be free of all evidence of endometriosis.

Isolation of Macrophages. Alveolar macrophages (AM) were isolated from bronchopulmonary lavage specimens which were obtained by fiberoptic bronchoscopy as we have described previously (25). Prior to isolation, the mononuclear cells from bronchopulmonary lavage specimens were greater than 90% macrophages based on morphology and latex ingestion. Following adherence in 96-well microtiter plates the adherent cells were greater than 99% macrophages.

PM were isolated from peritoneal aspirates which were obtained at the time of laparotomy or laparoscopy in patients with gynecological diseases. The fluids were processed as described for AM. Prior to isolation, the mononuclear cells from peritoneal fluids were greater than 60% macrophages based on morphology and latex ingestion. Following adherence in 96-well microtiter plates the adherent cells were greater than 99% macrophages.

PBM were isolated from venous blood as we have described (25). Prior to isolation, the mononuclear cells from venous blood contained 10–40% monocytes based on morphology and latex ingestion. Following adherence in 96-well plates the adherent cells were greater than 95% monocytes. To ensure the purity of monocytes in the PBM preparations, adherent cells were treated with anti-CD3 and anti-Leu 7 antibodies + complement to remove loosely adherent T-cells and/or large granular lymphocytes in representative experiments.

Macrophage Cytotoxicity Assay. Wells containing adherent cells were incubated overnight in the presence of IFN-γ (100 units/ml, Sigma). Following this incubation, the adherent cell cultures were washed and incubated with target cells overnight (18–24 h) in the presence of Escherichia coli lipopolysaccharide (2 μg/ml; Sigma) prior to the collection of supernatants by the Tietkalian system and the assessment of cytotoxicity. The target cells used in these studies were 51Cr-labeled Chang hepatoma cells; this target cell was chosen since it is insensitive to NK-mediated cytotoxicity. In preliminary studies, target cells were added to adherent cell monolayers at various E:T cell ratios depending on the cell yield (5:1 to 30:1) in a final volume of 200 μl and incubated overnight prior to collection of supernatants for quantitation of 51Cr release. Results are presented for E:T ratios of 20:1 since these were found to fall into the plateau range on the cytotoxicity curve for each type of macrophage (PBM, AM, PM) tested (i.e., E:T ratios of 30:1 did not yield greater cytotoxicity than E:T ratios of 20:1). The percentage of specific cytotoxicity was calculated as:

\[
\% \text{ of specific release} = \frac{E - S}{T - S} \times 100
\]

where \(E\) is cpm released from target cells in the presence of effector cells, \(S\) is cpm released from target cells in the absence of effector cells, and \(T\) is cpm released from target cells following treatment with 2% sodium dodecyl sulfate.

The concentration of IFN-γ used in this study was found to elicit maximal levels of cytotoxicity under the conditions of this assay. We have previously reported (25) that concentrations of IFN-γ up to 1000 units/ml did not elicit greater levels of cytotoxicity. Also, these concentrations of activators were nontoxic to adherent cells (based on total protein determinations of adherent cell cultures) or to target cells (based on trypan blue dye exclusion).

Inhibitors of AA Metabolism. The impact of inhibiting the cyclooxygenase and lipooxygenase pathways of AA metabolism on the development of macrophage tumoricidal function was investigated with the use of inhibitors of each pathway as we have described previously (24). Briefly, adherent mononuclear phagocyte cultures that were stimulated with IFN-γ were treated simultaneously with either indomethacin (2 μg/ml) to inhibit the cyclooxygenase pathway or NDGA (40 μm) to inhibit the lipooxygenase pathway. These concentrations of inhibitors were found to inhibit each pathway using HPLC analysis of [3H]JAA metabolism as a guide. Increasing the concentration of indomethacin beyond 10 μg/ml or of NDGA beyond 100 μm was found to lead to nonspecific inhibition of both pathways.

Assessment of AA Metabolism in Macrophages by HPLC. The metabolism of AA by macrophages was evaluated by quantitating the production of metabolites from [3H]AA. Mononuclear phagocytes were isolated in Petri dishes as described above for microtiter plates and incubated overnight with 3 μCi of [3H]AA. Following incubation, cells were washed and then treated with the ionophore, A23187 (2 μm), for 15 min prior to collection of supernatants in silanized 15-ml borosilicate glass tubes on ice. Supernatants (2 ml) were extracted with an acidified mixture of chloroform, methanol, and formic acid at a ratio of 12:12:1, respectively (5 ml). Tubes containing supernatants and extraction solvent were vortexed and centrifuged at 4°C for 10 min, at which time a distinct chloroform layer formed at the bottom of the tube. This layer was removed and placed in a silanized 10-ml test tube using a silanized Pasteur pipet. Samples were evaporated to dryness with nitrogen gas, reconstituted with 50 μl of methanol, transferred to 600-μl siliconized Eppendorf microtubes, and stored at −70°C until HPLC analysis. Prior to HPLC, tubes with sample were centrifuged in a microfuge to remove any precipitated material.

HPLC utilized a dual pump system. The mobile phase consisted of a mixture of 0.017 M phosphoric acid, HPLC grade H2O, and acetonitrile. One pump was connected to a flask with 0.017 M phosphoric acid and the other was connected to a flask containing acetonitrile. The flow rate was 0.6 ml/min for the phosphoric acid solution and 0.4 ml/min for the acetonitrile solution for a final flow rate of 1.0 ml/min. This ratio proved to be optimal for metabolite separation. Chromatography was with a Waters μBondapak 3.9 × 300-mm C18 reverse phase column; the column was attached to a variable wavelength detector (Milton Roy) which was attached to a Flo 1 beta detector (Packard). The wavelength used for detection was 195 nm. Individual AA metabolites were expressed as percentage of conversion of the total counts obtained during the run. Between runs, acetonitrile was used to wash the column until a consistently flat baseline was obtained.

Statistical Analysis of Data. The significance of differences between groups of data was evaluated by paired and unpaired t tests. The effect of inhibitors of AA metabolism on the development of tumoricidal function was evaluated with a paired test using values paired for identical cultures with and without the specific inhibitor. Differences between different groups utilized the unpaired t test.

RESULTS

The Effect of the Cyclooxygenase Inhibitor Indomethacin on the Development of Tumoricidal Function in AM, PM, and PBM from Cancer Patients. The impact of indomethacin on the development of tumoricidal function was tested in AM, PM, and PBM from cancer patients (Fig. 1). Indomethacin was found to have a differential effect on the development of tumoricidal function depending on the macrophage population tested. Thus, indomethacin significantly en-

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Fig. 1. Effect of the cyclooxygenase inhibitor, indomethacin, on the development of tumoricidal function in AM, PM, and PBM from cancer patients. Indomethacin (2 μg/ml) was added simultaneously with 100 units/ml IFN-γ to macrophage cultures. The effect:tor-target ratio used was 20:1.

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hanced the development of tumoricidal function in peripheral blood monocytes (mean percentages of cytotoxicity, 28.5 and 40.7% in the absence and presence, respectively, of indomethacin; *P* = 0.0005) and peritoneal macrophages (mean percentages of cytotoxicity, 8.6 and 18.4% in the absence and presence, respectively, of indomethacin; *P* = 0.002) from the cancer patient populations. In contrast, indomethacin had no significant effect on the development of tumoricidal function in alveolar macrophages from lung cancer patients (mean percentages of cytotoxicity, 7.4, and 6.8% in the absence and presence, respectively, of indomethacin; n.s.).

The results for individual samples were also analyzed by determining the magnitude of the change in cytotoxicity produced by indomethacin (expressed as a percentage of the cytotoxicity in the absence of indomethacin) and comparing this value to the change in cytotoxicity which is produced in normal PBM by indomethacin. On the basis of our previous studies utilizing normal PBM (24), we considered an effect of indomethacin in any individual sample which produced a change in cytotoxicity ≥ 50% to be significantly greater than normal (this cutoff represents an effect which is greater than 2 SD from the mean percentage change produced by indomethacin in normal PBM). By that criterion, indomethacin increased the development of cytotoxicity by an amount significantly greater than normal in 10 of 13 PM samples and in 11 of 18 PBM samples (5 of 9 lung cancer and 6 of 9 ovarian cancer patients). In contrast, indomethacin failed to significantly modify the cytotoxicity in 11 of 13 AM specimens from lung cancer patients; a significant increase and a significant decrease in cytotoxicity was observed in the other 2 AM samples tested.

**Effect of the Lipoxygenase Inhibitor, NDGA, on the Development of Tumoricidal Function in AM, PM, and PBM from Cancer Patients.** The impact of NDGA on the development of tumoricidal function was also tested in AM, PM, and PBM from cancer patients (Fig. 2). NDGA was found to inhibit tumoricidal function in each macrophage population tested. The mean percentages of cytotoxicity values in the absence and presence, respectively, of NDGA for each macrophage population were as follows: 29.3 and 19.6% for PM (*P* = 0.004); 12.2, and 8.6% for PM (*P* = 0.08); and 7.8 and 2.0% for AM (*P* = 0.02). Inhibition of cytotoxicity by at least 50% was found with 5 of 9 PM, 3 of 6 PM, and 3 of 6 AM specimens. Modest inhibitory effects were seen in the majority of the remaining samples. These inhibitory effects of NDGA were consistent with what has been found previously with normal PBM (24).

**Effect of AA Metabolic Inhibitors on the Development of Tumoricidal Function in AM, PM, and PBM from Control Patients.** For comparison with cancer patient macrophages, the effects of indomethacin and NDGA were also tested in AM, PM, and PBM from patients with nonmalignant lung or gynecological diseases. Indomethacin (2 μg/ml) or NDGA (40 μM) was added simultaneously with 100 units/ml IFN-γ to macrophage cultures. The effector/target ratio used was 20:1. The results presented are from variable numbers of donors (range, 6-20) for each type of macrophage and inhibitor. Bars, SD.

**Fig. 3.** Effect of indomethacin (Indo) and NDGA on the development of tumoricidal function in AM, PM, and PBM from control patients. For comparison with cancer patient macrophages, the effects of indomethacin and NDGA were also tested in AM, PM, and PBM from patients with nonmalignant lung or gynecological diseases (Fig. 3). These studies revealed that indomethacin did not significantly influence the development of tumoricidal function in any macrophage population tested. The mean percentage of cytotoxicity values in the absence and presence, respectively, of indomethacin for each macrophage population were: 40.1 and 47.7% for PBM (n.s.); 20.2 and 21.2% for PM (n.s.); and 29.9 and 28.1% for AM (n.s.). The lack of an effect with indomethacin was consistent when individual samples were considered.

It is of interest to note that in the absence of indomethacin, the cytotoxicity of each control (non-cancer patient) macrophage population was significantly greater than the cytotoxicity for the corresponding cancer patient macrophage population (*P* values were 0.0001, 0.023, and 0.005 for AM, PM, and PBM, respectively). However, in the presence of indomethacin, the cytotoxicity of PM and PBM from cancer patients was equivalent to (i.e., not significantly different from) the cytotoxicity of the PM and PBM from the control patients. This was not the case for the AM from lung cancer patients; in fact, no significant elevation of cytotoxicity was observed in AM treated with indomethacin.

NDGA, however, was found to significantly inhibit the development of tumoricidal function in each of the control macrophage populations tested, a result which was consistent with the effect of NDGA on the cancer patient macrophages. The mean percentages of cytotoxicity values in the absence and presence, respectively, of NDGA for each control macrophage population were: 40.1 and 20.3% for PBM (*P* = 0.002); 24.4 and 19.8% for PM (*P* = 0.03); and 29.9 and 20.3% for AM (*P* = 0.04). The effect of NDGA on macrophage tumoricidal function was consistent when individual samples were considered.

**Effect of PGE₂ and LTC₄ on the Development of Cytotoxicity in Indomethacin-treated and NDGA-treated AM, PM, and PBM from Cancer Patients.** To assess whether metabolites of AA could influence the development of tumoricidal function in cancer patient macrophages, the cyclooxygenase metabolite, PGE₂ (Fig. 4), and the lipoxygenase metabolite, LTC₄ (Fig. 5), were added to the different macrophage cultures that had been treated with the relevant metabolic pathway inhibitor. The effect of PGE₂ on indomethacin-treated cancer patient macrophages varied with the population of cells tested. PGE₂...
tently augmented the development of tumoricidal function in NDGA-treated AM from lung cancer patients.

![Graph showing effect of PGE2 on tumoricidal function](image)

The results revealed that PGE2 at a pharmacological dose (10^-7 M), a supraphysiological (i.e., inflammatory) dose (10^-9 M), and a physiological dose (10^-9 M) failed to inhibit the development of tumoricidal function in indomethacin-treated AM and in fact, augmented this function in 4 of 6 samples (mean percentages of cytotoxicity, 25.2 and 15.9% in the absence and presence, respectively, of PGE2, P = 0.018; 9.6 and 21.9% for PM (P = 0.05); and 2.2 and 5.6% for AM (P = 0.07).

**AA Metabolic Profiles in AM, PM, and PBM from Cancer Patients.** The apparent differential impact of cyclooxygenase metabolism on the tumoricidal function of AM, PM, and PBM from cancer patients was investigated further by evaluating the AA metabolism of these different macrophage populations (Table 2). The control values for these experiments consisted of 3 AM and 2 PM specimens from patients with nonmalignant diseases and 5 PBM specimens from normal donors since the number of mononuclear phagocytes required for HPLC limited the availability of PBM from all but 1 of the cancer patients.

When comparable types of macrophages were compared, it appeared that malignant disease affected cyclooxygenase-dependent AA metabolism in PBM and PM but not in AM. Thus, the relative amounts of PGE2 and 6-keto-PGF1α (a breakdown product of PGI2) were increased in PM from 5 ovarian cancer patients compared to PM from 2 control patients with infertility (P = 0.09). Similarly, these metabolites were increased in the single PBM sample from a patient with ovarian cancer compared to the 5 normal PBM samples, a finding which is consistent with our previous results (15). In contrast, the cyclooxygenase metabolic profiles from 3 AM samples of lung cancer patients were comparable to those of 3 AM samples of control patients.

When the metabolic profiles of different types of mononuclear phagocytes (i.e., AM, PM, and PBM from cancer patients) were compared, the AM of lung cancer patients (and, in fact, the AM of control subjects) were found to produce less thromboxane (P = 0.07) and 6-keto-PGF1α (P = 0.09) than the PM of ovarian cancer patients. A similar pattern was observed for these metabolites when comparing the AM of lung cancer patients with the single PBM sample from an ovarian cancer patient except for PGE2 which was greater in the PBM sample (12.1 versus 20.9%). This is consistent with a relatively greater activity of the cyclooxygenase pathway in PM and PBM versus AM of cancer patients. It is also important to note that the mean absolute amounts of AA metabolites produced by the different macrophage populations were comparable.

There was no apparent difference in the relative lipoxigenase activity of any of the cancer patient macrophages tested although it was difficult to clearly resolve the 5-HETE and 6-keto-PGF1α peaks in some specimens; thus these values were combined. Nevertheless, based on peak height, the relative amounts of 5-HETE were comparable in AM, PM, and PBM from the cancer patients, as were the amounts of LTC4; LTB4 was not readily detectable under these assay conditions.

### Table 1: Effect of different concentrations of PGE2 on the development of tumoricidal function in indomethacin-treated alveolar macrophages

<table>
<thead>
<tr>
<th>PGE2 concentration (M)</th>
<th>No.</th>
<th>% of cytotoxicity (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>5.2 ± 3</td>
</tr>
<tr>
<td>1 × 10^-7</td>
<td>3</td>
<td>8.4 ± 2</td>
</tr>
<tr>
<td>1 × 10^-8</td>
<td>3</td>
<td>6.2 ± 3</td>
</tr>
<tr>
<td>1 × 10^-9</td>
<td>3</td>
<td>5.5 ± 3</td>
</tr>
</tbody>
</table>

a. Alveolar macrophages were incubated with indomethacin plus γ-interferon in the presence and absence of PGE2 overnight followed by washing and target cell addition. An effector/target ratio of 20:1 was utilized.

b. Number of AM specimens tested from patients with non-small cell lung cancer.

In contrast to the differential effects of PGE2, the lipoxigenase metabolite, LTC4, at a physiological concentration (10^-10 m), consistently augmented the development of tumoricidal function in NDGA-treated cancer patient macrophages (Fig. 5). The mean percentages of cytotoxicity values in the absence and presence, respectively, of LTC4 for each NDGA-treated macrophage population were: 18.7 and 31.1% for PBM (P = 0.018); 9.6 and 21.9% for PM (P = 0.05); and 2.2 and 5.6% for AM (P = 0.07).

Inhibition of tumoricidal function in indomethacin-treated AM, induced by 100 units/ml IFN-γ and 40 µg/ml NDGA, was analyzed. The results presented are from variable numbers of donors (range, 4-8) for each type of macrophage. Bars, SD.
EICOSANOID REGULATION OF CANCER PATIENT MACROPHAGES

Table 2: Arachidonic acid metabolism by alveolar macrophages, peritoneal macrophages, and PBM from cancer patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Alveolar (%)</th>
<th>Peritoneal (%)</th>
<th>Peripheral blood (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cancer (n = 3)</td>
<td>Control (n = 3)</td>
<td>Cancer (n = 5)</td>
</tr>
<tr>
<td>Thromboxane</td>
<td>17.1 ± 2</td>
<td>11.9 ± 2</td>
<td>25.5 ± 3</td>
</tr>
<tr>
<td>PGF₂α</td>
<td>8.3 ± 1</td>
<td>12.1 ± 2</td>
<td>10.3 ± 2</td>
</tr>
<tr>
<td>PGE₂</td>
<td>14.5 ± 5</td>
<td>5.4 ± 1</td>
<td>8.3 ± 1</td>
</tr>
<tr>
<td>PGD₂</td>
<td>3.2 ± 1</td>
<td>1.0 ± 1</td>
<td>3.7 ± 1</td>
</tr>
<tr>
<td>LTC₄</td>
<td>3.6 ± 1</td>
<td>1.0 ± 1</td>
<td>3.7 ± 1</td>
</tr>
<tr>
<td>6-Keto PGE₂α + 5-HETE</td>
<td>10.7 ± 7</td>
<td>17.6 ± 10</td>
<td>13.5 ± 7</td>
</tr>
</tbody>
</table>

*Mean total counts metabolized, 10,476, 9,939, and 10,577 for AM, PM, and PBM, respectively, from cancer patients.

DISCUSSION

The results of this study demonstrate that mononuclear phagocytes from the peripheral blood, the peritoneal cavity, and the alveolar spaces of cancer patients differ in their sensitivity to inhibition of arachidonic acid metabolism via the cyclooxygenase but not via the lipoxygenase pathway. In the PBM and PM of ovarian cancer patients and in the PBM of lung cancer patients, inhibition of cyclooxygenase activity enhanced the development of tumoricidal function. The sensitivity of these macrophages to cyclooxygenase metabolism was also revealed by demonstrating suppression of tumoricidal function with the cyclooxygenase metabolite, PGE₂. However, in contrast to these results with PM and PBM, inhibition of cyclooxygenase activity had no modulatory effect on the tumoricidal function of AM from lung cancer patients even when the PBM of those same patients were significantly affected. Moreover, and quite unexpectedly, treatment of AM with PGE₂ was found to enhance rather than suppress the development of tumoricidal function. In contrast to these results in cancer patients, the AM, PM, and PBM from patients with nonmalignant lung or gynecological diseases showed no sensitivity to cyclooxygenase inhibition. Although the effects of cyclooxygenase inhibition were variable in this study, the effects of lipoxygenase inhibition were consistent. Thus, AM, PM, and PBM from cancer patients were inhibited by treatment with a lipoxygenase inhibitor and this inhibition was overcome when the lipoxygenase metabolite, LTC₄, was added to the culture. Taken together, these results suggest that cyclooxygenase metabolites such as PGE₂ may play a significant immunoregulatory role in the peripheral blood and the peritoneal cavity of cancer patients.

Although the effects of cyclooxygenase metabolism were more difficult to discern in AM of lung cancer patients due to the extent of cytotoxicity impairment in most specimens, it appears that AA metabolites may be less relevant to the immunoregulatory environment of the lung.

A satisfactory explanation for how malignant disease can affect the AA metabolism of different populations of macrophages remains to be elucidated. Numerous studies have revealed that macrophages are active secretors of eicosanoids (27, 28) but the absolute and relative quantity of each metabolite varies both with the source of the macrophage and with the stimulus. In general, peripheral blood monocytes and peritoneal macrophages are thought to metabolize AA preferentially via the cyclooxygenase pathway (29–31) while alveolar macrophages have a greater tendency to utilize the lipoxygenase pathway (32). The control of each pathway also may depend on whether the substrate is endogenous or exogenous (30, 32, 33). The results of the present study, therefore, may reflect shifts in macrophage populations in the peripheral blood and peritoneal cavity of relevant cancer patient groups. Although true lineage diversity has never been demonstrated in macrophages, diversity which is determined by the local environment has been observed. In the presence of malignant disease, a greater percentage of macrophages with a proclivity for cyclooxygenase metabolism may appear in the peripheral blood and in the peritoneal cavity than would be expected under conditions of normal homeostasis. In contrast, the alveolar spaces may be less subject to macrophage population shifts in the presence of malignant disease.

Although PM and PBM from most cancer patients were sensitive to cyclooxygenase inhibition, this was not the case with PM and PBM from control patients with non-malignant diseases. In those macrophages, tumoricidal function was largely unaffected by indomethacin. Because of this, the treatment of PM and PBM from cancer patients with indomethacin increased their tumoricidal function to a level which was comparable to that of the control macrophage populations. It appears, therefore, that impaired tumoricidal function in PM or PM from cancer patients is related to changes in AA metabolism. Although we have presented direct evidence for this association in circulating monocytes (15), this has not been well studied in macrophages from the peritoneal cavity of cancer patients. The results of this investigation suggest that PGE₂ may be an important modulator of peritoneal macrophage function in women with ovarian cancer.

However, the somewhat dogmatic notion that increased levels of PGE₂ should inevitably be associated with immunosuppression is challenged by the results obtained in this study with AM from lung cancer patients. Quite unexpectedly, the addition of even pharmacological concentrations of PGE₂ to these AM failed to suppress and in fact oftentimes enhanced the development of tumoricidal function. Also, the presence of malignant disease within the pulmonary cavity did not appear to affect the AA metabolism of the pulmonary macrophages themselves even though their cytotoxic function was reduced considerably compared to that of the controls. Taken together, these results are compatible with the observed failure of cyclooxygenase inhibition to improve tumoricidal function in the AM from lung cancer patients. They do not, however, provide an explanation for this striking impairment.

A satisfactory explanation for what appears to be differential sensitivity of macrophages from distinct anatomical/tumor environments to cyclooxygenase metabolism remains to be elucidated. Since multiple mechanisms for tumor cell cytotoxicity have been demonstrated in macrophages (34) it would not be surprising to find that some mechanisms are subject to cyclooxygenase regulation while others are not. Support for this possibility is provided by recent studies which demonstrate that indomethacin could enhance the development of monocyte tumoricidal function in response to IFN-γ but not in response to phorbol ester (24). It would also not be surprising to discover that macrophages in different environments may rely on separate regulatory or effector molecules for mediating cytotoxic events. Thus, while the synthesis of tumor necrosis factor and interleukin 1 is

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known to be sensitive to PGE₂ (28) and is thought to be fundamental to the tumoricidal function of some macrophage populations (35), these molecules may not be critical for the tumoricidal function of alveolar macrophages (25). Alternatively, there are multiple activation pathways for the elicitation of specific cytokines and/or effector molecules in tumoricidal macrophages, and these may vary in different macrophage populations. Evidence for multiple activation pathways for lipopolysaccharide-enhanced expression of interleukin I involving both protein kinase C and calmodulin kinase has recently been presented (36).

It is of interest to consider further the possibility that molecules such as PGE₂ may be utilized to suppress macrophage functions in certain environments and to stimulate macrophage functions in other environments. Direct evidence for this possibility was found in the present study in which PM and PBM were inhibited by PGE₂ whereas AM were often stimulated by PGE₂. Since the mechanism(s) by which PGE₂ can modulate cellular functions are diverse, these may in fact differ in specific macrophage populations. For example, PGE₂ is known to interact with a specific macrophage receptor but the presence and density of the putative PGE₂ receptor has not been well studied on different macrophage populations (37). PGE₂ is also known to activate adenylate cyclase leading to increased cytoplasmic levels of cyclic AMP (38) but the substrates for cyclic AMP-dependent kinases remain to be characterized in different macrophage populations. PGE₂ has also been shown to modulate ion flux across cellular membranes (39) but once again this activity of PGE₂ has not been investigated in different macrophage populations.

In contrast to the differential sensitivity of macrophages to cyclooxygenase metabolism, no such differential effects could be demonstrated for lipoxygenase metabolism suggesting that all macrophages may require products of the 5-lipoxygenase pathway for the development and/or expression of tumoricidal function. This possibility is strengthened further by the demonstration in the present study that the 5-lipoxygenase metabolite, LTC₄, can restore the tumoricidal function of NDGA-treated macrophages regardless of whether these macrophages came from the blood, the peritoneal cavity, or the alveolar spaces. This result is consistent with the results of studies which implicate products of the lipoxygenase pathway in other mononuclear phagocyte functions including phagocytosis, chemotaxis, respiratory burst activity, and enzyme secretion (40-44). However, it is also important to recognize that malignant disease did not appear to influence the lipoxygenase metabolism of the macrophages in this study even when those macrophages were obtained from a tumor environment. Thus, disturbances or abnormalities of the lipoxygenase pathway do not appear to be characteristic of cancer patient macrophages and do not contribute to their dysfunction.

The demonstration that the tumoricidal function of some macrophage populations can be suppressed by levels of PGE₂ that are often achieved in body fluids and tissue compartments of cancer patients might have important implications for cancer therapy. In particular, immunological function in patients with either systemic malignant disease or disease involving the peritoneal cavity might benefit from cyclooxygenase inhibition. This could be especially relevant to experimental therapies which are aimed at treating peritoneal disease with biological agents such as interferon or granulocyte-macrophage colony-stimulating factor. However, this study also suggests that the effect of cyclooxygenase inhibition in cancer patients is likely to be variable and individually specific. Clinical trials attempting to manipulate AA metabolism in cancer patients should take into account the differential sensitivity of macrophages, particularly those in the tumor environment, to cyclooxygenase metabolism.

**REFERENCES**

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