

The Role of Therapeutic Apheresis in the Treatment of Cancer: A Review

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Abstract: Immunosuppression is a hallmark of advanced malignancies in man. Over the past 40 years, many investigators have identified soluble immunosuppressive factors in blood, serum, ascitic fluid, and pleural fluid from cancers in man and other species. Suppressive factors have also been identified that are produced by tumors. The description of immunosuppressive factors in the blood of vertebrates who either have cancer or who are pregnant is significant, for only in pregnancy and cancer does a seemingly normal immune system tolerate immunogenic neoantigen. Tumor necrosis factors (TNFs) are known to be pleiotropic cytotoxic cytokines that are produced by macrophages and lymphocytes. These cells are thought to be suppressed in patients who have cancer or who are pregnant. Recently, elevated blood levels of soluble tumor ne-

crosis factor receptors (sTNFRs) have been reported in the blood in a variety of cancers and pregnancy. In 1990, after our initial publication of the discovery of sTNFRs in the serum and low molecular weight ultrafiltrates of serum from a variety of cancer patients, others confirmed significant elevations of sTNFRs in cancer patients. This elevation was found to correlate with a poor prognosis. The biologic activity of proinflammatory cytokines as well as the suppressive role of their shed receptors is herein reviewed. Work with cancer patients using ultrapheresis to reduce these suppressive molecules by the authors and others is reviewed. Several recommendations are made for future directions. **Key Words:** Ultrapheresis—Cancer—Immunosuppression.

IMMUNOSUPPRESSION: ITS ROLE IN CANCER

It is now well understood that immune suppression is a hallmark of advanced malignancies in man. Clinical cancers in man are characterized by a state of immunologic suppression or immunologic tolerance which is manifested by depression of cellular immunity; by the presence of circulating blocking factors that permit the tolerance of neoantigen; by the immunosuppressive effects of various hormones such as estrogen, progesterone, and beta human chorionic gonadotropin (beta HCG); by the presence of suppressor T cells; by the presence of leukocyte migration enhancement factors; and by decreased red cell immune adherence. The degree of suppression is directly proportional to tumor stage and tumor burden. The mechanisms which allow for immune tolerance and provide a state of acquired immune tolerance to the foreign proteins of the tumor cell are

soluble factors in blood and extracellular fluid that interfere with cellular immunity sufficiently to protect antigenic tumor cells from the immunosurveillance arm of the immune system (1). The cellular arm of the immune system in man and in all vertebrate life is responsible for the protection of the host from viral infections, the recognition of human leukocyte antigen (HLA) mismatched tissue and its rejection (as in transplantation), and the recognition and destruction of neoantigen as it arises in the form of alterations in the major histocompatibility antigens in neoplastic cells (2). It is by virtue of the proper functioning of the immunosurveillance arm of the immune system that the majority of vertebrate life does not develop cancers during a lifetime despite the mathematical probability of 4–6 cancer cells developing in each of us every day (3).

HISTORICAL REVIEW OF IMMUNOSUPPRESSION IN CANCER

Over the past 40 years, many investigators have identified soluble immunosuppressive factors in the

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blood, serum, ascitic fluid, and pleural fluid from cancers in man and other species. Previous observations made by Whittaker and Clark, and Suciu-Foca, et al. were that immunologic incompetence is quantifiable *in vitro* and is attributable to circulating blocking factors. The degree of incompetence advances as the disease progresses, and the quantitative level of blocking activity increases proportionately (4,5). This implies that a direct correlation exists between the tumor burden in the host and the degree of immunologic incompetence. Liechtenstein observed that pregnancy is accompanied by an advancing immunologic tolerance to a host of antigens, to which the cell mediated immune system responds under normal conditions (6). These observations were confirmed by our lab in 1980. Rosenstein, et al. isolated and partially characterized suppressive factors released from splenocytes in specific hapten sensitive mice. These factors were proteins of 65,000 to 70,000 kilodaltons and could be absorbed by macrophages (cells which can function as suppressor cells in this system) (7). Baldwin, et al. noted that serum from rats with hepatomas induced by aminoazo dye, when incubated with effector cells, specifically inhibited cytotoxicity against the tumor (8). Hellstrom and Hellstrom described inhibition by the autologous serum of cytotoxicity mediated *in vitro* by autologous mononuclear blood cells (9). Murray, et al. observed blocking activity against antibody dependent lymphocyte cytotoxicity in the serum of patients with melanoma. A glycoprotein appeared to be responsible for the blocking and a 15 kilodalton fraction was identified as causal (10).

Suppressor factors have also been identified that are produced by tumors. Sample, et al. observed that serum from cancer patients whose lymphocytes demonstrated low phytohemagglutinin reactivity suppressed the response of normal lymphocytes to phytohemagglutinin compared with pooled normal serum (11). A number of investigators have found immunosuppressive serum factors in cancer patients and have also proven that the degree of inhibition is directly proportional to the tumor burden (4,12-24). It is also noteworthy that Kasakura observed a similar suppressive effect on phytohemagglutinin reactivity as well as mixed lymphocyte culture reactivity in gravid serum (24). The description of immunosuppressive factors in the blood of vertebrate life which either have cancer or are pregnant is very important for the only 2 conditions in which a seemingly normal immune system tolerates immunogenic neoantigen are cancer and pregnancy (25). More recently, Muchmore and Decker isolated and purified an 85 kilodalton glycoprotein from the urine of preg-

nant women. This substance was found to block the early events required for T-cell proliferation *in vitro*. These investigators pose that *in vivo* it protects the placenta from maternal immunosurveillance (26). This same protein was later found to exist in the urine of people with a variety of cancers (27).

Finally, specific blocking factors to tumor necrosis factors (TNFs) (alpha and beta) were identified in the serum and ultrafiltrates of serum by Lentz and colleagues in 1989 (28). The specific inhibitors were later cloned and sequenced (29) and further characterized as solubilized receptors which function in tissue water as specific inhibitors of TNF (23).

PARADIGM OF IMMUNE REGULATION

The clinical features that characterize pregnancy and cancer are tolerance to fungal antigens, depression of delayed hypersensitivity reactions, prolonged allograft survival, acceleration of malignant conditions, the presence in the serum of the host of oncofetal antigens, and improvement in autoimmune disorders. These features, combined with the previous considerations, suggested to us in 1982 a paradigm of immune regulation whereby fetal cells or fetal like cells (those that were undifferentiated because they had not yet differentiated or had dedifferentiated from a mature form) must produce factor(s) that are able to downregulate normal cellular immunity in the local environment in which these cells lived sufficiently for them to escape and to survive immunosurveillance. This paradigm implies that a family of immune regulatory genes must exist in vertebrates. Vertebrates are the only family of organisms that possess both cellular and humoral immunity and must modulate this protective system if they are to reproduce by ovaparity, ovavivaparity, or vivaparity. We subsequently proposed that perhaps the impropitious turning on of this family of immune regulatory genes in specific tissues and the production of specific proteins to interfere with immunosurveillance might be essential to the development and subsequent immune tolerance to cancer cells. This paradigm suggests that lowering all soluble suppressive factors in blood and extracellular fluid by apheresis could prove very beneficial to the cancer patient (1).

TUMOR NECROSIS FACTOR, IL-1, IL-6, AND THEIR SHED RECEPTORS/INHIBITORS

TNFs are pleiotropic cytokines produced in response to injury or infection. These molecules are considered to be the primary effectors of the inflammatory and immune responses of animals. There are

2 distinct forms of TNF, TNF-alpha (TNF α) and TNF-beta (TNF β). TNF α and TNF β share 30% sequence homology and compete for binding to the same receptors on the surfaces of cells. TNFs play a necessary and beneficial role as mediators of host resistance to virus, bacteria, fungi, and cancer cells. It is important to note, however, that an overproduction of these factors can lead to a variety of pathological conditions including wasting, systemic toxicity, and septic shock (30,31). Two distinct TNF receptors (TNFR) have been identified and cloned. Virtually all cell types studied show the presence of one or both of these receptor types. One receptor type termed TNFR2 (type A, type alpha, 75 kilodaltons, or UTR antigen), shows an apparent molecular weight of 75 kilodaltons. The gene for this receptor encodes a presumptive transmembrane protein of 439 amino acid residues (32,33). The other receptor type, termed TNFR1 (type B, type beta, 55 kilodaltons, HTR antigen) shows an apparent molecular weight of 55 kilodaltons. The gene for this protein encodes a transmembrane protein of 426 amino acid residues (29,33,34). Both receptor types show high affinity binding of either TNF α or TNF β . The 2 receptor types are distinct immunologically, but their extracellular domains show similarities in the patterns of cysteine residue locations in 4 domains (32). The intracellular domains of the 2 receptor types are apparently unrelated, suggesting the possibility that the 2 receptor types employ different signal transduction pathways.

Several groups have identified soluble TNF binding proteins in human serum and urine that can neutralize the biological activities of TNF α and TNF β (27,35,36). Two types have been identified and designated, one for sTNFR1 and the other for sTNFR2. These soluble forms have now been shown to represent truncated forms of the 2 types of TNFR. The soluble receptor forms apparently arise as a result of the shedding of the extracellular domains of the receptors, and concentrations of about 1:2 ng/ml are found in the serum and urine of healthy subjects (37,38). The levels of the soluble receptors vary from individual to individual but are stable over time for a given individual (37,38).

Since their discovery, elevated levels of sTNFR have been reported for a variety of conditions. Elevated levels are found in the amniotic fluid and urine of pregnant women (39). Elevated levels of sTNFR in serum or plasma have been reported in association with pathological conditions such as endotoxemia (40), meningococemia (41), and HIV infection (42). High levels have also been found in the plasma and ascites of patients with infections and

malignancies (43). The mechanisms involved in the induction of shedding of the TNFR are not well understood. There are reports of correlations between increased TNF levels and soluble receptor levels, suggesting generally that stimuli which caused TNF levels to rise also induced shedding of TNFR (44,45). There is also evidence, however, that suggests that the shedding of the 2 types of soluble receptors are independently regulated (44). It is known that both types of soluble receptors can bind to TNF in vitro and inhibit its biological activity by competing with cell surface receptors for TNF binding. Consequently, it has been suggested that shedding of soluble receptors in response to TNF release could serve as a mechanism for binding and inhibiting the TNF not immediately bound to surface receptors, thus protecting other cells from the effects of TNF and localizing the inflammatory response (44,45). Conversely, it has been reported that at low concentrations, TNF binding to soluble receptors can stabilize TNF and augment some of its activities (37).

Cancer tissue studies suggest a different expression of sTNFR compared to that of normal tissues. In a study by Pusztai and McGee, 28 primary breast cancers and tissues from normal breast tissue obtained at reduction mammoplasty were stained by immunocytochemistry for TNFR1, TNFR2, and TNF α . Neither TNF α nor TNFR2 was detected in normal breast tissue or in nonmalignant breast tissue adjacent to the tumors. In contrast, TNFR1 was expressed by occasional stromal cells in normal tissue. TNF α was expressed focally in 50% of the tumors studied, being largely confined to macrophage like cells in the stroma. TNFR1 was expressed by a population of stromal cells in all the tumors examined, and a varying proportion of neoplastic cells was found in 75% of these tumors. TNFR2 was detected in 70% of the tumors (46).

In 1990, after our initial publication of the discovery of sTNFR in the sera and low molecular weight ultrafiltrates of sera from a variety of cancer patients (28), Aderka, et. al. studied 40 healthy subjects and 59 cancer patients with solid tumors. The mean $\pm$ SD concentrations of both the soluble type TNFR1 (p 55) and TNFR2 (p 75) receptors were significantly higher in the cancer patients than in the healthy controls (1.96 ± 1.19 vs. 0.79 ± 0.19 ng/ml [$p < 0.001$] and 6.43 ± 4.8 vs. 3.2 ± 0.6 ng/ml [$p < 0.001$], respectively). The extent of the increase in concentrations of these inhibitors correlated with the volume of disease. Sera of the cancer patients had a marked inhibitory effect on the in vitro cytotoxic activity of TNF. This inhibition was proportional to the concentration of sTNFRs and could be fully abolished by the addition

to the sera of specific antibodies against the receptors (47).

In the same year, Langkopf and Atzpodien investigated whether the prognosis of cancer patients could be predicted by the concentrations of their sTNFRs. They analyzed progression free survival times in subgroups of patients with sTNFR1 less than 6.5 ng/ml ($n = 76$, Group 1) or more than 6.5 ng/ml ($n = 23$, Group 2). Survival analysis according to the Kaplan-Meier method showed longer progression free survival in patients with sTNFR1 less than 6.5 ng/ml (Mantel Cox, $p = 0.0017$; TaroneWave, $p = 0.0039$; and Breslow, $p = 0.0101$). The median progression free survival time of patients with low pretreatment sTNFR1 concentrations was 10.6 months. Patients with high sTNFR1 levels (greater than 6.5 ng/ml) reached a median survival time of 3.6 months. These authors concluded that increased levels of sTNFR in cancer patients represent a tumor escape mechanism from the destructive effects of TNF α produced endogenously in response to tumor antigens (48).

INTERLEUKIN 1

There are 2 forms of interleukin 1 (IL-1), IL-1 α and IL-1 β . These are cytokines produced primarily by mononuclear phagocytes but also by a number of other cell types including skin keratocytes, some epithelial cells, and some cells of the central nervous system (49). These cytokines produce a wide variety of effects on numerous cell types. They also regulate the production of a great number of other proteins including other cytokines, IL-2, TNF, and colony stimulating factors. IL-1 α and IL-1 β are thus important mediators of inflammatory and immune responses in animals (49). Because of the early appearance of IL-1 during the inflammatory reaction and because of the variety of effects produced by IL-1 α and IL-1 β , these cytokines are believed to play important roles in the production of pathologic conditions including chronic inflammation, septic shock, and defects of hematopoiesis. The effects produced by IL-1 result from the binding of these cytokines to 2 distinct cell surface receptors, IL-1R types I and II (49). The type I receptor is an 80 kilodalton protein found on B cells and polymorphonuclear leukocytes. In general, the type I receptor binds to IL-1 α or IL-1 β with approximately equal affinity, and the type II receptor binds IL-1 β more strongly than IL-1 α . The type I receptor is capable of transducing the signal and can produce all the biologic effects attributable to IL-1 (50,51). The function of membrane bound type II receptors is to serve as the precursor for the soluble IL-1 binding factor that can be shed

under appropriate circumstances to antagonize and modulate IL-1 activity (50). A naturally occurring IL-1 binding protein has been described that seems to correspond to the soluble external portion of the type II receptor (52,53).

A different type of naturally occurring inhibitor of IL-1 was discovered and purified from the urine of patients with myelocytic leukemia (54,55). A cDNA encoding this polypeptide has been isolated from monocytes and found to code a 152 amino acid residue glycoprotein of 25,000 kilodalton (56,57). This molecule, known as secreted IL-1 receptor antagonist, shows 26% amino acid homology to IL-1 β and 19% homology to IL-1 α . Evidence indicates that the inhibitory action of sIL-1r α results from binding of IL-1 α to the IL receptor type I with an affinity comparable to that of the receptor. This binding, however, does not result in signal transduction. IL-1r α binds to the IL-1 receptor type II with considerably lower affinity than that shown by IL-1 β (58,59). This makes sense teleologically in that the 2 mechanisms designed to inhibit the actions of IL-1 β do not compete with each other.

Cells known to produce IL-1r α include monocytes, neutrophils, macrophages, and fibroblasts (60–62). Cytokines known to upregulate IL-1 α production include IL-13, IL-6, IL-4, interferon gamma, granulocyte-macrophage colony stimulating factor (GM-CSF) and tumor growth factor TGF β , the latter apparently by triggering IL-1 production, which itself triggers IL-1r α synthesis (60,63–67). IL-1r α is released in vitro during experimentally induced inflammation as a part of the natural course of many disease. It is thought to be part of a naturally occurring mechanism which limits, under most circumstances, the extent of the potentially deleterious effects of IL-1 (68,69). Administered experimentally, IL-1r α has been demonstrated to block IL-1 activity both in vitro and in vivo. In rabbits, pretreatment with IL-1r α has been shown to prevent death resulting from septic shock produced by lipopolysaccharide (LPS) injection (70) to prevent the development of immune complex mediated colitis (71,72) and to block cerebral spinal fluid inflammation induced by the cerebral ventricular administration of IL-1 (73). Preclinical and clinical studies are in progress on the possible therapeutic uses of IL-1r α in the treatment of sepsis, cachexia, rheumatoid arthritis, chronic myelogenous leukemia, and transplanted bone marrow induced graft versus host disease (74).

INTERLEUKIN 6

IL-6 is a multifunctional cytokine produced by a variety of cell types including T lymphocytes, mono-

cytes/macrophages, fibroblasts, hepatocytes, vascular endothelial cells, cardiac myxomas, bladder cell carcinomas, myelomas, astroglomas, and glioblastomas. The effects of IL-6 on different cell types are numerous and varied. These effects include the following: stimulation of B cell differentiation and antibody secretion; activation as a costimulant with phytohemagglutinin (PHA) and/or concanavalin A (ConA) to increase IL-2 production and IL-2 receptor expression T cells; and enhancement of the differentiation of cytotoxic T cells. They also include growth factor for mature thymic and peripheral T cells, myelomas, hybridomas, plasmacytomas, keratinocytes, and mesangial cells and colony stimulating activity on hematopoietic cells; the induction of neuronal cell differentiation; the induction of maturation of megakaryocytes; and the stimulation of the production of acute phase response proteins by hepatocytes. These varied effects indicate that IL-6 plays a major role in the mediation of the inflammatory and immune responses provoked by infection, injury, and malignancy. Elevated IL-6 levels have been reported to be associated with a variety of diseases including autoimmune diseases, mesangial proliferative glomerulonephritis, psoriasis, and malignancies (75–77).

The biologic activities of IL-6 are initiated by the binding of the cytokine to an affinity receptor complex consisting of 2 membrane glycoproteins. An 80 kilodalton receptor binds IL-6 with low affinity IL-6R and a signal transducing component of 130 kilodaltons (GP130) that does not bind IL-6 by itself but is required for the high affinity binding of IL-6 by the complex. Both components of the receptor complex, IL-6R and GP130, have been cloned, sequenced, and expressed (78–81).

IL-6 RECEPTOR

A soluble form of the IL-6 receptor with a molecular weight of approximately 50 kilodaltons has been found in the urine of healthy adult humans, in cultured medium conditioned by the growth of human myeloma cells (83), in culture supernatants from PHA stimulated peripheral blood mononuclear cells, in human T-cell leukemia/lymphoma virus (HTLV) 1 + T cell line supernatants, and in the sera of HIV sero positive blood donors (82,83). This soluble form of the receptor apparently arises from the proteolytic cleavage of membrane IL-6R. The regulation in vivo of the shedding of IL-6R as well as the function and significance of these soluble receptors in biologic fluids is not currently understood. It has been suggested, however, that the pathologic

states which involve elevated levels of IL-6 might also be associated with increased production of soluble IL-6R (83).

THE YIN AND YANG OF IMMUNE REGULATION

A paradigm is therefore suggested by the discoveries cited previously for the in vivo regulation of proinflammatory cytokines whereby the biologic action of proinflammatory cytokines induces the shedding of specific receptors to the cytokine in such a fashion that the proinflammatory cytokine itself is suppressed. This serves as an exquisite mechanism of control of the deleterious and injurious effects of this family of inflammatory mediators systemically and also in the microenvironment in which antigen is present. It certainly seems to be consistent with the literature that an increase in the soluble receptors so characteristic of the state of immunologic tolerances seen in advanced malignancies of various sorts may, in fact, be setting the immunologic stage so that neoantigens that can also characterize these same malignancies can be tolerated by an otherwise seemingly normal immune system.

Based on our studies using molecular sieving, we were convinced that the suppression of blastoid transformation reported by Whittaker was not caused by an antibody as suggested by Hellstrom and Hellstrom but was caused by material(s) under 150,000 kilodaltons molecular weight. In 1982, we designed a system to semiselectively pheresis all molecules from blood under 120,000 molecular weight. We used trak etched membranes in a parallel plate configuration. The membranes employed were made of polycarbonate and were 5 μ in thickness with a pore size of 0.05 μ . The sieving coefficient of albumin (69,000 molecular weight) was 40%, and the sieving coefficient of IgG was 8%. A dialysis machine was modified to serve as the filtration controller. The process was termed ultrapheresis because it was not plasmapheresis in the conventional centrifugal drum sense of fractionating blood at the platelet poor plasma interface and did not deplete blood of formed elements or clinically significant quantities of immunoglobulins (150,000–890,000 molecular weight), clotting factors, or complement.

The procedure was first used on pregnant animals to test the hypothesis that removal of the blocking factors would induce labor. Each of 6 pregnant goats of known gestational age was subjected to 1 ultrapheresis procedure. The filtration procedure in the goat was well tolerated, and no clinically significant adverse effects were observed. Each subject animal,

however, did experience the onset of labor at the end of the procedure. They then proceeded through a normal labor and delivery and gave birth to normal products of conception (84).

We then subjected 6 dogs with advanced cancers, 5 with mammary tumors and 1 with osteogenic sarcoma, to ultrapheresis for the purpose of provoking an inflammatory response in tumors by the removal of these blocking factors. After the first procedure, each dog's tumor became red, hot, swollen, and tender. After 2–3 procedures, the tumors demonstrated focal hemorrhagic and coagulative necrosis on biopsy. After 5 procedures, the tumors regressed. Residual tumor masses were resected and revealed scar only. The dogs were then observed until death (1–4 years), and none showed recurrence of the cancers.

CLINICAL STUDIES

A Phase 1 study of this procedure was approved by the United States Food and Drug Administration (FDA) in 1985 under IDE #G850101 and conducted at the University of California at Irvine. Each of the 16 human patients with a variety of advanced solid tumors for whom all conventional treatments had failed received 12 ultrapheresis procedures over a 1 month period. The procedures were well tolerated. Brisk tumor inflammation was observed consequent to the procedure. The amount of ultrafiltrated plasma removed was 10 cc/kg lean body mass (range: 5–25 cc/kg). This volume was removed 3 times a week and replaced with an equal volume of ultrafiltrate prepared from normal fresh frozen plasma obtained through the Red Cross from healthy donors. The procedure generally required 1 h but ranged from 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 or throbbing pain in tumor sites and/or increased redness, swelling, warmth, aching and tenderness in cutaneous tumors associated with a rise in serum C-reactive protein (CRP), and in lactate dehydrogenase (LDH). However, 1 patient with metastatic melanoma developed tumor specific hemorrhagic necrosis after 7 procedures, at volumes of 250 cc per procedure and demonstrated a tumor lysis syndrome. One patient with metastatic inflammatory breast cancer required an 8 L exchange in 1 procedure to elicit a tumor inflammatory response.

In this study, clinical response was defined as reduction in the sum of diameters of measurable lesions. Complete response was defined as resolution of all palpable disease and radiologically measurable disease. Partial response was defined as a reduction of the sum of the diameters of all measurable disease

greater than or equal to 50% or histologic necrosis and/or reparative fibrosis greater than 50% above baseline compared to pretreatment histologies. Minimal response was defined as a less than 50% reduction of the sum of tumor diameters or evidence of increased tumor necrosis, but less than 50% over pretreatment biopsies. Stable disease was defined as no tumor growth while on protocol when tumor doubling times could be reasonably calculated prior to treatment.

Tumor redness, tenderness, and swelling occurred in 14 of the 16 patients. Metastatic foci resolved in 1 patient with metastatic adenocarcinoma of the lung, 2 patients with melanoma, 3 patients with metastatic breast cancer, and 1 patient with transitional cell carcinoma of the bladder who had complete resolution of dysuria, urinary frequency, and urgency and an approximately 50% reduction in visible tumors in bladder mucosa at repeat cystoscopy. Three patients developed tumor lysis syndrome with complete histologic necrosis, 2 with melanoma and 1 with anaplastic Wilm's tumor. Six of the 16 patients had a total tumor reduction of 50% or greater. A less than 50% response was observed in 3 of 16 patients, and stable disease was documented in 2 of 16.

Several patients in this phase of the study had multiple cutaneous melanomas amenable to repetitive excisional biopsies. Consistent histologic changes were observed in this tumor type. Within 24 h of the first ultrapheresis procedure when tumors were not yet red or swollen, hypertrophy of the tumor vascular endothelial cells was observed. This was followed by a variable amount of perivascular mononuclear cell "cuffing" and developed within hours of the procedure at the onset of tumor edema and redness. Later, lymphocytes were seen infiltrating the tumor in a centrifugal fashion, producing piecemeal necrosis and tumor cell damage characterized by pyknosis of tumor cell nuclei, coagulative necrosis, and focal hemorrhagic necrosis of the tumor mass. As tumor necrosis progressed, the condensation of collagen was evident with an increase in reticulin staining. In several cases, this process progressed to complete scarring of the tumor.

Delayed hypersensitivity, measured by skin reaction to candida, trichophyton, and measles and mumps antigens, was followed monthly while on the study. Of 12 patients who were anergic at enrollment, 6 regained skin test positivity to 1 or more of the antigens tested.

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 were small, and the significance is unknown (85).

The FDA subsequently approved additional studies, which were conducted at National Medical Enterprises, John F. Kennedy Hospital, Palm Springs, California, and an additional 64 patients were treated. Tumor responses were observed in breast cancer, prostate cancer, melanoma, renal cell cancer, nonsmall cell lung cancer, ovarian cancer, endometrial cancer, Wilm's tumor, colon cancer, soft tissue sarcoma, osteogenic carcinoma, and squamous cell cancers of the head, neck, lung, and uterine cervix. In the course of these studies, we discovered the solubilized receptor/inhibitor to TNF itself in the serum and ultrafiltrates of serum of these patients (23,28,29). This inhibitor neutralizes the cytotoxicity of killer lymphocytes and macrophages and appears to be produced and secreted by tumor cells, stromal cells within tumors, and tumor vascular endothelial cells. We have also observed this inhibitor to be consistently elevated in gravid sera. Others have since confirmed these findings and described inhibitors to IL-1 and IL-6 in the same low molecular weight protein fraction removed by ultrapheresis. We are currently working on the identification of a possible specific inhibitor to gamma-interferon (g-IFN) and a molecule that appears distinct from the TNF inhibitors that perhaps is involved in interfering with blastoid transformation. Each of these molecules is less than 100,000 kilodaltons and is removed by ultrapheresis.

We developed a biologic assay to quantify the TNF inhibitors, based on their ability to inhibit recombinant TNF for killing the L-929 cell in vitro. This assay was later replaced by the ELISA based quantitative assay by R&D Systems, Inc., Minneapolis, MN, U.S.A. Using these assays, we demonstrated a direct correlation between the degree to which TNFR1 and TNFR2 were lowered and the degree of clinical tumor regression (86).

The current version of the ultrapheresis system employs hollow fiber membranes and is considerably more efficient than earlier versions and columns. We have completed a pilot study using this method and system in metastatic melanoma, breast, and colon cancers and have observed significant responses. Tumor specific inflammation provoked by apheresis of soluble TNF receptors suggested to us in this study the possibility that it is feasible to nonspecifically manipulate existent cell mediated and/or humoral immunity to achieve a specific tumor response. This type of tumor specific inflammation, associated with tumor necrosis in the relative absence of systemic toxicity, had not been clinically observed in our ear-

lier trials which had attempted to augment tumor immunity by employing super physiologic doses of exogenous proinflammatory cytokines. It was our observation that the apparent disparity between the predicted and actual postapheresis levels of TNFR1 and -R2 suggests that TNFR2 may be shed to a greater degree than TNFR1 in the condition of tumor tolerance. We are continuing work on more efficient ultrapheresis systems and devices to better control inhibitor concentrations to maximize clinical efficacy.

Recently, Munn and Mellor reported the discovery of indoleamine 2,3-dioxygenase (IDO), a tryptophan catabolizing enzyme expressed by trophoblasts and macrophages and suggested that the presence of this enzyme allows the placenta to destroy tryptophan, a requisite essential amino acid to the manufacture of TNF (88). It would not be unexpected that this enzyme can be demonstrated in cancer cells. This suggests yet another possible complementary mechanism to the evasion of immunosurveillance.

CLINICAL MANAGEMENT OF AUTOIMMUNE DISEASE

Immunex Pharmaceutical Corporation (Seattle, WA, U.S.A.) has made a fusion protein consisting of 2 sTNFR2 molecules and an Fc receptor. Trials are complete which document the ability of this material to stop inflammation in patients with rheumatoid arthritis, and a dose-response relationship exists whereby the higher the concentration of inhibitors infused, the greater the inhibition of arthritis (87). The finding of decreasing inflammation to altered native antigen as in rheumatoid arthritis by increasing blood concentrations of TNFR2 is consistent with our observations of increasing inflammation to altered native antigen by decreasing TNFR2 concentrations by ultrapheresis. Clearly, great caution must be used when giving these materials in the clinical management of autoimmune disease, and we must design a rest period in treatment so that blood levels can return to normal.

SUMMARY

Our current understanding of how the immune system reacts to antigen from an inflammatory standpoint is of a balancing act between the release and activation of TNF and other proinflammatory cytokines at one or more sites of antigen presentation with the production and biologic activation of their inhibitors. In this model, one can consider cancer and pregnancy as conditions in which inhibitor production and release occurs in that tissue's micro-environment in such a way as to produce an abnor-

mally high local concentration of inhibitors sufficient to protect the "graph" from a normal host attack. The genes for this biology are jealously conserved, possibly so that we can reproduce the way we do and live as long and successfully as we do, not so that we can tolerate cancers.

It now seems clear that soluble inhibitors of cellular immunity, specifically inhibitors of proinflammatory cytokines, are active in those clinical states in which antigenic tissues are tolerated by a seemingly intact immune system. There is no longer any doubt that inflammation can be upregulated by removing these inhibitors, and if the removal is sufficient, cancers can be destroyed. Similarly, pathogens and those antigens which characterize them immunologically can be the target of a more efficient, upregulated, immunologic/inflammatory response so that a state of tolerance to foreignness can be reversed. It is our belief and understanding based on our clinical and scientific work that complete tumor destruction is possible through this means and holds the added potential for the development of cellular memory post tumor kill that could decrease the potential for tumor recurrence. These goals should be the major focus for future cancer research.

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