Epidermal Growth Factor and Transforming Growth Factor α Induce Ascitic Fluid in Mice

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ABSTRACT

Recent studies have suggested that pleural or peritoneal effusion associated with metastatic tumors is induced by some mediators produced by the tumor cells. We studied the ability of well-characterized peptide growth factors to produce ascites in mice. Peritoneal administration of epidermal growth factor (EGF, 10 to 40 μg/mouse/wk) or transforming growth factor α (TGF-α, 10 to 40 μg/mouse/wk) via osmotic minipumps resulted in formation of bloody ascites. The amount of ascites produced was dependent on the dose of growth factors. Vehicle alone or insulin-like growth factor (IL-1, 40 μg/mouse/wk) was without effect. Indomethacin, a blocker of prostaglandin synthesis, significantly reduced the ascites accumulation induced by EGF, suggesting that prostaglandins are involved in ascites formation induced by EGF. Dexmethyladone was also effective in attenuating the effect of EGF. Thus, it is possible that peritoneal effusion associated with disseminated tumors is, at least in part, due to EGF-like mediators (most likely TGF-α) produced by tumor cells. The mechanism by which these peptides induce bloody ascites is not known for certain, but it may be due to the reported activity for neovascularization or increased vascular permeability.

INTRODUCTION

Recent studies have suggested that the peritoneal or pleural effusion associated with malignancy is induced by some mediators produced by the tumor cells (1, 2). For example, Senger et al. (3) have reported that a factor produced by tumor cells increases the permeability of the peritoneal vasculature, and they have postulated that the tumor-associated mediator may contribute to peritoneal effusion.

It is now well established that malignant cells produce a number of peptide growth factors, including transforming growth factors (3-6). It has recently been reported that TGF-α activity is present in ascites and pleural effusion obtained from patients with malignant tumors (7, 8). In addition, high concentrations of EGF were detected in cystic fluid of the breast (9, 10). Recently, we have found unexpectedly that EGF administered s.c. by an osmotic minipump induced fluid retention around the minipump in rats. TGF-α is structurally similar to EGF and competes with EGF for receptor binding (3). These observations suggest that locally produced EGF or TGF-α may be involved in the formation of abnormal fluid. To test this hypothesis, we examined the ascites-producing effect of EGF and TGF-α in mice.

MATERIALS AND METHODS

Male ICR mice (35 to 40 g) were used for the experiments. Recombinant human EGF was obtained from Earth Chemical Co. (Hyogo, Japan) and Wakunaga Pharmaceutical Co. (Osaka, Japan). Mouse EGF was purchased from Collaborative Research, Inc. (Waltham, MA). Recombinant human TGF-α and IGF-I were kindly supplied by Earth Chemical Co. and Fujisawa Pharmaceutical Co. (Tokyo, Japan), respectively. EGF (12.5, 25, and 50 μg), TGF-α (12.5, 25, and 50 μg), and IGF-I (50 μg) were dissolved with 0.22 ml of sterile normal saline solution containing 0.1% mouse serum albumin. The osmotic minipumps (Alzet, Palo Alto, CA) filled with the test materials were aseptically implanted into the peritoneal cavity of mice anesthetized with ether. According to the information supplied by the company, approximately 80% of the test materials (i.e., 10, 20, and 40 μg) can be released during the experimental period. After the operation, Penicillin G (10,000 units/mouse) was injected i.m. After 7 days, ascites fluid was aspirated, using a plastic tube with side pores, under ether anesthesia. The collected fluid was immediately heparinized and examined for the number of cells and differential cell counts after staining. Then the sample was centrifuged (2000 × g, 15 min), and the concentrations of total protein, amylase, and growth factors (EGF or TGF-α) in the supernatant were measured. The levels of EGF and TGF-α were determined by the specific radioimmunoassays for these peptides. IM (130 μg/day/mouse; Sigma, St. Louis, MO) or DEX (20 μg/day/mouse; Sigma) was injected i.p. daily from Day 2 to Day 6 after implantation of the minipump. The statistical significance of difference between groups was determined by the Student t test.

RESULTS

The effect of EGF on ascites formation is shown in Fig. 1. Recombinant human EGF (10, 20, and 40 μg/mouse/wk) induced fluid retention in the peritoneal cavity in a dose-dependent manner. The volume of ascites amounted to 1.5 ± 0.21 ml (mean ± SE; n = 7) in mice administered 40 μg of EGF/wk. Moreover, the EGF-induced ascites was bloody, and the red cell count was about 18% of that in peripheral blood (Table 1), irrespective of the dose of EGF. The number of white blood cells, most of which were macrophages/monocytes and neutrophils, in ascites was higher than that in peripheral blood. The concentration of EGF was 4.96 ± 5.1 ng/ml (mean ± SE; n = 20) in ascites obtained at the end of the experiment. No significant amount of ascites was found in mice that received the vehicle or IGF-I alone (Figs. 1 and 2). The administration of recombinant human TGF-α also induced the accumulation of bloody fluid in the peritoneal cavity (Fig. 2). The volume of ascites observed in mice given 40 μg of TGF-α/wk was as much as 5.11 ± 0.88 ml (mean ± SE, n = 6). The concentration of TGF-α in the ascites was 55.5 ± 11.9 ng/ml (mean ± SE; n = 6). The white blood cell count of ascites was lower compared with that of effusion induced by EGF. Serum amylase levels were not different between EGF- or TGF-α-treated mice (7287 ± 343 IU/liter; n = 8) and control mice (8721 ± 780 IU/liter; n = 5) and amylase levels in ascitic fluid were about 50 to 60% of those of serum levels.

To determine the involvement of prostaglandins in the induction of ascites by EGF, indomethacin, a blocker of prostaglandin synthesis, was injected i.p. daily. As shown in Fig. 3, IM (130 μg/day/mouse) markedly (73%) suppressed fluid re-
tension by EGF. Similarly, DEX (20 µg/day/mouse) reduced the EGF-induced ascitic fluid to 38%. IM or DEX alone was without effect on fluid retention.

DISCUSSION

We demonstrate that human EGF and TGF-α administered i.p. by osmotic minipump induce peritoneal effusion in mice. The amount of ascitic fluid stimulated by the peptides increased in a dose-dependent manner. Administration of vehicle alone or recombinant human IGF-I failed to produce ascites, suggesting that EGF- or TGF-α-induced ascites formation is not due to a nonspecific inflammatory effect of the peptides or the minipump itself. It is possible that minor contaminations in the preparations are responsible for ascites formation. This would be unlikely, however, because two other EGF preparations obtained from separate companies were able to produce ascites formation in a similar fashion (data not shown). We measured the concentrations of endotoxin in the solutions used in the experiment. There was no difference in endotoxin concentrations among EGF, TGF-α, and IGF-I solutions and the control vehicle (data not shown), indicating that endotoxin is not the causative substance for ascites formation. Since purified mouse EGF also induces ascites formation, it is not likely that the effect of EGF is due to an allergic reaction of the mouse to human EGF. The response to purified mouse EGF was smaller compared with that to human recombinant EGF. It might be explained by the difference of the purity between the two preparations. It is also unlikely that exudative ascites is caused by pancreatitis, since serum amylase levels were not different between EGF- or TGF-α-treated mice and control mice.

Analysis of the ascites revealed that the fluid was exudative and contained high concentrations of protein (approximately 70 to 80% of the level in serum) (Table 1). The WBC count was 0.8 to 3.5 × 10⁷/mm³, and the majority of the cells were macrophages. In mice administered 40 µg/mouse/wk of EGF, the number of WBC in ascites was slightly higher compared with that in TGF-α-treated mice. The cause(s) of this phenomenon is not clear at present. The number of RBC present in ascites amounted to approximately 20% of those in peripheral blood (Table 1). It is well known that pleural effusion or ascites associated with cancer contains high concentrations of protein and RBC. Seventy-five % of the sample fluids contain more than 2.5 g/dl of protein (11, 12). Although the causative factor remains to be clarified (1, 2, 13), it has been suggested that the increased permeability of vascular cells and/or the formation of fragile new vessels (neovascularization) may be responsible for the process. The present study shows that the property of both EGF- and TGF-α-induced ascites was similar to that seen in patients with cancer. Thus, EGF-like substances, most likely TGF-α, could be one of the factors responsible for ascites formation in such patients. The finding that TGF-α activity (1.56 to 50 ng/ml) is detected in ascites or pleural effusion associated with malignancy (7, 8) is compatible with this hypothesis.

The mechanism by which EGF or TGF-α stimulates ascites formation is not clear at present. Since EGF and TGF-α have been reported to stimulate the production of PGs, including PGE₂ and PGI₂ in several types of tissues and cells (14, 15), we examined the effect of indomethacin, a blocker of PG synthesis, on EGF-stimulated ascites formation. As shown in Fig. 3, indomethacin inhibited the effect of EGF, suggesting that PGs, especially PGE₂ and PGI₂, which have been reported to increase vascular permeability (16), are involved in EGF-stimulated ascites formation. However, we could not exclude the possibility that the suppressive effect of indomethacin is caused by some compensatory or other unknown mechanisms. Senger et al. (1) have reported that a factor produced by a number of tumor cells increases the permeability of the blood vessels. This factor, however, does not appear to be the same as EGF/TGF-α, since the effect was not abolished by indomethacin. EGF-stimulated ascites formation was also partially suppressed by dexamethasone (Fig. 3), which could inhibit the production and release of PGs (16). Thus, the data presented suggest that EGF or TGF-α induces ascites, at least in part, by increasing the vascular permeability, possibly through PG production. Heuser et al. (13) have reported that extravasation of erythrocytes in malignant ascites is due to the formation of new proliferating capillaries that lack an intact basement membrane. Since both EGF and TGF-α are angiogenic mediators (17) and induce the discontinuity of the basement membrane (18), they may induce bloody ascites through the formation of new fragile blood vessels. Very recently, Connolly et al. (19) have shown that vascular permeability factor, which has been purified from the conditioned medium of guinea pig line 10 tumor cells, has also

Table 1 The values of total protein, RBC, and WBC counts in ascitic fluid and peripheral blood obtained from mice given 40 µg/mouse/wk of EGF or TGF-α

<table>
<thead>
<tr>
<th></th>
<th>Ascitic fluid</th>
<th>Peripheral blood</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>EGF (40 µg/ mouse/wk)</td>
<td>TGF-α (40 µg/ mouse/wk)</td>
<td>Normal</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>3.2 ± 0.1*</td>
<td>3.2 ± 0.3</td>
<td>4.9 ± 0.3</td>
</tr>
<tr>
<td>RBC (×10⁸/mm³)</td>
<td>204 ± 86</td>
<td>230 ± 35</td>
<td>1267 ± 110</td>
</tr>
<tr>
<td>WBC (×10⁶/mm³)</td>
<td>2.50 ± 0.23</td>
<td>1.00 ± 0.18</td>
<td>1.10 ± 0.08</td>
</tr>
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<td>Macrophages/macrogamocytes (%)</td>
<td>54</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>25</td>
<td>28</td>
<td>11</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>6</td>
<td>6</td>
<td>69</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>12</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Plasma cells (%)</td>
<td>3</td>
<td>2</td>
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* Mean ± SE (n = 6 to 7 in each group).
angiogenic activity. Thus, it may be speculated that peptides which stimulate angiogenesis are able to induce ascitic fluid. In addition, the difference of potency between 40 μg/mouse/wk of EGF and TGF-α may be a reflection of their angiogenic activities, since TGF-α has been reported to stimulate angiogenesis more potently than EGF (17). Further studies are required to clarify the mechanism(s) of action of these peptides.

In conclusion, we have demonstrated that EGF or TGF-α induces ascitic fluid formation. The development of the abnormal fluid which contains EGF/TGF-α activity may be closely related to these growth factors. Any procedure that interferes with the action of these factors (i.e., antibody to EGF receptor, indomethacin, or corticosteroids) might be useful in inhibiting the accumulation of ascitic fluid or pleural effusion induced by EGF or TGF-α.

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