Immunosuppressive Activity of Ascitic Fluid from Patients with Cancer Metastatic to the Peritoneum

Alison M. Badger, Sidney R. Cooperband, Vincent J. Merluzzi, and Arthur H. Glasgow

SUMMARY

We have examined the immunosuppressive effects of ascitic fluids from patients with advanced cancer metastatic to the peritoneum and compared them with noncancerous abdominal or pleural effusions, serum from cancer patients, or the proteins human serum albumin and bovine γ-globulin. The ascitic fluids from cancer patients produce a nontoxic, dose-dependent suppression of DNA and protein synthesis of phytohemagglutinin-stimulated human peripheral blood lymphocytes. This suppression reached 100% with an ascitic protein concentration of 4 to 6 mg/ml, whereas control effusions, serum from cancer patients, or added extraneous proteins were not suppressive. Ascitic proteins also suppress the mixed lymphocyte reaction and the response of human peripheral blood lymphocytes to the antigen keyhole limpet hemocyanin. In vivo, the primary plaque-forming response to sheep red blood cells in mice could be suppressed, and this suppression was not due to antigenic competition.

INTRODUCTION

Nonspecific suppression of delayed hypersensitivity has been reported to occur in cancer-bearing patients. This suppression has been measured by a number of immunological parameters, such as a depressed reactivity to skin test antigens (3, 10, 16), a lowered response of lymphocytes to mitogens such as PHA (1, 14, 23, 28) and a decrease in spontaneous (E) rosette-forming cells (5, 30). The mechanism responsible for this phenomenon remains unclear, but some studies have suggested that this suppression of immune reactivity may result from circulating immunosuppressive factor(s) in the serum. Our laboratories and others have described nonspecific immunosuppressive activity in the serum of normal and cancer-bearing animals and humans (11, 13, 16, 27, 29). We have described an α-globulin fraction in normal serum (immunoregulatory α-globulin) which nonspecifically suppresses the immune reponse both in vitro and in vivo (6, 7, 15). More recently, we have isolated a peptide fraction from this α-globulin which is also immunosuppressive (26), and it appears in a higher concentration in cancer serum than in normal serum (16). Although the origin of the immunosuppressive factor(s) described by several groups has not been determined, it has been suggested that active substances might be produced by or induced by tumor cells and excreted into body fluid to effect immunosuppression. It would follow, therefore, that ascitic fluid bathing cancer cells might be a source of immunosuppressive activity. Studies reported here have been concerned with examining the cell-free ascitic fluid obtained from human patients with advanced cancer of different histological types that have metastasized to the peritoneal wall for evidence of humoral immunosuppressive activity. We have tested the immunosuppressive activity of these ascitic fluids in a number of assay systems both in vitro and in vivo. For comparison, we have examined transudate fluids from noncancer patients, serum obtained at the same time as the ascites from the cancer-bearing patients, and the proteins HSA and BGG.

MATERIALS AND METHODS

Patients and Preparation of Samples. Ascitic fluids, pleural fluids, and serum samples were obtained from cancer-bearing and control patients admitted to University Hospital and the Boston City Hospital of the Boston University Medical Center. Cancer ascites were obtained from: M. A., a 58-year-old white female with adenocarcinoma of the breast; W. H., a 72-year-old white female with adenocarcinoma of the breast; D. R., a 61-year-old white female with a metastatic adenocarcinoma of the stomach; S. T., a 58-year-old white female with oat cell carcinoma of the lung; B. R., an 83-year-old white female with endometrioid carcinoma of the ovary, Stage III; N. E., a 43-year-old black female with diffuse i.p. carcinomatosis from adenocarcinoma, origin undetermined; J. O., a 50-year-old white female with poorly differentiated adenocarcinoma of the ovary, Stage III; and E. M., a 54-year-old white male with intraabdominal adenocarcinoma, origin unidentified.

Control pleural or abdominal effusions were obtained from: A. W., a 62-year-old white female with congestive heart failure; H. G., a 73-year-old white male with congestive heart failure and sideroblastic anemia; S. O., whose records were lost, with myeloid metaplasia; and V. P., a 48-year-old white male with tuberculosis under treatment and with recurrent pleural effusions (sterile).

All the samples were centrifuged at 200 x g for 20 min, coded, sterilized by passage through Millipore HA filters.
(0.45 μm) and stored at −20°. Serum samples were also obtained simultaneously with the ascitic fluid whenever possible. We also utilized HSA and BGG (Miles Laboratories, Kankakee, Ill.) as protein controls.

The protein concentration of the fluid was determined by refractive index, and the concentrations were usually corroborated by Lowry determination (21) against an albumin standard. For any single experiment the fluids were adjusted so that their protein concentrations were identical. Dialysis of the ascitic fluids was carried out against 3 changes of Hanks' balanced salt solution, pH 7.2. The effect of pH on the immunosuppressive activity of the ascitic fluid was also accomplished by dialysis. Adjustment to pH 2.0 was done by dialysis against a hydrochloric acid-potassium chloride buffer and to pH 10.0 using a sodium bicarbonate-sodium carbonate buffer. The samples were readjusted to physiological pH by dialysis against Hank's balanced salt solution, pH 7.2. Studies on the heat stability of the fluid were done by heating the samples at 56°, 70°, and 80° for varying lengths of time.

**Assay for AFP.** Several control and ascitic samples were examined for AFP by 2 methods. Dr. Norman Zamcheck of the Mallory Institute of Pathology, Boston City Hospital, used a Laurell rocket immunoelectrophoresis method (19) to determine the presence of AFP in our effusions with a maximal sensitivity of 1 μg/ml. In addition, Dr. Eliot Alpert of the Massachusetts General Hospital examined our samples utilizing a radioimmunoassay with a maximal sensitivity of 10 ng AFP per ml.

**In Vitro Tissue Culture Methods.** Human peripheral leukocytes were cultured in Eagle’s minimal essential medium with Earle’s salts obtained from Flow Laboratories, Rockville, Md. This medium was supplemented with L-glutamine, 100 units of penicillin and 100 μg of streptomycin per ml, and 10% (v/v) heat-inactivated (56° for 30 min) fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.). HPBLS were prepared as described previously (6), and the cells were suspended to give a final concentration of 20 × 10⁴ lymphocytes/ml in Eagle’s minimal essential medium with 10% fetal calf serum. Microwells of HPBLS were established in Microtest plates (IS-FB-96 TC; Linbro Chemical Co., New Haven, Conn.) with culture medium, antigen, or mitogen, and ascitic or control fluid samples using 25- or 50-μl droppers (Linbro). The stimuli used to induce human lymphocyte proliferation were: PHA (Difco Laboratories, Inc., Detroit, Mich.) PWM (Difco), Con A (Miles Laboratories), and the antigen KLH (Bio-Marine Supply Co., Venice, Calif.).

Stimuli were used at a dose which provided optimum DNA and protein synthesis. The final concentration of HPBLS per well was 0.5 × 10⁴ in a final volume of 0.2 ml culture medium. The cultures were incubated in a humidified 5% CO₂ atmosphere at 37° for 3 or 6 days. For the determination of DNA-synthesis, cultures were pulsed for 16 hr with 0.5 μCi [³H]thymidine (Schwarz/Mann, Orangeburg, N. Y.; specific activity, 1.9 Ci/mmole). Cultures stimulated with antigen were pulsed from Day 2 to Day 3. Mixed lymphocyte cultures were established at a final concentration of 0.5 × 10⁴ lymphocytes/ml, and the cultures were pulsed with [³H]thymidine from Day 5 to Day 6. The incorporation of [¹⁴C]leucine (New England Nuclear, Boston, Mass.) was used to measure protein synthesis. 0.125 μCi of the isotope was added at the initiation of the experiment, and the cultures were terminated after 5 days of incubation for both antigen and mitogen stimulation. Harvesting of the cultures was accomplished on a multiple automated sample harvester (MASH II) obtained from Microbiological Associates, Bethesda, Md., as previously described (24). The samples were counted in a dioxane naphthalene-POPOP mixture in a Beckman liquid scintillation counter. The intensity of suppression was calculated in the following manner:

\[
% \text{suppression} = 100 - \left( \frac{cpm \text{ PHA stimulation of sample}}{cpm \text{ PHA stimulation of control}} \times 100 \right)
\]

We have previously shown (8) that the serum protein concentration in the culture medium plays an essential role in determining the intensity of lymphocyte response to PHA. Low concentrations of serum are essential for lymphocyte proliferation, but an excess of 25% (v/v) may inhibit proliferation. The maximal amount of ascitic fluid protein or serum protein added, therefore, was 8 mg/ml.

**Inhibition of Tumor Cell Growth.** In order to determine whether the ascitic fluid had any inhibitory activity on the proliferation of cells other than lymphocytes, we added a number of suppressive ascites fluids to HeLa cell cultures and assayed for changes in growth rate. HeLa cells were obtained from Flow Laboratories and were maintained according to accepted techniques (20). For the growth assay, single-cell suspensions were prepared by trypsinization (0.25%), and 10⁵ cells were then pipetted into each well of a microculture plate. The ascitic fluids and normal human serum controls were added at various concentrations. The cells were allowed to grow for 4 days, at which time they were pulsed with [³H]thymidine for 16 hr and the quantity of radioactive DNA was determined in the manner previously described for the lymphocyte cultures. In a few experiments the cells also were examined for changes in morphology.

**In Vivo Methods.** Three mouse strains were used in these experiments: CBA/J, male; DBA/2, male and female (obtained from the Jackson Laboratory, Bar Harbor, Maine); or CD1, male and female (obtained from Charles River Breeding Laboratories, Wilmington, Mass.). The animals were maintained on acidified (HCl) drinking water and routine mouse chow. Mice were 2 to 6 months old at the time of use. Within any given experiment only mice of the same age, strain, and sex were used. For determination of the primary immune response in vivo to SRBC, CBA/J or CD1 mice received injections of the samples of ascitic fluid or control proteins i.p. at various times before, at the same time, or after antigen. Each test or control sample was injected into 5 mice. Immunization was accomplished by injecting 4 × 10⁸ SRBC (obtained suspended in Alsever’s solution from Grand Island Biological Co. Diagnostics, Madison, Wis.; the erythrocytes were washed 3 times in Hanks’ balanced salt solution immediately before use). Antibody response to the SRBC was estimated by determining the number of antibody-forming cells (plaque-forming cells) in the spleens of these mice 4 days after SRBC injection using a modification of the Jerne plaque assay (18). In some of the in vivo experiments a possible toxic effect
of the ascitic fluid on the SRBC was abrogated by injecting the 2 samples by different routes, i.e., the ascites i.p. and the SRBC i.v. We also examined the ascites fluid for anti-SRBC antibody by a hemagglutination assay, but there was no visible agglutination. The potential cytotoxicity of the ascitic fluid in vitro was evaluated using the trypan blue exclusion test.

RESULTS

Effect of Human Ascitic Fluid on the Blast Transformation of HPBLS in Vitro. The data in Table 1 show the effect of a number of ascitic fluids on the incorporation of \(^{3}H\)thymidine into DNA and \(^{14}C\)leucine into protein of PHA-stimulated HPBLS. The data are presented as total counts incorporated into DNA in the presence of ascitic protein (4 mg/ml). The suppression ranged from 39 to 96% when compared to the untreated control. Similar inhibitory results without evidence of cytotoxicity were obtained when protein synthesis of lymphocytes stimulated with mitogen and treated with the same concentration of ascitic fluid was measured over a 5-day period. The mean suppression of protein synthesis was, however, lower (44%) than the inhibition of DNA synthesis (66%).

A number of experiments with dialyzed and nondialyzed ascites were performed to establish that this suppression was not the result of some small metabolite such as thymine or thymidine. We found no loss in the suppressive activity of cancer ascites after dialysis; in fact, sometimes there was a slight increase in activity. For example, in 1 experiment, samples from B. R. and J. O. were dialyzed against phosphate-buffered saline, pH 7.4, for 2 days. Four-mg/ml concentrations of these samples originally suppressed the PHA response 90 and 88%, respectively; after dialysis the suppression was 94 and 97%, respectively.

Three effusions obtained from noncancerous patients were also tested in this in vitro assay. These effusions were obtained from patients with tuberculosis, congestive heart failure, and myeloid metaplasia, and assayed at the same time as 3 fluids obtained from cancer patients. The results are shown in Table 2. Again we tested the ability of a standard concentration of ascitic protein (6 mg/ml) to suppress the PHA-induced proliferation of HPBLS as measured by the incorporation of \(^{3}H\)thymidine into DNA. Only the cancer ascites were suppressive (87 to 89%), there being no reduction in DNA synthesis in the presence of the noncancerous effusions.

The dose-dependent suppression of PHA stimulation of lymphocytes by a typical ascitic fluid sample is shown in Chart 1. The majority of our samples showed a similar activity, that is, almost 100% suppression being obtained with approximately 5 mg of ascitic protein.

The increased activity of this ascitic protein over serum protein is shown in Table 3. We used a serum protein concentration of 8 mg/ml (2 times the concentration of ascitic fluid used) from 4 cancer patients and 1 normal individual. Although it has been demonstrated that the serum from cancer patients is sometimes suppressive, none of our unfractionated samples was actively suppressive of the PHA stimulation of normal HPBLS in the concentrations we have assayed. We also examined the activity of the ascitic fluid on cells activated by the mitogens Con A and PWM. Using a standard concentration of exudate or transudate protein (4 mg/ml), HPBLS were stimulated with a range of doses of these 2 mitogens. The results in Chart 2 show that the entire dose-related response to Con A was completely suppressed by the ascitic fluid (Chart 2A), whereas the response to PWM was lowered by approximately 50% but over the entire dose curve (Chart 2B). We next examined the effect of these fluids on the re-

### Table 1

<table>
<thead>
<tr>
<th>Patient sample</th>
<th>Cancer origin</th>
<th>[H]Thymidine incorporation into DNA (cpm)</th>
<th>Suppression (%)</th>
<th>[14C]Leucine incorporation into protein (cpm)</th>
<th>Suppression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstimulated control</td>
<td></td>
<td>221 ± 62*</td>
<td>696 ± 68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHA-stimulated control</td>
<td></td>
<td>22,468 ± 2,219</td>
<td>14,242 ± 677</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. A., fluid + PHA</td>
<td>Breast</td>
<td>13,799 ± 1,857</td>
<td>39</td>
<td>6,918 ± 719</td>
<td></td>
</tr>
<tr>
<td>W. H., fluid + PHA</td>
<td>Breast</td>
<td>10,666 ± 1,972</td>
<td>53</td>
<td>8,363 ± 425</td>
<td></td>
</tr>
<tr>
<td>D. R., fluid + PHA</td>
<td>Stomach</td>
<td>7,167 ± 841</td>
<td>69</td>
<td>7,687 ± 163</td>
<td></td>
</tr>
<tr>
<td>S. T., fluid + PHA</td>
<td>Lung</td>
<td>6,378 ± 595</td>
<td>72</td>
<td>9,236 ± 415</td>
<td></td>
</tr>
<tr>
<td>B. R., fluid + PHA</td>
<td>Ovary</td>
<td>10,610 ± 1,283</td>
<td>53</td>
<td>11,097 ± 720</td>
<td></td>
</tr>
<tr>
<td>N. E., fluid + PHA</td>
<td>Colon</td>
<td>3,920 ± 764</td>
<td>83</td>
<td>4,732 ± 447</td>
<td></td>
</tr>
<tr>
<td>J. O., fluid + PHA</td>
<td>Ovary</td>
<td>7,737 ± 629</td>
<td>66</td>
<td>10,086 ± 1,024</td>
<td></td>
</tr>
<tr>
<td>E. M., fluid + PHA</td>
<td>Stomach</td>
<td>1,206 ± 60</td>
<td>96</td>
<td>ND*</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>7,685 ± 1,420</td>
<td>66 ± 6</td>
<td>8,302 ± 800</td>
<td>44 ± 6</td>
</tr>
</tbody>
</table>

* Mean ± S. E.
* ND, not determined.
All fluids were added at a final protein concentration of 6 mg/ml. 

Patient sample | Origin of effusion | Protein concentration of effusion (mg/ml) | [³H]Thymidine incorporation into DNA (cpm) | Suppression (%) |
--- | --- | --- | --- | --- |
Unstimulated control | | | 255 ± 32 \( \text{a} \) | |
PHA-stimulated control | | | 35,879 ± 4,675 | 89 |
N. E., fluid + PHA | Cancer | 40 | 4,196 ± 1,530 | 81 |
E. M., fluid + PHA | Cancer | 30 | 7,017 ± 2,170 | 81 |
M. A., fluid + PHA | Cancer | 50 | 4,831 ± 703 | 87 |
V. P., fluid + PHA | Tuberculosis | 51 | 37,737 ± 2,959 | None |
G. A., fluid + PHA | Congestive heart failure | 17 | 30,184 ± 2,343 | None |
S. O., fluid + PHA | Myeloid metaplasia | 55 | 37,493 ± 3,944 | None |

\( \text{a} \) Mean ± S.E.

Effect of ascitic fluids from cancer and control patients on PHA response of human peripheral lymphocytes

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| Patient sample | Protein concentration of effusion (mg/ml) | [³H]Thymidine incorporation into DNA (cpm) | Suppression (%) |
--- | --- | --- | --- |
Unstimulated cells | | 847 ± 117 | |
PHA-stimulated cells | 29,406 ± 2,845 | |
PHA-stimulated cells + serum protein (8 mg/ml) from: | | | |
B. R. | 27,583 ± 1,006 | None |
J. O. | 31,794 ± 1,675 | None |
W. H. | 20,802 ± 5,038 | 30 (NS) |
N. E. | 26,927 ± 3,935 | None |
Normal human serum control | 28,031 ± 3,533 | None |

\( \text{a} \) Mean ± S.E. 

\( \text{NS} \), nonsignificant; \( p > 1.0 \).

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Effect of human ascitic fluid on the primary plaque-forming response of mice to SRBC in vivo. 

As seen in Chart 3, 100% suppression of DNA synthesis was obtained with an ascitic protein concentration of 1 mg/ml (4 to 5 times less than that required to achieve the same degree of suppression when the cells received mitogen stimulation). The effect of 5 concentrations of ascitic protein on the mixed lymphocyte reaction between histoincompatible donors is shown in Table 4.

Effect of Human Ascitic Fluid on the Primary Plaque-forming Response of Mice to SRBC in Vivo. 

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Effect of human ascitic protein on 2-way mixed lymphocyte reactions between histoincompatible donors. The data are presented as mean percentage of suppression ± S.D. obtained in the presence of ascites as compared with the untreated mixed lymphocyte culture.

<table>
<thead>
<tr>
<th>Ascitic fraction (mg/ml)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>90.5 ± 5.6</td>
<td>100.0 ± 0</td>
<td>92.7 ± 7.2</td>
</tr>
<tr>
<td>2.5</td>
<td>83.5 ± 5.6</td>
<td>85.8 ± 8.9</td>
<td>99.0 ± 0.6</td>
</tr>
<tr>
<td>1.25</td>
<td>53.6 ± 11.3</td>
<td>81.3 ± 10.3</td>
<td>71.8 ± 10.1</td>
</tr>
<tr>
<td>0.6</td>
<td>44.4 ± 8.0</td>
<td>65.5 ± 9.4</td>
<td>80.6 ± 6.3</td>
</tr>
<tr>
<td>0.3</td>
<td>44.1 ± 7.0</td>
<td>56.0 ± 9.0</td>
<td>36.0 ± 20.3</td>
</tr>
</tbody>
</table>

Chart 3. Suppression of antigen stimulation of HPBLS by ascitic protein. Dose curve of ascitic protein on KLH-sensitized lymphocytes stimulated with an optimal dose of KLH. Data are presented as the mean cpm ± S.E.

Chart 2. Effect of a single suppressive dose (4 mg/ml) of an ascitic effusion (N. E.) on the response of HPBLS to Con A (A) and to PWM (B). The mitogens were added at a number of concentrations to give both suboptimal and optimal stimulation and high dose suppression of lymphocyte transformation. Data are presented as the mean cpm ± S.E.

Immunosuppressive activity of the ascitic fluid was not lost by dialysis or by heat treatment at temperatures up to 70° for 30 min. Dialysis of the fluid against buffers at pH 2 and pH 10 for 2 hr, however, resulted in some loss of activity. These results are shown in Table 8. Ascitic fluid, originally 84% suppressive at 4 mg/ml, was 55% suppressive after treatment for 2 hr at pH 10 and only 24% suppressive after treatment at pH 2. Quantitative and qualitative immunochemical studies of AFP in these ascites failed to demonstrate the presence of this protein down to a detectable limit of 10 ng/ml, a level below that found to be immunosuppressive (25). Our in vitro experiments never utilized more than 0.1 ml of fluid (<1 ng AFP), and the in vivo experiments did not utilize more than 2 ml (<20 ng AFP).

The effect of the ascitic fluid on cells other than human peripheral lymphocyte cultures was examined by treating HeLa cells with several concentrations of ascites protein and normal human serum (heat inactivated at 56° for 30 min) as a control. There was no effect on HeLa cell growth as measured by incorporation of [3H]thymidine into DNA or by enumeration of total cells after 4 days incubation. Incubation for this time period did not appear to produce any effect on the morphological appearance of the HeLa cells.

DISCUSSION

The experiments presented in this paper demonstrate that the ascitic fluids from patients bearing a variety of cancers contain a nontoxic, humoral factor(s) that will suppress immunological responsiveness both in vitro and in vivo. We have demonstrated that the ascitic fluid will inhibit in vitro the blast transformation of human peripheral lymphocytes induced by mitogens (PHA, Con A, and PWM) and by antigen (the mixed lymphocyte reaction between 2 histoincompatible donors and KLH). The suppression in these last 2 instances was effected by one-fifth the concentration of ascitic fluid required to suppress the mitogen response. Controls for these in vitro experiments utilized normal hu-
Effects of human ascitic fluid administered in vivo before and after antigen (SRBC)

**Table 6**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plaque-forming cells/spleen (× 10⁴)</th>
<th>Suppression (%)</th>
<th>Statistical significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no ascites)</td>
<td>40.1 ± 7.7a</td>
<td>86</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Day −2</td>
<td>5.6 ± 2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0 (With antigen)</td>
<td>35.5 ± 3.9</td>
<td>2</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Day +1</td>
<td>39.9 ± 2.2</td>
<td>0</td>
<td>&gt;0.1</td>
</tr>
</tbody>
</table>

* Each treatment was compared to the untreated control using Student's t test.

* Mean ± S.E.

**Table 7**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plaque-forming cells/spleen (× 10⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRBC</td>
<td>55.0 ± 6.3a</td>
</tr>
<tr>
<td>Ascites</td>
<td>14.7 ± 2.3</td>
</tr>
<tr>
<td>Ascites + cyclophosphamide</td>
<td>8.6 ± 2.5</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>37.2 ± 4.1</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

**Table 8**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>[³H]Thymidine incorporation into DNA (cpm)</th>
<th>Suppression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstimulated control cells</td>
<td>542 ± 110a</td>
<td></td>
</tr>
<tr>
<td>PHA-stimulated cells</td>
<td>27,384 ± 1,928</td>
<td>84</td>
</tr>
<tr>
<td>PHA with untreated ascitic fluid (N. E.)</td>
<td>4,907 ± 777</td>
<td></td>
</tr>
<tr>
<td>PHA- and heat-treated ascitic fluid 56°, 30 min</td>
<td>3,266 ± 1,316</td>
<td>90</td>
</tr>
<tr>
<td>70°, 30 min</td>
<td>1,780 ± 515</td>
<td>96</td>
</tr>
<tr>
<td>PHA- and pH 10.0-treated ascitic fluid</td>
<td>12,326 ± 1,660</td>
<td>55</td>
</tr>
<tr>
<td>PHA- and pH 2.0-treated ascitic fluid</td>
<td>21,152 ± 3,652</td>
<td>24</td>
</tr>
</tbody>
</table>

* Mean ± S.E.
as well as DNA synthesis from thymidine, and we have also observed a direct effect on the development of antibody-producing cells. In addition, they have demonstrated that the factor responsible for this phenomenon is dialyzable, and our suppressive material is not dialyzable.

The recent finding that AFP is immunosuppressive in several in vitro and in vivo systems (25) led us to carefully examine the ascitic fluids for AFP. Utilizing the Laurell rocket immunoelectrophoresis method, we first found that these fluids contained less than 1 μg AFP per ml. Dr. E. Alpert of the Massachusetts General Hospital, utilizing a radioimmunoassay, subsequently demonstrated that there were less than 10 ng of AFP per ml ascitic fluid in each of the samples we used in this study. This is far less than Murgita and Tomasi have demonstrated to be immunosuppressive.

The immunosuppressive activity of the ascitic fluids that have been examined to date appears to be similar to the immunoregulatory α-globulin found in normal and cancer plasma and the peptide that has been isolated from it (7, 15, 16, 26). Both the immunoregulatory α-globulin and the substances in human cancer ascites appear to be nonspecifically immunosuppressive and active across species barriers and to act on the early events of lymphocyte activation. In this study we have not determined whether the suppressive effects in ascites are caused by the same substance that is present in serum, and currently ongoing studies do not yet give a clear answer to this question.

The origin of this suppressor factor also remains to be determined. However, the biological activity is easily observed in the fluid bathing a variety of peritoneal metastatic cancers and may be observed in ascites when it cannot be observed in whole serum. These observations suggest that the factor(s) is being produced at the tumor site, either from tumor cells or from other host cells stimulated by the presence of cancer. This may be of some significance in understanding the mechanisms by which some tumor cells evade immune surveillance or immunologically mediated removal.

ACKNOWLEDGMENTS

We thank Max Roth, Melvin Hecht, and Stephan Pearlman, for their technical assistance, and Dr. Norman Zamcheck and Dr. Eliot Alpert, for the AFP assays.

REFERENCES

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