Continuous Whole Blood UltraPheresis Procedure in Patients with Metastatic Cancer

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Summary: Sixteen patients with metastatic cancer, each with bidirectionally measurable disease, were treated with a total of 24 membrane UltraPheresis procedures each to remove a low molecular weight (less than 150,000 daltons) plasma fraction. No other oncologic treatment was applied during the 2 months of study. The procedure was generally well tolerated, and no clinically significant adverse effects were observed from the procedure. A consistent tumor-specific inflammatory response was observed following the UltraPheresis procedure and was associated with lymphocytic infiltration of tumor and tumor necrosis that was demonstrated in those patients evaluable by repeat biopsy. In some patients, anergy was reversed and Karnofsky status improved. Six of the 16 patients had reduction of the sum of mean cross-sectional diameters of measurable lesions by 50% or more. Key Words: UltraPheresis—Cancer—Tumor inflammation—Tumor necrosis.

Immunosuppressive factors in the sera of cancer patients have been demonstrated to effect inhibition of lymphocyte blastoid transformation as well as specific cytotoxicity in vitro. This suppression has been ascribed to immune complexes by Theofilapoulas et al. (1) and Hellstrom et al. (2), “acute phase reactants” by Israel et al. (3), “prostaglandins” by Goodwin et al. (4), and “immunoglobulin” by Hellstrom (5). Others have demonstrated low molecular weight inhibitors, both in the mouse and human systems, that may play a regulatory role at the local cellular level in “desensitizing” the helper-effector arm or boosting the suppressor arm of both antibody-dependent and antibody-independent immune cytotoxicity. It is noteworthy that each of these suppressor regulators is in the molecular weight range of less than 150,000 daltons.

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UltraPheresis is a trademark of Anisa Medical, Inc. The UltraPheresis procedure is patented in the U.S.A. and has patents in other countries.
vein and changed monthly. It was filled with heparinized saline between UltraPheresis procedures and maintained under a sterile occlusive dressing. During the procedure, blood was withdrawn via the proximal limb of the catheter and pumped through standard dialysis-type blood tubing (Life Med, Travenol). Blood was directed through a plate filter and returned, via tubing, to the distal port of the catheter, where it was returned to the patient. Negative pressure was established on the nonblood surface of the membranes within the filter by a second roller pump, thus causing a controlled transmembrane pressure gradient that produced the ultrafiltrate.

A third roller pump metered replacement solution into the postfiltered blood at the same rate as ultrafiltrate was removed from the filtered blood. The patient’s central volume was thus maintained constant.

Membrane used in this trial was the Nuclepore 0.05 µm polycarbonate membrane. It was chosen because, of the commercially available membranes tested, it demonstrated the steepest performance curve of permeability coefficient versus molecular weight, and thus the best discrimination between molecules of different sizes. The performance curve of this membrane is described in Fig. 3.

The average amount of ultrafiltrate removed was 10 cc/kg lean body mass per procedure (range 5–25 cc/kg); this volume was removed three times per week. Time for the procedure averaged 1 h (range 30 min to 2 h). This frequency and volume appeared to be sufficient to establish and maintain a "tumor inflammatory response," defined in this study as heightened aching or throbbing pain in tumor

FIG. 1. Schematic diagram of the UltraPheresis procedure.
sites or increased redness, swelling, warmth, and tenderness in cutaneous tumors associated with a rise in serum C-reactive protein (CRP). However, one patient with metastatic melanoma developed tumor-specific hemorrhagic necrosis after seven procedures, at a volume of 250 cc total per procedure, and one patient with metastatic inflammatory breast cancer required an 8 L removal in one procedure to elicit a tumor inflammatory response. "Clinical response" was defined as objective reduction in the sum of mean cross-sectional diameters of measurable disease where complete response was disappearance of all palpable or radiographic evidence of disease or complete necrosis of all measurable lesions histologically. Partial response was defined as >50% reduction of the sum of mean cross-sectional diameters of measurable disease or >50% histologically demonstrable necrosis and a reparative fibrosis above baseline pretreatment histologies. Minimal response was defined as <50% reduction in the sum of mean cross-

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sectional diameters or evidence of an increased amount of tumor cell destruction in biopsiable lesions over baseline histologies. Stable disease was defined as no tumor growth while on protocol where tumor doubling times could be reasonably calculated prior to treatment, suggesting a true stabilization of disease. Progressive disease was defined as tumor growth on protocol. Three types of ultrafiltrate replacement solution were employed. The most intense tumor inflammation and necrosis was observed in those patients whose ultrafiltrate was replaced with ultrafiltrate from normal plasma, appropriately ABO-matched to the patient (4/16 patients). Albumin (2.5%) in normal saline and fresh frozen plasma (50%) in normal saline were also employed in the remaining patients, and no significant differences in tumor inflammatory response were observed in this group. Sodium heparin was delivered in the afferent blood tubing line to maintain an activated whole blood clotting time of 180-250 s throughout the procedure.

Patients

Sixteen patients were studied, each with metastatic, bidirectionally measurable lesions: adenocarcinoma of the lung—two patients; oat-cell carcinoma—one patient; melanoma—four patients; breast, infiltrating ductal—two patients; inflammatory carcinoma of the breast in a premenopausal woman—one patient; ana-
plastic Wilms tumor—one patient; adenocarcinoma of the pancreas—one patient; bladder (transitional cell carcinoma)—one patient; adenocarcinoma of the colon—three patients. Previous treatment had included surgery and radiation therapy. All but two had had previous chemotherapy. None had had chemotherapy within 2 months of beginning the UltraPheresis procedure. Karnofsky status ranged from 50 to 80%. There were 5 men and 11 women, mean age 47.2 years (range 11–67 years). Only one patient had demonstrated central nervous system (CNS) metastases (melanoma). All patients except two were felt to be terminally ill and to have an anticipated survival of 2–6 months at protocol entry.

Immunologic Studies

Recall antigen skin tests (candida, trichophytin, and mumps) were tested prior to the first procedure, after 1 month, and after 2 months on protocol.

Candida was given at 1:500 wt/vol. Readings were performed at 24, 48, and 72 h; the test was done 0.1 ml intradermally in the solar aspect of the forearm.

Trichophytin was used at 1:500 wt/vol. Reading times were 24, 48, and 72 h; 0.1 ml was given intradermally in the solar aspect of the forearm.

The mumps skin test antigen (Connaught) was used. Reading times were 24, 48, and 72 h; 0.1 ml was given intradermally in the solar aspect of the forearm.

A positive response was identified if 5 mm or more induration was obtained and a negative response if no induration developed. An induration of <5 mm was considered equivocal and recorded as negative.

Lymphoid marker studies were also performed monthly and included total lymphocyte count, T4, T8, total T, and total B using cytoflorometry with fluorescent Coulter monoclonal antibody (Smith-Kline Reference Laboratory). Total immunoglobulin determinations were assessed monthly.

RESULTS

In 12 patients, a subjective improvement was noted during therapy that lasted throughout the 2 months of treatment. Appetite increased, and objective weight gain occurred in seven patients, or 43% of the total. Karnofsky status improved on therapy by an average of 25%, with a range of 15–30% in six patients.

Tumor redness, tenderness, swelling, and/or reported inflammatory pain was observed in 14 of the 16 patients. Metastatic foci in lymph nodes resolved in one patient with metastatic adenocarcinoma of the lung, in two patients with melanoma, and in three patients with adenocarcinoma of the breast. One patient with transitional cell carcinoma of the bladder had a complete resolution of dysuria, urinary frequency and urgency, and a marked improvement in the appearance of his intravesicular disease on repeat cytoscopy. His symptoms returned, however, and cystoscopy findings were positive 8 weeks after completing the course of treatment.

Three patients developed tumor lysis syndrome. One of these patients had metastatic melanoma with numerous cutaneous, visceral, and CNS metastases. Massive hemorrhagic necrosis began 72 h after the seventh UltraPheresis proce-
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dure and progressed to complete hemorrhagic necrosis of all lesions within 1 week of its onset. There was no clinical evidence of associated infection.

The second patient with metastatic melanoma (K-status 60%) had complete resolution of bulky cervical and supraclavicular node metastases over the first 6 weeks of UltraPheresis. Subsequently, the amount of filtrate removed per procedure was incrementally increased, and the patient, subsequent to the 21st procedure, developed severe pain in all tumor sites, followed by fever to 102°. His CRP rose within 24 h to 33 and his lactic acid dehydrogenase (LDH) to >2,000 within the first 24 h of this procedure. The patient subsequently developed low-grade disseminated intravascular coagulopathy (DIC) followed by adult respiratory distress syndrome (ARDS) and renal failure. Numerous cultures of blood, sputum, urine, and cerebral spinal fluid failed to reveal a septic etiology as causal or associated with the response.

A third patient with metastatic anaplastic Wilms tumor (K-status 40%) developed massive tumor necrosis after three UltraPheresis procedures and, despite emergency debulking of large amounts of necrotic tumor in the pelvis, developed low-grade DIC, ARDS, and renal failure and died.

Autopsy examination of each of these patients revealed virtually complete destruction of tumor.

In summary, the clinical responses observed in this pilot study included three tumor lysis syndromes with histologic evidence of complete response. Two of these patients had metastatic melanoma and one anaplastic Wilms tumor. Partial responses, meaning >50% reduction of the sum of cross-sectional diameters of measurable disease were seen in three patients, one with premenopausal inflammatory carcinoma of the breast who demonstrated new tumor growth 2 months after completing therapy, one with adenocarcinoma of the lung with a response duration of 3 months, and one with metastatic adenocarcinoma of the colon with 1 year duration of response. Less than 50% responses were seen in transitional cell carcinoma of the bladder, with a mean duration of response to demonstrated new tumor growth of 2 months, and in two patients with adenocarcinoma of the breast; the duration of these responses is unknown, as both of these patients were lost to follow-up. Stabilization of disease was seen in two patients with metastatic melanoma and in one patient with colon cancer. Progressive disease was observed in one patient with pancreatic cancer, one with metastatic colon cancer, one with metastatic oat cell carcinoma, and one with metastatic melanoma.

Histopathology

Consistent histologic changes in a variety of malignant tumors was observed following the application of the UltraPheresis procedure as described. A single case (P.G.) is shown in temporal sequence for illustration.

The initial tumor shows a highly pleomorphic, amelanotic melanoma from a biopsy taken prior to the initiation of therapy (Fig. 4). After the initiation of therapy, variable amounts of perivascular, lymphocytic cuffing are observed in tissue surrounding the tumor site (Fig. 5). Lymphocytes are then seen infiltrating into the tumor, in a centrifugal fashion, producing piecemeal necrosis and cell

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damage characterized by pyknosis of tumor cell nuclei followed by a coagulative necrosis (Figs. 6 and 7). Subsequently, the areas of piecemeal necrosis coalesce, forming a large field of coagulative necrosis surrounded by tumor cells showing pyknotic nuclei. The lymphocytic infiltrate persists in the areas of tumor necrosis, with progression of the necrosis in a centrifugal fashion (Fig. 8). Immunoperoxidase studies for T-cell specificity show the majority of these tumor-infiltrating lymphocytes to be of T-cell origin (Fig. 9) As tumor necrosis and loss of parenchyma progresses, condensation of collagen can be seen (Figs. 10–13). In several cases, this latter process has progressed to complete scarring of the tumor site.

This apparent immunologic response was histologically demonstrated in 12 of the 16 patients studied. The tumor histologies involved in this response included adenocarcinoma of the lung, melanoma, anaplastic Wilms tumor, adenocarcinoma of the breast, adenocarcinoma of the colon, inflammatory adenocarcinoma of the breast, and transitional cell carcinoma of the bladder.

Adverse Effects

Overall, the procedure was well tolerated. Three patients, two with widely metastatic melanoma and one with widely metastatic anaplastic Wilms tumor, demonstrated a tumor lysis syndrome, with sudden onset of extreme pain in the tumor sites, followed by a rapidly rising uric acid, CRP and LDH. Despite hospitalization in the intensive care unit and vigorous supportive measures, these patients died in tumor lysis syndrome. Two had estimated tumor burdens $>$1 Kg.
and one had extensive CNS metastases. Histologic examination of all the tumors in each case revealed massive hemorrhagic necrosis (one melanoma) and massive coagulation necrosis, (one melanoma, one Wilms).

The clinical and inflammatory response associated with the UltraPheresis procedure consisted of fever (99.6–102°) that began within 1–8 h of completing the procedure. The temperature elevation lasted from 2 to 12 h (average 4 h) and was consistently observed in this patient population. Fever promptly responded to an acetaminophen. Pain in tumor sites was described by patients as a deep, burning ache, or a warm, throbbing pain, developing during fever but lasting up to 36 h. Pain was often severe and required narcotic analgesics for control. No significant changes in general physical examination parameters were observed. Hematologic and biochemical tests showed no significant changes, except for an absolute monocytosis and an increase in CRP and fibrinogen. Interestingly, total white blood count did not rise with, or following, the fever. There was no significant change in blood cellular elements or immunoglobulins associated with the procedure.

Immunologic Studies

Delayed hypersensitivity, measured by skin reaction to candida, trichophytin, measles, and mumps antigens were followed monthly while on study. Of 12 patients who were anergic at enrollment, 6 regained skin test positivity to one or more antigens tested.

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Lymphoid marker determinations were obtained each month and revealed a relative increase in T4/T8 ratios. This change appeared to be due to a decrease in T8 lymphocytes as opposed to an increase in T4 lymphocytes, but the numbers are small, and the significance is unknown (Table 2).

Immune complex-like material was not tested, as previous investigations had suggested that these complexes were greater in size than that which could be cleared by this membrane.

**Tumor Markers**

Six patients showed prestudy elevation of tumor markers. One patient had an elevation of alpha-fetoprotein (molecular weight 70,000 daltons), membrane permeability coefficient 35%. One patient had an elevation of beta-human chorionic gonadotropin (HCG) to 71 (molecular weight 45,000 daltons), permeability coefficient 50%. Five patients had elevations of carcinoembryonic antigen (CEA) (molecular weight 200,000 daltons), permeability coefficient 0. Beta-HCG and alpha-fetoprotein levels fell to normal ranges with the UltraFheresis procedure. CEA levels rose during the first month of treatment, and then fell in subsequent months. A particularly interesting observation was a rapid increase in serum CEA from 19.1 to 171 in 3 weeks, associated with objective regression of palpable intraabdominal metastases of adenocarcinoma of the colon. One month later the serum CEA had fallen to 19.2.
FIG. 7. Melanoma following seven treatments, showing extensive lymphocytic infiltration with transition to an area of extensive coagulative necrosis. ×67.

DISCUSSION

In this study, we were interested in the effect, if any, of the UltraPheresis removal of a low molecular weight fraction (less than 150,000 daltons molecular weight) on patients and on tumors of patients with a variety of objectively measurable metastatic solid tumors, with no interference from any other oncologic treatment. From our observations on 16 patients with measurable advanced disease, it can be reasonably concluded that the removal of this protein fraction is not attended by a clinically significant adverse effect and that it is well tolerated. Subjective improvement in general physical condition and a sense of well-being was reported in the majority of cases (65%). This was attended by an increase in appetite and gain in weight in a significant number of patients. Objectively, three tumor lysis syndromes occurred, with massive necrosis of all measurable disease (18.7%). An objective reduction in tumor of 50% or more was observed in 6 of the 16 patients (37.5%). Although survival was not a study parameter initially, 9 of the 16 have since survived for >1 year, 6 of the 16 for 2 years. This we feel is significant, given the average performance status of 70% of protocol enrollment. Contrary to the observations of Wulfrank et al. (17), who observed a reduction in T4 (helper) lymphocytes using membrane plasma exchange, we observed a reduction of T8 (suppressor) lymphocytes associated with the UltraPheresis procedure in several patients, but the numbers are too small to approach significance;
future work will attempt to determine whether or not this initial suggestion is significant.

These findings might suggest the presence of soluble mediators of helper/effectector function, which are also present in cancer sera, but which have a lower permeability coefficient than suppressor mediators in the system of membrane plasma ultrafiltration. Our experience in observing enhanced nonspecific, as well as specific, cellular immunity is more consistent with the observations of Israel et al. in 1978 and 1981 (16). This disparity may well be due to technical differences between the systems used in each study. A centrifugal plasma cell separator creates centrifugal bands based on density of cellular and molecular elements. The site of the interface for plasma skimming is generally based on a crudely estimated difference between leukocytes and platelet-rich plasma, or platelet-rich and platelet-poor plasma. Variables between centrifuge speed, radius of the tub, and time create uncontrollable variables that make for differences in banding of plasma proteins from procedure to procedure. Membrane plasma ultrafiltration might be more selective, but here thick, woven, and gas-blown membranes may be too variable in their performance characteristics to achieve predictable clinical results when attempting to obtain a significant differentiation of clearance in this molecular size range, between molecules that might be inhibitory or facilitatory to immunologic injury.

In our own system, in preclinical trials, we found that transmembrane pressures in the range of 75–300 mm Hg produced the performance curves described above.
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Below 75 mm Hg transmembrane pressure, the curve shifted to the left. Transmembrane pressures >300 mm Hg shut down the membrane by closing its pores. Also noted was that increasing blood flow rate through the filter, above 200 cc/min shifted the curve to the left, and blood flow rates less than 75 cc/min shifted the curve to the right. Recognizing this type of variability in systems and acknowledging that soluble mediators of both cytotoxic suppression and facilitation may exist in the serum of the patient, we might well expect variations in results between ultrafiltration systems. In studying melanoma, Hersey reported one objective response in seven patients using membrane plasma apheresis (18). Cupissol reported three responses in 32 patients (19). MacDonald (20), Salinas (21), and Retsas et al. (22) were all unable to register objective responses using cell separators. At present the role of various replacement solutions in providing factors that may be facilitory in the immune response is unclear.

In the future, we plan to study in vitro the role of the material removed in the ultrafiltrate, for blocking activity both to cell-mediated cytotoxicity as well as to antibody-dependent cytotoxicity. We additionally plan to investigate the role of specific cellular augmentation, using known lymphokines in an effort to augment the biologic responses thus far observed.

It is far too early, based on this preliminary report, to make any suggestions on therapeutic effect in the classical clinical sense, but the basic observations made would suggest that this form of ultrafiltration alone, or in combination with other modalities, may offer some benefit in the treatment of certain forms of cancer in
FIG. 10. Collapse of tumor with condensation of collagen following 13 ultrafiltration procedures. Note continued presence of lymphocytic infiltrate. x67.

FIG. 11. High-power view of tumor collapse with condensation of collagen. x268.

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FIG. 12. Transition zone between tumor, showing lymphocyte infiltrate with early necrosis and extensive coagulative necrosis. ×67.

FIG. 13. Close-up of transition zone between lymphocytic infiltrate and coagulative necrosis. ×268.
### TABLE 2. Phytohemagglutinin (PHA) stimulation tests and lymphoid subsets

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*Not performed on cases 1, 2, 5, and 6.*

The future. Extensive additional research needs to be directed, not only to lymphokines and other soluble mediators of immune enhancement in patients, but also to the role of soluble mediators of immune suppression that may be protective of cancer cells in the tumor-bearing patient, either by masking tumor-associated antigen, or by directly deregulating cells capable of cytotoxicity, or by competing with soluble mediators of immune augmentation.

### REFERENCES


