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NEPHROLOGY FORUM

Acute renal failure in the setting of bone marrow transplantation

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Case presentation

A 28-year-old white man, first demonstrated to be human immunodeficiency virus (HIV) positive 2.5 years ago, presented 6 months later with an expanding scalp mass. Biopsy of this lesion, which was shown by CT scan to extend into the dura, revealed high-grade non-Hodgkin's lymphoma. Radiochemotherapy for the lesion, consisting of 300 cGy, plus adriamycin, bleomycin, vincristine, and dexamethasone, produced a good response. Over the next 6 months, the patient had two episodes of *Pneumocystis carinii* pneumonia, which responded first to trimethoprim/sulfamethoxazole and then to pentamidine therapy. In addition, an intrahepatic mass noted on an abdominal CT scan was consistent with recurrent lymphoma. Because of these problems, he was referred to the Fred Hutchinson Cancer Research Center for treatment with an experimental protocol comprising lethal doses of radiochemotherapy designed to eradicate both the malignancy and the lymphohematopoietic system, the principal reservoir of HIV; the cytoreductive therapy is followed by bone marrow transplantation. Following marrow infusion, zidovudine (AZT) and cloned CD8+ lymphocytes, obtained from the patient pretransplantation, are administered to combat residual HIV. The CD8+ cells, selected for their reactivity to the HIV gag protein, are expanded in vitro and transduced with a retrovirus, thereby introducing a thymidine kinase "suicide gene." This gene confers susceptibility to ganciclovir, so that these cells can be ablated in vivo should severe immunologic complications develop.

The patient received 120 mg/kg cyclophosphamide and 1200 cGy total-body irradiation prior to marrow transplantation, which was accomplished using an HLA-identical graft from his brother. The cloned T-cells (CD8+) were infused subsequently. Within one week after cytoreductive therapy, marrow aplasia developed, which was complicated by corynebacterium bacteremia manifested by severe rigors and profound hyperthermia (up to 41.3°C). Myoglobinuria resulted, which, in concert with tobramycin therapy, induced oliguric acute renal failure. A diagnosis of acute tubular necrosis was made. Cyclosporine, administered for graft-versus-host disease prophylaxis, was stopped. The patient regained renal function after 3 days of hemodialysis. His hospital course was further complicated by hepatic insufficiency, progressive jaundice, and mild azotemia, however. During his fourth week of hospitalization, his renal function again rapidly deteriorated. A microangiopathic hemolytic anemia, worsening thrombocytopenia, proteinuria, and red blood cell casts in the urine sediment were noted, and a diagnosis of hemolytic-uremic syndrome (HUS) was made. The patient underwent 14 plasma exchanges, but without hematologic or renal functional improvement, and he required alternate-day hemodialysis. Despite adequate marrow engraftment, multiple infectious complications ensued; these included bacterial and cytomegalovirus pneumonitis as well as central nervous system aspergillosis. The patient succumbed to these infections after 2 months of hospitalization. Renal tissue obtained at autopsy revealed multiple arteriolar and glomerular capillary thrombi, consistent with the clinical diagnosis of HUS.

Discussion

DR. RICHARD A. ZAGER (*Professor of Medicine, Fred Hutchinson Cancer Research Center, Seattle, Washington*): Worldwide, approximately 5000 bone marrow transplants (BMT) are conducted annually, and the list of indications continues to grow. Currently, indications include aplastic anemia, hematologic and non-hematologic malignancies, hereditary enzyme deficiencies, immunodeficiency states, and selected hemoglobinopathies. As illustrated by the patient presented here, the therapeutic utility of BMT continues to be tested. Aggressive investigation of the utility of BMT should provide us with new insights into disease mechanisms and treatment-related toxicities.

One of the most frequent and potentially life-threatening complications of marrow transplantation is acute renal failure (ARF). Approximately 40% of patients develop renal insufficiency early in the course of this treatment, and approximately 50% of these individuals require dialysis (Fig. 1). This high incidence of ARF is by no means unexpected, given the life-threatening nature of the underlying diseases and the toxicities inherent to the cytoreductive-radiochemotherapeutic preparatory regimens. Indeed, the patient presented here developed three distinct ARF syndromes during his 2-month hospitalization. First, sepsis, profound hyperthermia, myoglobinuria, and tobramycin therapy resulted in acute tubular necrosis. Next, hepatic insufficiency led to mild renal insufficiency, probably on a hemodynamic basis. Finally, the patient developed classic manifestations of HUS. Thus, this case graphically depicts the varied forms of ARF that frequently befall this patient population.

The purpose of this review is to present an overview of several renal syndromes that are either unique to, or occur with a disproportionate frequency in, BMT patients. These syndromes

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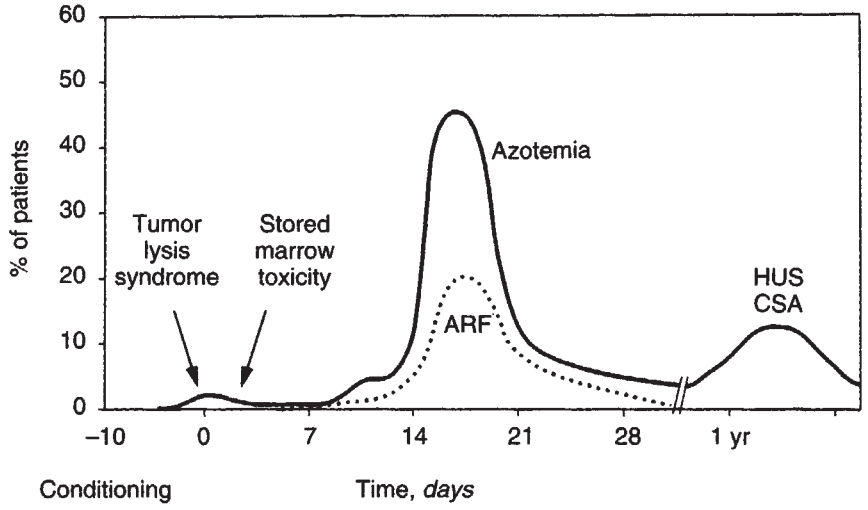

Fig. 1. *Time distribution and frequency of renal syndromes in the setting of bone marrow transplantation.* The *solid line* depicts the approximate frequency of renal insufficiency, as defined by at least a doubling of the baseline serum creatinine concentration (azotemia); the *dotted line* represents the frequency of dialysis-requiring ARF [32]. During the period of conditioning, tumor lysis syndrome and stored marrow-infusion toxicity are most common; between 10–28 days, the peak incidence of ARF is observed, most notably due to a veno-occlusive-disease-associated hepatorenal-like syndrome. Beyond one month, the hemolytic-uremic syndrome, and less commonly chronic cyclosporine toxicity, can be observed. (The frequency with which these require dialytic support is not known.) HUS, hemolytic-uremic syndrome; CSA, cyclosporine A.

can best be subdivided clinically according to characteristic times of onset, and I will present them in that order. First, immediate ARF syndromes (within the first 5 days) will be discussed, most notably the tumor lysis syndrome and stored marrow-infusion-associated toxicity. Next, we will look at a hepatorenal-like syndrome that generally occurs within the first month post transplantation. Finally, I will present the relatively late (after 4 weeks) post-BMT-associated renal disorders, notably the hemolytic-uremic syndrome and chronic cyclosporine nephrotoxicity.

Immediate renal syndromes

Tumor lysis syndrome. The tumor-lysis syndrome (TLS) is a potential life-threatening complication of cytoreductive therapy in patients with rapidly growing and hence highly radiochemotherapy-sensitive tumors [reviewed in Ref. 1, 2]. This syndrome is of particular concern in individuals who have extensive, rapidly growing, and radiochemosensitive tumors, because the release of tumor-derived intracellular constituents into the systemic circulation, with their subsequent filtration into tubular lumina, is believed responsible for the resulting ARF. The best-documented tumor-derived products thought to play pathogenetic roles in the renal injury are uric acid, phosphate, and xanthine [1]. Xanthine accumulates when xanthine oxidase inhibitors (such as allopurinol) are given to prevent urate production as a result of purine breakdown. Since uric acid, xanthine, and phosphate have low urinary solubilities, intratubular precipitation can result, causing intratubular obstruction and hence ARF [1].

Although lethal doses of radiochemotherapy are given to BMT patients in an attempt to eradicate malignancy, the incidence of TLS in this patient population is surprisingly low, with only a few cases reported [3, 4]. At our institution, approximately one in 400 patients develops overt TLS and attendant renal failure. The reasons for this low incidence are unclear, but two possible explanations exist. (*1*) The majority of patients undergoing BMT have disease in relapse, not de-novo disease, and hence their tumor burden usually is not extreme. (*2*) Given the widespread recognition of this syndrome, patients routinely receive prophylaxis with vigorous volume expansion/hydration, urinary alkalinization, and allopurinol therapy. If extreme leukocytosis exists prior to radiochemotherapy, leukapheresis can be used. Finally, if TLS does develop, early hemodialysis is initiated to remove circulating purine by-products and phosphate in an attempt to limit renal toxicity.

Marrow infusion toxicity. Bone marrow transplantation has increasingly employed autologous marrow for reconstituting the hematopoietic system following cytoreductive therapy, thereby avoiding the need for marrow donors, related or unrelated [5]. However, cryopreservation of the harvested marrow is usually required, since hematopoietic stem cells generally die within a few days of storage at 4°C [6]. Cryopreservation is generally performed at −80°C, with 10% dimethyl sulfoxide (DMSO) added as a cytoprotectant. Although progenitor cells are well maintained during freezing, storage, and thawing, granulocytes and red blood cells are disrupted [7]. Thus, during marrow infusion, patients are exposed to potentially toxic cell lysis products, as well as to DMSO. Reported side effects include nausea, vomiting, abdominal pain, dyspnea, headache, flushing, fever, chills, hyper- and hypotension, as well as bradycardia with 1st, 2nd, or 3rd degree heart block [6–9]. In addition, overt hemoglobinuria occurs in approximately 75% to 100% of such patients [8, 10, 11]. The free hemoglobin load results from cryopreservation-induced red blood cell disruption, as well as from in-vivo hemolysis. The latter is a frequent complication of DMSO infusion, particularly when high concentrations (>10%) or large total amounts are employed [10, 12, 13]. In-vivo hemolysis also can occur with peripheral stem cell grafts, as their collection via apheresis produces a dilute stem cell population and necessitates large-volume DMSO infusions [10].

In 1987, Smith et al noted the potential for marrow infusion to cause ARF [11]. These investigators reviewed 33 consecutive cases of autologous BMT and identified 3 patients who developed ARF immediately after administration of cryopreserved marrow. They concluded that the ARF was hemoglobin induced because the onset of the ARF was not associated with hypotension or nephrotoxic drug exposure, gross hemoglobinuria occurred in all 33 patients, and the renal histology showed dilated tubules with hemoglobin casts and acute tubular necrosis. I should note that DMSO-induced intravascular hemolysis can cause ARF even in the *absence* of BMT [12], so marrow products are not essential for the induction of this syndrome.

The mechanisms by which heme proteins (hemoglobin/myoglobin) induce ARF have been extensively studied for many years, but as of yet, no single, unifying hypothesis has emerged. However, at least three interrelated pathophysiologic alterations probably are involved (reviewed in Ref. 13). *First*, following their filtration, heme proteins precipitate within distal nephron segments, particularly in the presence of an acid pH (<6.5). Because heme proteins and Tamm-Horsfall protein each precipitate and co-aggregate under aciduric conditions [14, 15], cast formation with early tubular obstruction results. That cast formation is a critical event in the evolution of heme-protein-induced ARF is underscored by innumerable studies documenting that prevention of heme precipitation, either by urinary alkalinization or by administration of impermeant solute (for example, mannitol, Na_2SO_4), can protect against subsequent ARF [13–18].

Second, proximal tubular heme protein uptake occurs via endocytic reabsorption and leads to cellular heme overload. The presence of heme protein casts undoubtedly increases this process because tubular obstruction prevents heme protein excretion, allowing more time for proximal tubular endocytic reabsorption to occur. To demonstrate this point, our laboratory compared the amount of proximal tubular heme protein endocytic uptake by normal rats and by rats subjected to extrarenal (ureteral) obstruction [14]. Whereas relatively mild endocytic heme uptake occurred in normal rat kidneys, rats with ureteral obstruction developed rapid and massive proximal tubular heme protein overload, as denoted by giant heme-stained endolysosome formation [14]. Thus, the amount of proximal tubular heme uptake, a critical determinant of subsequent heme protein cytotoxicity, does not merely depend on the magnitude of hemoglobinuria, but also on the presence of obstructing casts.

Third, proximal tubular heme loading eventuates in tubular cell necrosis. However, the mechanisms involved remain ill defined. Of note, neither hemoglobin nor myoglobin is acutely toxic to either cultured proximal tubular cells [19] or to freshly isolated proximal tubular segments [20]. Indeed, these proteins paradoxically can exert cytoprotective influences [20, 21], possibly because of their ability to scavenge nitric oxide, which is produced within proximal tubular cells [21]. It is generally believed that heme proteins, once they have gained an intracellular location, must be metabolically "processed," and that this process culminates in the generation of toxic by-products. The most commonly discussed is inorganic iron, released from the porphyrin ring. Once freed, it can catalyze the formation of highly toxic hydroxyl radical (·OH) via the Fenton/Haber Weiss reactions and can initiate a chain of oxidative reactions that culminate in cell death. In support of this theory are several reports that deferoxamine, an iron chelator, can mitigate in-vivo myohemoglobinuric ARF [22–24]. But deferoxamine can prevent iron-induced tubular cell killing in vitro despite paradoxically increasing ·OH generation [20, 24]. Furthermore, free-radical-induced lipid peroxidation can exert cytoprotective, as well as injurious, proximal tubular effects [25]. Therefore, the mechanisms by which heme proteins induce tubular cell necrosis remain speculative. Study of this issue is further complicated by the fact that non-iron-containing, low-molecular-weight proteins also can cause renal injury; heme protein nephrotoxicity thus is not simply a result of iron-dependent oxidant stress [26, 27].

Despite the frequency of hemoglobinuria during cryopreserved marrow infusion, ARF uncommonly results, undoubtedly because a prophylactic solute and bicarbonate diuresis is routinely induced [7]. This diuresis prevents cast formation and hence the initiating step in heme protein nephrotoxicity. Of further note is that DMSO, contained within the marrow infusate, might exert a cytoprotective influence by contributing to the solute diuresis, and possibly by functioning as a free-radical scavenger. Indeed, DMSO's potential for protecting against acute tubular necrosis (ATN) is underscored by a study in which its administration mitigated post-ischemic renal injury in rats [28]. However, this protective influence is by no means universal; Bennett et al showed that it had no effect on potassium-dichromate-induced ARF [29].

An unresolved issue is whether marrow-infusion-induced proximal tubular cell heme protein loading alters the kidney's susceptibility to ischemic or toxic insults sustained later in the post-transplant period. In this regard, Nath and colleagues have demonstrated that administration of nontoxic amounts of heme proteins increase heme oxygenase and ferritin concentrations within renal cortex [30]. These proteins function as antioxidants, so theoretically they could protect the kidney from subsequent tubular insults [30]. Alternatively, our laboratory has found that heme infusions raise intratubular cell free-iron content, sensitizing these cells to superimposed oxidant (H_2O_2) stress [31]. Thus, the long-term effect of renal heme/iron loading on post-BMT renal function remains an interesting, but unresolved, issue.

Early hepatorenal-like syndrome

By far the most common time frame in which BMT-associated ARF develops is within the first 10 to 21 days post transplantation. It is during this period that most of the acute complications of radiochemotherapy become manifest, including marrow aplasia, life-threatening infections, pneumonitis, and gastrointestinal and hepatic toxicities. At our institution, approximately 40% of patients develop renal insufficiency during this period (as defined by a doubling at least of the baseline serum creatinine concentration), and one-half of these individuals require dialysis (Fig. 1) [32]. Our experience is not unique. For example, Johns Hopkins University School of Medicine reported a 64% incidence of early BMT-associated renal insufficiency [33], and a recent study from Madrid, Spain, noted an 84% occurrence rate [34]. The development of ARF during this period has profound implications for patient survival. For example, a doubling of the serum creatinine is associated with a twofold increase in mortality (from 17% to 37%) [32], whereas the need for dialysis predicts a mortality rate of 84% to 88% [32, 34]. Thus, it is clear that early BMT-associated renal dysfunction has ominous implications.

To discern the types of ARF that develop during this early period after BMT, we conducted an extensive retrospective review of 272 patients who received bone marrow transplants in 1986 at our institution [32]. À priori we assumed that patients who developed ARF during the early post-transplant period would manifest a broad spectrum of renal diseases, not a specific syndrome, because of the multiple and diverse risk factors for renal dysfunction that exist in these critically ill patients. As examples, nausea, vomiting, and diarrhea due to radiochemotherapy and to acute graft-versus-host disease (GVHD) can cause intravascular volume depletion, and hence, a pre-renal state. Frequent nephrotoxic drug exposure (such as with aminoglycosides, amphotericin B) and hemorrhagic and septic shock would seem likely causes of ATN. These patients are exposed to a wide variety of drugs, such as semisynthetic penicillins, cephalosporins,

and allopurinol, which have the potential for inducing allergic interstitial nephritis. As I will discuss in a moment, BMT is a frequent cause of the hemolytic-uremic syndrome (HUS). Finally, high-dose methotrexate [35], hemorrhagic cystitis with clot formation, and fungal involvement of the collecting system each can cause obstructive nephropathy. Thus, a wide range of possibilities exists, and a complete differential diagnostic evaluation is needed in these patients.

Despite this broad range of diagnostic possibilities, our experience indicates that approximately 90% of patients who develop early BMT-associated ARF manifest a single disorder that mimics the hepatorenal syndrome (HRS) [32]. Its clinical characteristics are as follows. Within one to 7 days after marrow infusion, a sodium-retentive state develops that leads to weight gain (approximately 2–6 kg within 2 weeks), peripheral edema, and sometimes ascites. Concomitant hepatic dysfunction due to veno-occlusive disease almost always occurs and is manifested by progressive hyperbilirubinemia. The onset of azotemia characteristically begins between 10 and 16 days post transplantation and, within an additional 5 to 7 days, approximately one-half of the affected individuals require dialysis for control of ARF. More than 80% of these patients have a persistently low urinary sodium concentration (<20–40 mEq/liter) despite the use of diuretics. Urinalysis typically demonstrates only trace protein but the urine sediment often contains large numbers of "muddy brown" granular casts. These casts are also present in comparably jaundiced BMT patients who do not have ARF; thus the casts are likely a consequence of bile salt/bilirubin effects on tubules [36], rather than indicators of ATN. Approximately two-thirds of the patients are nonoliguric; however, substantial fluid requirements for medications and nutritional support plus refractoriness to diuretics often lead to progressive volume expansion and pulmonary vascular congestion. Indeed, volume overload with pulmonary vascular congestion, not severe azotemia, is the usual indication for initiating dialysis [32]. The mean BUN and creatinine concentrations at this time are approximately 100 mg/dl and 3.5 mg/dl, respectively [32]. After dialysis is begun, oliguria frequently supervenes.

The central role for hepatic disease in this form of ARF is underscored by the facts that jaundice, portal hypertension, and renal sodium avidity precede the onset of azotemia, and that the severity of these abnormalities predicts the likelihood that the patient will require dialysis [32, 34]. For example, a bilirubin of <4 mg/dl portends a relatively good renal prognosis; conversely, a bilirubin of >7 mg/dl at the time of serum creatinine doubling predicts a high likelihood for the subsequent need for hemodialysis. Multiple potential causes for hepatic disease exist in this patient population. These include drug-induced and viral hepatitis, hyperalimentation-induced fatty liver, and acute graft-versus-host disease. By far the most common cause, accounting for 90% of cases, is veno-occlusive disease [37–41] (Fig. 2). Veno-occlusive disease is caused by acute radiochemotherapy-induced endothelial cell injury of hepatic venules, with its resultant venular thrombosis, possible collagen and reticulum fiber deposition, and ultimately sinusoidal and portal hypertension. In addition, variable degrees of zone-three hepatocyte necrosis occur, and the serum transaminases rise modestly. Jaundice results from intrahepatic portal vein-systemic venous shunting and hepatocyte necrosis. With the onset of ARF, the degree of hyperbilirubinemia is exacerbated because bilirubin is not excreted, and serum concentrations of greater than 50 mg/dl are frequently observed.

A number of pre-transplant risk factors for veno-occlusive disease can predict which individuals are at risk of developing the associated ARF. These include pre-existing hepatic disease, fever during cytoreductive therapy, and selected medications (estrogen-progestin, amphotericin, methotrexate) [38, 42]. Additional risk factors for ARF include mismatched grafts, age greater than 25 years, and a mildly elevated baseline serum creatinine concentration [32]. The vast majority of patients undergoing BMT have normal pre-transplant serum creatinine levels. Small differences within the normal range seem to predict different renal outcomes, however. For example, mean pre-transplant serum creatinine levels (measured after vigorous volume expansion) for groups that subsequently do or do not develop ARF are 0.75 ± 0.02 and 0.65 ± 0.02 mg/dl, respectively ($P < 0.001$) [32]. Thus, even a slight reduction in renal functional reserve appears to predict an increased risk of early BMT-associated acute renal failure [32].

The clinical similarities between this syndrome and hepatorenal syndrome are striking. (*1*) Jaundice and portal hypertension precede the onset of ARF. (*2*) Sodium avidity is almost always present, even when severe azotemia exists. (*3*) The BUN/creatinine ratio is typically high (~30/1), again suggesting a hemodynamic form of ARF. (*4*) Mild hyponatremia, a common feature of hepatorenal syndrome, is frequently observed [43]. (*5*) Systemic arterial blood pressure is usually low (~90–100 mm Hg systolic), suggesting peripheral vasodilation, as in hepatorenal syndrome [43]. (*6*) Autopsy material obtained from 36 patients dying with this syndrome at our institution failed to reveal any structural or morphologic basis for the ARF [32]. Although postmortem autolysis precluded definitive exclusion of ATN, when these negative morphologic results are interpreted in light of the pertinent clinical and laboratory parameters, a hemodynamic, rather than structural, form of ARF appears highly likely.

Although veno-occlusive disease sets the stage for most cases of early BMT-associated ARF, a superimposed clinical event usually triggers the development of renal failure (Fig. 3). The most common, in our experience, is sepsis syndrome. For example, in our retrospective analysis, fever and positive blood cultures were observed in 95% and 63% of patients, respectively, within the 48 hours prior to the onset of renal insufficiency [32]. Conversely, the incidence of hypotension did not increase during this period. Since more than 50% of the positive blood cultures contain fungus, amphotericin B therapy is generally initiated at this time; indeed, its use is the single strongest clinical association with the onset of ARF. Whether amphotericin B is directly involved in triggering ARF (for example, due to its vasoconstrictive properties) [44], or whether it is merely a marker for sepsis, which then precipitates the renal failure, remains unknown. However, that amphotericin B is typically administered for only one to 3 days, not weeks, prior to the development of azotemia strongly suggests that its adverse influence is not due to direct tubulotoxicity, which requires more prolonged administration to develop [45]. In our experience, neither aminoglycosides nor cyclosporine (CSA) exposure significantly increases the frequency of acute renal failure soon after BMT [32, additional unpublished data]. This is surprising because liver disease is a major risk factor for aminoglycoside toxicity [46, 47], and because CSA would be expected to exacerbate a hemodynamic form of ARF given its vasoconstrictor effects. Thus, while these drugs might *contribute* to ARF in

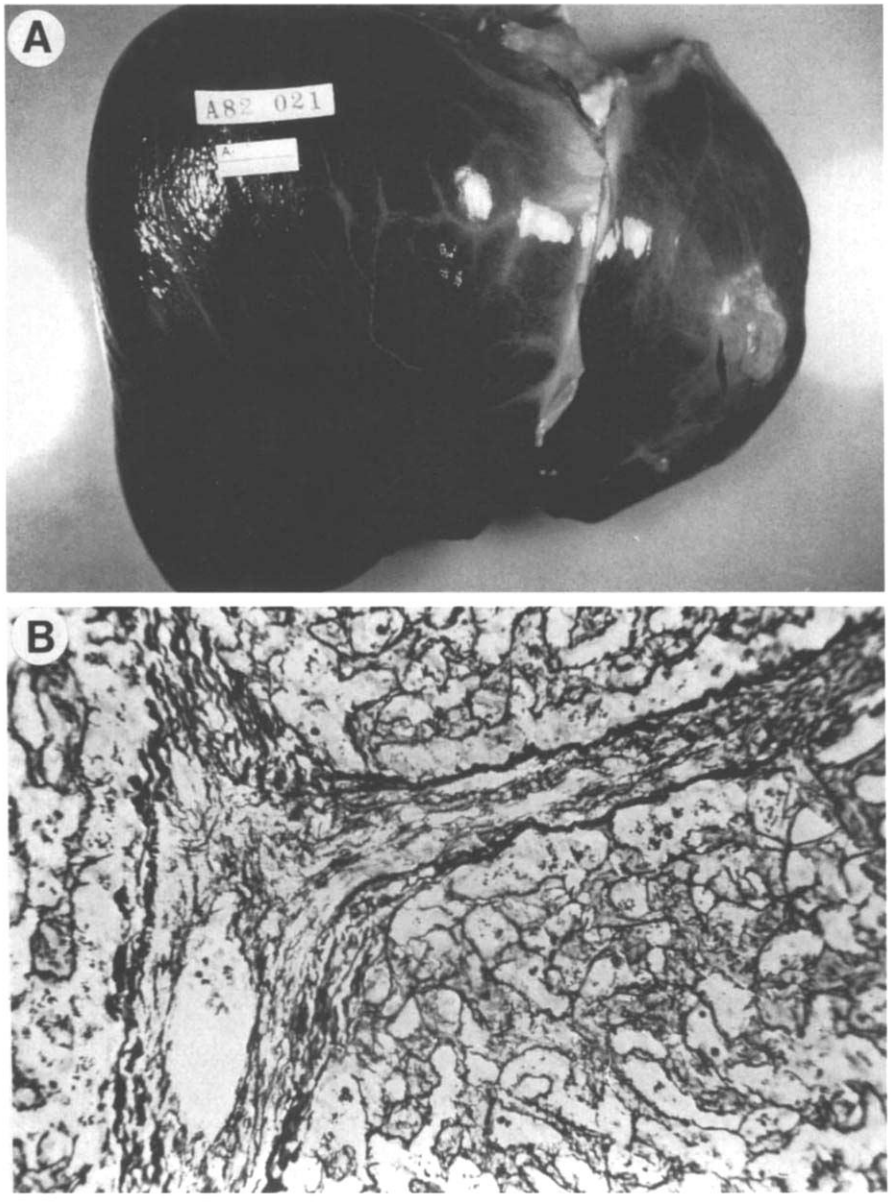

Fig. 2. A *Gross appearance of a liver with severe veno-occlusive disease.* Marked vascular congestion, swelling, and bile staining are apparent. **B** *Histologic reticulum-stained section of liver showing a hepatic venule and its tributary, which are largely occluded with a network of reticulum fibers.*

selected patients, these compounds are not simple explanations for the vast majority of cases at our institution.

Because this form of ARF resembles hepatorenal syndrome clinically, it probably is hemodynamic in origin, being mediated by a primary increase in renal vascular resistance. That sequential isotopic blood flow measurements, conducted on 4 patients, have documented approximate 50% decrements in renal perfusion with the onset of renal insufficiency further supports this view (personal unpublished data). The mediators of this vasoconstriction remain unknown. By analogy with hepatorenal syndrome, multiple possibilities exist, including endotoxemia, false neurotransmitters, excess catechol production, increased renal sympathetic tone, altered prostaglandin synthesis, excess angiotensin II generation, and defects in the kallikrein-kinin system [reviewed in 43]. Endothelin recently was implicated in hepatorenal syndrome because circulating levels were preferentially increased in cirrhotic patients who developed this form of renal failure [48]. With these diverse pathogenetic possibilities, and given that sepsis [49], amphotericin B [44], and cyclosporine each can induce renal vasoconstriction, it will be extremely difficult to identify the specific mediators of this syndrome in BMT patients.

Veno-occlusive disease, sepsis, and amphotericin B are the principal risk factors for early BMT-associated acute renal failure, and a number of prophylactic and therapeutic approaches have been developed to combat each. Various modifications of cytoreductive therapy have been tried in an attempt to minimize the hepatic injury that gives rise to veno-occlusive disease [37]. Continuous heparin [50–53] and PGE_1 infusions [54, 55] also have been used to prevent hepatic venular thrombosis and, hence, portal hypertension. Unfortunately, these approaches have met with inconsistent success. In an attempt to reverse severe veno-occlusive disease, Bearman and colleagues at this instititon conducted a trial of tissue plasminogen activator (tPA) plus heparin therapy [56]. This combined therapy reduced jaundice

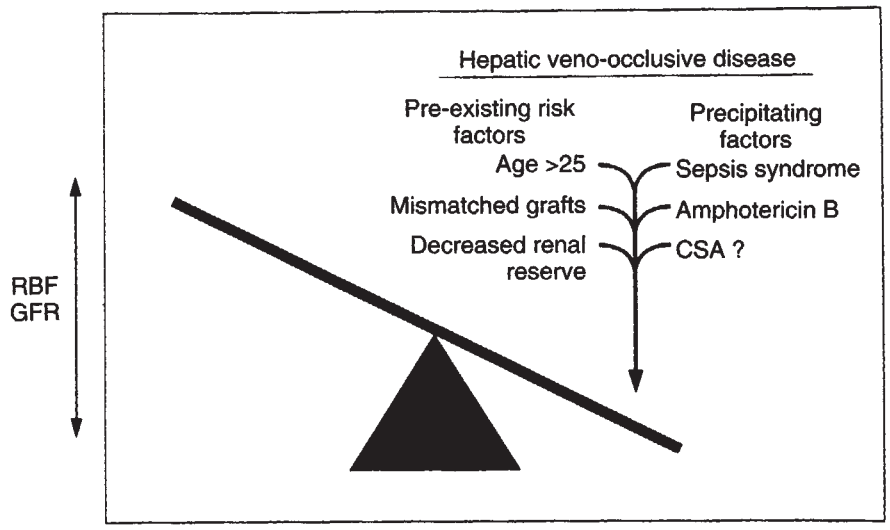

Fig. 3. *Schematic representation of the major pre-existing risk factors and acute precipitating factors for veno-occlusive-disease-associated ARF.* These factors are believed to predispose to, or cause, renal vasoconstriction, which culminates in decreasing renal blood flow (RBF) and hence lowered glomerular filtration rate (GFR).

and increased urine output in 5 of 7 patients. But the high risk of hemorrhage in these patients limits broad application of this approach. Because protein C and anti-thrombin-III deficiencies might be involved in the pathogenesis of veno-occlusive disease [57], therapeutic trials with these naturally occurring anticoagulants are planned.

A number of approaches for preventing sepsis and its complications also have been undertaken. For example, treatment with granulocyte-macrophage colony stimulating factor (GM-CSF) might accelerate recovery from neutropenia, potentially decreasing infectious complications, and possibly reducing the incidence of ARF. Because regimen-related toxicities are associated with TNFα generation [37], and since the latter is responsible for many components of sepsis [49], research has centered on blocking its production with pentoxifylline (PTX). When PTX was given to 30 BMT recipients in a phase I-II trial, lessening of the early BMT-associated hepatic and renal dysfunction appeared to result [58]. However, a more recent randomized trial of PTX has shown no beneficial effects of the drug [59].

Approaches taken to eliminate the risk of amphotericin B nephrotoxicity include combined fluconazole and low-dose amphotericin B prophylaxis [60, 61]. It is hoped that preventing fungal sepsis will obviate the need for full therapeutic, and hence nephrotoxic, amphotericin B doses. However, the utility of this approach remains to be determined. Alternatively, new amphotericin B formulations might decrease its toxicity. One such compound (ABCD; Liposomes Technology, Inc., Menlo Park, California) is currently undergoing clinical trials at our institution. In this formulation, amphotericin is complexed with cholesteryl sulfate; its ability to insert into mammalian cell membranes is decreased and its toxicity thereby reduced [62, 63]. A recent in-vitro study from our laboratory illustrates this point [64]. Freshly isolated rat proximal tubular segments were incubated with either traditional amphotericin B (Fungizone) with sodium deoxycholate (the bile salt dispersing agent used in the Fungizone preparation) or with ABCD. Under the conditions employed, Fungizone caused approximately 40% cell death and about 90% cellular ATP depletion during 60 minutes of incubation. Approximately one-half of this toxicity could be directly attributed to the deoxycholate carrier, undoubtedly because of its detergent effect. In contrast, ABCD caused absolutely no cytotoxicity, even when the concentrations were increased threefold above the Fungizone levels. The ABCD was completely nontoxic when the proximal tubules were subjected to superimposed hypoxic injury. Experimental in-vivo studies also suggest an absence of nephrotoxicity [62, 63]. Nevertheless, the utility and safety of this promising agent in the clinical setting remain to be defined.

Once ARF develops, management is supportive. Attempts at maintaining normal fluid balance with sodium and water restriction and loop diuretics generally have met with little success. Low-dose dopamine infusion has been largely abandoned at our institution because of poor therapeutic responses. Dialysis thus remains the mainstay of treatment.

Late renal syndromes

Hemolytic-uremic syndrome. In 1981, Shulman et al reported 3 patients who developed arterial hypertension, microangiopathic hemolytic anemia, and severe renal failure within 6 weeks after bone marrow transplantation [65]. Histologic evaluation of renal tissue samples obtained at autopsy revealed similar findings in each of the 3 patients. The most prominent changes were afferent arteriolar and glomerular capillary thrombi, tubular dilation with intraluminal debris, red blood cell/hemoglobin casts, and focal tubular necrosis. The microangiopathy and the characteristic clinical presentation led these researchers to conclude that these individuals had developed hemolytic-uremic syndrome. That each of these patients had received cyclosporine, that CSA had previously been reported to cause vascular injury, and that microangiopathy had not been observed in non-CSA-treated BMT patients led the authors to hypothesize that this syndrome was CSA-induced.

Since Shulman's report, BMT-associated HUS has been widely recognized, with more than 20 reports appearing in the literature [66–85]. Its most characteristic presentation is an acute nephritic syndrome, with moderate to severe hypertension, hematuria/red blood cell casts, proteinuria, and renal failure (usually noted 4 to 12 months post BMT). In addition, central nervous system abnormalities (that is, perturbations triggered by hypertensive

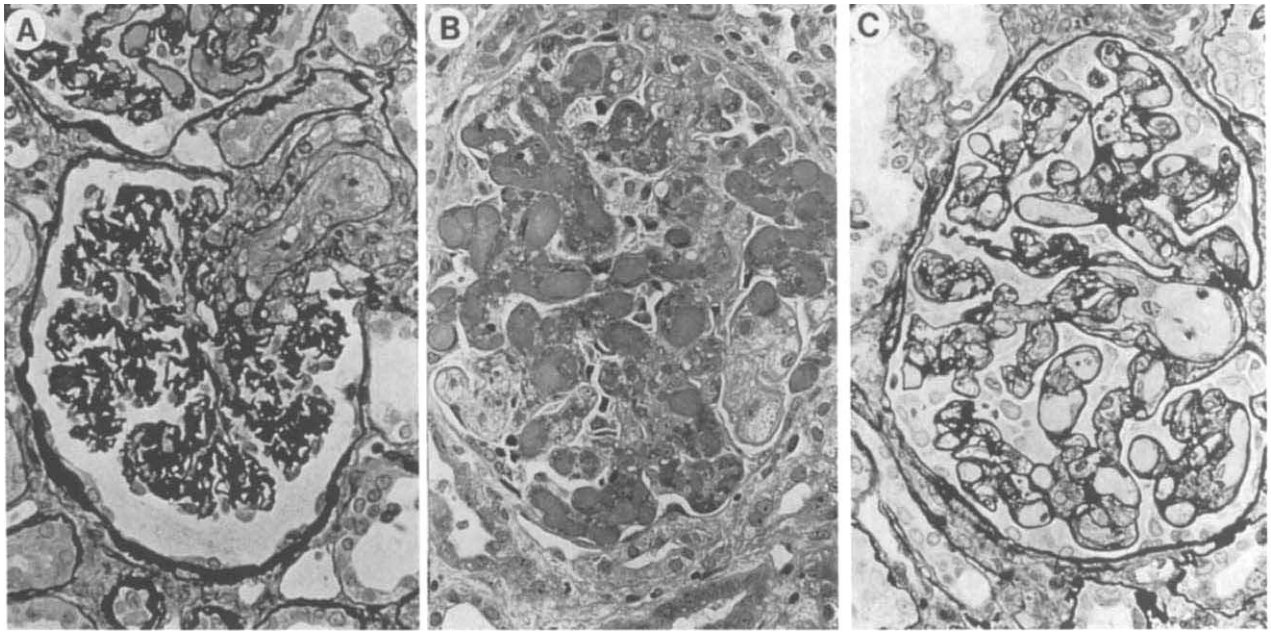

Fig. 4. *Characteristic glomerular pathology in renal biopsy samples obtained from a patient with BMT-associated HUS.* **A** An afferent arteriolar intraluminal thrombus and collapse of glomerular capillary tufts are observed (*silver methenamine stain*). **B** Widespread glomerular capillary thrombi are depicted, which appear to contain fragmented red blood cells and hemoglobin (*Mason trichrome*). **C** Mesangial dissolution ("mesangiolysis"), splitting of glomerular basement membranes, and intraluminal clots are present (*silver methenamine stain*).

encephalopathy, intracranial hemorrhage, and/or thrombotic microangiopathy) have been commonly observed, often dominating the disease. The nephritic presentation and the frequency of infection in this patient population sometimes lead to a presumptive diagnosis of postinfectious glomerulonephritis. However, microangiopathic hemolytic anemia and its consequences (schistocytes, depressed haptoglobin, elevated lactic dehydrogenase concentrations) plus the onset, or worsening, of thrombocytopenia typically clarify the diagnosis. Thus, a renal biopsy usually is not required.

Over the past 10 years, retrospective and prospective studies have ascertained the frequency and potential causes of BMT-associated hemolytic-uremic syndrome. An overall incidence of approximately 15% to 20% has been found; one study reported a 71% occurrence rate [71]. Documented in all age groups, its incidence appears unrelated to the disease for which transplantation is performed, and it has been observed following both allogeneic and autologous grafting. Other than exposure to cytoreductive therapy, no consistent preexisting or precipitating conditions have been identified. Enough cases of BMT-associated HUS have been documented in the absence of cyclosporine treatment [72, 75, 80] to indicate that the drug either is not responsible for the syndrome or is not a prerequisite for its development. These retrospective and prospective analyses also have more completely defined the clinical spectrum of this disease. Whereas early reports stressed a fulminant nephritic presentation, it has become clear that more subtle cases are at least as frequent. For example, the syndrome can manifest as subacute or insidious renal failure; furthermore, microangiopathic hemolytic anemia and thrombocytopenia may be minimal or not immediately apparent. Under these circumstances, a diagnosis of chronic cyclosporine nephrotoxicity may be entertained. Alternatively, HUS may remain entirely subclinical, its presence only being documented by careful laboratory assessments for microangiopathic hemolytic anemia, by transient thrombocytopenia, or by subtle abnormalities on urinalysis.

The clinical course of BMT-associated HUS is quite variable. Because the early literature stressed fulminant cases, death or irreversible renal failure has been reported most frequently. However, as the spectrum of the disease has become more apparent, it is clear that this disease also can run a self-limited course with resolution of the microangiopathic hemolytic anemia and thrombocytopenia. Most series have noted that renal insufficiency or renal failure, once it develops, usually remains fixed and frequently necessitates chronic dialysis. Some cases of reversible acute renal failure also have been noted, however. As Shulman et al pointed out [65], acute tubular necrosis can complicate this disease, and its resolution may explain many of these reversible cases. Indeed, the development of ATN during the course of HUS is not particularly surprising, because one consequence of thrombotic microangiopathy is tubular ischemia. Furthermore, hemoglobinuria, a potential consequence of microangiopathic hemolytic anemia, can directly cause ATN.

The central histopathologic features of BMT-associated HUS are arteriolar and glomerular capillary injury with associated thrombus formation (Fig. 4). Antignac and colleagues published a detailed description of the microangiopathy [75]. In brief, the arteriolar changes comprise marked luminal narrowing due to mucoid intimal thickening, and fibrinoid material accumulation. The underlying myocytes can be swollen or necrotic. The characteristic glomerular lesion is "mesangiolysis" [86], a term denoting dissolution of the mesangial cells and mesangial matrix with accumulation of a spongiform material that can extend into

glomerular capillary loops, producing a "double contour" appearance of the peripheral capillary walls. Immunofluorescence microscopy characteristically demonstrates glomerular and arteriolar fibrin deposition. Coarse granular C3 deposits are frequently observed in the muscular layer of arterioles. Electron microscopy reveals edematous infiltration of the mesangium, matrix destruction, and accumulation of electron-lucent material within the mesangial and sub-endothelial spaces with associated capillary wall thickening and luminal encroachment. Endothelial cell swelling, but not necrosis, is typically observed. Repeat biopsies performed on a limited number of patients after the acute phase of their disease have demonstrated that the disease can eventually lead to increased mesangial matrix production, glomerular fibrosis, and chronic ischemic changes, characterized by wrinkling of the glomerular basement membrane [75]. In addition, arteriolar sclerosis can result. These changes provide a morphologic explanation for persistent renal failure.

Although HUS can have diverse causes (for example, toxins, infections, immunologic insults), increasing evidence strongly suggests that in BMT patients, HUS is primarily a consequence of cytoreductive therapy, particularly total-body irradiation (TBI). The reasons for this assumption are as follows: (*1*) The histopathologic changes described, although not specific for radiation nephritis, are completely consistent with this diagnosis [75, 87–90]. (*2*) Acute radiation nephritis typically has its onset 6 to 12 months after radiation, and this natural history is consistent with the usual latency period seen in most cases of BMT-associated HUS. (*3*) The presentation of radiation nephritis can be indistinguishable from that of classic HUS [75, 90]. (*4*) Although a number of BMT-associated cases of HUS have presented within a few weeks after cytoreductive therapy (as illustrated by the patient described here), the literature clearly indicates that the expression of radiation injury can be substantially shortened by concomitant, or previous, chemotherapy (such as cyclophosphamide, bleomycin, adriamycin, actinomycin D) or corticosteroid administration [88, 89]. (*5*) Lawton and coworkers reported that partial renal shielding during TBI reduced the incidence of post-BMT HUS from 26% to 6% [85]; this finding strongly supports a cause-and-effect relationship. (*6*) Total-body irradiation fractionation, which is known to mitigate organ toxicity, appears to decrease the incidence of post-BMT HUS [75; unpublished observations from the Fred Hutchinson Cancer Center]. (*7*) The incidence of BMT-associated HUS is approximately the same in CSA- versus non-CSA-treated patients [80]. Thus, although cyclosporine might contribute to HUS in selected clinical circumstances [91], it does not appear to be a prime cause of HUS in patients who have undergone bone marrow transplantation.

The mechanism by which radiation injury causes HUS is not completely understood. It is widely recognized, however, that radiation's biologic effects are due to tissue energy deposition, with differing susceptibilities being expressed by various cell types, as well as by different intracellular constituents [92]. Within the kidney, endothelial cells appear to be the most susceptible. With relatively low-dose exposure, single-stranded DNA breaks occur. These can be repaired, but normal cells can lose their ability to replicate; after 2 to 3 cell cycles, defective cells, or no cells, replace the irradiated parent. The damage to the DNA and its ensuing alterations probably explain the usual 4- to 12-month latency period between total-body irradiation and the onset of HUS. Presumably, the defective endothelium is predisposed to microthrombus formation (for example, because of decreased prostacyclin production [93]); this process culminates in HUS. If high-dose radiation injury occurs, or if radiosensitizers are present, such as radiomimetic chemotherapeutic drugs, double-stranded DNA breaks and other critical cellular damage result, potentially culminating in a more rapid expression of this syndrome. Hydroxyl radical (·OH), a byproduct of radiation's interaction with cell water, is thought to be a prime mediator of radiation injury [92]. Once formed, it triggers a cascade of oxidative reactions, the results of which range from sublethal injury to cell death, depending on the target's susceptibility to oxidative damage. Tissue resistance depends on the integrity of the cell's antioxidant defense systems and whether superimposed stresses exist.

Regarding this latter possibility, we recently found that free (non-protein-bound) iron frequently exists in the plasma of many BMT patients. We obtained plasma samples from 38 patients 2 to 138 days following marrow infusion; we measured free ("catalytic") iron levels by the bleomycin assay technique [94]. This assay is based on the principle that bleomycin addition to biologic samples chelates the free iron that is present, and subsequently the iron-bleomycin complex can be detected by its ability to induce in-vitro oxidant DNA damage. Of the patients tested, 55% had detectable free iron, with concentrations ranging from 0.1 to 5.0 μmol/liter. Because normal plasma contains no free iron (because it is rapidly bound to transferrin), the finding of free iron by this technique represented a distinctly unusual result. To ascertain its cause and/or clinical relevance of the presence of the iron, we conducted a retrospective chart review; the presence of free iron strongly correlated with jaundice, particularly when it was due to acute GVHD. Since the liver is an important iron depot, we hypothesized that acute GVHD causes free-iron liberation and that the iron circulates in a form capable of catalyzing free-radical reactions. The clinical implications of the free-iron liberation, and whether it increases the risk of HUS, remain unknown. But the latter possibility seems plausible. Once within the circulation, "catalytic" iron should have direct contact with endothelial cells, and potentially can induce ·OH-driven oxidant damage.

Given that TBI and oxidant stress are likely causes of BMT-associated HUS, a number of strategies theoretically can be employed to decrease radiation injury and hence the incidence of HUS. These include partial renal shielding during TBI [85], hyperfractionation of the radiation dose, slow radiation administration (for example, from a cobalt source rather than from a linear accelerator), and the provision of exogenous antioxidants/·OH scavengers. But a potential problem exists with each of these: decreased tumor cell killing might result, which is also free-radical dependent. Provision of exogenous antioxidants also could impair marrow engraftment and subsequent proliferation. For example, a number of transcription factors are highly cell-redox-sensitive [95, 96]; therefore, provision of antioxidants (·OH scavengers, iron chelators) theoretically could exert antiproliferative effects. Recent experiments from this laboratory using a newly established immortalized adult human kidney proximal tubular cell line, HK-2 [19], illustrate this point [97]. When subconfluent cultures of these cells were incubated with a xanthine oxidase inhibitor (oxypurinol) to decrease superoxide production, HK-2 outgrowth was impaired. Proliferation also was inhibited when ·OH scavengers (sodium benzoate or dimethylthiourea) were added. Conversely, when the cells were challenged with sublethal oxidant stress

($FeSO_4$-driven ·OH production), proliferation was increased [97]. Although these results might have no direct relevance to therapy given to prevent HUS, these data do indicate that antioxidants could have unexpected and potentially undesirable effects in marrow-transplant patients.

Once established, BMT-associated HUS is largely unresponsive to all but supportive therapy. While plasma exchange, with or without vincristine therapy, has been widely used because of its reported efficacy in non-BMT-associated cases of HUS and thrombotic thrombocytopenic purpura, its results have been disappointing in BMT patients. For example, in one prospective uncontrolled trial conducted on 8 patients, 4 were completely unresponsive, while 4 showed some hematologic improvement [83]. However, of these 8 patients, 7 died within one to 3 months of disease onset. Thus, the therapeutic efficacy of plasma exchange remains unconvincing. Nevertheless, we usually employ a trial of it (5 to 10 treatments) in the hope of achieving a clinical response. Another unresolved issue is whether cyclosporine should be withdrawn in these patients. Although it is unlikely that CSA is responsible for hemolytic-uremic syndrome in the vast majority of BMT-associated cases, its vasculotoxicity generally prompts dose reduction or temporary discontinuation of the drug. No proof exists that this is absolutely necessary and, if GVHD appears to be life-threatening, CSA still might be indicated. Some patients are left with end-stage renal failure, and hence require maintenance dialysis. An interesting therapeutic possibility is renal transplantation with a kidney from the marrow donor. Since the donor marrow presumably has reconstituted the patient's immune system, the kidney theoretically should be extremely well tolerated. But no reports of this intriguing biologic experiment have yet been reported.

Cyclosporine A nephrotoxicity. Both acute and chronic graft-versus-host disease represent major complications of bone marrow transplantation, and moderate to severe GVHD is associated with an approximate 10% to 50% mortality rate [98–100]. Since its introduction in the early 1980s, cyclosporine has been a mainstay of GVHD prophylaxis and treatment largely because, unlike most immunosuppressive agents, it has no marrow inhibitory effect. When used alone for prophylaxis against acute GVHD, CSA has not been found superior to traditional methotrexate therapy. In combination with methotrexate or prednisone, however, CSA decreases the incidence of acute GVHD; hence cyclosporine is widely used within the first year following BMT [99, 100]. At our institution, 3 mg/kg/day is given intravenously initially in divided doses and subsequently 12.5 mg/kg/day is given orally in divided doses as soon as the patient can tolerate it. After 50 days, the CSA is tapered by approximately 5% per week and, in the absence of active GVHD, is discontinued at day 180.

Shortly after CSA's introduction for bone marrow transplantation, it became apparent that nephrotoxicity was a limiting complication. For example, in one early trial comparing CSA to conventional methotrexate prophylaxis in patients with acute non-lymphoblastic leukemia, 33 of 36 CSA-treated patients experienced at least a doubling of their baseline serum creatinine levels (to a mean peak value of 2.4 ± 0.3 mg/dl), whereas only 18 of 39 methotrexate-treated patients did so (peak value of 1.2 ± 0.1 mg/dl) [100]. Similar results have been noted in several other studies from our institution and others [101–104]. The risk of acute CSA-induced renal dysfunction has generally correlated reasonably well with serum/plasma CSA concentrations [105–107]. For instance, in one study from London [107], the correlations between trough CSA levels (as measured with a polyclonal radioimmunoassay) and the BUN and creatinine concentrations were 0.88 and 0.93 (r values) respectively. Concomitant nephrotoxin use, most notably amphotericin B, appears to markedly increase the risk of early CSA-associated renal dysfunction. In a study from our institution, renal insufficiency, as defined by a doubling of the serum creatinine, was noted in 19%, 38%, and 80% of patients treated with amphotericin plus methotrexate, CSA alone, and amphotericin plus CSA, respectively [108].

Monitoring of trough CSA concentrations has been standard practice in BMT patients because CSA levels clearly correlate with the associated toxicities (for example, renal, hepatic, and infectious complications; diastolic hypertension) as well as with the risk of acute GVHD [98, 106]. But no consensus has been reached as to which assay should be employed—high-performance liquid chromatography (HPLC), radioimmunoassay, fluorescence polarization immunoassay (FPIA); whether polyclonal or monoclonal assays are preferable; or whether whole blood, serum, or plasma should be used. Although it seems reasonable that measurement of the parent compound (for example, by HPLC, monoclonal immunoassay) would be superior to the use of a polyclonal assay (which detects CSA and its metabolites), this hypothesis has not been convincingly demonstrated. Indeed, one group reported that trough serum CSA levels, as assessed by polyclonal radioimmunoassay, correlated better with the risk of renal dysfunction than did parent CSA monitoring by HPLC [105, 109]. In 1986, our institution began monitoring plasma levels using an FPIA (TDx; Abbott Laboratories, Chicago, Illinois); since that time, CSA therapy has not had a statistically significant adverse effect on early (≤1 month) post-transplant renal function. Whether this reduction in acute CSA toxicity is due to the use of this nonspecific assay (suggesting that CSA metabolites are involved in the nephrotoxicity) or whether it reflects some other undefined clinical variable remains unclear. However, it does point out that parent drug monitoring currently is not mandatory and, indeed, it might even be inferior to nonspecific assays for predicting nephrotoxicity. Whole-blood assays have a theoretical advantage over plasma/serum assays, as they can better gauge the total-body CSA burden by providing an index of the drug's lipid partitioning. No firm data exist to support this hypothesis in BMT patients, however.

Given the frequency with which cyclosporine causes early BMT-associated renal insufficiency, it is surprising that CSA has not been implicated in the development of dialysis-requiring ARF, at least at our institution. This lack of association has been noted in three studies [32, 100, 101], and we recently confirmed this finding in a prospective analysis of 355 patients undergoing bone marrow transplantation in 1987 and 1988 (unpublished data). Our most recent information suggests that neither CSA use, daily dosage, total amount administered, nor plasma concentrations predict the development of acute renal failure. A number of potential explanations exist. *First*, CSA levels are routinely monitored and dose adjustments are made for high levels. *Second*, if unexplained azotemia develops, CSA doses are routinely reduced; we thus presumably remove this contributing factor to ARF. *Third*, since early BMT-associated ARF appears to be of hemodynamic origin and is triggered by veno-occlusive disease, sepsis, and amphotericin B, the vasoconstrictive effects of CSA may be superfluous rather than additive in this situation. Nevertheless,

when azotemia does develop in this patient population, CSA should be temporarily reduced or discontinued, as there is no other way to exclude its participation in causation of ARF in any given individual.

Cyclosporine typically is given in full therapeutic doses for only 2 months in stable patients; not surprisingly, therefore, chronic CSA nephrotoxicity has rarely been observed. For example, in a comparison of our experience with patients treated with prophylactic methotrexate versus methotrexate plus CSA, no significant difference in serum creatinines was observed at one year post BMT [104]. A group from Basel, Switzerland, reported that patients tolerated very well 6 months of full CSA therapy, followed by taper and withdrawal by one year [103]. These observations have led to the assumption that patients who have undergone bone marrow transplantation have a low risk of chronic CSA-induced renal failure. But typical changes of chronic CSA nephropathy (striped interstitial fibrosis, tubular atrophy, arteriolopathy) have been documented in CSA-treated BMT patients, particularly in those receiving prolonged therapy for chronic GVHD [103, 110]. Since unrelated grafts are increasingly being used for marrow transplantation, it is likely that more chronic GVHD will result, leading to more prolonged CSA therapy. Thus, more chronic CSA nephrotoxicity can be expected.

Conclusion

As the patient presented here demonstrates, bone marrow transplantation is being applied more and more for therapy of a broad variety of malignant and nonmalignant diseases. Its increasingly frequent use will undoubtedly teach us more about its therapeutic potential, the diseases for which it is used, and its attendant complications. Bone marrow transplantation confers a very high risk of acute renal failure, most notably due to a hepatorenal-like syndrome and hemolytic-uremic syndrome. Their occurrence, while life-threatening, offers nephrologists unique opportunities to study the pathogenesis of these diseases and their treatment in a prospective fashion. The effects of sepsis on renal function also can be assessed, given an approximate 50% incidence of sepsis within the first month following transplantation. Thus, in addition to its expanding therapeutic utility, bone marrow transplantation offers unique research opportunities to basic scientists and clinicians with highly varied investigative interests.

Questions and answers

DR. NICOLAOS E. MADIAS, M.D. (*Chief, Division of Nephrology, New England Medical Center, Boston, Massachusetts*): It is surprising that there seems to be a low incidence of acute tubular necrosis in the marrow transplant population, particularly during the early transplant period, when sepsis and hypotension are so frequent. Why do you think this might be so?

DR. ZAGER: That is an extremely interesting question. Unfortunately, I can only speculate about the answer. As you point out, marrow transplant patients have many risk factors for ATN, particularly during the first 3 to 4 weeks following marrow infusion, when most cytoreductive-therapy-associated complications develop. Some of these risk factors are directly related to marrow aplasia, such as sepsis syndrome and, at times, thrombocytopenia-related hemorrhagic shock. Severe liver disease is another risk factor for ATN [111], and as I mentioned, hepatic veno-occlusive disease is a frequent complication during this period. Another potential risk factor is hyperthermia which, in the experimental setting, profoundly worsens renal tubular injury even in the absence of bacteremia [112, 113]. For example, if body temperature is varied in rats from 32.0° to 39.5°C during renal ischemia, the severity of post-ischemic tubular necrosis and filtration failure directly and dramatically increase [112]. We have documented this same injury-potentiating effect directly at the cellular level with the use of isolated proximal tubular segments: the higher temperature, the greater the amount of hypoxic tubular injury [113]. This relationship partly can be explained by thermal effects on cellular metabolic rates, since increasing the temperature decreases cellular ATP depletion during oxygen deprivation [112, 113]. But because all cellular reactions are temperature dependent, a change in energy profiles undoubtedly only partially explains this phenomenon. Intermittent fevers are extremely common within the first few weeks following marrow transplantation; one therefore would presume that alterations in renal susceptibility to injury would result.

Given these multiple risk factors, it is tempting for us to postulate that the apparent infrequency of ATN is due to the emergence of counterbalancing cytoprotective pathways that allow the kidney to withstand frequent and diverse tubular insults. In fact, it has been recognized for at least 75 years that the kidney can acquire cytoresistance to acute tubular injury [114]. For example, following recovery from different types of nephrotoxic acute renal failure, animals can withstand further toxic insults [114]. Our laboratory has shown that renal tubular cytoresistance also can emerge within 24 hours of ischemic renal injury [115]. Some of this cytoresistance can be attributed to reductions in functional renal mass, as surgical renal ablation (1½ nephrectomy) confers cytoresistance on the remaining nephrons [116, 117].

We are currently investigating the mechanisms underlying acquired cytoresistance. To date, we know that it exists directly at the cellular level, because proximal tubules extracted from rats with ARF are protected against diverse forms of in-vitro injury [117]. One attractive possibility is that cytoprotectant heat-shock proteins (HSPs), synthesized by the kidney in response to diverse forms of tissue injury, are responsible [117–120]. The fact that hyperthermia is so common following marrow transplantation could help explain why these patients are resistant to ATN. In my view, however, it remains to be proven that HSPs actually protect the kidney from acute tubular injury. For example, we have induced hyperthermic heat shock in rats and produced marked HSP synthesis (HSP-70); yet the severity of post-ischemic ARF has not been affected (unpublished observations). Furthermore, if proximal tubules are extracted from these "heat-shocked" kidneys, only trivial cytoresistance to in-vitro hypoxic injury is observed [117]. These negative results have led us to explore whether the increased expression of alternative cytoprotectant molecules, such as antioxidant enzymes [30, 121, 122] are responsible, or whether functional/structural alterations induced within tubular cells in the aftermath of injury account for the cytoprotection.

DR. MADIAS: What is the natural history of the veno-occlusive-disease-induced hepatorenal syndrome? Second, is there an animal model that has been used to study it?

DR. ZAGER: The outcome of veno-occlusive-disease (VOD)-associated renal failure largely depends on the severity of the underlying hepatic disease. If the hepatic disease is relatively

modest, patients generally recover from it and from the frequently accompanying renal insufficiency. Severe VOD, which is graded according to a number of clinical and laboratory parameters [42], confers an ominous prognosis. For example, McDonald et al prospectively analyzed the incidence of VOD in 355 patients undergoing BMT [42]. The incidence of mild, moderate, and severe VOD in these patients was 12%, 26%, and 15% respectively, whereas the incidence of ARF for these groups was 10%, 10%, and 54%, respectively. Of the 54 patients who had severe VOD, 53 died, most with ARF. Overall, ARF requiring dialysis is associated with an approximate 85% risk of death [32]. In many ways, the natural history of VOD-associated renal failure mimics that of the hepatorenal syndrome: if the patient has reversible liver failure, recovery from ARF also occurs. However, if the hepatic disease is unremitting, death in ARF can be expected.

Regarding the second part of your question, a few animal models of hepatic VOD have been established; these involve giving hepatic irradiation and chemotherapy [123, 124]. These models have not produced ARF, however. Perhaps this indicates that VOD alone is insufficient to produce ARF, and that additional precipitating factors, such as sepsis syndrome and amphotericin B, are important. This is certainly our clinical experience, as most patients with VOD develop acute renal failure only after the onset of sepsis.

DR. JOHN T. HARRINGTON (*Chief of Medicine, Newton-Wellesley Hospital, Newton, Massachusetts*): Is there a way of blocking the cytokines thought to be involved in the pathogenesis of marrow-transplant-associated VOD? If so, have cytokine-blocking agents been administered clinically to see whether they protect the kidney?

DR. ZAGER: The cytokine most implicated in the pathogenesis of VOD, and in other forms of chemoradiotherapy toxicities, is TNFα. Early post-transplant plasma levels of TNFα correlate with the development of VOD as well as with GVHD and noninfectious pneumonitis [125]. Synthesized both by activated phagocytes and nonphagocytic cells, TNFα has diverse biologic actions. In regard to VOD, it is thought to exert a procoagulant effect, stimulate the production of inflammatory prostanoids, and cause the generation of superoxide radicals. Together these processes can lead to hepatic venular damage, thrombosis, and reticulum and collagen deposition [37]. Pentoxifylline is a synthetic xanthine derivative that downregulates TNFα production [58]. It had been suggested that its use can decrease the severity of both sepsis syndrome and radiation-induced lung injury [126]. These considerations led to clinical trials in marrow transplant patients. In a phase I-II trial, 30 patients were treated with PTX from the start of conditioning therapy through hospital discharge, and hepatic disease and renal insufficiency decreased [58]. Unfortunately, a more recent randomized trial showed no benefit [59].

DR. AJAY SINGH (*Division of Nephrology, New England Medical Center*): Is there any evidence for endothelial cell damage outside the liver in VOD?

DR. ZAGER: Chemoradiotherapy likely induces widespread endothelial damage, but to my knowledge, the only other organ that develops venous occlusions is the lung. In one autopsy study, the incidence of pulmonary endothelial swelling and localized venous thromboses was approximately 50% [127]. Yet clinically significant pulmonary VOD is thought to be quite uncommon, although it has been reported [128, 129]. Why the liver appears to be uniquely susceptible to this complication of cytoreductive therapy is unknown.

DR. SINGH: Has anyone measured endothelin levels in these patients?

DR. ZAGER: Not to my knowledge. We are just starting a prospective analysis of circulating endothelin and nitric oxide levels to assess whether they predict, or correlate with, the development of VOD and renal failure. Our working hypothesis is that chemoradiotherapy induces endothelial damage, which leads to increased endothelin or decreased nitric oxide production, and which culminates in renal vasoconstriction and a hemodynamic form of ARF. That patients with hepatorenal syndrome have increased circulating levels of endothelin [48] offers support for this possibility.

DR. SINGH: Has TNF been injected into normal animals? To my knowledge, it doesn't cause any damage and therefore might only predispose the kidney to other forms of injury.

DR. ZAGER: I believe that you are correct. We have addressed this issue experimentally by injecting either purified endotoxin or *E. coli* into rats; both substances cause TNF release. Although renal blood flow decreased, we noted no tubular necrosis or sustained reduction in GFR. If we induced endotoxemia at the time of one-shot gentamicin administration, however, severe ARF and focal ATN resulted [130]. Although the endotoxemia increased proximal tubular gentamicin uptake, this change alone could not explain the induction of renal failure. Endotoxemia and TNF also increase the severity of postischemic and myohemoglobinuric ARF [131, 132] and likely potentiate diverse forms of proximal renal tubular cell injury. Alternatively, induction of endotoxin tolerance can protect against subsequent renal injury [130, 131].

DR. RONALD PERRONE (*Division of Nephrology, New England Medical Center*): The few marrow transplant patients I've seen are beyond the marrow aplasia phase and have been on 5 to 10 different drugs, some of which are potentially nephrotoxic. It's always hard to pin down exactly what's causing azotemia in these patients. Do you have any thoughts regarding these patients?

DR. ZAGER: I'm sympathetic to that plight. It can be extremely difficult to identify the underlying cause. If the onset of the renal insufficiency can be identified by reviewing the serum creatinine concentrations, the medication history immediately preceding that date might be helpful in implicating a specific agent. The onset of renal insufficiency during this time raises the possibility of the hemolytic-uremic syndrome. We would look carefully at the urinalysis for proteinuria, new-onset hematuria or RBC casts, and hematologic markers of HUS, such as schistocytes, a falling hematocrit, a rise in LDH, or a decrease in the platelet count. If none of these approaches suggested a cause for the renal insufficiency, we would try to exclude cyclosporine toxicity by temporarily reducing the dose, particularly if the blood levels were not low and there were no active GVHD.

DR. MADIAS: As I recall, some people have speculated as to whether the incidence of membranous nephropathy increases in the aftermath of bone marrow transplantation, possibly related to the development of GVHD. What information is available regarding this issue?

DR. ZAGER: Since 1988, 5 patients with chronic GVHD have been described who have heavy proteinuria and in whom renal biopsies have shown light and/or electron microscopic changes suggestive of membranous nephropathy [133–136]. Given that a

murine model of chronic GVHD is associated with immunologic glomerular disease [137], these cases might have been GVHD induced. That 3 of these patients experienced reductions in proteinuria with immunosuppressive treatment of their GVHD also suggests a pathogenetic link [133, 135]. Despite this evidence suggesting a causal relationship, a number of potential caveats need to be considered. *First*, 2 of the reported patients did not demonstrate immunoglobulin deposition in glomerular basement membranes; therefore, the nature of the electron-dense basement membrane deposits remains unresolved [135]. *Second*, 2 of the patients had prominent mesangial proliferation [135], which is inconsistent with typical membranous nephropathy. *Third*, nonspecific glomerular changes, including mesangial proliferation and sclerosis, thickening and splitting of basement membranes, and GBM deposits have been observed following BMT in the absence of clinical renal disease, possibly as delayed consequences of cytoreductive therapy [138]. *Fourth*, a remission of glomerulopathy during immunosuppression of GVHD does not necessarily link the two diseases; idiopathic membranous nephropathy frequently responds to immunosuppressive therapy. Therefore, I think it is unclear whether a causal or a chance association between GVHD and membranous nephropathy exists.

Dr. Andrew King (*Division of Nephrology, New England Medical Center*): Let me return to Dr. Singh's earlier question. Endothelial damage and renal vasoconstriction raise the question of a disproportionate increase in endothelium-derived vasoconstrictors, compared to vasodilators. One of the characteristics of radiation nephritis is severe hypertension. Could you comment on whether endothelium-derived vasoactive compounds play a role?

Dr. Zager: The form of endothelium-derived vasoconstrictors and vasodilators in radiation-nephritis-induced hypertension has not been defined. We see a significant amount of mild hypertension in marrow transplant patients, but it usually is due to either cyclosporine or corticosteroid therapy. Accelerated or malignant hypertension, characteristic of radiation nephritis, is quite unusual in marrow transplant patients, except if severe HUS is present, probably because patients undergoing marrow transplantation generally receive only 8–14 Gy of radiation. In contrast, typical radiation nephritis requires approximately 23–25 Gy to develop. The use of cytotoxic drugs such as cyclophosphamide as part of cytoreductive therapy reduces the amount of irradiation needed and probably prevents the development of classic radiation nephritis and its attendant hypertensive complications.

Dr. Richard Lafayette (*Division of Nephrology, New England Medical Center*): You excluded acute tubular necrosis as the diagnosis in these patients who have many risk factors. It is well known that patients can have ATN with a low urinary sodium concentration if they have either nonoliguric ATN or an ineffective circulating blood volume. What else have you done to exclude the possibility of tubular necrosis in the marrow-transplant patients?

Dr. Zager: We too have been impressed that many patients with nonoliguric ATN have low urinary sodium concentrations. Therefore, renal sodium retention alone does not exclude ATN in patients with VOD-associated acute renal failure. In BMT patients, however, sodium avidity always precedes the onset of azotemia by many days. Thus it is the first evidence of the renal failure rather than a secondary manifestation of it. The observation by Bearman et al that partial resolution of hepatic VOD with t-PA and heparin therapy improves renal function [56] also suggests a hemodynamic form of ARF. If the renal failure were due to ATN, a reduction in portal hypertension would not immediately improve renal function. We also have reviewed autopsy material from 36 patients who died with ARF to look for histologic evidence of ATN. Although post-mortem autolysis makes it difficult to definitively exclude ATN, we found only one patient who had morphologic evidence of tubular necrosis [32].

Dr. Madias: Although you separated temporally the veno-occlusive renal failure syndrome from HUS, my own experience suggests that these two entities might co-exist. Indeed, the same factors that induce endothelial damage in hepatic venules also might damage the endothelium of arterioles.

Dr. Zager: I agree, and the patient presented today illustrates your point. Veno-occlusive disease is generally seen one to 3 weeks after transplantation, and HUS most often occurs 4 to 12 months later, but the two entities can co-exist. As I said earlier, previous exposure to chemoradiotherapy can accelerate the onset of post-transplant HUS, potentially allowing it to overlap with the VOD syndrome.

Dr. Harrington: I believe you said that patients whose serum creatinine levels fell in response to volume loading were not likely to develop acute renal failure compared with patients whose serum creatinine levels did not fall. Could you quantitate your comment?

Dr. Zager: In our retrospective analysis of 272 patients who underwent marrow transplantation, the mean serum creatinines just prior to marrow infusion for patients who subsequently did or did not develop ARF were 0.75 and 0.65 mg/dl respectively [32]. This was a highly significant, but quantitatively trivial, difference. It was interesting that both groups of patients had relatively low serum creatinine levels, compared with the normal range of 0.6–1.4 mg/dl. We ascribe these low values to the fact that these patients undergo vigorous volume expansion as part of their conditioning regimen, possibly calling into play their renal functional reserve. If this assumption is true, the possibility exists that seemingly trivial decrements in baseline renal function, or in the amount of renal functional reserve, might confer on these patients an increased risk of developing VOD-associated acute renal failure. But this remains a hypothesis that has not been directly tested in a prospective fashion.

Dr. Svetlozar N. Natov (*Clinical Research Fellow in Nephrology, New England Medical Center*): I have a question regarding viral infections in bone marrow transplantation. Herpes viruses, particularly CMV, can be transmitted with the allograft, and CMV is a major cause of morbidity and mortality in allogeneic bone marrow recipients [139]. Transmission with the transplant also has been shown for hepatitis B and hepatitis C viruses. Would you please comment on the impact of these viral infections on liver failure, and whether some of these viruses can cause any specific type of renal injury, as this has been reported for CMV in renal transplantation [140].

Dr. Zager: Viral hepatitis, particularly due to hepatitis C, affects approximately 20% of bone-marrow-transplant candidates [37]. Most of the attendant risks for post-transplant hepatitis come from these pre-existing infections, rather than from new viral transmission during the transplant or post-transplant period. The existence of pre-transplant hepatitis does impose significant risks [37]: (*1*) it is a major risk factor for hepatic VOD; (*2*) a fulminant flair of the hepatitis can result in the post-transplant period; and (*3*) chronic progressive liver disease can ensue. I know

of no specific post-marrow-transplant renal syndromes with a direct viral cause. Because viral hepatitis is a major risk factor for VOD, however, it undoubtedly threatens post-transplant renal function, even if only indirectly.

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