Pathology of the Liver With Bone Marrow Transplantation

Effects of Busulfan, Carmustine, Acute Graft-Versus-Host Disease, and Cytomegalovirus Infection

William E. Beschorner, MD, Jose Pino, MD, John K. Boitnott, MD, Peter J. Tutschka, MD, and George W. Santos, MD

The morphologic changes in the livers of autopsy specimens from recipients of 62 allogeneic bone marrow transplants were reviewed and characterized in specimens from patients who had had apparent drug toxicity, graft-versus-host disease (GVHD), and disseminated cytomegalovirus (CMV) infection. Two conditioning protocols were associated with significant hepatic toxicity. Two of 3 recipients on whom autopsies were performed who had been prepared with high doses of carmustine, cyclophosphamide, and total body irradiation had undergone acute hepatic failure and submassive necrosis with periportal sparing. Seven of 9 patients prepared with busulfan (16 or 20 mg/kg) and cyclophosphamide had moderate to marked centrilobular sinusoidal fibrosis and associated hepatocellular atrophy and necrosis. Twenty of the patients had acute cutaneous GVHD with associated hepatic dysfunction, including 8 with disseminated CMV infection. Of the 12 patients without concomitant CMV infection, 5 had an early onset of GVHD and had predominantly periportal and focal midzonal hepatocellular necrosis, and 7 had acute GVHD with later onset with predominantly bile duct injury. Fifteen patients had evidence of disseminated CMV infection. Whereas CMV infection alone was associated with both hepatocellular and bile duct injury, detectable virus infection was not a requirement for hepatocellular or bile duct injury associated with GVHD. (Am J Pathol 1980, 99:369–386)

WHILE ALLOGENEIC bone marrow transplantation is of proven value in the treatment of patients with aplastic anemia,[1,2] severe combined immune deficiency,[3] and acute leukemia,[1,2,4] many systemic complications have prevented the full realization of its theoretical potential. These often fatal complications include graft-versus-host disease (GVHD),[5–12] transient immune deficiency with opportunistic infections,[13–15] most commonly involving cytomegalovirus,[16,17] and non-marrow toxicity of the conditioning regimen used before the transplant.[18] Hepatic dysfunction is frequently associated with these complications.

To achieve a better understanding of the pathophysiology of hepatic complications, the biopsy and autopsy specimens of 62 recipients of allogeneic bone marrow transplants were retrospectively reviewed. These pa-

From the Department of Pathology and the Bone Marrow Transplant Unit, Oncology Center, The Johns Hopkins Medical Institutions, Baltimore, Maryland.

Supported by Grant CA-15396 from the National Cancer Institute, Department of Health, Education and Welfare.

Accepted for publication December 19, 1979.

Address reprint requests to William E. Beschorner, MD, Department of Pathology, The Johns Hopkins Hospital, Baltimore, MD 21205.

0002-9440/80/0508-0369$01.00

tients were subdivided and compared on the basis of conditioning protocol and various complications. Significant morphologic changes were observed and characterized in specimens from those conditioned with very high doses of busulfan or carmustine, those in whom early or late-onset acute GVHD developed, and those with a systemic CMV infection. Where appropriate, the histopathologic features of the liver were compared at autopsy to those of patients who had had similar drug therapy or infections and who had not been candidates for bone marrow transplantation.

Materials and Methods

From 1968 through December 1978 autopsies were performed on 63 patients who had died following bone marrow transplantation performed at The Johns Hopkins Bone Marrow Transplant Unit as therapy for severe aplastic anemia and various malignancies. Of those 63 patients, 62 received allogeneic grafts (58 from genotypically HLA-identical siblings, 4 from mismatched donors), while 1 received a syngeneic graft from a monozygotic twin. Of the 62 patients given allogeneic grafts, 44 had been given transplants for acute leukemia, 11 for aplastic anemia, 4 for chronic myelogenous leukemia in blast crisis, 2 for widespread lymphoma, and 1 for small-cell anaplastic carcinoma of the lung. Several conditioning regimens were used (Table 1). Eighteen patients received 200 mg/kg Cy given in 4 daily doses at 50 mg/kg (Regimen I, Cy), 16 patients received 200 mg/kg followed by 1000 rads or 800 rads TBI given in one single exposure or 2 fractions of 400 rads each (Regimen II, Cy + TBI), 11 patients received 8–20 mg/kg busulfan given over 4–8 days followed by 200 mg/kg Cy given in 4 daily doses of 50 mg/kg (Regimen III, Bu + Cy). Two patients received, in addition to Bu + Cy, 3 doses of 12.5 mg/kg procarbazine (P), alternated with 3 doses of 12.5 mg/kg Upjohn horse-antihuman-thymocyte globulin (A) (Regimen IV, PA Bu + Cy) and 4 patients the same PA regimen followed by 200 mg/kg Cy given in 4 daily doses of 50 mg/kg (Regimen V, PA Cy). One patient was given PA and 800 rads TBI, (Regimen VI, PA TBI). Three patients were given 3 daily doses of 150 mg/sq m carmustine (BCNU) followed by 120 mg/kg Cy and 1000 rads TBI (Regimen VII, BCNU-Cy-TBI), and 2 patients received 1 mg/kg Daunomycin and 15 mg/kg cytosine arabinoside daily for 3 days followed by 200 mg/kg Cy given in 4 daily doses of 50 mg/kg each (Regimen VIII, Ara-C-Dauno-Cy).

In most instances the autopsy was performed less than 6 hours postmortem. Routine cultures taken at this time included viral cultures of liver, lung, kidney, spleen and jejunum, fungal cultures of lung, spleen, and kidney, and bacterial cultures of lung and spleen. The tissues for viral cultures were plated on multiple cell cultures within 60 minutes. Between 5 and 15 sections of liver from each case were placed in formalin shortly after the opening incision. These sections were stained with hematoxylin and eosin, Masson trichrome, periodic acid–Schiff with diastase digestion, and bile stain with Fouchet's reagent.

The histologic changes in the hepatic lobule were systematically reviewed with respect to the following parameters: preservation of sinusoidal architecture; relative amount, location, and profile of inflammation and hepatocellular necrosis; fat accumulation; bile stasis; appearance and relative number of Kupffer cells; degree of congestion; central venous sclerosis; and sinusoidal fibrosis. The histologic changes in the portal and periportal regions were reviewed with regard to these parameters: degree of piecemeal necrosis and periportal fibrosis; extent and character of triad inflammation; vascular changes; bile duct injury; and bile duct epithelial atypia. Semiquantitative analysis included the determination of foci of hepatocellular necrosis per 20 high-power fields, excluding areas of centrilobular necrosis; the percentage of these foci with associated lymphoid mononuclear cell

Table 1—Conditioning Regimens of Bone Marrow Transplant Recipients on Whom Autopsies Were Performed

		Acute leukemia	Aplastic anemia	Chronic leukemia	Lymphoma	Small cell carcinoma	Total
I	Cy	11*	5	0	1	1	18
II	Cy-TBI	16	0	0	0	0	16
III	Bu-Cy	11†	0	1‡	0	0	12
IV	PA Bu-Cy	2	0	0	0	0	2
V	PA Cy	2	5	2	0	0	9
VI	PA TBI	0	1	0	0	0	1
VII	BCNU-Cy-TBI	2	0	0	1	0	3
VIII	Ara C-Dauno-Cy	0	0	2	0	0	2
TOTAL		44	11	5	2	1	63

* Including 3 patients with a mismatched donor.
† Including 1 patient with a mismatched donor.
‡ Transplanted with marrow from a monozygotic twin.

infiltrate; the number of bile ducts per 10 small triads; the number of triads per 10 small triads containing injured bile ducts; and the number of ducts with associated lymphoid mononuclear cells.

For appropriate comparisons, the hepatic histopathologic characteristics of patients who had had CMV infection of the liver or treatment with busulfan but who were not treated by the bone marrow transplant unit were reviewed. Seven such patients upon whom autopsies were performed between 1962 and 1978 had culture or histologic evidence of CMV in the liver. Included were 2 patients with acute leukemia, 1 with thrombotic thrombocytopenic purpura, 1 with mixed cryoglobulinema, 1 with Hemophilus influenza meningitis, 1 with chronic renal failure and a cadaveric renal transplant, and 1 patient with a postperfusion syndrome after repair of an abdominal aortic aneurysm. The hepatic histologic features of 11 patients each with chronic myelogenous leukemia treated with busulfan (2 to 6 mg per day) for over 2 years on whom autopsies were performed between 1963 and 1978 were reviewed. In addition, 1 patient with CML in blast crisis who had not been given a transplant received 400 mg busulfan 59 days before death.

Results

Hepatic Toxicity Associated With Conditioning Regimens

Fourteen of the patients on whom autopsies were performed were conditioned with protocols using busulfan (Regimens III and IV, Table 1). In these patients there was a very high frequency of centrilobular sinusoidal fibrosis (Table 2), including all 5 patients prepared with 20 mg/kg busulfan and 2 of 4 patients prepared with 16 mg/kg. This centrilobular sinusoidal fibrosis (Figure 1A) was generally accompanied by cellular necrosis and atrophy. While occasionally there was prominent fibrosis of the central veins, usually the central vein fibrosis was overshadowed by the dense sinusoidal scarring. Centrilobular bile stasis was prominent in 5 patients, and periportal bile stasis was prominent in 1 patient, who also had bile duct injury with GVHD.

Busulfan is insoluble in water and must be administered orally. Recrystallized busulfan suspended in saline or serum consists of irregularly shaped birefringent crystals. In 5 patients with busulfan-associated sinusoidal fibrosis, similar crystals were evident in the centrilobular sinusoids, present in Kupffer cells, and occasionally present in hepatocytes undergoing ballooning degeneration (Figure 1B). Birefringent material was evident in frozen unstained sections of 2 of these patients and could be dissolved easily with absolute alcohol.

Six patients with acute leukemia were conditioned with carmustine, used in conjunction with total body irradiation (1000 rads) and cyclophosphamide (120 mg/kg). One died prior to transplant; the others died 31 to 56 days after the transplant, 3 with acute hepatic failure. Of the autopsy material available (3 cases), the two with acute hepatic failure had submassive hepatocellular necrosis involving the centrilobular and midzone regions. All 3 patients had significant central vein sclerosis with endothelial cell proliferation, subintimal edema, and active fibrosis.

Table 2—Frequency of Sinusoidal Fibrosis in Busulfan-Conditioned Patients

Protocol	Frequency of sinusoidal fibrosis
No busulfan (patients with AML)	3/14 (21%)
Busulfan, 8 or 12 mg/kg	1/5 (20%)
Busulfan, 16 or 20 mg/kg	7/9 (78%)*†

* $P < 0.05$, chi square test, compared with 8–12 mg/kg for the busulfan group; $P < 0.01$, compared with the group that received no busulfan.

† Includes 2 of 4 patients pretreated with 16 mg/kg busulfan and 5 of 5 patients pretreated with 20 mg/kg busulfan.

After excluding patients with known causes of hepatic injury (GVHD, CMV, hepatitis B antigenemia), there were no distinctive hepatic changes associated with conditioning protocols using high doses of cyclophosphamide alone (200 mg/kg), cyclophosphamide with total body irradiation, with procarbazine and antithymocyte globulin or total body irradiation, procarbazine, and antithymocyte globulin (Regimens I, II, V, and VI). In these patients there was only acute centrilobular congestion or necrosis, which was consistent with their terminal course of septic, hypovolemic or cardiac shock, or respiratory failure.

Following allogeneic transplant for aplastic anemic, low doses of methotrexate were administered prophylactically for GVHD. While there was frequently a mild increase in associated periportal fibrosis extending into the sinusoids, in no case was there evidence of cirrhosis.

Acute Graft-Versus-Host Disease

To characterize acute GVHD histologically, we performed autopsies on a subgroup of patients defined as those with *a*) a skin rash clinically and histologically characteristic of acute GVHD, Grade 2 or greater,[19] and *b*) significant elevation of total bilirubin (> 2.5 mg%) and SGOT (> 100 IU) within 10 days of the onset of cutaneous GVHD. Of the 20 patients fulfilling these criteria, 8 had either culture, serologic, or histologic evidence of disseminated herpesvirus infection including CMV or hepatitis B antigenemia and were excluded from consideration for the purpose of characterizing the pathologic features of GVHD.

Five patients were clinically distinct in that they had a very early and fulminant onset of GVHD (4–13 days, median 6 days after transplant) and survived only 8–32 days (median 15 days after transplant). This group included 4 patients whose donor was HLA-mismatched and 1 patient whose donor was genotypically HLA-identical but sex-mismatched (female donor, male recipient).

In contrast, the onset of GVHD in the other 7 patients occurred at 16–56 days (median 28) after transplant, and they survived 32–65 days (median 49). Each of these recipients received a transplant from an HLA-

Table 3—Relative Hepatocellular and Bile Duct Injury With Graft-Versus-Host Disease

	Hepatocellular necrosis Mean foci/20 HPF (range)	Bile duct injury Triads with injured bile duct/10 triads (range)
Early onset (0–14 days, n = 5)	18.0 (9–24) †	4.0 (2–6) ‡
Late onset (16–56 days, n = 7)	4.7 (1–6) †	9.1 (7–10) †
Other patients (n = 28)*	1.3 (0–5)	0.3 (0–3)

* Excluding patients with submassive necrosis, disseminated CMV (see Table 4).
† $P < 0.001$, Mann-Whitney test.
‡ $P < 0.01$, Mann-Whitney test.

identical sibling donor with bidirectionally negative mixed lymphocyte reactions. For 6 of the patients, the donor was sex-matched. The seventh recipient was a male recipient whose transplant was from a female donor.

The types of liver injury associated with these two clinical patterns are contrasted in Figure 2 and Table 3. Whereas the patients with early-onset GVHD had predominant hepatocellular necrosis, those with late-onset acute GVHD had predominant bile duct injury.

The hepatocellular necrosis was characterized by periportal acidophilic cells and hepatocellular dropout as well as small foci of acidophilic cells in the midzone region. With the exception of 1 patient, the necrotic foci were associated with a mild to moderate infiltrate of lymphoid mononuclear cells. One patient was treated with horse antithymocyte globulin just prior to death and had a notable absence of inflammation.

The bile duct injury, particularly in the recipients with late-onset acute GVHD, was evinced by epithelial dysplasia with nuclear and cellular polymorphism, hyperchromasia, angulation of the nuclear membrane, and frequent pyknotic nuclei. Cellular debris was often evident in the ductal lumen. Although periductal lymphocytes were observed in every patient, they were noticeably sparse. The portal triads contained a mild to moderate mononuclear cell infiltrate. Bile plugs were regularly observed, distending the periportal canaliculi.

In most patients with late-onset acute GVHD there was endothelial prominence, but with only a mild to moderate degree of sclerosis of the central veins. The sclerosis was most evident in veins smaller than 75 μ in diameter. Of the 3 patients with greater than 50% occlusion of the small veins, one had had a pretransplant biopsy with evidence of central vein sclerosis, while a second patient had been conditioned with a high dose of busulfan and had had sinusoidal fibrosis similar to the busulfan-associated changes described above. Two patients with GVHD had had portal fibrosis with triad–triad and triad–central vein bridging. The increased portal

Table 4—Relative Hepatocellular and Bile Duct Injury With CMV and GVHD

	Hepatocellular necrosis Foci/20 HPF (range)	Bile duct injury Triads with injured bile ducts/10 triads (range)	
GVHD alone (Late onset acute, n = 7)	4.7 (1–6)	9.1 (7–10)	*
CMV alone (n = 7)	7.3 (0–16)	5.3 (2–8)	*
GVHD and CMV (n = 8)	6.6 (0–11)	8.2 (7–10)	†
CMV, no bone marrow transplant (n = 7)	6.7 (1–25)	3.4 (1–7)	†

* $P < 0.02$, Mann-Whitney test.
† $P < 0.0002$, Mann-Whitney test.

fibrosis was not evident in a pretransplant biopsy from one of these two recipients.

Disseminated Cytomegalovirus Infection

Fifteen of the autopsy specimens from recipients of allogeneic transplants had culture or histologic evidence of active disseminated CMV infection. Eleven had had multiple postmortem viral cultures taken, and CMV had been cultured from 9 of these patients. There were positive cultures for lung (5 patients), liver (4 patients), spleen, bone marrow, or blood (4 patients), and kidney (1 patient). Diagnostic viral cytopathic changes were evident at autopsy in each case, consisting of cytomegaly with large eosinophilic to amphophilic intranuclear inclusions with peri-inclusion chromatin clearing and margination of the nuclear chromatin. Often but not invariably, eosinophilic cytoplasmic inclusions were also observed. Organs containing these cells included lung (12 patients), liver (6 patients, including 3 with positive liver cultures and 2 in which the liver was not cultured), spleen (5 patients), gastrointestinal tract (2 patients), and pancreas (1 patient). Diagnostic cytopathic cells were rare in the liver in each case, requiring multiple sections for their detection. Cytomegaly was observed in the bile ducts in 5 patients (Figure 3), and within the lobule in only 1 patient, even though hepatocellular injury was present in most patients.

Because the time of onset of clinically apparent CMV infection, 30–100 days after transplant,[16,17] is also a time of high risk for late-onset acute GVHD, the relative contribution of CMV to the liver injury associated with hepatic GVHD was determined by comparing 4 groups of patients (Table 4).

Hepatocellular and bile duct injury was evident both in patients with only CMV infections as well as recipients with GVHD. A total of 7 pa-

tients with late-onset GVHD and no detectable CMV had marked bile duct injury. Thus, a detectable CMV infection does not appear necessary for bile duct injury associated with GVHD.

Qualitative differences were also noted in the type of hepatic injury associated with GVHD as compared with CMV. The bile duct injury evident with GVHD or with GVHD and CMV had much more epithelial atypia as compared with those with CMV alone. Within the lobule, small foci of acidophilic cells and mononuclear cell infiltrate were evident both with GVHD and with CMV infection. Piecemeal necrosis was more commonly observed with GVHD (5 of 7 patients, compared with 1 of 7 with CMV alone), whereas large areas of patchy hepatocellular necrosis with minimal inflammation were observed only in patients with CMV infection.

Other Hepatic Complications Following Bone Marrow Transplantation

Additional complications following bone marrow transplantation that affected the liver included recurrent leukemia (3 patients), systemic Candidiasis (1 patient), and hepatitis B antigenemia (3 patients). None of the latter 3 patients had massive or submassive hepatic necrosis. These patients were excluded from the above studies for the purpose of characterizing the respective histopathologic characteristics.

Discussion

The systemic complications with hepatic injury that follow bone marrow transplantation can be initially considered to be of three general causes: the hepatic injury by some cytoreductive agents used for eliminating tumor cells and preventing graft rejection; the consequences of graft-versus-host disease; and opportunistic infections, especially herpesviruses such as CMV, associated with the transient immune deficiency. Clearly, many patients have complications from more than one cause, with additive and perhaps synergistic consequences. Through comparisons of clinically defined subgroups we have attempted to determine the extent and type of liver injury associated with the hepatic insult of the above causes. While the morphologic features associated with some complications appear characteristic, the descriptions have not so much importance in diagnosis as in the suggestion of pathogenetic mechanisms of injury.

Busulfan is an excellent antimyelocytic alkylating agent with a good antitumor affect [20,21] and rapid post-transplant engraftment.[21] The major nonmarrow toxicity described by others has been interstitial pulmonary fibrosis [22–24] and liver chemistry abnormalities [25,26] in patients with a cumulative dose of 1–4 g.

Recipients prepared with very high doses of busulfan (16 or 20 mg/kg)

in addition to cyclophosphamide showed at autopsy a high frequency of moderate to marked sinusoidal fibrosis in the centrilobular region with associated hepatocellular atrophy and necrosis and often sclerosis of the central veins. This pattern resembles that observed after cytosine arabinoside, 6 thioquanine, *Crotolaria fulva* (Jamaican bush tea), and urethane.[27-31] It appears to be different from that described with high cumulative doses of abdominal irradiation [32-34] and that of transplant recipients treated for acute leukemia who later develop GVHD.[35] In this second pattern, the primary injury appears to be to the central veins, with subendothelial edema, intimal proliferation, and sclerosis with occlusion of the small central veins.

The pattern of sinusoidal fibrosis appears unrelated to GVHD, because it was found in those who received no transplants and who were (4 of 11) treated with low doses of busulfan for an extended period and in 1 patient who did not receive a transplant and who received a high dose of busulfan (a total of 400 mg).

Because of its insolubility in aqueous medium, busulfan is an orally administered drug. Rodent studies have indicated that orally administered busulfan was absorbed into the portal venous system with eventual deposition in the centrilobular sinusoids. In the human marrow recipients prepared with a high dose of busulfan and in whom sinusoidal fibrosis developed, birefringent crystals with solubility and morphologic characteristics similar to those of purified busulfan were observed in the centrilobular sinusoids, within both Kupffer cells and degenerating hepatocytes. The chemical nature of this birefringent material is presently unknown. While it is possible that entrapment of insoluble busulfan or a metabolite may be contributory to the development of sinusoidal fibrosis, it is probably not necessary. Histologically similar sinusoidal fibrosis has been observed by the Seattle marrow transplant group in patients with leukemia prepared with several different regimens that contain a high dose of cytosine arabinoside, carmustine, or dimethylbusulfan in combination with cyclophosphamide (60 mg/kg ×2) plus 1000 rads total body irradiation or following dimethylbusulfan (10 mg/kg) given as a single agent for second transplants following leukemia relapse.[36] Dimethylbusulfan is a soluble analog of busulfan that is administered systemically.

Another conditioning regimen with significant associated hepatic toxicity was that utilizing carmustine. Three of the six patients so conditioned died with acute liver failure not associated with known virus infection. Massive centrilobular necrosis and central venous subintimal edema were present at autopsy. An early clinical trial with lower doses of carmustine noted reversible hepatic and renal toxicity,[39] while in another study 1 of 2 patients had marked centrilobular necrosis at autopsy.[40] Because of

our experience with a high frequency of acute hepatic failure, this protocol has been abandoned.

Methotrexate [37,38] has been associated in the past with increased portal fibrosis, often progressing to cirrhosis. In the recipients who received methotrexate for a relatively short period for the prevention of GVHD, only a slight to moderate increase in the portal fibrosis was evident. In no patient, however, was there evidence of cirrhosis.

The liver is one of the major target organs involved in acute GVHD, along with the skin, intestines, and bronchi.[5–12,41,43] There is, however, variability in the described pathologic characteristics and debate concerning the pathogenesis of liver injury. Some investigators have emphasized that bile duct injury is the common denominator with a variable degree of hepatocellular necrosis,[8,11,42] while others have found that periportal and lobular hepatocellular necrosis represents the hallmark of hepatic GVHD.[7,10] We have observed a pattern of predominant periportal and midzone hepatocellular necrosis in those with acute GVHD with a very early onset (most of whom received grafts from mismatched donors), as compared with a pattern of bile duct injury seen in those with a late-onset acute GVHD (all of whom received transplants from HLA-identical, MLC-negative siblings).

There are several hypothetical pathogenetic mechanisms consistent with these two patterns of acute GVHD. It is unlikely that they represent consecutive stages of the disease or differences in severity, because 2 of the patients with early-onset GVHD had a duration of disease exceeding the median duration of the patients with late-onset GVHD.

First, the two patterns may reflect differences in the cellular distribution, density, or immunogenicity of major HLA antigens and some minor antigens, as compared with most minor HLA antigens. Thus, early-onset GVHD may represent a reaction against antigens expressed on the hepatocytes or Kupffer cells, whereas late-onset GVHD would forgo this reaction and show a later reaction against minor antigens on the bile duct epithelium.

Second, the early-onset pattern could represent an early bystander injury [44,45] of the hepatocytes following lymphocyte-associated injury of residual host sinusoidal leukocytes or Kupffer cells.[46]

Third, a later developing humoral response [47,48] may alter late-onset GVHD by modifying the response to either the bile duct epithelial cells or hepatocytes.

Finally, it is possible that an as yet undetected virus infection triggered a generalized GVHD against minor previously nonimmunogenic antigens shared by the epidermis and bile duct epithelium.

The most frequent known virus infection associated with fatal complications of bone marrow transplantation is cytomegalovirus.[16,17,49] While associated pneumonia is the most frequent fatal manifestation, associated hepatitis and jaundice can also be prominent manifestations. The pathologic characteristics of the liver with disseminated CMV infection and no cutaneous GVHD were similar to those of the liver in patients who received no transplants and those of patients with CMV hepatitis,[50,51] with multiple foci of midzone hepatocellular necrosis, often with mononuclear or mixed inflammatory infiltrate but occasionally with only minimal inflammatory infiltrate. Although bile duct injury was mild, with mild periductal mononuclear cell infiltrate, occasional injured epithelial cells, and intraluminal debris, the cytomegaly in the liver was most frequently identified within the bile duct epithelium.

Because of the cytomegaly observed in the bile ducts and the similar time of onset of GVHD and CMV infection,[17,49] the suggestion has been made that bile duct injury associated with GVHD may be secondary to a CMV superinfection of GVHD.[52] We have demonstrated that whereas CMV infection by itself is associated with both hepatocellular injury and some bile duct injury, more extensive bile duct injury was observed in patients with late-onset GVHD and no detectable CMV infection.

In allogeneic recipients of bone marrow transplants, the liver is susceptible to injury by the chemotherapy used for conditioning the patients, particularly high doses of busulfan and carmustine, by graft-versus-host disease, and by herpesvirus infections, particularly cytomegalovirus infections. While the histologic patterns are not necessarily unique for a particular cause, they are sufficiently characteristic to suggest the cause. More important, they also help us explain the clinical manifestations and understand the pathologic mechanisms of injury.

References

1. Santos GW: Bone marrow transplantation. Adv Intern Med 1979, 24:157–182
2. Thomas ED, Storb R, Clift RA, Fefer A, Johnson FL, Neiman PE, Lerner KG, Glucksberg H, Buckner CD: Bone-marrow transplantation. N Engl J Med 1975, 292:832–843
3. Good RA, Bach FH: Bone marrow and thymus transplants: Cellular engineering to correct primary immunodeficiency, Clinical Immunobiology. Vol 2. Edited by FN Bach, RA Good. New York, Academic Press, 1974, pp 63–114
4. Sanders JE, Seattle Marrow Transplant Group: Marrow transplantation for children with acute leukemia. Exp Hematol 1978, 7 (Suppl 6):86
5. Van Bekkum DW, De Vries MJ: Radiation Chimeras. New York, Academic Press, 1967, pp 125–165
6. Grebe SC, Streilein JW: Graft-versus-host reactions: A review. Adv Immunol 1976, 22:119–221

7. Woodruff JM, Eltringham JR, Casey HW: Early secondary disease in the Rhesus monkey: I. A comparative histopathologic study. Lab Invest 1969, 20:499–511
8. Krüger GRF, Berard CW, DeLellis RA, Graw RG Jr, Yankee RA, Leventhal BG, Rogentine GN, Herzig GP, Halterman RH, Henderson ES: Graft-versus-host disease: Morphologic variations and differential diagnosis in 8 cases of HL-A matched bone marrow transplantation. Am J Pathol 1971, 63:179–202
9. Kersey JH, Meuwissen HJ, Good RA: Graft versus host reactions following transplantation of allogeneic hematopoietic cells. Human Pathol 1971, 2:389–402
10. Slavin RE, Santos GW: The graft versus host reaction in man after bone marrow transplantation: Pathology, pathogenesis, clinical features, and implication. Clin Immunol Immunopathol 1973, 1:472–498
11. Lerner KG, Kao GF, Storb R, Buckner CD, Clift RA, Thomas ED: Histopathology of graft-versus-host reaction (GVHR) in human recipients of marrow from HL-A matched sibling donors. Transplant Proc 1974, 6:367–371
12. Beschorner WE, Saral R, Hutchins GM, Tutschka PJ, Santos GW: Lymphocytic bronchitis associated with graft-versus-host disease in recipients of bone-marrow transplants. N Engl J Med 1978, 299:1030–1036
13. Halterman RH, Graw RG Jr, Fuccillo DA, Leventhal BG: Immunocompetence following allogeneic bone marrow transplantation in man. Transplantation 1972, 14:689–697
14. Storb R, Ochs HD, Weider PL, Thomas ED: Immunologic reactivity in marrow graft recipients. Transplant Proc 1976, 8:637–639
15. Noel DR, Witherspoon RP, Storb R, Atkinson K, Doney K, Mickelson EM, Ochs HD, Warren RP, Weiden PL, Thomas ED: Does graft-versus-host disease influence tempo of immunologic recovery after allogeneic human marrow transplantation? An observation on 56 long-term survivors. Blood 1978, 51:1087–1105
16. Meyers JD, Spencer HC Jr, Watts JC, Gregg MB, Stewart JA, Troupin RH, Thomas ED: Cytomegalovirus pneumonia after human marrow transplantation. Ann Intern Med 1975, 82:181–188
17. Beschorner WE, Burns WH, Hutchins GM, Saral R, Santos GW: Miliary and diffuse forms of cytomegalovirus pneumonia in patients with allogeneic bone marrow transplantation (abstr). Lab Invest 1979, 40:240–241
18. Slavin RE, Woodruff JM: The pathology of bone marrow transplantation. Pathol Ann 1974, 9:291–344
19. Sale GE, Lerner KG, Barker EA, Shulman HM, Thomas ED: The skin biopsy in the diagnosis of acute graft-versus-host disease in man. Am J Pathol 1977, 89:621–636
20. Dunn CDR: The chemical and biological properties of busulfan ('Myleran'). Exp Hematol 1974, 2:101–117
21. Tutschka PJ, Santos GW, Elfenbein G: Marrow transplantation in acute leukemia following conditioning with busulfan and cyclophosphamide. Blut (In press)
22. Koss LG, Melamed MR, Mayer K: The effect of busulfan in human epithelia. Am J Clin Pathol 1965, 44:385–397
23. Smalley RV, Wall RL: Two cases of busulfan toxicity. Ann Intern Med 1966, 64:154–164
24. Pearl M: Busulfan lung. Am J Dis Child 1977, 131:650–652
25. Underwood JCE, Shahani RT, Blackburn EK: Jaundice after treatment of leukemia with busulfan. Br Med J 1971, 1:556–557
26. Hast R: Increase in serum alkaline phosphatase (S-ALP) in chronic myelocytic leukemia: Sign of drug-induced cholestasis? Acta Med Scand 1978, 203:93–94
27. Griner PF, Elbadawi A, Packman CH: Veno-occlusive disease of the liver after chemotherapy of acute leukemia. Ann Intern Med 1976, 85:578–582

28. Bras G, Jelliffe DB, Stuart KL: Veno-occlusive disease of liver with nonportal type of cirrhosis, occurring in Jamaica. Arch Pathol 1954, 57:285–300
29. Stuart KL, Bras G: Veno-occlusive disease of the liver. Q J Med 26:291–316
30. Putzke H-P, Persaud TVN, Bienengräber A: Fine structure of the liver in experimental veno-occlusive disease. Exp Pathol 1972, 6:325–332
31. Brodsky I, Johnson H, Killman S-A, Cronkite EP: Fibrosis of central and hepatic veins, and perisinusoidal spaces of the liver following prolonged administration of urethane. Am J Med 1961, 30:976–980
32. Scott RB, Budinger JM, Prendergast RAM, Nydick I: Hepatic veno-occlusive syndrome in an American adult. Gastroenterol 1962, 42:631–636
33. Reed GB Jr, Cox AJ Jr: The human liver after radiation injury: A form of veno-occlusive disease. Am J Pathol 1966, 48:597–611
34. Kim TH, Panahon AM, Friedman M, Webster JH: Acute transient radiation hepatitis following whole abdominal irradiation. Clin Radiol 1976, 27:449–454
35. Berk PD, Popper H, Krueger GRF, Decter J, Herzig G, Graw RG Jr: Venoocclusive disease of the liver after allogeneic bone marrow transplantation. Ann Intern Med 1979, 90:158–164
36. Shulman HM: Personal communication.
37. Nyfors A, Poulsen H: Morphogenesis of fibrosis and cirrhosis in methotrexate-treated patients with psoriasis. Am J Surg Pathol 1977, 1:235–243
38. Nesbit M, Krivit W, Heyn R, Sharp H: Acute and chronic effects of methotrexate on hepatic, pulmonary, and skeletal systems. Cancer, 1976, 37:1048–1054
39. DeVita VT, Carbone PP, Owens AH Jr, Gold GL, Krant MJ, Edmonson J: Clinical trials with 1,3-bis(2-chlorethyl)-1-nitrosourea, NSC-409962. Cancer Res 1965, 25:1876–1881
40. Lokich JJ, Drum DE, Kaplan W: Hepatic toxicity of nitrosourea analogues. Clin Pharmacol Ther 1974, 16:363–367
41. Uyeki EM, Palotay JL: Some studies on liver infiltrates in radiation chimeras. Br J Exp Pathol 1965, 46:577–582
42. Sale GE, Storb R, Kolb H: Histopathology of hepatic acute graft-versus-host disease in the dog: A double blind study confirms the specificity of small bile duct lesions. Transplantation 1978, 26:103–106
43. Shulman HM, Sale GE, McDonald GB: Histopathology of the liver in human acute and chronic graft-versus-host disease (abstr). J Exp Hematol 1978, 6:43
44. Elkins WL, Guttmann RD: Pathogenesis of a local graft versus host reaction: Immunogenicity of circulating host leukocytes. Science 1968, 159:1250–1251
45. Elson CO, Reilly RW, Rosenberg IH: Small intestinal injury in the graft versus host reaction: An innocent bystander phenomenon. Gastroenterol 1977, 72:886–889
46. Gale RP, Sparkes RS, Golde DW: Bone marrow origin of hepatic macrophages (Kupffer cells) in humans. Science 1978, 201:937–938
47. Merritt CB, Mann DL, Rogentine GN Jr: Cytotoxic antibody for epithelial cells in human graft versus host disease. Nature 1971, 232:638–639
48. Tsoi M-S, Storb R, Jones E, Weiden PL, Shulman H, Witherspoon R, Atkinson K, Thomas ED: Deposition of IgM and complement at the dermo-epidermal junction in acute and chronic cutaneous graft-vs-host disease in man. J Immunol 1978, 120:1485–1492
49. Neiman PE, Reeves W, Ray G, Flournoy N, Lerner KG, Sale GE, Thomas ED: A prospective analysis of interstitial pneumonia and opportunistic viral infection among recipients of allogeneic bone marrow grafts. J Infect Dis 1977, 136:754–767
50. Henson DE, Grimley PM, Strano AJ: Postnatal cytomegalovirus hepatitis: An autopsy and liver biopsy study. Human Pathol 1974, 5:93–103

51. Rosen PP: Cytomegalovirus infection in cancer patients. Pathology Annals. Edited by SC Sommers and PP Rosen. New York, Appleton-Century-Crofts, 1978, pp 175–208
52. Woodruff JM, Hansen JA, Good RA, Santos GW, Slavin RE: The pathology of the graft-versus-host reaction (GVHR) in adults receiving bone marrow transplants. Transplant Proc 1976, 8:675–684
53. Kanich RE, Craighead JE: Cytomegalovirus infection and cytomegalic inclusion disease in renal homotransplant recipients. Am J Med 1966, 40:874–882

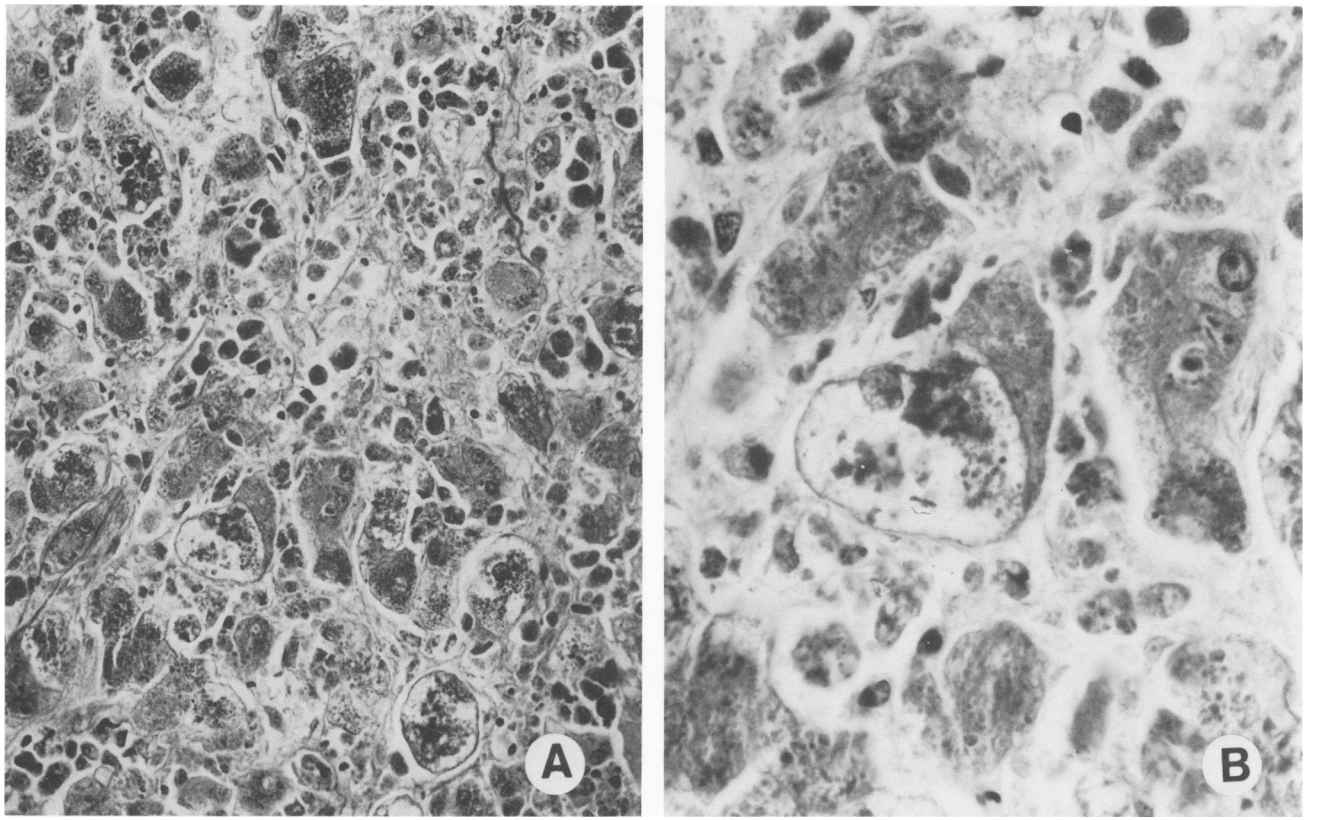

Figure 1A—Centrilobular region of a recipient conditioned with a high dose of busulfan (20 mg/kg) and cyclophosphamide. There was extensive fibrosis extending from the central vein into the sinusoids, with associated hepatocellular necrosis. **B**—Degenerating hepatocytes and sinusoids containing cellular debris. (**A**, H&E, ×250; **B**, H&E, ×625)

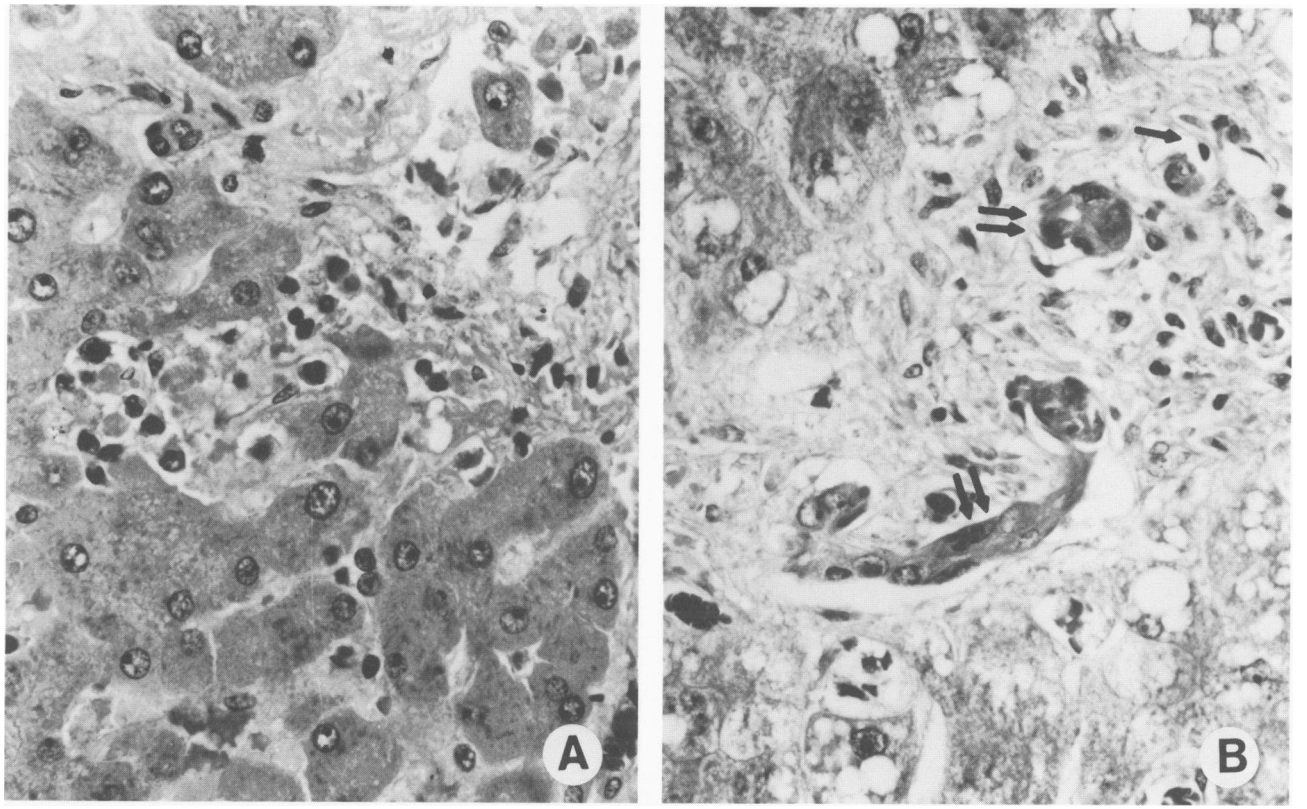

Figure 2A—Periportal region of recipient with early-onset acute GVHD (Day 4). Small foci of hepatocellular necrosis and associated mononuclear cells were present. **B**—Portal triad of recipient with late-onset acute GVHD (Day 21). The degenerating bile duct epithelium (*double arrows*) demonstrated marked bile duct dysplasia but mild periductal inflammation with rare periductal lymphocytes (*arrow*). (H&E, ×425)

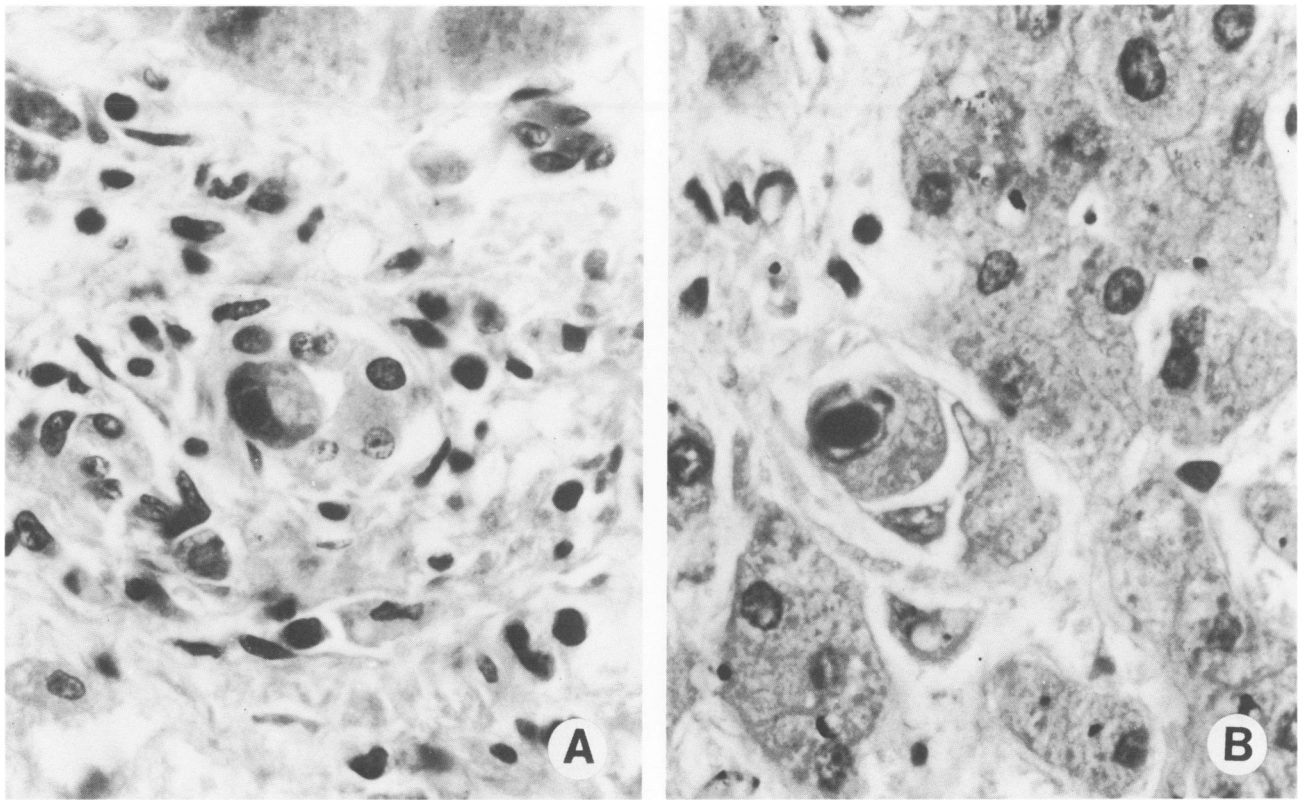

Figure 3A—Portal triad of a recipient with disseminated CMV infection. Within the bile duct was a cell with cytomegaly and a large amphophyllic intranuclear inclusion characteristic of cytomegalovirus. The remaining epithelial cells had only mild dysplasia. **B**—Periportal region of recipient with disseminated CMV infection. Cell resembling hepatocyte with cytomegaly and characteristic intranuclear inclusion. (H&E, ×900)

[*End of Article*]