Acute and Chronic Graft-Versus-Host Disease in Dogs Given Hemopoietic Grafts From DLA-Nonidentical Littermates

Two Distinct Syndromes

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We recognized two distinct clinical and histologic syndromes of acute and chronic graft-versus-host disease (GVHD) in irradiation chimeric dogs given hemopoietic grafts from DLA-nonidentical littermate donors. Clinically acute GVHD developed, with a median onset of 13 days after the transplant, and was characterized by skin erythema, jaundice, diarrhea, and gram-negative infections; the median survival of these dogs was 29.5 days. Chronic GVHD developed a median of 124 days after the transplant and was characterized by generalized skin ulcerations, massive ascites, and gram-positive infections; the median survival of these dogs was 150 days. Chronic GVHD could be distinguished histologically from acute GVHD by epidermal atrophy and dermal fibrosis and by bile duct proliferation, bridging, piecemeal necrosis, and portal fibrosis in the liver. Questions related to GVHD in man can be investigated in this model of acute and chronic GVHD in a large outbred species. (Am J Pathol 1982, 108:196–205)

DOGS given hemopoietic grafts from DLA-nonidentical littermate donors usually die within 100 days of transplantation from acute graft-versus-host disease (GVHD).[1,2] A small fraction of these chimeric dogs will survive beyond 100 days, but most eventually die with delayed or chronic GVHD. The clinical and pathologic features of chronic GVHD in these dogs and the distinction of these findings from those in dogs dying earlier with acute GVHD are the subject of the present report.

Methods

The donor-recipient pairs were littermates from random-bred litters of various breeds. The dogs were 7–14 months old and weighed 4.3–29.0 kg. They had been observed for disease for at least 2 months and were dewormed and vaccinated against measles, distemper, hepatitis, and leptospirosis. Recipients were chosen on the basis of DLA nonidentity with their respective littermate donor as determined by serotyping[2,3] and mixed leukocyte culture (MLC).[4] Whenever possible, the recipient was of the opposite sex.

Recipients were conditioned for transplantation by 8.5–9.2 Gy (midline tissue dose) total body irradiation (TBI) from two opposing ^{60}Co sources at a rate of 0.07 Gy/minute,[5] followed by hemopoietic grafts consisting of donor marrow and peripheral blood leukocytes.[6] The day of transplantation was designated Day 0. The mean number of bone marrow cells

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was 6.7 ± 6.8 (range, 0.3–23.9) and that of blood leukocytes 3.3 ± 8.2 (range, 1.1–28.6) × 10^8/kg. All recipients were given methotrexate (MTX) 0.4 mg/kg intravenously on Days 1, 3, 6, 11, and once a week until Day 102 to ameliorate GVHD.[6] Supportive care included parenteral Ringer's lactate, antibiotics, and transfusions as clinically indicated. Criteria for marrow engraftment[7] included sustained rises in white blood cell (WBC) and platelet counts after the irradiation-induced nadirs, the demonstration of donor sex chromosomes in metaphase spreads of cells from marrow and peripheral blood, and the development of clinical and histologic evidence of GVHD.

Criteria for the clinical diagnosis of acute GVHD in dogs have been described.[7] Acute GVHD characteristically begins with erythema of the ears and injection of the conjunctival vessels. With progressive GVHD the ears become ulcerated and suppurated, and conjunctivas develop purulent exudates. Generalized erythema of the skin may develop. Rhinorrhea is common and may become purulent. Manifestations of gut involvement are anorexia and diarrhea. Acute hepatic GVHD is manifested clinically by jaundice.

The tentative clinical diagnosis of chronic GVHD was made in dogs with generalized skin ulcerations, massive ascites, or both, occurring either 1) 100 or more days after the transplant or 2) before Day 100 but after a period of good health in a survivor of an episode of clinically typical acute GVHD.

The dogs underwent autopsy within 16 hours of natural death. Specimens for histologic examination were obtained from the skin of the ear, trunk, and paw; liver; small and large intestines; lungs; heart; pancreas; kidneys; lymph nodes; spleen; and thymus. The specimens were fixed in either 10% neutral buffered formalin or Millonig's fixative, sectioned, and stained with hematoxylin and eosin and in some instances Gomori or Masson trichrome.

Coded autopsy sections from 8 dogs that met the clinical criteria of chronic GVHD and from 16 dogs with clinical acute GVHD were reviewed and interpreted by one of us (H.S.). Coded sections of liver, skin, and gut were examined separately from those of marrow. So as not to bias the interpretation of GVHD, we reviewed liver sections first, and recorded impressions without knowledge of the findings in the skin or gut. The skin and gut sections were reviewed next, and the salient findings were described. Finally, an overall impression of the severity and duration (acute versus chronic) of the GVHD was made. When the overall impression was inconclusive, the GVHD was labeled "indeterminate type."

The histopathologic criteria of GVHD were based on our experience with both human and canine GVHD. These criteria have been described previously.[8–15] We have also described changes in the dog due to TBI alone or TBI followed by autologous bone marrow transplantation.[3,8,16]

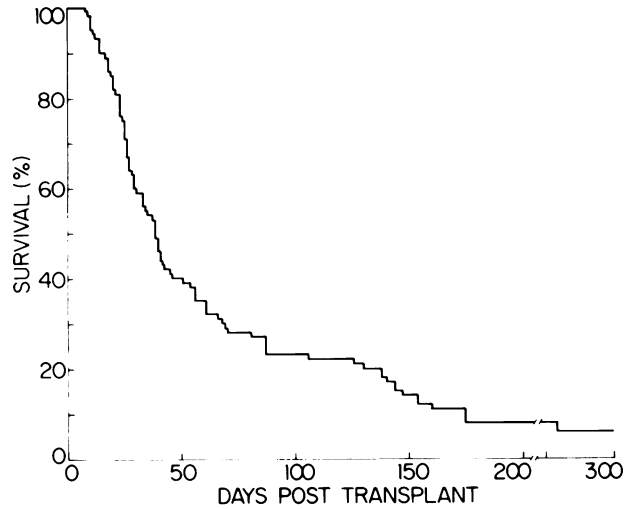


Figure 1 – Survival of 80 recipients with sustained engraftment.

Autologous Controls

Histologic sections of pancreas were examined from 12 dogs given similar conditioning with TBI, followed by autologous hemopoietic grafts during the same time period as the above-described dogs. These sections were compared with those from the DLA-nonidentical allogeneic recipients with GVHD.

Results

Clinical Studies

Engraftment

Among the 95 dogs, 13 died of infection or hemorrhage between Day 6 and Day 16 (median, Day 8), either too early for us to assess engraftment or with marrow aplasia. Two dogs had transient engraftment but then rejected the graft and died on Days 32 and 51, respectively. Autopsy revealed marrow aplasia and no gross or histologic evidence of GVHD. Five dogs (5.3%) became healthy long-term survivors and remain alive >1½, >1¾, >3, >6¾, and >9¾ years after the transplant. Eighty dogs achieved sustained engraftment; their survival is shown in Figure 1.

Table 1—Clinical and Laboratory Characteristics of Dogs That Died of Acute or Chronic GVHD

	Acute GVHD (50 dogs)	Chronic GVHD (10 dogs)
Median day of onset (range)	13 (3-42)	124 (59-150)
Median survival in days (range)	29.5 (9-87)	150 (69-275)
Median weight loss (% decrease from pretransplant weight)	32	23
Clinical organ involvement (number of dogs)		
Skin	47	6
Liver	14	6
Gut	25	2
Extent of clinical involvement (number of dogs)		
Skin, liver, gut	6	1
Skin, liver	7	1
Skin, gut	17	0
Liver, gut	0	1
Skin only	17	4
Liver only	1	3
Gut only	2	0
Clinical signs of skin GVHD (number of dogs)		
Erythema	47	0
Generalized ulceration	12	6
Clinical signs of liver GVHD (number of dogs)		
Jaundice	14	2
Clinically detectable ascites	0	4
Clinical signs of gut GVHD (number of dogs)		
Diarrhea	21	2
Diarrhea and vomiting	3	0
Anorexia	1	0
Liver function tests	*2 dogs*	*4 dogs*
Bilirubin (Normal 0-0.1 mg/dl)	3.2, 14.5	0.1, 0.1, 1.4, 2.1
Alkaline phosphatase (Normal 19-128 units/l)	700, 1650	118, 165, 229, 337
SGOT (Normal 16-52 units/l)	30, 600	20, 54, 63, 103
Bacterial culture of lung tissue and/or heart blood at autopsy		
Gram-negative rods	17/42*	1/10*
Gram-positive bacilli	2/42	0/10
Gram-positive cocci	1/42	5/10
No growth	22/42	4/10
Immediate cause of death (number of dogs)		
Sepsis		
Culture-proven	16	4
Clinically suspected but culture negative	13	
Pneumonia (autopsy-proven)	11	2
Not determined	8	2
Miscellaneous	2	2

* Number of dogs with positive cultures per number of dogs studied.

Acute GVHD

Of the 80 dogs with sustained engraftment, 68 (85%) experienced acute GVHD, and 50 of these died with acute GVHD. Clinical and laboratory details are shown in Table 1. In addition to these 50, transient acute GVHD was seen in 2 of 5 healthy long-term survivors, in 8 of 15 dogs dying of miscellaneous causes between Days 8 and 198, and in 8 dogs in which chronic GVHD later developed. Among the 50 dogs that died of acute GVHD, the median onset and survival was 13 and 29.5 days, respectively. The frequency of organ involvement was as follows: skin, 94%; liver, 28%; and gut, 50%. In 60% of the dogs more than one organ system was involved by GVHD. Clinical manifestations included skin erythema, jaundice, and diarrhea. Liver function tests were markedly abnormal in jaundiced dogs. Gram-negative infections were the most common cause of death. At autopsy these organisms were grown from lung and heart blood.

Chronic GVHD

Ten of the 80 dogs with sustained engraftment developed chronic GVHD. Clinical and laboratory details are summarized in Table 1. Six dogs recovered from an episode of acute GVHD (onset Day 7-14, median, Day 13), and chronic GVHD later developed. In 2 dogs acute GVHD remained active and progressed slowly to a chronic syndrome. In 2 dogs chronic GVHD developed (*de novo*) without a preceding episode of clinically evident acute GVHD. The clinical manifestations of chronic GVHD developed 100 or more days after the transplant in 9 dogs and before Day 100 in 1 dog. The median onset and survival of the dogs with chronic GVHD was 124 and 150 days, respectively. The frequency of organ involvement was as follows: skin, 60%; liver, 60%; and gut, 20%. Clinical features were ulceration or ascites, or both. The majority (70%) of dogs with chronic GVHD had only single-organ involvement. Liver involvement was associated with minimally abnormal liver function tests. Infections with gram-positive cocci were common and usually the immediate cause of death.

Dogs With Engraftment Dying of Causes Other Than GVHD

Fifteen dogs showed evidence of engraftment but died of causes other than GVHD between Days 8 and 198 (median, Day 37) after transplant. Eight had survived an episode of acute GVHD. The cause of death was culture-proven septicemia in 6, clinically suspected septicemia in 2, autopsy-proven pneumonia in 4, pancreatic atrophy and malnutrition in 2, and undetermined in 1.

Table 2—Histologic Features Noted on Blind Examination of Skin Taken at Autopsy From Dogs With Acute or Chronic GVHD*

Skin	9 dogs with clinical involvement of skin by acute GVHD	4 dogs with clinical involvement of skin by chronic GVHD	3 dogs with chronic GVHD *without* clinical skin involvement
Skin atrophy or hyperkeratosis	5/9	4/4	3/3
Marked basilar inflammation	5/9	4/4	0/3
Necrotic keratinocytes (eosinophilic bodies)	6/9 (all marked)	4/4 (3 marked)	0/3
Basal layer irregularity	5/9	3/4	0/3
Glandular inflammation	5/9	3/4	0/3
Inflammation of sweat gland in footpad	1/3	1/2	0/2
Inflammation of pilar unit	1/9	2/3	2/3
Dermal fibrosis	1/9	1/4	2/3

* Number positive per number evaluated.
Boxed figures indicate histologic features that appeared to be more characteristic of chronic than of acute GVHD.

Histologic Features of Acute and Chronic GVHD

Skin

The histologic findings of GVHD were present in the skin, liver, and gut. The remaining organs were either normal or lacked histologic findings that could be separated from the effects of irradiation and infection. Sections of skin taken at autopsy were examined from 9 dogs with acute cutaneous GVHD and from 7 with chronic cutaneous GVHD, 4 of which had gross cutaneous involvement and 3 of which did not. Findings are shown in Tables 2 and 5. Histologic signs of cutaneous GVHD were present in 8 of 9 dogs with cutaneous acute GVHD and in all 7 dogs with chronic GVHD, regardless of clinical findings. There was considerable histologic overlap between the two groups; both had lymphocytic inflammation and necrosis of individual epidermal basilar cells, particularly in sections from the ears (Figure 2). However, chronic GVHD could be distinguished from acute GVHD in some dogs by epidermal atrophy and fibrous remodeling of the dermis and subcutaneous fat in the footpads (Figure 3). Inflammation about the deeper portions of pilar units was also more frequent with chronic GVHD (Table 2).

Liver

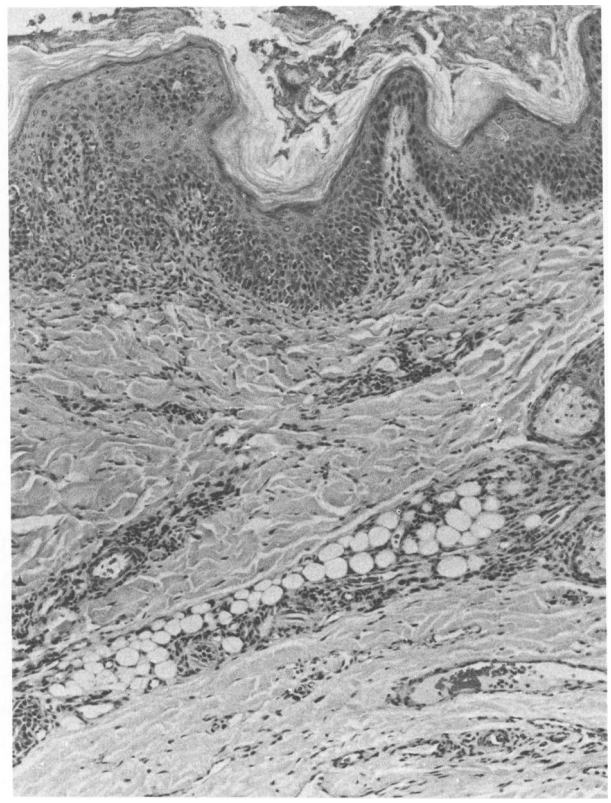
Liver from 12 dogs with acute GVHD was histologically examined, 7 with and 5 without clinical signs of involvement (icterus), and from 8 with chronic GVHD, 5 with and 3 without clinical signs of involvement (ascites). The results are given in Tables 3 and 5. Histologic features of liver GVHD, triaditis, injured bile ducts, and cholestatic hepatitis were present in all 7 icteric dogs and in 4 of 5 nonicteric dogs with acute GVHD. All dogs with chronic GVHD had histologic evidence of GVHD. None of the dogs with ascites had gross or histologic cardiac abnormalities. The histologic appearance of acute and that of chronic hepatic GVHD were not mutually exclusive, although chronic GVHD of the liver was often distinguished by the presence of bile ductule proliferation, piecemeal necrosis, bridging necrosis and portal fibrosis (Figures 4 and 5). The lymphoplasmacytic infiltrates surrounding central veins were present in dogs with acute and chronic GVHD. Two dogs also had veno-occlusive disease of the liver with subintimal fibrous thickening of sublobular central venules.

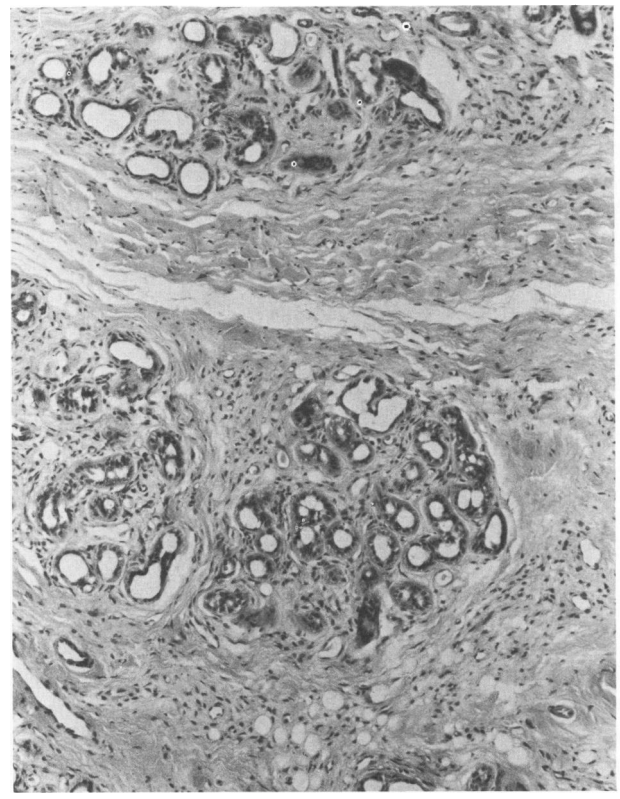
Gut

The gut was examined histologically in 11 dogs with acute GVHD, 8 with and 3 without clinical involvement (diarrhea); and results are given in Tables 4 and 5. These changes consisted of spotty individual and multiple crypt cell degeneration (Figure 6). Of 8 dogs with clinical acute GVHD of the gut, all had histologic changes of GVHD in sections of small bowel, while in 4 of 6 dogs with available sections of colon had GVHD.

The gut was examined histologically in 7 dogs with chronic GVHD, 2 with clinical signs of gut involvement (diarrhea) and 5 without. None of the dogs had histologic changes of GVHD, including the 2 dogs with diarrhea.

Several dogs had histologic lesions in the pancreas. Two dogs, one with acute and the other with chronic GVHD, had large pancreatic ducts with mild lymphocytic exocytosis and individual cell necrosis or degeneration (apoptosis) of ductal epithelium (Figure





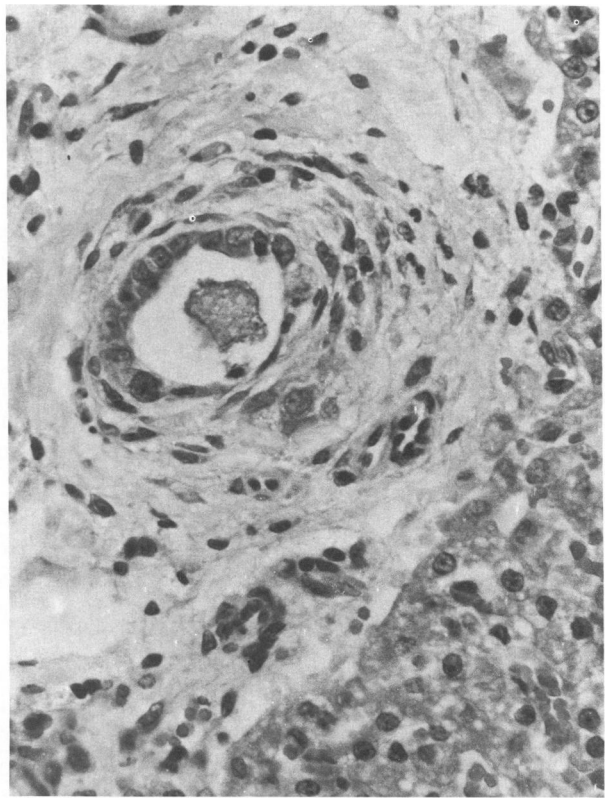


Figure 2—Ear skin of a dog with chronic GVHD showing an extensive lymphocytic lichenoid reaction and periappendigeal inflammation indistinguishable from acute GVHD. (H&E, × 63) **Figure 3**—Atrophic eccrine coils and fibrous replacement of fat in footpad characteristic of dogs with chronic GVHD. (H&E, × 250) **Figure 4**—Interlobular bile duct lined by flattened and focally ulcerated atypical epithelium in a dog with acute GVHD. (H&E, × 425)

Table 3—Histologic Features Noted on Blind Examination of Liver Taken at Autopsy From Dogs With Acute or Chronic GVHD*

Liver	7 dogs with clinical involvement of liver by acute GVHD	5 dogs with clinical acute GVHD but without clinical liver involvement	5 dogs with clinical involvement of liver by chronic GVHD	3 dogs with clinical chronic GVHD but *without* clinical liver involvement
Decreased number of bile ducts	4/7	3/5	4/5	1/3
Proliferation of bile ductules	0/7	1/5	3/5	2/3
Triaditis	6/7 (Plasmacytic 2; Lymphocytic 2; Lymphoplasmacytic 1; Not determined 1)	2/5 (Lymphocytic 2)	5/5 (Plasmacytic 2; Lymphocytic 2; Lymphoplasmacytic 1)	3/3 (Plasmacytic 1; Lymphoplasmacytic 1; Not determined 1)
Piecemeal necrosis	0/7	1/5	2/5	2/3
Bridging/portal fibrosis	0/7	0/5	3/5	2/3
Hepatocyte unrest, ballooning	1/7	0/5	2/5	1/3
Hepatocyte degeneration (acidophilic bodies), lobular disarray	2/7	1/5	1/5	1/3

* Number positive per number evaluated.
Boxed figures indicate histologic features that appeared to be more characteristic of chronic than of acute GVHD.

7) without other changes in the glandular acini or small ducts. Two additional dogs with chronic GVHD and diarrhea had histologically normal gut and pancreatitis. We analyzed the significance of pancreatic duct cell necrosis with lymphocytic inflammation *vis-à-vis* GVHD by reviewing postmortem sections of pancreas from 12 control dogs given similar doses of TBI and autologous grafts. Among sections of pancreas from the controls given autografts, 1 dog had a few degenerative or necrotic epithelial cells in the large pancreatic duct, 1 contained a few lymphocytes in the wall of the duct, 1 had pancreatitis, and the remaining 9 had no remarkable findings.

Other Organs

The lymphoid organs had moderate to marked lymphoid depletion of germinal cords. These changes were no different from those seen in similarly conditioned dogs with autografts that survived comparable periods.

Discussion

We found that canine radiation chimeras transplanted from DLA-nonidentical littermates developed distinct syndromes of acute and chronic GVHD. Acute GVHD was a disease of early onset manifested by cutaneous erythema, jaundice, diarrhea, and short survival from gram-negative infections. In contrast, the chronic syndrome was of a later onset, made manifest chiefly by cutaneous ulceration and ascites, with death from infections with gram-positive cocci. Neither TBI before nor the administration of MTX after autologous marrow transplants produced such

Table 4—Histologic Features Noted on Blind Examination of Gut Taken at Autopsy From Dogs With Acute or Chronic GVHD*

Gut	8 dogs with clinical involvement of gut by acute GVHD	3 dogs with clinical acute GVHD but *without* clinical gut involvement	2 dogs with clinical involvement of gut by chronic GVHD	5 dogs with clinical chronic GVHD but *without* clinical gut involvement
Crypt epithelial cell degeneration/ crypt dilatation & abscess formation				
Small bowel	8/8	0/3	0/2	0/5
Colon	4/6	0/3	0/2	0/5

* Number positive per number evaluated.

Table 5—Correlation of Clinical and Histologic Findings

Type of clinical GVHD	Number of dogs evaluated histologically	Histological findings: Acute GVHD	Chronic GVHD	GVHD type not determined	No Abnormality	Miscellaneous findings
Skin						
Acute GVHD involving skin	9	7	1	0	1	
Acute GVHD without skin involvement	0	–	–	–	–	
Chronic GVHD involving skin	4	1	2	1	0	
Chronic GVHD without skin involvement	3	0	2	1	0	
Liver						
Acute GVHD involving liver	7	2	1	4	0	1 dog with changes suggestive of GVHD also had VOD*
Acute GVHD without liver involvement	5	1	2	1	0	VOD 1
Chronic GVHD involving liver	5	1	3	1	0	
Chronic GVHD without liver involvement	3	1	2	0	0	
Gut						
Acute GVHD involving gut	8	Small bowel 8 Colon 4/6 evaluable	0	0	Small bowel 0 Colon 2/6 evaluable	
Acute GVHD without gut involvement	3	0	0	0	3	
Chronic GVHD involving gut	2	0	0	0	2	
Chronic GVHD without gut involvement	5	0	0	0	5	

* VOD = veno-occlusive disease of the liver.

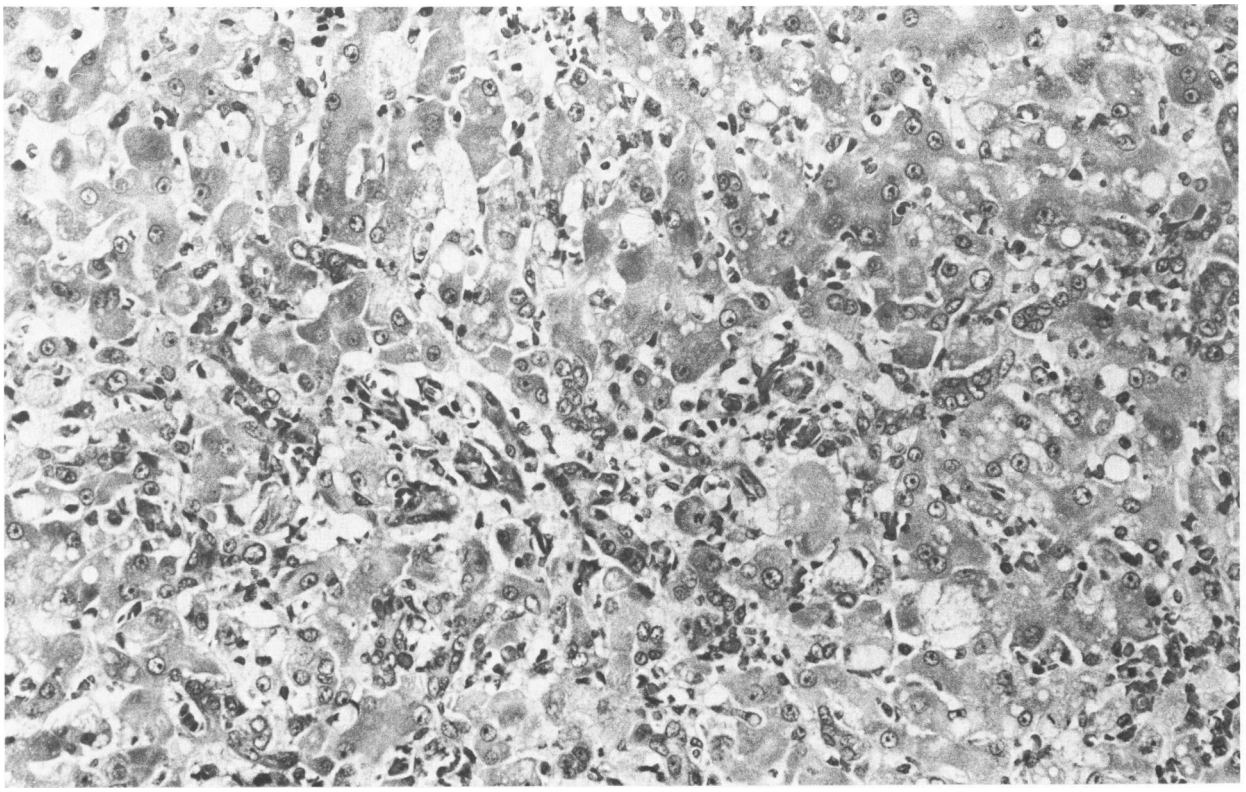
sustained or characteristic clinical or histologic features.[8,15,16]

Histologically, acute and chronic GVHD have some differences, possibly because of the longer duration of active GVHD in the chronic syndrome. With chronic GVHD the skin more commonly showed epidermal atrophy and fibrous widening of the papillary and reticular dermis; the liver showed bile ductule proliferation, bridging, piecemeal necrosis, and portal fibrosis. Moderate portal triaditis with injured interlobular bile ducts were present in dogs with either acute or chronic GVHD. Lymphocytic or plasmacytic infiltrates around the central veins also occur in dogs receiving autologous transplants and must be considered nonspecific. Dogs with chronic GVHD lacked gut alterations found consistently in dogs with acute GVHD of the gut; the diarrhea in 2 dogs with chronic GVHD was probably the consequence of pancreatitis.

We could not confirm the impression by Zurcher et al[17] that lymphocytic inflammation and necrosis of individual cells in the large pancreatic duct found in 2 dogs is a manifestation of GVHD. Similar changes were seen in dogs given autologous grafts, suggesting that these alterations were related to the conditioning. Furthermore, pancreatitis and pancreatic atrophy are recognized complications of TBI in autologously transplanted dogs.[18]

We noted many similarities between chronic GVHD in dogs and in humans. In both species, the chronic syndrome presents by 1 of 3 modes: Skin and gut involvement are common in the acute syndrome, while gut involvement is rare in the chronic syndrome. Gram-negative rods are the commonest organisms causing bacterial infections in acute GVHD, whereas the predominant infectious complications of chronic GVHD are caused by gram-positive cocci.[19]

Some manifestations of chronic GVHD differ between man and dogs. Ascites was a primary manifestation of chronic GVHD in dogs, whereas it occurs infrequently among humans with chronic hepatic GVHD. The cutaneous type of chronic GVHD re-



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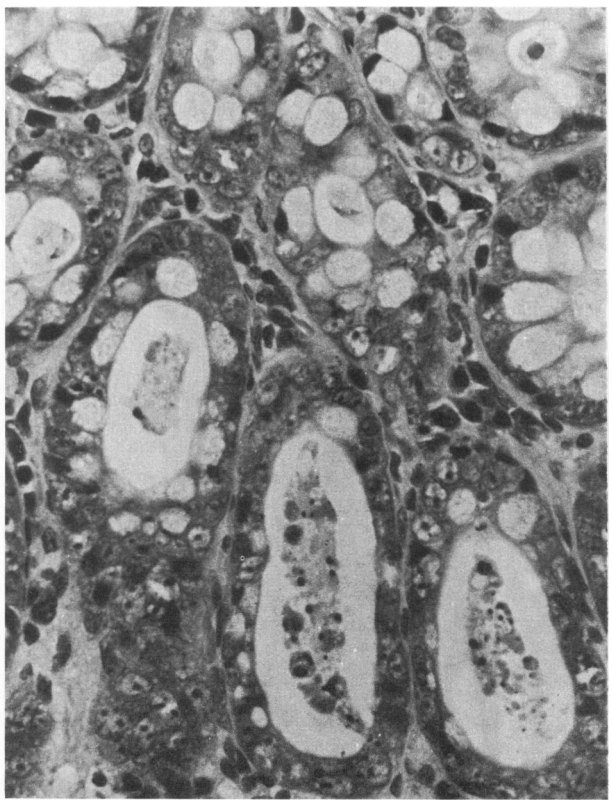

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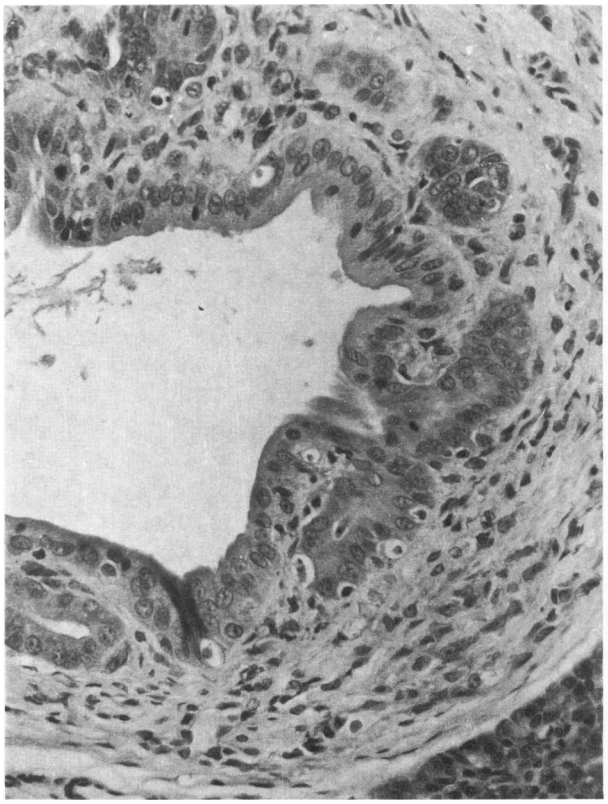

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Figure 5—Chronic hepatic GVHD showing early portal-to-portal bridging with proliferating bile ductules and hepatocyte unrest with lobular disarray. (H&E, ×250) **Figure 6**—Acute GVHD of colon showing dilated, debris-filled crypts lined by karyorrhectic and atypical epithelium. (H&E, ×425) **Figure 7**—Large pancreatic duct from a dog with acute GVHD showing nonspecific findings of mild lymphocytic infiltration and degeneration of individual duct cells (apoptosis). (H&E, ×250)

sembling localized scleroderma (limited form) was not observed in the dogs. Both species may develop a florid lymphocytic lichenoid reaction of the skin, often generalized in man but most prominent on the ears of dogs. This reaction is often associated with acanthosis in man but not in dogs, which have a thinner epidermis. Inflammation of eccrine sweat coils is more frequent in persons with chronic GVHD than in dogs, where such glands are located mainly in the footpads. On the other hand, inflammation of apocrine glands is seen in both acute and chronic GVHD in dogs. In both man and dogs, the variable changes of liver GVHD appear to reflect the duration of activity. Hepatic changes of chronic GVHD in man involve more profound changes of cholestasis with periportal bile thrombi and dilated cholangioles.

Some differences in the severity and expression of chronic GVHD in canine and human marrow graft recipients may be related to the degree of allogeneic disparity between donor and recipient; ie, all dogs in the present study received DLA-nonidentical littermate grafts, while, until recently, human transplants have been restricted almost entirely to HLA-identical sibling donors. Furthermore, some patients with chronic GVHD had received immunosuppressive therapy. As a result, chronic GVHD in humans may sometimes be mild and certainly is not universally fatal, whereas all dogs in the current study that developed chronic GVHD succumbed.

Other manifestations of chronic GVHD in humans include polymyositis, serositis, synovitis (unpublished data), a desquamative esophagitis,[21] and a sicca syndrome with dryness of the eyes, mouth, large airways, and vagina.[10] Microscopically, these organs show chronic inflammation and/or destruction of secretory glands. Beshorner et al have suggested that the respiratory epithelium lining the tracheobronchial airways are also affected by a lymphocytic bronchitis secondary to GVHD.[21] We have been unable to confirm this in our studies of human[22] and canine acute GVHD (unpublished observations). We did not examine these tissues in this retrospective study since most of these dogs were given transplants before we recognized the chronic GVHD syndrome in man or dog. Since that time, our postmortem examinations also include samples from these organs.

The similarities of chronic GVHD in humans and dogs, both outbred species, suggest that dogs can be used as a model of chronic GVHD. Other models of chronic GVHD have used inbred strains of rodents.[23,24] In only a few of the animals in this study did chronic GVHD develop. More recent experiments indicate that modifications of the posttransplant immunosuppressive regimen including MTX and Cyclosporin A result in more long-term survivors with or without chronic GVHD.[25]

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