Staphylococcal Protein A Column: Correlation of Mitogenicity of Perfused Plasma with Clinical Response


ABSTRACT

Eleven patients with advanced breast cancer and four with astrocytoma were treated with plasma perfused over columns containing staphylococcal Protein A (SPA). Doses of 5 to 20 mg of SPA were bound to collodion charcoal particles, and this treatment resulted in partial remissions in one patient with astrocytoma and in two patients with breast cancer.Remission duration was 6 wk to 6 mo. Resolution of lymphadenopathy and a decrease in cancer embryoanticörper were noted in an additional two breast cancer patients. Systemic reactions to infused plasma consisted of fever, chills, and rigors. In brain cancer patients, increased intracranial pressure was also noted.

A mitogenic substance was generated in plasma of 11 patients after it was perfused over the SPA charcoal matrix. The mitogenic material induced lymphoproliferation comparable to concanavalin A and required the presence of SPA on the collodion charcoal but was not due to leakage of SPA from the column during plasma perfusion. Of considerable significance was that only patients whose column perfused plasma contained this mitogenic activity exhibited systemic reactions, and five of these patients obtained antitumor responses. This striking correlation implies that the mitogenic factor is an active component of SPA therapy. The ability to demonstrate mitogenicity in column perfused plasma might also be useful for selecting patients amenable to SPA therapy.

These findings attest to the therapeutic value of this mode of treatment and provide an initial definition of a mediator of SPA antitumor activity.

INTRODUCTION

Over the past several years a variety of biological response modifiers has been used for the investigational treatment of cancer. One modality, treatment of patients with plasma perfused over Staphylococcus aureus Cowans I or its active component SPA,2 has attracted particular attention. Antitumor responses were reported by five independent investigators in cancer patients (3, 4, 21, 26) and experimental animals (17, 19, 27).

A variety of mechanisms has been suggested to explain SPA-induced tumor regressions including removal of serum inhibitors (7), activation of complement (25), induction of tumor-directed antibodies (17), leakage of SPA (16), and the antitumor effects of pyrexia (9). Unfortunately little direct evidence supports any of these hypotheses. Removal of immune complexes or other immunosuppressive material seemed an especially attractive possibility, since immobilized SPA is known to bind and thus remove such complexes (12). However, infusion of even small aliquots of perfused plasma has also resulted in significant clinical responses (26) as has infusion of treated plasma from one individual to another (26). Under these conditions removal of immune complexes would be incomplete and thus could not entirely explain the antitumor activity.

Presence of immunostimulatory material in CPP might be a better explanation for the biological activities of SPA therapy. In fact we have detected such an immunostimulatory component and have reported that CPP from cancer patients (5) or from normal donors (6) is highly mitogenic to normal lymphocytes. We now present the complete results of a Phase I study including toxicities, hematological changes, and tumor regressions observed and correlate the presence of mitogenic material with clinical symptomatology and antitumor activity. An initial characterization of this mitogenic component is also provided.

MATERIALS AND METHODS

Patients. Eleven patients with histologically confirmed breast cancer and four patients with astrocytoma were enrolled in this study. All patients were refractory to standard therapy including surgery and radiotherapy for breast cancer patients, and standard chemotherapy or hormonal therapy for breast cancer patients. None of the patients received chemotherapy or radiotherapy within 4 wk before, during, or after completion of the study. Signed informed consent was obtained in all cases, according to National Cancer Institute and institutional policies.

Preparation of the SPA Column and Schema of Treatment. SPA columns were prepared according to our modification of the procedure of Terman et al. (26). Six to 14 mesh charcoal particles (Fisher Scientific) were washed extensively with distilled water and PBS, autoclaved, dried at 45°C for 1 wk, and then divided into 30-g aliquots. Five to 40 mg of SPA dissolved in 2 ml of 0.1 M Tris buffer (pH 7.4) were added dropwise to a rapidly stirred mixture of 4 ml of alcohol, 4 ml of collodion, and 48 ml of ether under a fume hood. Thirty g of charcoal were then added and stirred with a wooden applicator until dry. The coated particles were allowed to dry in a fume hood for 12 h at 27°C, washed 3 times with 100 ml of sterile PBS, and then loaded into 60-ml plastic syringes (Fenwal) which had a stainless steel (No. 40) mesh placed at the outflow tract.

Autologous plasma was obtained by a single phlebotomy using a double plasmapheresis double blood pack containing ADC-Formula I anticoagulant (Fenwal). This procedure yielded approximately 500 ml of plasma which was divided into 100-ml aliquots and stored at -30°C. Autologous RBC were reinfused on the day of the phlebotomy.

On the day of treatment, a 100-ml aliquot of autologous plasma was thawed and perfused at 27°C over the SPA column in a laminar flow hood at a rate of 0.8 ml/min. Perfused plasma was collected in a sterile transfer bag and reinfused into the patient i.v. over a 2-h period. Samples of perfused plasma were tested for sterility, for endotoxin contamination by Limulus assay, and for pyrogenicity in rabbits. All patients were closely

1 To whom requests for reprints should be addressed.
2 The abbreviations used are: SPA, staphylococcal Protein A; CPP, column perfused plasma; PBS, phosphate-buffered saline; CT, computerized tomography; IL-1, interleukin 1; IL-2, interleukin 2.

Received 12/18/84; revised 5/21/85; accepted 6/3/85.

[CANCER RESEARCH 45, 4486-4494, September 1985]
observed for at least 12 h after plasma reinfusion.

Patients were treated weekly with 100 ml of autologous plasma perfused over columns containing increasing amounts of SPA for a total of five treatments. The amounts of SPA on the column were 5, 10, and 20 mg during the first three treatments, respectively. During the final two treatments, 20 or 40 mg were used.

Construction of Miniature SPA Columns. Plasma samples were also perfused over miniature SPA columns constructed as described above, except that columns contained only 5 g of SPA matrix within a 10-ml syringe, and 10 ml of plasma were perfused.

Assays of Lymphoproliferation. Mononuclear cells from normal individuals were prepared from heparinized venous blood by Ficoll-Hypaque density-gradient centrifugation. Cells were dispensed into microtiter wells at a concentration of 2 x 10⁶/well in 200 µl of RPMI 1640 medium. Twenty µl of normal serum or patients' pre- or post-perfusion plasma were then added to the wells. In certain experiments, concanavalin A (20 µg/well), SPA (1 µg/well), or pre- and post-perfusion SPA charcoal particles were used instead of plasma or serum.

Plates were cultured in a humidified atmosphere of 5% CO₂ in air at 37°C. Wells were then pulsed with [³H]thymidine (1 µCi/well). Cells were harvested 18 h later onto glass fiber strips, and thymidine uptake was measured by scintillation counting. Data are expressed as the mean of quadruplicate wells.

Heat Inactivation. In some instances SPA or CPP was incubated at 56°C for 30 min prior to addition to the microtiter wells in lymphoproliferation assays.

Determination of Binding and Detachment of SPA from the Charcoal Matrix. SPA was radioactively labeled with ¹²⁵I by the chloramine T method (22). Approximately 5.4 x 10⁶ cpm of ¹²⁵I-labeled SPA were attached to the charcoal matrix. ¹²⁵I levels were determined in triplicate on 3-ml aliquots of the three PBS washings and CPP. Radioactivity was also measured on 1-g triplicates of the charcoal matrix immediately after attachment of SPA, after the third wash with PBS and after perfusion of plasma. The quantities of bound and subsequently detached SPA were calculated based on retained and released radioactivity.

RESULTS

Fifteen patients (Table 1) were entered into this Phase I trial of treatment with autologous plasma perfused over columns containing SPA. Eleven patients had advanced breast cancer, and four had astrocytoma. Patients with breast cancer were chosen because this tumor has been reported to be responsive to SPA therapy (26). Antitumor responses obtained in this group could both validate our methodology and confirm the work of others. Patients with primary brain tumors were included into this trial because the recent demonstration of glioma-associated antigens might suggest responsiveness of this tumor type to immunological maneuvers (1). All patients were evaluable for toxicity. Antitumor responses were also carefully noted, even though this was a Phase I trial.

Antitumor Activity of SPA Therapy. Antitumor activity of various degrees was observed in 5 of the 15 patients with Patients 1, 3, and 7 meeting the classical criteria for partial remission.

Increased central tumor necrosis was observed in the two responding brain cancer patients. This occurred immediately after the first treatment in Patient 10 and required surgical drainage of mostly necrotic tumor tissue. Follow-up CT scans revealed slow regrowth of the left temporal lesion after completion of therapy, and the patient expired 18 mo thereafter. Patient 1 manifested low grade seizure activity during all treatments, and a CT scan obtained at the end of the fifth treatment demonstrated partial response with more than 50% tumor necrosis (Fig. 1). Stereotactic brain biopsies were repeated at that time also and revealed necrosis of the initially viable tumor tissue. Remission duration was 2 mo.

A considerable degree of antitumor activity was also observed in 3 of the 11 patients with breast cancer. Patient 3 who presented with left axillary lymphadenopathy and a single brainstem metastasis had a partial remission with complete resolution of the axillary adenopathy and necrosis of the brainstem mass (Fig. 2). Patient 7 had chest wall involvement and axillary adenopathy. She experienced pain in tumor-bearing areas during each treatment with subsequent complete resolution of axillary lymphadenopathy. Patient 12 similarly experienced pain in tumor-bearing areas during therapy; there was some shrinkage of the chest wall disease, and the peripheral blood carcinoembryonic antigen levels had decreased at the end of therapy from 856 to 34 ng/ml. Signs of clinical response were evident in all five patients after the first three treatments, and response was complete after five treatments.

Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Primary tumor</th>
<th>Tumor sites involved</th>
<th>Previous therapy</th>
<th>Antitumor response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28</td>
<td>Brain</td>
<td>Right frontalpolar lobe</td>
<td>Surgery, XRT⁴</td>
<td>Central necrosis</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>Breast</td>
<td>Lung, left cervical nodes</td>
<td>L-PAM, 5-FU, ADR</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>Breast</td>
<td>Brainstem, left axillary nodes</td>
<td>CMF</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>49</td>
<td>Breast</td>
<td>Brain</td>
<td>Surgery, XRT</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>37</td>
<td>Breast</td>
<td>Left parietal lobe</td>
<td>Surgery, XRT</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>47</td>
<td>Breast</td>
<td>Bone, bone marrow</td>
<td>L-PAM, 5-FU, ADR, TAM</td>
<td>Resolution of adenopathy</td>
</tr>
<tr>
<td>7</td>
<td>58</td>
<td>Breast</td>
<td>Chest wall, left axillary nodes</td>
<td>L-PAM, 5-FU, XRT, ADR</td>
<td>Resolution of adenopathy</td>
</tr>
<tr>
<td>8</td>
<td>59</td>
<td>Breast</td>
<td>Bone</td>
<td>CMF, TAM, IFN</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>54</td>
<td>Breast</td>
<td>Brain</td>
<td>CMF, TAM, XRT</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>36</td>
<td>Brain</td>
<td>Left temporal lobe</td>
<td>Surgery, XRT</td>
<td>Central necrosis</td>
</tr>
<tr>
<td>11</td>
<td>33</td>
<td>Brain</td>
<td>Brain stem</td>
<td>Surgery, XRT</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>56</td>
<td>Breast</td>
<td>Chest wall, lungs, retroperitoneal nodes</td>
<td>L-PAM, IFN</td>
<td>Decrease of CEA</td>
</tr>
<tr>
<td>13</td>
<td>48</td>
<td>Breast</td>
<td>Lung</td>
<td>CMF, TAM, IFN, XRT</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>47</td>
<td>Breast</td>
<td>Lung</td>
<td>ADR, XRT</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>68</td>
<td>Breast</td>
<td>Chest wall, right axilla</td>
<td>CMF, ADR, Velban</td>
<td></td>
</tr>
</tbody>
</table>

⁴XRT, radiotherapy; L-PAM, L-phenylalanine mustard; 5-FU, 5-fluorouracil; ADR, adriamycin; CMF, cyclophosphamide, methotrexate, and 5-fluorouracil; TAM, tamoxifen; IFN, interferon; CEA, carcinoembryonic antigen.
Three patients (Patients 6, 14, and 15) had tumor progression during therapy and did not complete the treatment cycle, while tumor progression at the end of therapy was noted in Patient 4. The remaining patients had stable disease during and usually for at least 1 mo after therapy.

Toxicity of SPA Therapy. Clinical reactions to infusion of CPP were observed in 11 of the 15 patients: in 7 of 11 breast cancer patients and 4 of 4 patients with brain cancer. They consisted of chills, rigors, and fever in the breast cancer patients and also of increased intracranial pressure in brain cancer patients. These constitutional symptoms seemed to be linked to antitumor response. All patients whose tumors regressed exhibited strong systemic reactions to CPP. In contrast the four patients who were asymptomatic after SPA therapy had no signs of antitumor activity. These four patients were also the only individuals with tumor progression during or at the end of therapy. Systemic reactions thus appear to identify patients who may be amenable to this treatment modality.

Systemic reactions to infusion of CPP generally occurred in a predictable sequence and increased with increasing amounts of SPA bound to the charcoal. Chills and rigors usually developed within 1 to 3 h after beginning the plasma infusion and were followed 30 to 60 min later by the rapid onset of fever (Chart 1). Fever reached peak levels as high as 40°C and persisted for more than 18 h in several patients. The full magnitude and duration of the febrile reaction, however, were not determined since antipyretics were given for temperatures above 38.3°C. This fever was not due to bacteremia, since blood cultures obtained during the febrile episodes were negative for growth. Bronchospasm, easily controlled with bronchodilators and nasal oxygen, was noted in one patient (Patient 7) who had metastatic lung involvement. Additional side effects were noted in three of four patients with primary brain tumors and consisted of headaches, agitation, or severe disorientation. Such symptomatology occurred almost instantly after start of therapy and was most pronounced in Patient 10 (Chart 2) who had nausea, vomiting, and disorientation associated with bilateral mild papilledema. In this patient a CT scan confirmed the presence of cerebral edema localized to the tumor site (Fig. 3). Hypotension was noted in this patient during each treatment and in Patient 9 as well during her fourth therapy. Hypotension, however, did not occur in the breast cancer patients who revealed mild transient hypertension or no blood pressure changes during and after therapy (Chart 1). Increased intracranial pressure rather than SPA-induced peripheral vascular dilatation thus might have been the cause for these hypotensive episodes.

Hematological and Biochemical Changes Induced by SPA Therapy. Profound changes in peripheral blood counts and T-cell subsets were noted in blood specimens obtained serially from three of the "reacting" patients, i.e., those with fever and rigors. Striking lymphocytopenia developed with each treatment in these patients. The nadir was reached approximately 16 h after the beginning of therapy at the time when fever and other constitutional symptoms had already subsided (Chart 3).
absolute lymphocytopenia was usually accompanied by a marked granulocytosis resulting in an overall leukocytosis. T-cell subsets were determined concomitantly with monoclonal antibodies T3, T4, T8, and T11. Profound depletion of all determined T-cell subsets occurred, and the kinetics of the decrease closely resembled the total lymphocyte curve (Chart 3). In contrast, no significant changes in peripheral blood counts and lymphocyte subsets occurred in the two examined patients not reacting to therapy. Serum chemistries were unchanged in all patients during all treatments.

Correlation of Toxicity, Antitumor Activity, and Mitogenicity of Perfused Plasma. The apparent linkage between systemic reactions and antitumor response prompted us to investigate the immunomodulatory potential of CPP with a variety of immunological assays. We found that plasma from a majority of our patients became highly mitogenic after perfusion over SPA columns (Chart 4). Small doses of charcoal-attached SPA (5 and 10 mg) generally caused low levels of mitogenicity, while higher doses (Treatments 3 to 5) resulted in higher levels. CPP of Patients 9, 10, and 13, however, was exceptionally mitogenic during all treatments and did not appear to follow this dose-response curve. Finally doses above 20 mg of charcoal-bound SPA did not further increase mitogenicity.

Mitogenic activity correlated strongly with the occurrence of systemic symptoms and to a lesser degree with antitumor activity (Table 2). Plasmas from 11 of the 15 patients were strongly mitogenic in vitro. All 11 patients showed constitutional symptoms of fever, chills, and rigors after infusion of CPP, and 5 of these patients experienced antitumor responses. Strength of mitogenicity in CPP also appeared to correlate with the degree of clinical symptoms. Low levels (Treatments 1 and 2) induced only mild temperature elevations and chills, while high levels (Treatments 3 to 5) gave rise to the full clinical symptomatology. The CPP from the four patients who exhibited no systemic symptoms and no antitumor effects was devoid of mitogenicity.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mitogenicity of CPP</th>
<th>Clinical reactions</th>
<th>Antitumor response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
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<td>7</td>
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<td>12</td>
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<tr>
<td>2</td>
<td>+</td>
<td>+</td>
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<tr>
<td>5</td>
<td>+</td>
<td>+</td>
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<td>8</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Plasma was considered to be mitogenic if it stimulated incorporation of more than 10,000 cpm [3H]thymidine by mononuclear cells.

b Clinical reactions consisted of fever (38.3°C), chills, and rigors in brain cancer patients also of increased intracranial pressure.

c Type of response is listed in Table 1.
The relationship of antitumor activity, constitutional symptoms, and mitogenicity of perfused plasma was recognized early in this trial during the treatment of Patients 3 and 4. Patient 3 experienced strong constitutional symptoms to infusion of CPP, while Patient 4 treated with identical columns had no side effects at all. CPP from these patients was incubated with autologous or homologous normal mononuclear cells, and the proliferative response was determined (Chart 5). Perfused plasma from Patient 3 was highly mitogenic to lymphocytes, while unperfused plasma was devoid of mitogenicity. Marked proliferation of this patient's own mononuclear cells after exposure to his CPP was also observed, eliminating allogeneic differences as an explanation for the observed mitogenicity. In contrast neither perfused nor unperfused plasma from the other patient (Patient 4) induced any mitogenicity against homologous or autologous mononuclear cells. Patient 3 had an antitumor response, while the disease of Patient 4 progressed during therapy.

**Relationship of CPP to SPA.** Mitogenicity in CPP might have been induced, among others, by SPA, which is a potent lymphocyte mitogen (24) or the collodion charcoal matrix itself in which SPA is embedded. We tested this latter possibility by perfusion of stored highly mitogenic plasma samples over charcoal alone, charcoal coated with collodion, or the complete SPA-collodion matrix. Plasma perfused over charcoal alone or collodion charcoal remained nonmitogenic (1,568 cpm versus 1,743 cpm of \(^{3}H\)thymidine incorporation, respectively), while plasma perfused over the complete SPA-collodion charcoal complex became highly mitogenic (124,384 cpm of \(^{3}H\)thymidine incorporation). SPA thus appeared to be required for induction of mitogenicity.

This dependence of mitogenicity on the presence of immobilized SPA might have been explained by leakage of small quantities of SPA from the column during the perfusion process. We tested this possibility with heat inactivation experiments. SPA containing solutions or CPP was incubated at 56°C for 30 min, and mitogenic capability of the pretreated specimen was determined (Chart 6). Indeed mitogenicity of SPA which is known to be heat stable (13) was not affected, whereas the mitogenicity of CPP was almost totally abolished by heat. Detachment of SPA during washing and plasma perfusion was then quantitated with columns containing traces of \(^{125}\)I-labeled SPA (Table 3). Leached radioactivity was measured in the 3 PBS exchanges used for washing the SPA-collodion charcoal and subsequently in perfused plasma. Leakage of column bound SPA occurred mainly in the first PBS wash and was 8% for the 40-mg dose. Each subsequent wash contained approximately one-tenth of the radioactivity present in the previous wash, so that the final wash contained less than 0.1% of the radioactivity. No radioactivity was detected in subsequently perfused plasma. Therefore perfused plasma contained no detectable leached SPA (sensitivity of this assay, <2 μg of SPA).

All eluates were concomitantly assayed for mitogenicity and the heat stability of such mitogenicity (Table 3). Mitogenicity was present only in washes containing radioactive SPA and in CPP. As expected, mitogenicity in the PBS washings which contained leached SPA was heat stable, while mitogenicity in CPP was heat labile. These data collectively confirm that mitogenicity of CPP was not due to leakage or whole SPA and also virtually exclude contaminants of SPA preparations, such as enterotoxins as the source of the mitogenicity in CPP.

**Mitogenic Capabilities of Plasma-perfused SPA, Charcoal Particles, and of CPP.** Since PBS perfused over a prewashed matrix was devoid of mitogenic material while plasma passed over an identical matrix was highly mitogenic, mitogenicity might...
Normal mononuclear cells were incubated with either CPP, was thus generated or retained on the SPA charcoal particles. Cells after plasma perfusion, however, had at least 3 times the mitogenic activity of unperfused SPA charcoal. Mitogenic activity in CPP was also determined (Table 3). Data for mitogenicity before and after plasma perfusion are shown in Chart 7.

Table 3
Quantitation of SPA leakage from the SPA-charcoal column during washing and subsequent plasma perfusion, and relationship to mitogenicity in the eluates

<table>
<thead>
<tr>
<th>Column perfused with</th>
<th>Quantity of leaked SPA (µg/100 ml)</th>
<th>Heat-treated (56°C, 30 min) eluate</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First wash</td>
<td>3.126</td>
<td>137,343 ± 6,984*</td>
</tr>
<tr>
<td>Second wash</td>
<td>300</td>
<td>6,287 ± 527</td>
</tr>
<tr>
<td>Third wash</td>
<td>24</td>
<td>244 ± 53</td>
</tr>
<tr>
<td>Plasma</td>
<td>2</td>
<td>72,920 ± 6,358</td>
</tr>
</tbody>
</table>

*Mean ± SE.

Mitogenic assay (cpm [3H]thymidine)

Unperfused Charcoal


Table 4
Comparison of mitogenic activity of CPP with that of concanavalin A or alloantigen

<table>
<thead>
<tr>
<th>Stimulant</th>
<th>[3H]Thymidine incorporation (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPP</td>
<td>91,484 ± 6,842*</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>89,380 ± 5,712</td>
</tr>
<tr>
<td>Alloantigen</td>
<td>129,680 ± 8,119</td>
</tr>
</tbody>
</table>

*Mean ± SE.

have been created by interaction of plasma molecules with immobilized SPA. We thus tested SPA-coated charcoal particles for mitogenicity before and after plasma perfusion (Chart 7). Uncoated charcoal particles which did not contain SPA were used as control and were nonmitogenic. SPA-colloidon charcoal particles before perfusion were mitogenic, presumably due to the presence of immobilized SPA. SPA-colloidon charcoal particles after plasma perfusion, however, had at least 3 times the mitogenic activity of unperfused SPA charcoal. Mitogenic activity was thus generated or retained on the SPA charcoal particles.

The mitogenic capability of CPP was also determined (Table 4). Normal mononuclear cells were incubated with either CPP, concanavalin A, or alloantigens. Twenty µl of CPP induced proliferation comparable to 20 µg of concanavalin A per ml or 2 x 10^5 of allogeneic cells. CPP in small amounts was a potent lymphocyte mitogen.

DISCUSSION

Our studies have both confirmed the effectiveness of treatment with the SPA column and provided a lead as to its mechanism of action.

Objective antitumor activity was seen in 5 of the 15 patients and consisted of central tumor necrosis in those with brain cancer and resolution of lymphadenopathy, decrease in carcinembryonic antigen, and necrosis of metastatic lesions in breast cancer patients. Antitumor responses were rapid in onset, sustained as long as 6 mo (Patient 7), and fulfilled criteria for partial response in three of the responding patients. Toxicities consisted of fever, rigor, and chills in the breast cancer patients and also of increased intracranial pressure in the responding patients with brain cancer. Hypotension was also noted in these two patients and might represent a consequence of the brain edema rather than a direct SPA-mediated toxicity. These hypotensive episodes responded well to volume expansion and Dopamine and were generally quite manageable. The febrile response could be successfully attenuated with antipyretics when given at the onset of fever, and the frequently observed rigors could be easily abolished with the judicious use of Demerol. Brochospasm which was seen in a patient with pulmonary metastasis could be reversed with the use of bronchodilators.

The mechanism of action of SPA therapy remains an important question. Removal of immune complexes seems an unlikely explanation, since small volumes of CPP were sufficient to induce substantial antitumor effects. SPA perfused plasma thus could contain immunostimulatory molecules which are either derived from the matrix or formed from plasma after contact with the SPA matrix. These molecules might be directly tumoricidal or act indirectly by stimulation of immunocompetent cells. The demonstration of a strong mitogenic activity in CPP supports the latter possibility as does the finding that this activity apparently is important for effectiveness of SPA therapy. All patients with mitogenic CPP experienced constitutional symptoms such as chills, rigors, and fever, and a subset of these patients had antitumor effects. In contrast the four asymptomatic patients not only lacked antitumor activity but were the only individuals of this trial with tumor progression during therapy. Mitogenic activity thus was linked with systemic reactions and appeared to be important for antitumor response. Testing for generation of mitogenicity in CPP might therefore allow prediction of patients amenable to SPA therapy.

Interleukins may be important mediators of mitogenicity-induced antitumor activity. Dumonde et al. (11) performed a clinical trial in advanced cancer patients with a crude lymphokine preparation which was rich in IL-1. Chills, rigors, and fever, and high spiking temperatures were observed shortly after administration of the preparation. Changes in peripheral blood counts were also identical to those observed by us and consisted of profound lymphocytopenia with accompanying granulocytosis and leukocytosis. This striking similarity, the fact that IL-1 is a major cause of nonendotoxin fever (10), and the recent demonstration that an SPA-related enterotoxin can induce interleukin production (18) all support liberation of interleukins as an important mechanism of SPA therapy. Finally the clinical finding that SPA therapy might be beneficial in the treatment of acquired immune deficiency syndrome (20), a disease where IL-2 production is severely depressed, might further support this concept. Perfusion of
expansion of preimmunized lymphocyte clones by IL-2 which is
with immobilized SPA. Such material would then interact with
molecules and function, as powerful mitogens in the immune
complexed form. In this context, demonstration of SPA-immu-
(23) and frequent contaminants of SPA preparations.3 However,
determined. This activity was neither due to leakage of SPA nor
complement component C3b had a similar antitumor effect.4
Indeed Cooper recently has tentatively identified Clq in
plasma perfused over SPA-Sepharose as the antitumor principle
(14). The partially purified complement component C3b could be identical with this complement component.
Decay of C3b then would lead to generation of C5a and subsequent
production of interleukins. However, the antitumor activity in
Cooper’s system was highly unstable, while CPP of our
patients was still strongly mitogenic after more than a yr of
storage at ~30°C. The fact that not all patients reacted and
responded to therapy also argues against the participation of
complement in the SPA-induced tumoricidal response. Since
complement is not allotypically regulated, the lack of mitogenic
activity in some plasma would require either defects of activation
or the presence of inhibitors of complement.
Isolation and identification of the mitogenic component in CPP
will provide a more rational base for future research on this
immunomodulatory treatment modality. Even now, however, the
immunological correlates provide some insight into a possible
mechanism of SPA therapy.

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Fig. 1. CT scan of head of Patient 1 prior to the first SPA treatment (left) and after completion of therapy (right). A large lesion with focal enhancement causing considerable mass effect was initially present in the right frontoparietal region. After completion of therapy, large areas of the tumor appeared necrotic, and areas of tumor enhancement representing solid tumor were diminished.

Fig. 2. Photomicrograph of a representative section of the brainstem metastasis from Patient 3. Areas of tumor necrosis (left upper and right lower corner), collagen deposition with neovascularization (left lower corner), and some viable tumor cells (middle section) are seen.
Fig. 3. CT scan of head of Patient 10 immediately prior to (left) and 16 h after (right) CPP infusion. A bilobed cystic tumor was present in the left parietal region prior to therapy. After CPP infusion, the margins of the tumor were less well seen, and there were increased edema and mass effect medially and posteriorly of the tumor.
Staphylococcal Protein A Column: Correlation of Mitogenicity of Perfused Plasma with Clinical Response

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