Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

CLINICAL IMMUNOLOGY AND IMMUNOPATHOLOGY 22, 203–224 (1982)

Sequential Morphology of Graft-versus-Host Disease in the Rat Radiation Chimera[1]

WILLIAM E. BESCHORNER,[2] PETER J. TUTSCHKA, AND GEORGE W. SANTOS

Department of Pathology and The Bone Marrow Transplant Unit, Oncology Center, The Johns Hopkins Medical Institutions, Baltimore, Maryland 21205

INTRODUCTION

Acute graft-versus-host disease (GVHD) is both a complex expression of cell-mediated immunity against host antigens and a clinically challenging complication of bone marrow transplantation. The pathology in radiation chimeras has been described for man (1–6), the monkey (7, 8), the dog (9), and the Syrian hamster (10). The most commonly studied models have been in rodents: rats (11–15), mice (16–20) and rabbits (21). GVHD is readily induced by injecting bone marrow or bone marrow and lymphoid cells into newborn or thymectomized semiallogeneic recipients or into lethally immunosuppressed (total body irradiation or high-dose cyclophosphamide) allogeneic recipients. Within days of the transplant, splenomegaly and lymph node enlargement is observed in most semiallogeneic models and some allogeneic systems. Later the host becomes emaciated with variable degrees of weight loss, dermatitis, and diarrhea. GVHD has been monitored through mortality (22), splenomegaly (11), popliteal lymph node enlargement (23–26), and through histopathology of the target tissues including skin, liver, and intestines.

An increasingly important although poorly understood complication in long-term chimeras is the development of chronic graft-versus-host disease. The histology in man (27–30) and rats (31) appears distinct from acute GVHD in that it is associated with scleroderma-like cutaneous changes, injury to salivary glands and lacrimal glands leading to a sicca syndrome, and chronic active hepatitis.

The following sequential description addresses and attempts to clarify several points. The kinetics of lymphoid proliferation as well as lymphoid depletion in recipients of allogeneic marrow are distinguished from the effects of total body irradiation and reconstitution of the recipient by syngeneic marrow. The kinetics of injury to nonlymphoid tissues and to cellular components of tissues such as liver and intestines are characterized in the allogeneic host. The frequency and pattern of GVHD-associated injury to additional organs are presented. Finally, the patterns of lymphoid proliferation and tissue injury in recipients of additional

[1] This investigation was supported by PHS Grants CA-28701 and CA-15396, awarded by the National Cancer Institute, DHHS.

[2] To whom requests for reprints should be addressed.

0090-1229/82/020203-22$01.00/0

allogeneic spleen cells are compared to the patterns in recipients of allogeneic bone marrow only.

MATERIALS AND METHODS

Ten- to twelve-week-old female Lewis rats (Rt-l^1, 150–200 g, Microbiological Associates) were lethally irradiated with 960 rad and from a cesium-137 small-animal irradiator 1 day prior to transplant. At Day 0, the rats received either (A) no transplant (radiation control); (B) 6×10^7 nucleated bone marrow cells from pooled 10- to 12-week-old Lewis rats; (C) 6×10^7 nucleated bone marrow cells from pooled 24- to 30-week-old female ACI (Rt-l^a) rat donors; or (D) 6×10^7 nucleated marrow and 3×10^7 spleen mononuclear cells from the same pool of ACI donors. Bone marrow and spleen cells were prepared as decribed previously (32). All rats were injected with intraperitoneal Bactrim (generously supplied by Hoffman–La Roche) (33) and subcutaneous gentamicin for 10 days as well as drinking acidified water supplemented with nonabsorbable polymyxin and neomycin (−2 days to +8 days) followed by acidified water for the duration of the experiment.

The animals were examined and weighed three times per week. Three animals from each transplant group were sacrificed at Days 2, 5, 8, 14, 17, 21, and 28. Sections of the following tissues were removed and fixed in 10% neutral-buffered formalin: thymus, cervical lymph node, mesenteric lymph node, spleen, tibia, skin, tongue, upper and lower esophagus, liver, intestines, lungs, mainstem bronchi, serous and mucinous salivary glands, lacrimal gland, harderian gland, eye, kidney, adrenal gland, pancreas, ovary, uterus, urinary bladder, heart, brain cortex and cerebellum, skeletal muscle, thyroid gland, and synovium from the knee. Five-micron sections were cut from paraffin-embedded tissues and stained with hematoxylin and eosin and in some tissues, with Masson trichrome stains.

RESULTS

Bone Marrow

The lethally irradiated rats which did not reaceive a bone marrow transplant showed persistence of scattered neutrophils at 2 and 5 days post-transplant and megakaryocytes at 2 days post-transplant. There was no evidence of repopulation at 8 days.

The rats which received syngeneic bone marrow after lethal irradiation had persistence of neutrophils, basophils, and megakaryocytes at 2 days post-transplant. By 5 days there were multiple small colonies of erythroid precursors, myeloid precursors, and immature megakaryocytes. By Day 14, there was 60 to 80% cellularity of the bone marrow with maturation of all three hematopoietic elements. Rats who received either allogeneic bone marrow or marrow and spleen cells did not have any delay in repopulation compared to rats receiving syngeneic bone marrow.

Thymus

At 2 days following irradiation, the thymus was similar in all groups. The cortex was replaced with large histiocytes. The medulla contained scattered small lym-

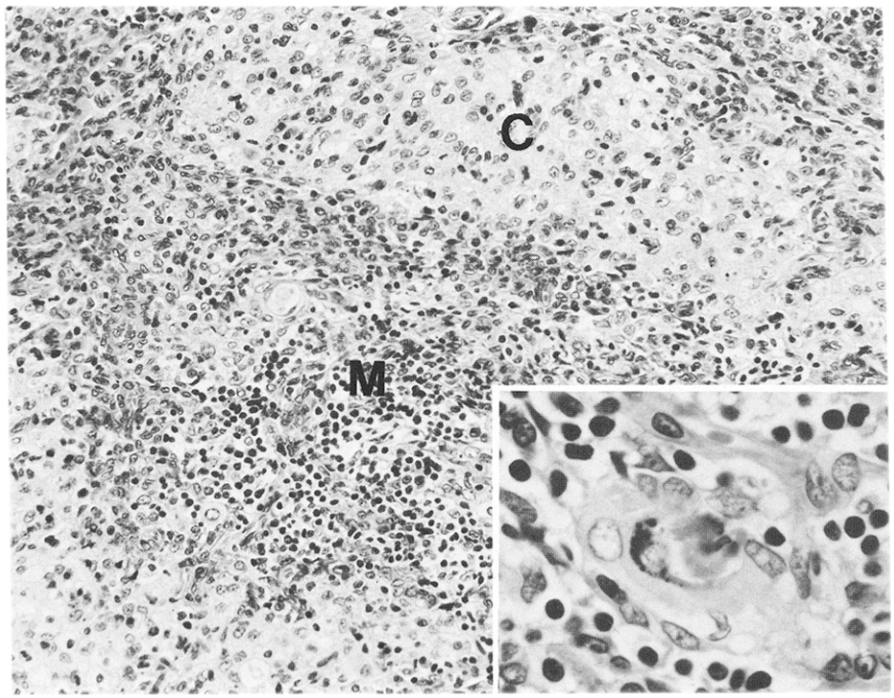

FIG. 1. Thymus, radiation control rat, Day 2. The cortex contains histiocytic cells. The medulla has scattered small lymphocytes, epithelium and Hassall's corpuscles (inset). C, Cortex; M, medulla. (H + E, 200×, inset, 600×.)

phocytes and prominent epithelial cells with elongated bland appearing nuclei. Hassall's corpuscles, normally not readily identified in the rat, were prominent at this stage (Fig. 1).

The rats which did not receive a transplant showed limited repopulation of the cortex and medulla by Day 8 with small lymphocytes.

With syngeneic bone marrow, lymphoblasts were evident by Day 5 in both the cortex and medulla. These were replaced with small lymphocytes in the cortex by Day 8. By Day 14 there were nearly normal numbers of small lymphocytes in the cortex and medulla with sharp demarcation (Fig. 2).

The thymus in rats receiving allogeneic bone marrow transiently contained medium-sized lymphocytes in the cortex at Day 8. From Days 14 through 28, however, the cortex contained only large foamy histiocytes, while the medulla showed increased perivascular fibrosis (Fig. 3). The epithelial cells and Hassall's corpuscles seen at Day 2 were no longer evident.

With allogeneic bone marrow and spleen, the thymus had a more prolonged repopulation of the cortex and medulla with medium sized lymphocytes (Days 8 to 21). Throughout this period, both regions were hypocellular compared to the normal thymus and rats with syngeneic transplants. By Day 28, the thymus glands in these rats also were markedly hypocellular with fibrosis and depletion of medullary epithelial cells.

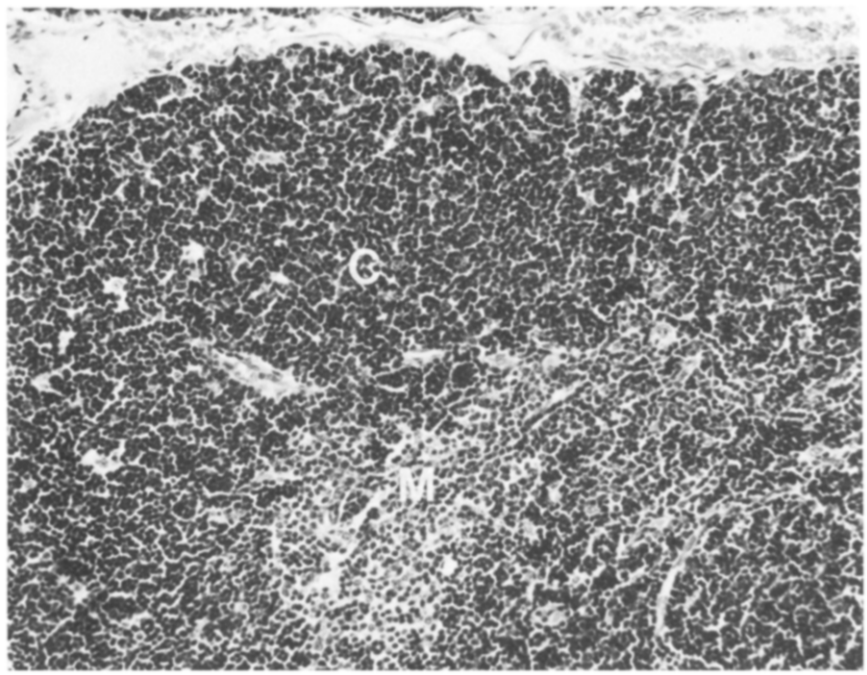

FIG. 2. Thymus, syngeneic marrow recipient, Day 14. Numerous small lymphocytes are present in the cortex and medulla. C, Cortex; M, medulla. (H + E, 200×.)

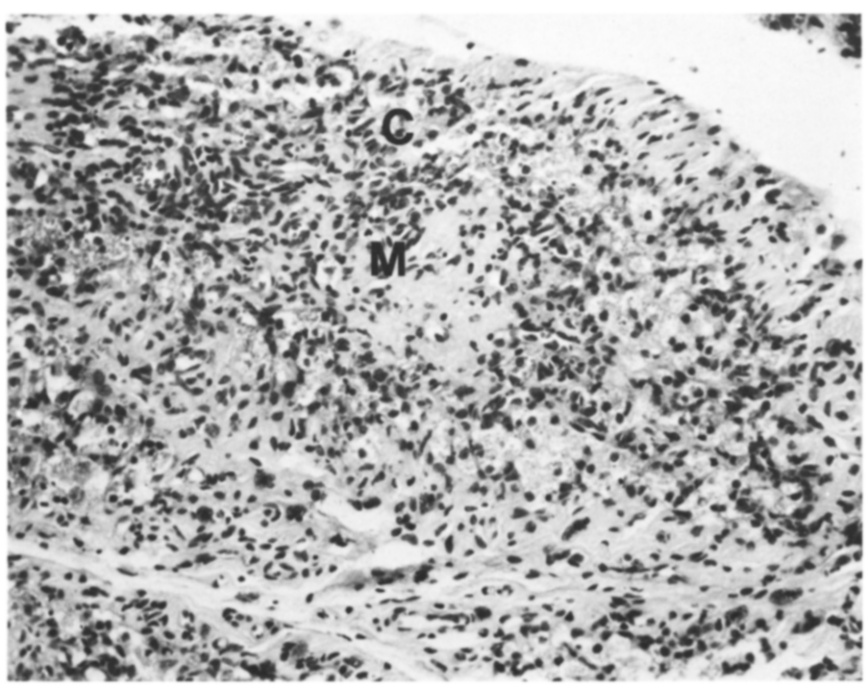

FIG. 3. Thymus, allogeneic marrow recipient with GVHD, Day 14. The cortex contains predominantly histiocytic cells. The medulla has only vessels and increased fibrosis. Previously evident epithelium is no longer evident. C, Cortex; M, medulla. (H + E, 200×.)

Spleen

The developing morphology of the spleen after irradiation and marrow transplantation is detailed in Table 1.

Two days after irradiation, the white pulp was quite hypocellular, with a mixture of small lymphocytes and large histiocytic cells. Mitoses were not present. Occasionally, pyknotic cells and karyorrhexis were observed. No follicles were evident. The red pulp contained many histiocytes (some with hemosiderin), stromal cells, and red cells. The endothelial cells of the arterioles were vacuolated, appreciably decreasing the diameter of the lumen.

By Day 8 the spleen of rats which received no transplant was nearly acellular. The stroma of the red pulp extended up to the arteriolar adventitia. Scattered histiocytes were present in the red pulp.

In contrast, recipients of syngeneic bone marrow had increased numbers of blasts and medium-sized lymphocytes in the white pulp as early as Day 5. Many cells had a single prominent nucleolus and basophilic cytoplasm. By Day 14, these were replaced with predominantly small lymphocytes. Well-defined follicles were present with small lymphocytes, but were still hypocellular and without germinal centers. Germinal centers were evident by Day 21 and appeared quite distinct at Day 28. Extramedullary hematopoiesis was evident in the Day 5 spleens and was prominent with marked erythropoiesis from Days 14 through 21.

The recipients of allogeneic bone marrow with major Rt-l discrepancy also demonstrated an immunoblast response in the periarteriolar region by Day 5. The morphologic characteristics and intensity at this time did not differ from syngeneic rats. Instead of maturing, however, the periarteriolar blasts persisted through Day 21, and eccentric follicles did not develop. By Day 28, the white pulp was quite hypocellular. In the red pulp, extramedullary hematopoiesis persisted through Day 28 with prominent erythropoiesis.

With allogeneic bone marrow plus spleen cells, the spleen was qualitatively similar to that seen with bone marrow alone. The intensity of blast response was greater at 5 through 17 days. At 28 days however, the periarteriolar region was markedly hypocellular and eccentric follicles failed to develop.

Lymph Nodes

The lymph node morphology is described in detail in Table 2.

Two days following radiation, the lymph nodes were similar in all four groups of animals. The nodes were hypocellular but not acellular. Primary follicles (recognized here as large deep cortical follicles) contained small lymphocytes while the secondary follicles (recognized as smaller superficial cortical follicles) contained predominantly concentrically arranged histiocytes. Numerous plasma cells were present in the medullary cords. The interfollicular region had numerous prominent postcapillary venules and increased fibrosis but only scattered histiocytes. The endothelial cells of capillaries were swollen and vacuolated in the venules. In the rats which did not receive a transplant, the small lymphocytes in the primary follicles and the plasma cells persisted through Day 8 when the last of these animals were sacrificed.

TABLE 1
SPLEEN MORPHOGENESIS AFTER BONE MARROW TRANSPLANTATION

Transplant group	Day 2		Days 5, 8	
	White pulp	Red pulp	White pulp	Red pulp
I. Radiation control	Very hypocellular with periarteriolar histiocytic cells, small lymphocytes, karyorrhexis. Vacuolization of arteriolar endothelium.	Histiocytes, stroma, congestion.	Occasional histiocytic cell, stroma extends to adventitia.	Stroma.
II. Syngeneic bone marrow	Very hypocellular with periarteriolar histiocytic cells, small lymphocytes, karyorrhexis. Vacuolization of arteriolar endothelium.	Histiocytes, stroma, congestion.	Moderately hypocellular with periarteriolar and eccentric blasts with prominent nucleoli and basophilic cytoplasm.	Colonies of extramedullary hematopoiesis, prominent erythropoiesis.
III. Allogeneic bone marrow	Very hypocellular with periarteriolar histiocytic cells, small lymphocytes, karyorrhexis. Vacuolization of arteriolar endothelium.	Histiocytes, stroma, congestion.	Mildly hypocellular with periarteriolar blasts and mitotic figures. A few small lymphocytes in eccentric region.	Colonies of extramedullary hematopoiesis, prominent erythropoiesis.
IV. Allogeneic bone marrow and spleen	Very hypocellular with periarteriolar histiocytic cells, small lymphocytes, karyorrhexis. Vacuolization of arteriolar endothelium.	Histiocytes, stroma, congestion.	Increased cellularity with many periarteriolar blasts and mitotic figures, moderate follicular blasts, and small lymphocytes.	Extramedullary hematopoiesis with prominent erythropoiesis.

Transplant group	Days 14, 17		Days 21, 28	
	White pulp	Red pulp	White pulp	Red pulp
I. Radiation control	N.D.		N.D.	
II. Syngeneic bone marrow	Mildly hypocellular with small lymphocytes in the periarteriolar region and eccentric follicles but without germinal centers.	Increased extramedullary hematopoiesis, prominent erythropoiesis.	Normal cellularity with small lymphocytes in periarteriolar region well formed follicles with germinal centers, peripheral mantle with medium lymphocytes, histiocytes.	Occasional colony of extramedullary hematopoiesis.
III. Allogeneic bone marrow	Mildly hypocellular with periarteriolar blasts and mitotic figures. No follicles present.	Extramedullary hematopoiesis with prominent erythropoiesis.	Very hypocellular with few periarteriolar and eccentric small lymphocytes.	Increased dense extramedullary hematopoiesis with prominent erythropoiesis.
IV. Allogeneic bone marrow and spleen	Increased cellularity with many periarteriolar blasts and mitotic figures, few follicular small lymphocytes.	Increased dense extramedullary hematopoiesis with prominent erythropoiesis.	Very hypocellular with occasional periarteriolar blast and small lymphocytes.	Extramedullary hematopoiesis with prominent erythropoiesis.

TABLE 2

LYMPH NODE MORPHOGENESIS AFTER BONE MARROW TRANSPLANTATION

	Day 2	Days 5, 8	Days 14, 17	Days 21, 28
I. Radiation control	Primary follicles: Small lymphocytes, edema. Secondary follicles: Histiocytes, karyorrhexis. Paracortical region: Prominent venules, fibrosis, histiocytes. Medullary cords: Numerous plasma cells, edema. Vacuolated endothelium.	Primary follicles: Few small lymphocytes, stroma, edema. Secondary follicles: Occasional histiocytes, fibrosis. Paracortical region: Numerous venules, loose fibrosis. Medullary cords: Numerous plasma cells, edema.	N.D.	N.D.
II. Syngeneic bone marrow	Primary follicles: Small lymphocytes, edema. Secondary follicles: Histiocytes, karyorrhexis. Paracortical region: Prominent venules fibrosis, histiocytes. Medullary cords: Numerous plasma cells, edema. Vacuolated arteriolar endothelium.	Primary follicles: Many round blasts. Secondary follicles: Moderate small lymphocytes. Paracortical region: Occasional blasts, many venules. Medullary cords: Many plasma cells.	Primary follicles: Many blasts and medium lymphocytes, cuff of histiocytic cells. Secondary follicles: Many small lymphocytes. Paracortical region: Few round and polygonal blasts. Medullary cords: Many plasma cells.	Primary follicles: Many small lymphocytes, peripheral mantle with histiocytic cells. Secondary follicles: Many small lymphocytes, around well defined germinal centers. Paracortical region: Increased numbers of round and polygonal blasts, small lymphocytes. Medullary cords: Many plasma cells.
III. Allogeneic bone marrow	Primary follicles: Small lymphocytes, edema. Secondary follicles: Histiocytes, karyorrhexis. Paracortical region: Prominent venules, fibrosis, histiocytes. Medullary cords: Numerous plasma cells, edema. Vacuolated arteriolar endothelium.	Primary follicles: Few small lymphocytes, edema. Secondary follicles: Not observed. Paracortical region: Expanded with many round and polygonal blasts, medium lymphocytes, vascular proliferation. Medullary cords: Not distinct, rare plasma cells, histiocytes.	Primary follicles: Not observed. Secondary follicles: Not observed. Cortex: Few blasts, histiocytes, small lymphocytes, karyorrhexis, many venules. Medullary cords: Histiocytes, few plasma cells, edema.	Primary follicles: Few blasts, small lymphocytes, edema. Secondary follicles: Occasional groups of small lymphocytes. Paracortical region: Occasional small lymphocyte, many venules, edema. Medullary cords: Moderate numbers of plasma cells.
IV. Allogeneic bone marrow and spleen	Primary follicles: Moderate small lymphocytes, edema. Secondary follicles: Histiocytes, karyorrhexis. Paracortical region: Prominent venules, edema, histiocytes. Medullary cords: Moderate plasma cells. Vacuolated arteriolar endothelium.	Primary follicles: Moderate round blasts, small lymphocytes, karyorrhexis. Secondary follicles: Small groups of small lymphocytes. Paracortical region: Mildly expanded with round blasts, occasional polygonal blasts, many venules. Medullary cords: Histiocytes, edema.	Primary follicles: Small lymphocytes, few blasts, edema. Secondary follicles: Rare groups of small lymphocytes. Paracortical region: A few round blasts, histiocytes, many capillaries, venules, edema. Medullary cords: Histiocytes, edema.	Primary follicles: Few small lymphocytes, edema. Secondary follicles: Not observed. Paracortical region: Variable numbers of round and polygonal blasts, small lymphocytes, histiocytes, karyorrhexis, many venules. Medullary cords: Histiocytes, small lymphocytes, rare plasma cells, edema.

With syngeneic bone marrow, the lymph nodes had evidence of repopulation within five days with increased immunoblasts and medium lymphocytes in the primary follicles and repopulation of secondary follicles with small lymphocytes. Plasma cells were prominent throughout the post-transplant period. By 21 days, the primary follicles were filled with small and medium lymphocytes. Around the periphery was a cuff of histiocytic cells. The secondary follicles had a densely cellular mantle of small lymphocytes around a distinct germinal center containing round immunoblasts and mitotic figures. In contrast to the follicles, the interfollicular regions remained hypocellular until 17 days post-transplant when they became moderately populated with immunoblasts including occasional blasts with hyperchromatic polygonal nuclei. By Day 28 these regions contained mostly small lymphocytes with scattered immunoblasts.

The lymph nodes in recipients of allogeneic marrow were strikingly different. Although a few small lymphocytes persisted in the primary follicles until Day 8, there were no immunoblasts evident, and secondary follicles and plasma cells were only rarely observed after Day 2. On the other hand, by Day 5, the interfollicular regions contained many immunoblasts (some with dark angular nuclei), histiocytes, cellular debris, and additionally these regions showed prominent neovascularization. By Day 8 the lymph nodes were enlarged and the above-described proliferative process was diffuse throughout the cortex. The lymphoid proliferation was more prominent in the mesenteric lymph nodes than in the cervical lymph nodes. By Day 14, the number of immunoblasts was considerably diminished although the increased vascularity persisted. By Day 28, the interfollicular regions as well as the follicles were very hypocellular with increased interstitial edema.

When lethally irradiated rats received allogeneic spleen in addition to bone marrow, the primary follicles contained increased small lymphocytes at 2 days compared to recipients of marrow alone. Scattered immunoblasts were present at Days 5 and 8 in the primary follicles. Groups of small lymphocytes were present in the secondary follicles. From Days 14 through 28 the primary follicles remained hypocellular and did not develop a peripheral cuff. Secondary follicles were not observed in any of the Day 21 or Day 28 lymph nodes. While not as intense as in rats receiving only allogeneic bone marrow, there was a moderate degree of lymphoid and vascular proliferation in the interfollicular regions at Days 5 and 8. By Day 28 these regions were quite hypocellular and edematous.

Injury to Nonlymphoid Tissues

The target tissues which had significant injury in recipients of Rt-1 mismatched allogeneic bone marrow at Days 8 to 28 but not in recipients of syngeneic bone marrow or in the radiation control group at Day 8 are listed in Table 3. In the early transplant period (Days 8 and 14), most animals had lymphocyte associated injury of the tongue, intestines, conjunctivas and corneas. By 17 days most animals had evidence of injury to the skin, lobular hepatocytes, and pancreas. By 28 days, injury was also evident in the lacrimal and serous salivary glands, bronchial mucosa, urinary bladder, renal pelvis, and esophagus.

Rats receiving both marrow and spleen cells in this particular study did not have

a more severe GVHD as judged by mortality (6 of 27 vs 10 of 27 initial recipients) or weight loss (18 ± 8% vs 30 ± 9%). The GVHD was, however, different with an altered morphology of skin GVHD from the onset at 8 days. Furthermore there was injury to the hepatic bile ducts, salivary and lacrimal glands, and bronchial mucosa from the onset at 8 days.

Tongue

At 2 days the papillary capillaries in all groups had prominent swollen endothelium. These changes were resolved by Day 5. In radiation controls and syngeneic recipients, there were no other histologic changes.

At 8 days, with allogeneic marrow transplantation, a lymphocytic infiltrate was present in the submucosa with focal exocytosis. In these regions, vacuoles were present within the epidermal mucosa containing cellular debris and one or more lymphocytic cells. By 17 days, the necrotizing process was diffuse. The submucosa had many small lymphocytic cells, histiocytes, and capillaries and was edematous. There was a moderate to marked lymphocytic exocytosis with vacuolization of some basal and epidermal cells and scattered large eosinophillic necrotic cells. Many of the epidermal cells contained vesicular nuclei with a single prominent nucleolus and mitotic figures were evident. With increased histologic severity, there were infrabasallar bullae, ulceration with bacterial overgrowth, and edema of the muscularis. There was no consistent difference of the mucosa between those receiving bone marrow alone and those receiving bone marrow and spleen. With additional spleen cells, however, there was greater perivascular infiltration and muscular atrophy.

TABLE 3
PERIPHERAL TISSUES INJURED WITH GVHD

Recipients of	Allogeneic bone marrow		Allogeneic marrow and spleen	
	Early (Days 8, 14)	Later (Days 17–28)	Early (Days 8, 14)	Later (Days 17–28)
Tissue				
Tongue	5/5	5/5	6/6	8/8
Skin	1/5	5/5	5/6	8/8
Esophagus	0/5	3/5	2/6	7/8
Liver	2/5	4/5	6/6	7/8
Hepatocytes	2/5	4/5	2/6	4/8
Bile ducts	0/5	2/5	6/6	7/8
Intestines	5/5	5/5	5/5	8/8
Karyolytic bodies	3/5	4/5	1/5	6/8
Plasma cell depletion	5/5	5/5	5/5	8/8
Villous atrophy	2/5	3/5	3/5	8/8
Bronchial mucosa	0/5	2/4	5/6	7/8
Serous salivary glands	0/5	2/5	4/6	8/8
Lacrimal glands	0/4	2/4	4/6	3/7
Conjunctivae	3/5	2/5	2/6	4/8
Renal pelvis	0/5	2/5	2/6	4/8
Urinary bladder	0/5	3/4	3/6	6/7
Pancreas	2/5	5/5	2/6	7/8
Heart	0/5	3/5	3/6	5/8

Skin

At Day 2 post-transplant rats from all four groups had mild edema of the superficial dermis and prominent capillary endothelium. These mild changes were no longer evident by Day 5.

Recipients of allogeneic bone marrow at 14 days had vacuolization of basal epidermal cells and scattered eosinophilic necrotic cells in the epidermis, some containing pyknotic nuclei. There was a mild lymphocytic infiltrate in the dermis with exocytosis into the epidermis. The necrotic epidermal cells only occasionally had a satellite lymphocyte. Later there was greater liquifaction of epidermis associated with lymphocytes (Fig. 4). Similar Liquifaction was evident in the hair follicles, particularly at the junction with epidermis. The sebaceous glands and hair bulbs were generally spared. In more severe cases, there was suprabasalar epidermolysis, dermal edema, and perivascular infiltrates of lymphocytes and histiocytes about the superficial arterioles.

Recipients of marrow and spleen cells had from the onset of GVHD at 8 days patchy acanthosis and often keratosis of the epidermis (Fig. 5). There was less exocytosis of lymphocytes and less liquifactive degeneration than recipients of marrow alone. The dermis was relatively thicker with collagen extending to the basal cell layer. There was greater infiltration of the dermis with lymphocytes, histiocytes and fibroblasts. The arterioles and arteries in the deep dermis had greater perivascular infiltration with lymphocytes and histiocytes.

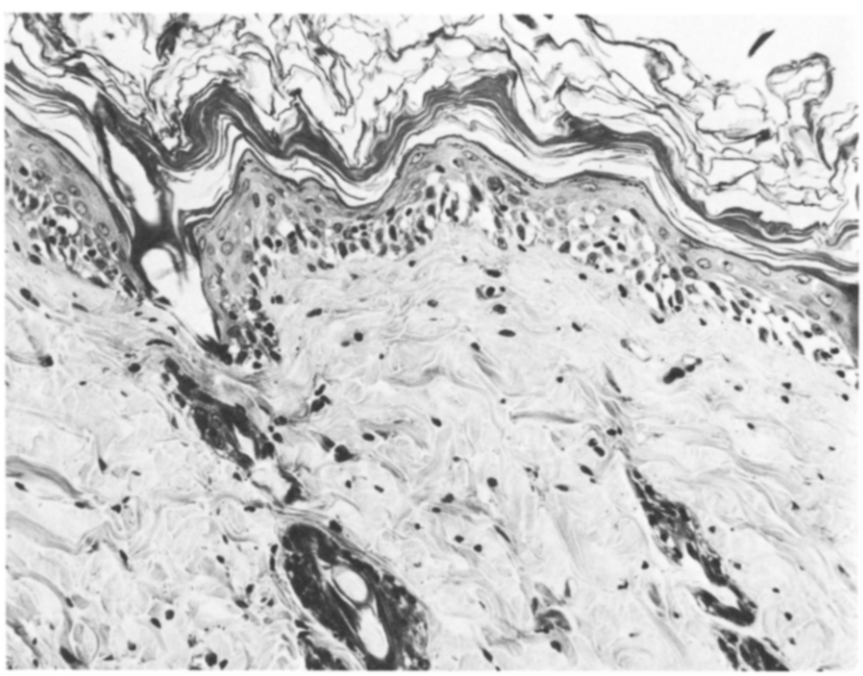

FIG. 4. Graft-versus-host disease, skin, recipient of allogeneic bone marrow, Day 21. Note lymphocyte exocytosis of epidermis with associated vacuolization. (H + E, 200×.)

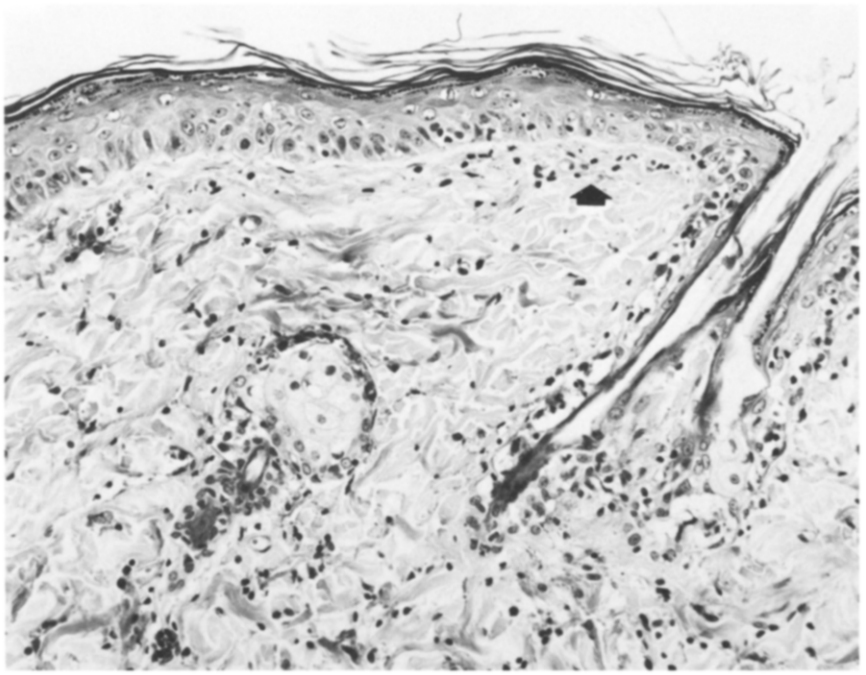

FIG. 5. Graft-versus-host disease, skin, recipient of allogeneic bone marrow and spleen, Day 21. Compared to Fig. 4, note decreased infiltration and vacuolization of epidermis, band-like infiltrate of superficial dermis (arrow), and thickened collagen in the superficial dermis. (H + E, 200×.)

Eye

No significant histologic changes were evident in radiation controls or syngeneic marrow recipients. The majority of chimeric rats had injury to the scleral limbus adjacent to the cornea. As early as Day 8, there was mild submucosal lymphocytic infiltate, epidermal exocytosis, and associated vacuoles containing eosinophilic cells and cellular debris (Fig. 6). The submucosa was edematous and had prominent capillaries.

Esophagus

The esophagus in all groups of rats at Day 2 had vascular changes as described above. The radiation control group and syngeneic recipient groups had no additional pathology.

In contrast to the tongue, skin, and conjunctiva, the esophagus was involved relatively late in the course of GVHD, with injury first observed at Day 17 in the allogeneic marrow recipients and Day 14 of the allogeneic marrow and spleen recipients. There was a mild submucosal infiltrate of small lymphocytes with focal lymphocytic exocytosis and vacuolization of the basal cells. By Day 28, the submucosal infiltrate was moderately dense and the associated epidermal changes were diffuse. Often small bullae were present. At this time, gram-positive cocci were numerous along the corium. There were no consistent pathologic differences between the upper and lower esophagus.

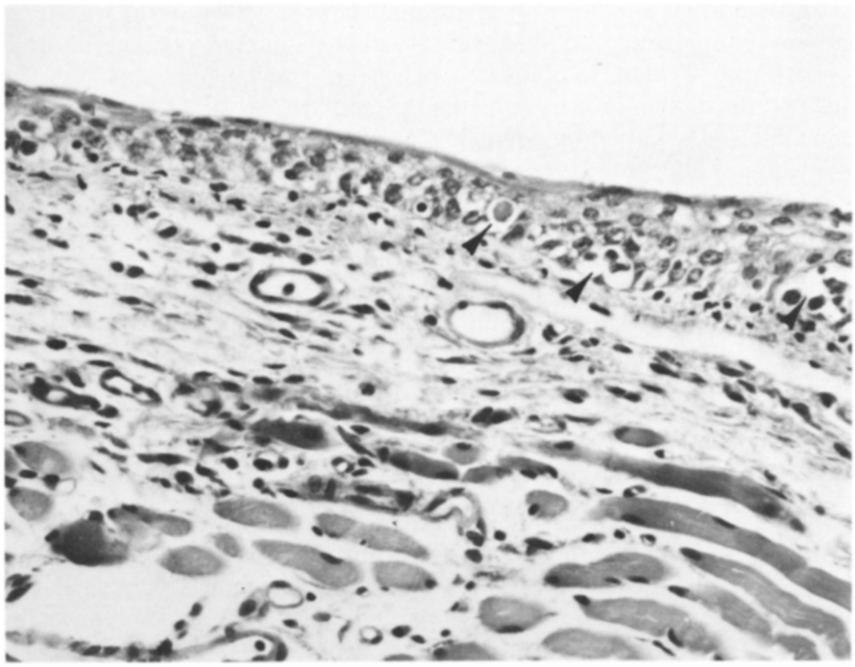

FIG. 6. Graft-versus-host disease, scleral limbus near the cornea, recipient of allogeneic bone marrow, Day 21. There is a submucosal infiltrate of mononuclear cells with exocytosis of epidermis and associated vacuoles containing pyknotic cells and debris (arrows). (H + E, 300×.)

Small Intestine

At 2 days post-transplant, the Peyer's patches were quite hypocellular. In the radiation control group and syngeneic marrow recipients, the remaining cells consisted of small lymphocytes. In recipients of allogeneic marrow or marrow and spleen cells, there were, in addition, occasional immunoblasts present. The small lymphocytes persisted in the radiation control rats. With syngeneic marrow transplantation there were increased numbers small lymphocytes and immunoblasts containing a prominent nucleolus by Day 5. Around the periphery there was focal cellular debris but no overall organization. By Day 21, follicles were evident containing densely packed small lymphocytes but no germinal centers.

In recipients of allogeneic bone marrow, or marrow and spleen, there were numerous immunoblasts by Day 5 with only scattered small lymphocytes. By Day 17, the patches were again hypocellular with scattered small lymphocytes and rare immunoblasts. By Day 28, they were very hypocellular with fibrosis and an occasional small lymphocyte.

The mucosa and lamina propria were similar for all groups of animals 2 days after irradiation. The villi were moderately flattened and the crypts were quite abnormal with atypical columnar cells having large vesicular nuclei, absence of mitotic figures and occasional vacuoles (karyolytic bodies) containing pyknotic cells and cellular debris. The lamina propria contained numerous plasma cells, a few small lymphocytes and occasional neutrophils and mast cells. At Day 5, there was resolution of the nuclear atypia, increased numbers of crypt mitotic figures

and villi of normal height. Karyolytic bodies were still present focally. In the radiation control group and syngeneic marrow recipients, the karyolytic bodies were no longer evident subsequent to Day 8. The mucosa was histologically normal and the lamina propria contained slightly decreased to normal numbers of plasma cells and small lymphocytes.

Recipients of allogeneic bone marrow or bone marrow and spleen had mild to moderately flattened villi, numerous crypt mitoses, and rare or focal karyolytic bodies on Days 8 and 14. The lamina propria contained only rare plasma cells and scattered small and medium lymphocytes and histiocytes. On Days 21 and 28, there was variably flattened villi, numerous crypt mitoses, and moderate numbers of karyolytic bodies. Some sections appeared to have increased numbers of intraepithelial lymphocytes in the crypts. The lamina propria still was depleted of plasma cells, but had increased numbers of small lymphocytes and histiocytes as well as neutrophils.

Liver

At Day 2, the liver demonstrated moderate centrilobular congestion which had resolved by Day 5. In the radiation control group at Days 5 and 8, there were no histologic abnormalities. From Days 8 through 28, syngeneic recipients occasionally had a few small lymphocytes in the portal triads. There was not, however, any associated hepatocellular or bile duct injury.

The liver at Day 8 in recipients of mismatched bone marrow or bone marrow and spleen had numerous foci of lobular necrosis consisting of vacuoles containing one or more eosinophilic cells with pyknotic nuclei and several small or medium hyperchromatic lymphocytes. By Day 28 of the marrow allogeneic recipients and Day 8 of the allogeneic marrow and spleen recipients, there was evidence of bile duct injury with periductal lymphocytes, nuclear pleomorphism, and cytoplasmic vacuolization of the columnar cells. Occasionally cellular debris was evident in the lumen. Periportal bile stasis was seen in some sections but was never prominent. Piecemeal necrosis of the periportal hepatocytes was not prominent in either group until 21 days. Sections of liver taken late in the course also had increased numbers of hepatocytes with mitoses or multiple nuclei. At this time the Kupfer cells appeared prominent.

Trachea and Mainstem Bronchi

Sections of the upper trachea and mainstem bronchi had moderate nuclear atypia with large vesicular nuclei and rare round eosinophillic cells with pyknotic nuclei at 2 days. These changes were no longer evident by Day 5. Thereafter, the radiation control rats and syngeneic recipients had no histologic changes.

From Days 8 through 28, lymphocyte associated injury was more evident among recipients of allogeneic bone marrow and spleen cells than recipients of allogeneic bone marrow alone (12 of 14 vs 2 of 9, $P < 0.005$). This consisted of a moderate submucosal infiltrate of small and occasional medium lymphocytes, prominent capillaries, and edema, with exocytosis into the mucosa and associated round necrotic cells and focal hyperplasia. A similar infiltrate was present in the submucosal glands and ducts.

Salivary Glands

Sections of serous salivary glands examined at Day 2 showed extensive injury to the acini with decreased secretions, scattered cellular debris, and interstitial edema. No inflammatory cells were present. These changes were no longer evident by Day 5. Radiation control rats and syngeneic marrow recipients had no other changes.

Like the bronchi, the serous salivary glands were injured more frequently in recipients of Rt-1 mismatched bone marrow and spleen than in recipients of mismatched bone marrow alone (12 of 14 vs 2 of 10, $P < 0.005$). This consisted of mild or moderate periductal infiltrate of small lymphocytes with migration within the duct basement membrane. The mucosal cells were vacuolated and the nuclei disoriented. The acini also had an infiltrate of small lymphocytes. The columnar cells were variable in size with many being vacuolated. Later, the glands appeared atrophic and increased amounts of interstitial edema was present. The mucinous salivary glands had minimal changes, consisting of occasional ducts with lymphocytic infiltrates.

Lacrimal Glands and Harderian Glands

Sections of intraorbital lacrimal glands and Harderian glands from radiation control rats and syngeneic marrow recipients were histologically unremarkable.

In the allogeneic chimeras, these glands had a mild lymphocytic infiltrate of the ducts with vacuolization of the columnar cells. This was present in 7 of 13 recipients of allogeneic bone marrow and spleen compared to 2 of 8 recipients of bone marrow alone ($P < 0.2$) but was evident much earlier. Focal injury to the acini was evident in only 3 rats.

Kidney

In all rats sacrificed at Day 2 there were vascular changes of the small arterioles as described previously. The radiation control rats had no histologic changes at Days 5 and 8.

In the syngeneic marrow recipients as well as both groups of allogeneic chimeras there was a mild interstitial infiltrate of lymphocytes in the perivascular region. The glomeruli in all groups were not significantly changes by light microscopy. Thus we cannot identify any lesions in the renal parenchyma definitely associated with GVHD.

In the renal pelvis, however, 6 of 13 allogeneic recipients had lymphocytic infiltration and injury to the renal pelvis at Days 17 through 28, which was not evident in the syngeneic recipients. The transitional epithelium was mildly hyperplastic with vacuolization of the basal cells and occasional eosinophillic necrotic cells.

Urinary Bladder

The bladder at Day 2 showed the previously described vascular changes and vacuolization of individual transitional cells. These changes were resolved by Day 5. There were no other changes in the radiation control rats or syngeneic marrow recipients.

In allogeneic recipients with GVHD, there was patchy infiltration of the mucosa with small lymphocytes and associated liquifaction producing small bullae. This process was more prevalent at 17 to 28 days.

Pancreas

The pancreas from rats of the radiation control group and from syngeneic marrow recipients had no histologic changes. Sections of pancreas from rats with allogeneic marrow or marrow spleen cells showed focal infiltrates of lymphocytes and histiocytes in the periductal regions extending to the islets of Langerhans (Fig. 7). Around the periphery of these lesions were vacuoles containing necrotic cells and cellular debris and acini with vacuolated cytoplasm. These lesions were more frequently seen 17 to 28 days post-transplant ($P < 0.01$).

Heart

At Day 2, the heart in all groups of animals had vascular changes as previously detailed. Thereafter, the radiation control group and syngeneic recipients had no cardiac changes.

Half of the rats with acute GVHD had increased lymphocytes and edema in the interstitium. However, there was not any associated necrosis of the myofibers evident.

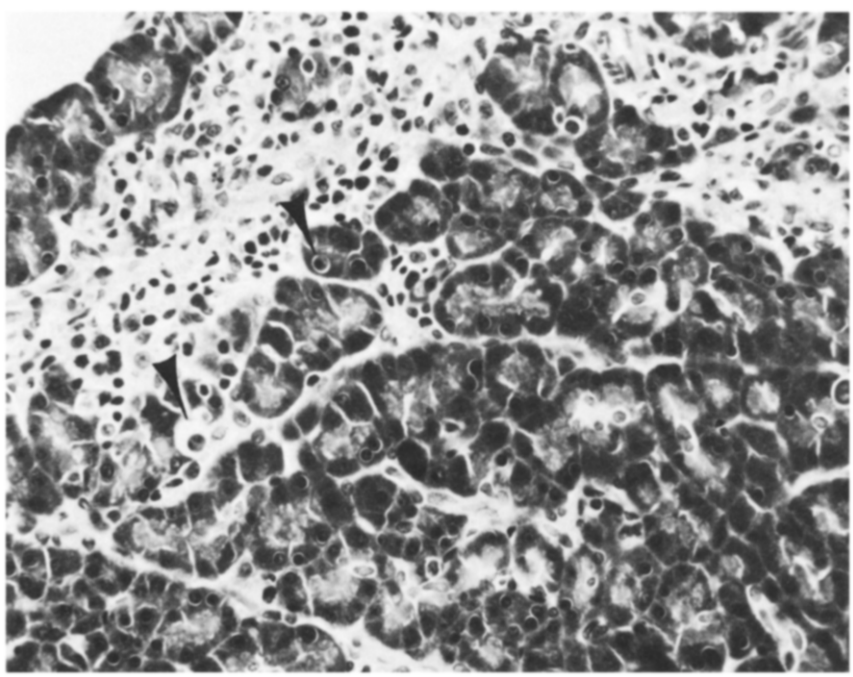

FIG. 7. Graft-versus-host disease, pancreas, recipient of allogeneic bone marrow, Day 17. A perivascular infiltrate of mononuclear cells extends into the interstitial areas of the excocrine pancreas with lose of acini. Around the periphery note the vacuoles containing pyknotic cells and debris (arrows). (H + E, 300×.)

Other Tissues

Tissues not noticeably or consistently altered in allogeneic recipients included brain, adrenal glands, thyroid, ovaries, uterus, skeletal muscle, synovium, and articular cartilage.

DISCUSSION

Acute graft-versus-host disease has been studied extensively in the mouse radiation chimera and in several reports of rat radiation chimeras. Rats offer a number of advantages as a model of acute GVHD in humans, including its size, a similar high mortality rate, similar organ involvement, and independence from strain variability among various Rt-1 mismatched, mixed-lymphocyte reactive strain combinations (14, 34). The present systematic study of rats sacrificed periodically extends previous descriptions and raises additional issues.

Although the thymus, spleen, and lymph nodes all had marked lymphoid atrophy and histiocytosis immediately after irradiation, these tissues varied greatly in the subsequent early course with respect to the other lymphoid tissues and whether their hosts received no transplant, syngeneic, or allogeneic bone marrow.

As observed in other systems, there was marked depletion of thymic lymphocytes and relative sparing of medullary epithelium immediately after irradiation (34, 36). In contrast to syngeneic recipients with rapid subsequent repopulation and even with the radiation control rats with partial repopulation by thymic lymphocytes, recipients of allogeneic marrow had severe lymphoid atrophy and apparent loss of epithelium. Thymic involution has been observed with GVHD in man, mice, rats, and rabbits (13, 17, 19, 35). Observations with adrenalectomized mice indicate that the involution, particularly in the medulla, is not the result of endogenous glucocorticoteroids (37). The radiation control rats, which should have had greater stress-related involution, actually had better repopulation than the allogeneic marrow recipients. When GVHD is prevented in Rt-l mismatched chimeras, there is good reconstitution with donor lymphocytes, indicating that the atrophy is not caused by a failure of alloantigen recognition by donor cells (38). Possible causes of prolonged atrophy would include destruction or inhibition of radioresistant lymphocytes (39), and alterations of the microenvironment determined by the epithelium.

Whereas the spleen in syngeneic marrow recipients had a transient immunoblast phase followed by apparent maturation by 21 days, the spleen in recipients of allogeneic marrow had a prolonged immunoblast response in the periarteriolar region through Day 17, followed by a dramatic lymphoid depletion. While the periarteriolar region in the normal spleen contains predominantly T lymphocytes (40, 41), we cannot necessarily extrapolate this organization to the early chimeric spleen. The marked depletion which followed shortly thereafter suggests that these immunoblasts were not immediate precursors of normal spleen T lymphocytes. Could the subsequent atrophy of the white pulp be due to failure of the thymus or to a type of allogeneic restriction? These spleens were much more atrophic, particularly in the eccentric regions, than in patients with DiGeorge's syndrome or thymectomized rats reconstituted with syngeneic marrow, suggesting that thymic atrophy alone was not responsible. The good reconstitution ob-

served in allogeneic recipients when GVHD was prevented also suggests that GVHD-associated atrophy was not just a failure of alloantigen distinct lymphocytes to recognize the appropriate splenic regions. The severe atrophy in the radiation control group indicates that most of the host's spleen lymphocytes were radiosensitive and that the atrophy in allogeneic recipients was due to suppression or failure of reconstitution rather than a cytotoxic effect of donor lymphocytes against necessary host cells.

As was the case with the thymus, the lymph nodes had a number of lymphoid cells which were radioresistant at this level of therapy. All radiation controls (Days 2–8) had small lymphocytes in the primary follicles and many plasma cells about the medullary cords. Populations of resistant lymphoid cells have been observed in other rat systems receiving lethal irradiation (42) or high-dose cyclophosphamide (43). These cells could be important in stimulating allogeneic cells or in providing residual immune protection to radiation controls and syngeneic recipients. Allogeneic typing in rats recovering from GVHD and in allogeneic chimeras in whom GVHD is prevented indicate that these cells are eliminated in the chimera (32, 38). Following syngeneic marrow transplantation, there was an early immunoblast response in the region of the primary follicles with increasing numbers of small lymphocytes in the superficial secondary follicles. The paracortical regions (putative T lymphocyte region) (40, 41, 44, 45), which were distinguished from the deep primary follicles by the presence of numerous venules, was hypocellular until 17 days post-transplant when it underwent a blast phase. By 21 days the primary and secondary follicles had a nearly normal appearance.

The early response in allogeneic recipient lymph nodes was strikingly different with an early immunoblast response accompanied by small vessel proliferation in the paracortical region. On the other hand, there was no blast response in the primary follicles. Later the lymphoid proliferation became diffuse, obliterating the discernable architecture. If in fact the paracortical blast response does represent a T-lymphoblast response, then the early lymph nodes in allogeneic marrow recipients would be characterized not only by a T-cell proliferation, but also by absent or suppressed B-cell proliferation. This would distinguish the radiation chimera from newborn semiallogeneic models in which there is a host B-cell proliferation (46, 47).

As in other models and studies, the target organ injury clearly followed the lymphoid proliferation. While all rats sacrificed at Day 5 had an established immunoblastic process in the lymph nodes and spleen, target tissues showed mild changes at Day 8 and well established injury at Day 14. The tongue had the earliest injury and was the most sensitive nonlymphoid tissue for monitoring GVHD. Along with the tongue, there was in some rats injury to lobular hepatocytes, intestinal crypt epithelium, conjuctivae epithelium, and exocrine pancreas. Additional tissues consistently injured later than 14 days included skin epithelium and hair follicles, esophagus, urinary bladder, and less frequently, hepatic bile ducts, the bronchial mucosa, salivary and lacrimal gland duct epithelium, and renal pelvis. One cannot exclude a role for infection, such as coronavirus, rat hepatitis virus, or mycoplasma pulmonis contributing to injury of various target tissues

(48–50). We believe, however, that these tissues are injured by the same mechanism for the following reasons. The lesions were absent in syngeneic recipients, Day 8 radiation controls, and (excluding the intestine) allogeneic recipients before Day 8. The histologic pattern was similar with lymphocytic infiltration, intersitial edema, and vacuolization and necrosis of target cells. Finally, the presence and severity of injury was proportional to the degree of inflammation and injury in the tongue.

Tissues which were lined with squamous epithelium were not all injured in the same manner or at the same time. While the tongue was most consistently and severely effected, injury was less frequent and severe in the skin and conjunctivas and least evident in the esophagus. This could reflect either differences in antigen density such as the number of Langerhans cells bearing Ia like or organ specific antigens (51, 52), differences in vascularity, differences in antigen accessability, or even differences in susceptability of epithelial cells to injury.

The liver in allogeneic marrow recipients had predominantly lobular foci of hepatocellular necrosis in the early injury phase and bile duct injury and periportal piecemeal necrosis by Day 28. In this respect, the rat more closely parallels liver GVHD in the human (53) than in the dog (54) which had early bile duct injury. In human recipients of HLA mismatched marrow there is an earlier onset of GVHD with predominantly hepatocellular injury as compared to a later onset of GVHD and predominantly bile duct injury in recipients of marrow from HLA identical siblings.

A notable clinical distinction between the rat and human GVHD has been the relatively mild extent of intestinal involvement. While our rats with GVHD did have diarrhea or loose stools, we, as have others, did not observe the same extent of mucosal and crypt destruction often present in man (1–4, 14, 55). One possible explanation is a greater capacity for repair in the rat. Whereas the human intestine can require as much as 3 weeks to repair from radiation injury (56) the irradiated rats had nearly complete repair with tall villi and numerous crypt mitoses by Day 5 and the syngeneic recipients and radiation controls had no evidence of mucosal injury by Day 8.

It has been proposed that the crypt karyolytic bodies in GVHD are due to an impaired regeneration from radiation damage (57). Indeed, they are morphologically very similar and in this study there were no rats which demonstrated complete intestinal repair prior to the onset of tongue and intestinal GVHD.

The significance of these karyolytic bodies in the rat model must be questioned, however, because they were very sparse (one to two per 10 high power fields) compared to the numerous crypt mitoses and moderate to severe villous atrophy and crypt hyperplasia. A second contributing factor to mucosal damage would be an associated deficiency of local intestinal immunity in the conventionally raised rat. In contrast to the syngeneic recipients and even radiation controls, there was marked plasma cell depletion by Day 5 and absence after Day 8 in the allogeneic marrow recipients. GVHD has been observed to prevent the normal development of intestinal immunity in newborn mice (58) and to deplete intestinal plasma cells in man (55). The significance of eliminating host plasma cells from the intestine is suggested by studies which observe karyolytic bodies but minimal diarrhea or

mortality in germ-free chimeras (59, 60) and increased mortality with defined bacterial contamination (61). While the infectious agents were not identified in the present model, superinfection was suggested by increased neutrophillic infiltrate and villous flattening in the later periods.

Additional tissues which had injury consistently associated with GVHD in the allogeneic marrow recipient included exocrine pancreas, urinary bladder, renal pelvis, and by Day 28, the bronchi, serous salivary glands, and lacrimal glands. Involvement of the pancreas and urinary bladder has been described in mouse chimeras (20, 62), the bronchi, pancreas, and urinary bladder in canine chimeras (63), and the bronchi, salivary glands, and lacrimal glands in human chimeras (27, 28, 64).

In a dose–response manner, transplantation of allogeneic spleen lymphocytes added to marrow leads to a more severe GVHD as indicated by earlier onset and higher mortality (22). In this study, the added spleen cells did not produce an earlier onset, greater weight loss, or higher mortality. It is thus likely that the cell dose was insufficient for a clinically more severe GVHD. Compared to recipients of allogeneic marrow alone, however, the lymphoid and target tissues were altered in some unexpected ways.

The thymus had a more prolonged partial repopulation of the cortex and medulla and lymphocytic atrophy was delayed until Day 28. The spleen qualitatively resembled that of allogeneic marrow recipients although the immunoblast response appeared more intense. The lymph nodes had an early mild immunoblast response in the primary follicles and persistence of primary and secondary follicular lymphocytes. Lymph nodes of allogeneic marrow recipients, on the other hand, contained only small lymphocytes in the primary follicles early and no follicular immunoblasts until Day 28.

Added spleen cells altered the tissue injury both by histologic pattern and kinetics. From the onset of GVHD at 8 days the skin had more acanthosis, dermal and perivascular infiltrates, and thickened dermal collagen resembling early chronic GVHD. Also glandular tissues, including salivary glands, lacrimal glands, bronchial mucosa, and glands and hepatic bile ducts, which otherwise were not involved until Day 28, were effected from the onset of GVHD at Day 8.

The resemblance of these changes in the skin, salivary glands, and lacrimal glands to chronic GVHD as described in the long-term rat (31) and human (27–30) chimeras was striking. Chronic GVHD, with associated nonspecific suppressor cells, "autoantibodies," and organ manifestations resembling autoimmune disorders such as progressive systemic sclerosis and keratoconjunctivitis sicca is usually considered to be an expression of immune dysregulation (28, 30, 65). In contrast, acute GVHD is thought to result from a cytotoxic immune response to alloantigens (46). The cellular makeup of the donors' spleens was not defined in our study. They did, however, contain either effector or regulator cells capable of modulating the marrow lymphocytes to produce a chronic-type GVHD in the short-term chimera.

The goal of this description of GVHD in the rat radiation chimera was to illustrate the value of histology in delineating the kinetics of lymphoid changes and tissue injury with GVHD, in dissecting the cellular components of tissue injury, in

recognizing injury in tissues not classically associated or clinically apparent, and in distinguishing patterns of GVHD under different transplantation conditions.

REFERENCES

1. Slavin, R. E., and Santos, G., *Clin. Immunol. Immunopathol.* **1**, 472, 1973.
2. Lerner, K. G., Kao, G. F., Storb, R., Buckner, C. D., Clift, R. A., and Thomas, E. D., *Transplant. Proc.* **1**, 367, 1974.
3. Kruger, G. R. F., Berard, C. W., DeLellis, R. A., Graw, R. G., Jr., Yankee, R. A., Leventhal, B. G., Halterman, R. H., and Henderson, E. S., *Amer. J. Pathol.* **63**, 179, 1971.
4. Kersey, J. H., Meuwissen, H. J., and Good, R. A., *Human Pathol.* **2**, 389, 1971.
5. Claudy, A. L., Schmitt, D., and Freycon, F., *Acta Derm.-Venereol.* **59**, 7, 1979.
6. Sale, G. E., Lerner, K. G., Barker, E. A., Shulman, H. M., and Thomas, E. D., *Amer. J. Pathol.* **89**, 621, 1977.
7. deVries, M. J., Crouch, B. G., van Putten, L. M., and van Bekkum, D. W., *J. Nat. Canc. Inst.* **27**, 74, 1961.
8. Woodruff, J. M., Eltringham, J. R., and Casey, H. W., *Lab. Invest.* **20**, 499, 1969.
9. Kolb, H., Sale, G. E., Lerner, K. G., Storb, R., and Thomas, E. D., *Amer. J. Pathol.* **96**, 581, 1979.
10. Streilein, J. W., and Billingham, R. E., *J. Exp. Med.* **132**, 163, 1970.
11. Simonsen, M., *Prog. Allergy* **6**, 349, 1962.
12. Nisbet, N. W., Heslop, B. F., and Zeiss, I. M., *Proc. Univ. Otago. Med. Sch.* **38**, 11, 1960.
13. Billingham, R. E., Brown, J. B., Defendi, V., Silvers, W. K., and Steinmuller, D., *Ann. N. Y. Acad. Sci.* **87**, 457, 1960.
14. Balner, H., de Vries, M. J., and van Bekkum, D. W., *J. Natl. Canc. Inst.* **32**, 419, 1964.
15. Stastny, P. Stembridge, V. A., and Ziff, M., *J. Exp. Med.* **118**, 635, 1963.
16. Vos, O. de Vries, M. J., Collenteur, J. D., and van Bekkum, D. W., *J. Nat. Cancer Inst.* **23**, 53, 1959.
17. de Vries, M. J., and Vos, O., *J. Nat. Canc. Inst.* **23**, 1403, 1959.
18. Sandberg, J. S., Owens, A. H., Jr., and Santos, G. W., *J. Nat. Cancer Inst.* **46**, 151, 1971.
19. Gorer, P. A., and Boyse, E. A., *J. Immunol.* **2**, 182, 1959.
20. Rappaport, H., Khalil, A., Halle-Pannenko, O., Pritchard, L., Dantchev, D., and Mathe, G., *Amer. J. Pathol* **96**, 121, 1979.
21. Porter, K. A., *Brit. J. Cancer* **14**, 66, 1959.
22. O'Kunewick, J. P., Meredith, R. F., Brozovich, B. J., Seeman, P. R., and Magliere, K., *Transplantation* **29**, 507, 1980.
23. Yoshida, Y., and Osmond, D. G., *Transplantation* **12**, 121, 1971.
24. Monie, H. J., and Everett, N. B., *Anat. Rec.* **179**, 19, 1974.
25. Twist, V. W., and Barnes, R. D., *Transplantation* **15**, 182, 1973.
26. Bonney, W. W., and Feldbush, T. L., *Transplantation* **15**, 215, 1973.
27. Shulman, H. M., Sale, G. E., Lerner, K. G., Barker, E. A., Weiden, P. L., Sullivan, K., Galluci, B., Thomas, E. D., and Storb, B., *Amer. J. Pathol.* **91**, 545, 1978.
28. Shulman, H. M., Sullivan, K. M., Weiden, P. L., McDonald, G. B., Striker, G. E., Sale, G. E., Hackman, R., Tsoi, M. S., Storb, R., and Thomas, E. D., *Amer. J. Med.* **69**, 204, 1980.
29. Lawley, T. J., Peck, G. L., Haralampos, M. M., Gratwohl, A. A., and Deisseroth, A. B., *Ann. Int. Med.* **87**, 707, 1977.
30. Graze, P. R., and Gale, R. P., *Amer. J. Med.* **66**, 611, 1979.
31. Beschorner, W. E., Tutschka, P. J., and Santos, G. W., *Transplantation*, 1982, in press.
32. Tutschka, P. J. Hutchins, G. M., and Santos, G. W., *In* "Experimental Hematology Today, 1979" (S. J. Baum and G. P. Ledney, Eds.), pp. 113–121, Springer-Verlag, New York, 1979.
33. Loew, F. M., Tutschka, P. J., Strandberg, J. D., and Hopp, D. H., *In* "29th Annual Session, American Association Laboratory Animal Science, New York, N. Y." 1978.
34. Cramer, D. V., David, B. K., Shonnard, J. W., and Gill, T. J., III, *Transplantation* **23**, 498, 1977.
35. Beschorner, W. E., Hutchins, G. M., Elfenbein, G. J., and Santos, G. W., *Amer. J. Pathol.* **92**, 173, 1978.
36. Swasdikul, D., and Block, M., *Radiat. Res.* **50**, 73, 1972.

37. Seemayer, T. A., Lapp, W. S., and Bolande, R. P., *Amer. J. Pathol.* **93**, 325, 1978.
38. Tutschka, P. J., Beschorner, W. E., Allison, A. C., Burns, W. H., and Santos, G. W., *Nature (London)*. **280**, 148, 1979.
39. Kadish, J. L., and Basch, R. S., *J. Immunol.* **114**, 452, 1975.
40. Schoorl, R., de la Rivière, A. B., von dem Borne, A. E. G. K., and FeltkampVroom, T. M., *Amer. J. Pathol.* **84**, 529, 1976.
41. Knowles, D. M., II, and Holck, S., *Lab. Invest.* **35**, 70, 1978.
42. Miller, J. J., and Cole, L. J., *J. Immunol.* **98**, 982, 1967.
43. Miller, J. J., and Cole, L. J., *J. Exp. Med.* **126**, 109, 1967.
44. Poppema, S., Bhan, A. K., Reinherz, E. L., McCluskey, R. T., and Schlossman, S. F., *J. Exp. Med.* **153**, 30, 1981.
45. Seymour, G. J., Greaves, M. F., and Janossy, G., *Clin. Exp. Immunol.* **39**, 66, 1980.
46. Grebe, S. C., and Streilein, J. W., *Advan. Immunol.* **21**, 119, 1976.
47. Gleichmann, E., Gleichmann, H., and Wilke, W., *Transplant Rev.* **31**, 156, 1976.
48. McIntosh, K., *Curr. Top. Microbiol. Immunol.* **63**, 85, 1974.
49. Margolis, G., Kilham, L., and Ruffolo, P. R., *Exp. Mol. Pathol.* **8**, 1, 1968.
50. Lindsey, J. R., Baker, H. J., Overcash, R. G., Cassell, G. H., and Hunt, C. E., *Amer. J. Pathol.* **64**, 675, 1971.
51. Rowden, G., Lewis, M. G., and Sullivan, A. K., *Nature (London)* **268**, 247, 1977.
52. Steinmuller, D., and Lofgreen, J. S., *Transplant. Proc.* **9**, 1385, 1977.
53. Beschorner, W. E., Pino, J., Boitnott, J. K., Tutschka, P. J., and Santos, G. W., *Amer. J. Pathol.* **99**, 369, 1980.
54. Sale, G. E., Storb, R., and Kolb, H., *Transplantation* **26**, 103, 1978.
55. Beschorner, W. E., Yardley, J. H., Tutschka, P. J., and Santos, G. W., *J. Infect. Dis.* **144**, 38, 1981.
56. Epstein, R. S., McDonald, G. B., Sale, G. E., Shulman, H. M., and Thomas, E. D., *Gastroenterology* **78**, 764, 1980.
57. Nowell, P. C., and Cole, L. J., *Transplant. Bull.* **8**, 94, 1961.
58. Gold, J. A., Kosek, J., Wanek, N., and Baur, S., *J. Immunol.* **117**, 471, 1976.
59. van Bekkum, D. W., de Vries, M. J., and van der Waay, D., *J. Nat. Cancer Inst.* **38**, 223, 1967.
60. Jones, J. M., Wilson, R., and Bealmear, P. M., *Radiat. Res.* **45**, 577, 1971.
61. Pollard, M., Chang, C. F., and Srivastava, K. K., *Transplant. Proc.* **8**, 533, 1976.
62. Nizze, H., *Exp. Pathol.* **16**, 254, 1978.
63. Zurcher, C., Van Kessel, A. C. M., and Vrisendorp, H. M., *In*. "Annual Report to the Trustees, Radiobiological Institute, Rijswijk, 1978," pp. 278–280.
64. Beschorner, W. E., Saral, R., Hutchins, G., Tutschka, P. J., and Santos, G. W., *N. Engl. J. Med.* **299**, 1030, 1978.
65. Tsoi, M. S., Storb, R., Dobbs, S., Kopecky, K. J., Santos, E., Weiden, P. L., and Thoimas, E. D., *J. Immunol.* **123**, 1970, 1979.

Received July 28, 1981; accepted August 26, 1981.