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Pathology of the Thymus After Allogeneic Bone Marrow Transplantation in Man

A Histologic Immunohistochemical Study of 36 Patients

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A major hypothesis to explain the immunodeficiency associated with bone marrow transplantation states that thymic epithelial damage due to graft-versus-host disease (GVHD) abrogates or delays the recovery of normal immunologic function. This study evaluated the thymus glands of 36 human bone marrow transplant recipients dying between 4 and 1742 days after transplant using histology, histochemistry, and immunohistology. The observations lead to a model of thymic damage by irradiation, chemotherapy, and GVHD in which early injury by all three of these agents results in profound thymic atrophy followed by long-delayed restitution. Patients undergoing total body irradiation showed more severe damage to thymic cortical and medullary epithelium than did patients undergoing chemotherapy alone as preparation for transplantation. Patients with GVHD showed additional damage in the form of individual thymic epithelial cell death and showed HLA-DR surface protein expression on thymic epithelium during GVHD. Longer-term survivors showed a profoundly delayed restitution of normal thymic epithelium and delayed evidence of restored lymphopoiesis. A few patients dying late after transplant showed evidence of reconstitution of normal thymic structure or nodules of lymphopoiesis in focal areas of epithelial-cell reconstitution. Evidence of such lymphopoiesis was seen at times ranging between 90 and 1742 days after grafting. The data are consistent with a model of long-standing thymic damage caused by GVHD which is reversible after the development of tolerance. (Am J Pathol 1987, 129:242–256)

BONE MARROW transplantation (BMT) in man is now established as an effective therapeutic approach to many diseases, most characterized by severe hemopoietic insufficiency or hematologic neoplasia.[1–5] Recovery of hematopoiesis after lethal total body irradiation (TBI), chemotherapeutic conditioning, and allogeneic BMT usually does not take longer than 3 weeks. The main morbidity and mortality of BMT patients is due to their susceptibility to infections and to graft-versus-host disease (GVHD).[6] Therefore, recovery of the host's immunologic reactivity after BMT has to be considered as one of the decisive problems for the ultimate outcome of this therapy. Although peripheral lymphocyte counts rapidly return to normal or only slightly subnormal values after BMT, the analysis of T-cell subtypes and immune function reveals long-lasting abnormalities that are most pronounced in older patients and those with GVHD.[7–16] Significant numbers of T cells of the helper subtype with normalization of the T4/T8 ratio of peripheral blood lymphocytes return very slowly.

The function of the thymus as the central lymphoid organ of T-cell lymphopoiesis is now considered lifelong.[17] Because thymectomized animals or animals

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with genetic thymic defects do not seem to show recovery of normal immunologic function after lethal irradiation and syngeneic or allogeneic BMT, immunologic reconstitution in human BMT recipients may also be a function of thymus gland regeneration.

Studies of the thymus microenvironment in recent years show that lymphopoiesis is generated and influenced within the epithelial cell-defined space of the thymic cortex and medulla by a variety of differentiated epithelial cells which may be recognized in man by cytologic and ultrastructural features and by different immunologic phenotypes.[18-20] During ontogenesis, this complicated structural interaction is formed by the primordial thymus anlage derived partially from the endoderm of the third pharynygeal pouch. Only the competence and interaction of ectoderm and epithelial anlage lead to normal lymphoid colonization and lymphopoiesis.[21-23]

So far histologic data on thymic restitution after BMT have been reported in only a few studies.[24-27] In all cases severe atrophy of the thymus gland was reported without evidence of significant lymphopoiesis. Because clinical studies have shown recurrent immune function in long-term survivors of allogeneic BMT,[28] the lack of reconstituted specimens was attributed largely to the paucity of specimens from longer-term survivors in these studies. Moreover, thymic atrophy may also be caused by other factors. It has been known to be a major sign of GVHD for some time.[29-31] Seemayer and colleagues[32] showed that the destructive action of the graft-versus-host reaction (GVHR) is focused on thymic epithelial cells in mice.

The inability of the thymus after GVHD damage to generate normal lymphopoiesis thus eventually may lead to an indefinite period of immunodeficiency. Furthermore, pretransplantation treatment of patients with hematologic neoplasia or aplastic anemia may damage thymic epithelial cells to such an extent that effective lymphopoiesis may become impossible, especially if age involution has already caused a significant reduction of its physiologic reserve.

The present study of thymus pathology after BMT considers a large sample of cases ranging from Day 4 until 5 years after BMT. The different effects of conditioning and pretreatment regimens in aplastic anemia and leukemia patients are described. Definite cytodestructive effects of lymphocytes on the thymic epithelial cells in the early phase after BMT may be part of a generalized or localized GVHR and may lead to severe irreparable thymic atrophy. On the other hand, even after a moderate GVHR eventually some restitution of thymic lymphopoiesis may be found. The histologic and immunohistologic findings of individual cases suggest a definite time course of sequentially occurring changes leading to thymic lymphopoiesis within small epithelial defined areas as early as 3 months after BMT.

Materials and Methods

All autopsy reports done at the Fred Hutchinson Cancer Research Center have been reviewed if thymus histology was reported. Then available autopsy reports of patients dying later than Day 80 after transplantation were reviewed. For selected cases undergoing autopsy elsewhere after leaving Seattle, slides and blocks were requested.

In order to obtain controls, we reviewed autopsy reports of BMT in identical twins and autologous BMT. However, only one specimen of thymus tissue Day 26 after autologous BMT was available that was not considered as representative. Two identical twin transplant cases were excluded because of extensive mediastinal irradiation and cytostatic treatment for relapse of the original leukemia.

Thirty-six autopsies with tissue blocks and slides of thymus were collected (Table 1). Since the beginning of the study, 7 of the autopsies also had thymus processed for immunohistology. Patients older than 35 years and those after the second or third BMT for leukemia have been excluded. Because conditioning and pretreatment of these cases collected over several years were not exactly comparable, stratification was made only between patients with aplastic anemia, where conditioning does not usually include TBI, and acute leukemia, where patients received cytotoxic treatment prior to BMT as well as TBI.

Light Microscopy

Each case had formalin-fixed paraffin-embedded tissue available for investigation using several special stains hematoxylin and eosin ([H&E], periodic acid–Schiff [PAS], Giemsa, silver impregnation, trichrome).

Immunohistology

Immunohistology on cryostat sections was performed on 7 cases. Table 2 details the primary monoclonal antisera as well as their specificity and source. For detection of immunoreactivity, single or double indirect immunoperoxidase techniques using peroxidase-conjugated rabbit-anti-mouse Ig and goat-anti-rabbit Ig were used. The standard peroxidase technique of Graham and Karnovsky (1966) using diaminobenzidine (Sigma, St. Louis) as coupling reagent was used.

Table 1—List of Patients

No.	UPN	Autopsy	Day after transplant	Age at death (yr)	Sex	Diagnosis	GVHD (clin) clin grade II–IV*	GVHD (path)	Infections
1	2766	OA85-13	4	22	M	AML	–	–	–
2	2428	OA84-26	14	36	F	CML	+	+	–
3	2701	OA85-06	17	15	F	ALL	–	–	*Pseudomonas* *Candida*
4	2529	OA85-38	20	13	M	PML	–	–	–
5	554	OA76-02	19	4	F	AL	–	–	*Aspergillus* *Candida*
6	2551	OA84-48	19	24	F	ANL	–	+	CMV
7	621	OA76-37	30	19	M	AA	Reject	–	*Pseudomonas* *Klebsiella* *Aspergillus*
8	1212	OA80-64	59 (30)	20	M	AA	+	+	CMV
9	966	OA79-16	32	23	M	AL	–	–	–
10	1270	OA80-74	33	3	M	AL	–	–	*Candida*
11	755	OA77-41	34	32	M	AA	–	–	*Candida*
12	790	OA78-14	49	5	M	AL	+	–	CMV
13	2963	OA85-68	54	5	M	ALL	–	–	CMV
14	713	OA77-34	59	28	M	CML blast	–	–	CMV *Pneumocystis carinii*
15	990	OA79-23	60	10	F	AML	–	–	CMV
16	939	OA79-10	62	16	M	AA	+	+	*Escherichia coli* *Candida*
17	761	OA78-05	63	4	F	AML	+	+	*E coli*
18	392	OA75-17	87	32	M	AMML	+	+	–
19	2470	OA84-47	84	5	M	CML	+	(–)	CMV
20	1079	OA80-09	88	21	M	AML	+	+	–
21	887	OA78-38	93	13	M	ALL	–	–	CMV, *E coli*
22	1568	OA82-20	95	21	M	ALL	–	–	CMV
23	1624	OA82-34	98	14	F	AML	+	+ Severe	Adenovirus
24	1765	OA82-62	98	10	M	ALL	+	+	*Staphylococcus*
25	1402	OA81-43	99	24	M	ALL	+	+	Varicella-zoster *Aeromonas* *Clostridium perfringens*
26	609	OA76-44	120	19	M	ALL-T	+	+	CMV toxoplasmosis
27	2343	OA84-43	177	37	F	AML	+	+	CMV
28	260	OA77-11	346	12	F	ALL	+	–	CMV adenovirus Herpes virus *Pneumococcus* *Haemophilus*
29	2569	OA85-58	349	15	M	AMML	+	+	–
30	394	OA76-16	350	13	F	AA	+ Severe	+ Chron	Diverse bacteria *Candida Giardia*
31	677	OA79-04	651	30	M	ALL	+	+	gram-positive bacteria *Pseudomonas*
32	366	OA76-38	719	36	F	AA	+	+ Chron	–
33	585	OA78-24	726	32	M	AA	+	+ Chron	*Pseudomonas* *Pneumocystis*
34	873		1020	14	F	ALL	–	–	Septicemia, not specified
35	510	OA83-26	1725	18	M	ALL	–	–	–
36	644	OA83-12	1742	16	M	ALL	(+)	–	–

*GVHD of histologic Grade II of at least one of the 3 major target organs (skin, liver, and gut) at the time of autopsy. In some chronic cases, active inflammation may be absent at autopsy. In other cases, ongoing inflammatory activity is evident. Case 644, for example, had prior histologically proven chronic GVHD, which had resolved well before accidental death.

Table 2—List of Antibodies Used in This Study

Antibody	CD no.	Antigen or cells identified	Source
Anti-Leu-1	5	All T cells	Becton Dickinson, Oxnard, CA
Anti-Leu-2a	8	Suppressor, cytotoxic subset of T cells	Becton Dickinson, Oxnard, CA
Anti-Leu-3a	4	Helper/inducer subset of T cells	Becton Dickinson, Oxnard, CA
Anti-Leu-4	3	Sheep erythrocyte receptor positive cells	Becton Dickinson, Oxnard, CA
Anti-Leu-7		Human natural killer cells	Becton Dickinson, Oxnard, CA
Anti-HLA-DR	n/a	HLA-DR antigens	New England Nuclear, Cambridge, MA
IV/82	n/a	Squamous epithelium keratin	Institute of Pathology, Kiel, FRG
35 beta H 11	n/a	54-kd keratin(s) in most nonsquamous epithelia	Gown and Vogel J Cell Biol 1982, 95:414
KiM8	n/a	Macrophages	Dr. Radzun, Kiel, FRG
OKT6	1	Human common thymocytes Interdigitating cells Langerhans cells of epidermis	Ortho Diagnostics
MASO 36C	1	Human common thymocytes	Sera-lab
Anti-B cells	22	All human B cells	DAKO

Results

We here describe the general features of thymic histology, first by comparing recipients of differing conditioning regimens (aplastic anemia [AA] versus leukemia patients) and then in general terms by temporal group (eg, early or late). The data on patients with and without GVHD are discussed within each temporal group.

Effects of Different Types of Pretransplant Treatment and Conditioning on Thymus Structure after BMT (AA versus Leukemia)

In the early posttransplant phase, differences of thymus structure of AA patients and leukemia patients are evident: In AA patients the epithelial structure appears to be largely preserved. It consists of a row of (prismatic) subcapsular epithelial cells that are overlain by basement membrane. Toward the medullary region medium-sized, densely packed epithelial cells show a more irregular three-dimensional orientation. In the medulla, large Hassall's corpuscles are found that may even be larger than before transplantation (Figure 1a). These solid strands of epithelial cells are surrounded by a moderately dense connective tissue capsule and mature fatty tissue. In leukemia patients the epithelial thymus is more atrophic. Hassall's bodies are infrequent. Only small areas of concentrically layered epithelial cells in the center of epithelial strands reveal their previous locations (Figure 1b). Up to 3 months after BMT, no thymic lymphopoiesis is found in either AA or in leukemia patients. Some cases already show in the early posttransplant phase an extreme atrophy leaving only thin strands of epithelial cells and connective tissue remnants of thymic tissue.

Alterations of Thymic Remnant During the First and Second Month After BMT

The first alterations within the epithelial remnant are seen at Day 15–20 after BMT. These consist of changes of the epithelial structure as well as of the lymphoid cells. In the epithelial remnant of very early cases after BMT (Day 4) epithelial cells are morphologically diverse. In specimens obtained beyond Day 18–20 after transplantation, the epithelial structure becomes more uniform (Figure 2). It consists mainly of central dense strands of fusiform epithelial cells sometimes surrounded by a prismatic layer of subcapsular epithelial cells. This layer is frequently lacking at later stages. A cortical area cannot be distinguished. The connective tissue surrounding the dense epithelial strands contains many dilated capillaries. In later stages (several weeks), increased collagen deposition leads to a thickening of the thymus capsule. At Day 18–20 an increase of lymphoid cells is found in the central (medullary) areas of the thymus. These lymphocytes are mainly small- to medium-sized and contain pleomorphic nuclei. The infiltration appears to be denser around Hassall's corpuscles, sometimes with infiltration of the outer layer of epithelial cells by lymphoid cells. Individual epithelial cell necrosis may be seen as well as a spongiotic change of Hassall's corpuscles. These features, however, are more prominent in specimens at 30 days after transplantation, when lymphocytes may also be seen in the thymic capsule and among the capillary-rich subcapsular region. After Day 20 after transplantation (Day 20–40) the lymphocytes within the epithelial remnant are diffusely distributed. In many cases individual epithelial cell necrosis within the Hassall's corpuscles may be seen. In some specimens also an increase of foam cells containing cellular debris is localized among epi-

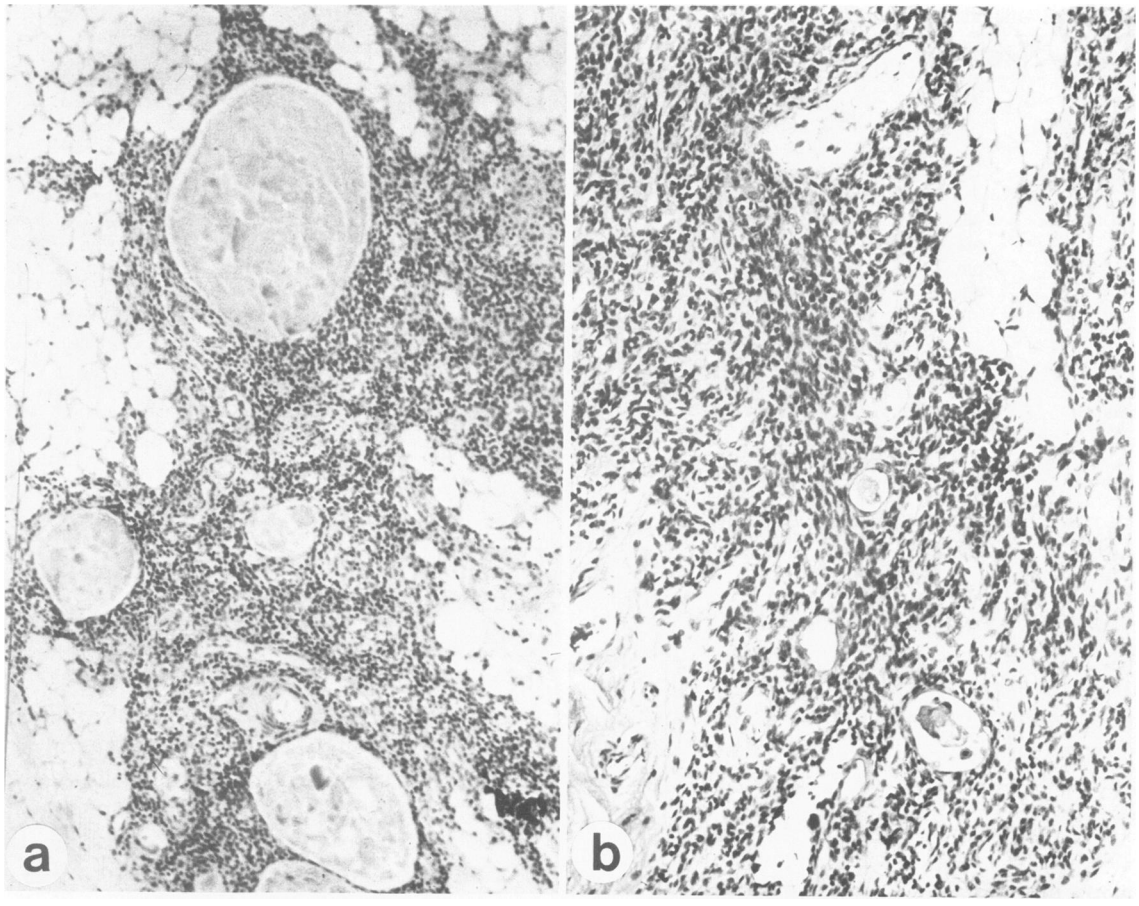

Figure 1—Early changes of thymus after BMT. The thymus consists of almost purely epithelial component, showing some lobular arrangement. Epithelial cells are spindle-shaped and isomorphic. **a**—UPN 621, Day 30 after BMT, aplastic anemia. Note large cystic Hassall's corpuscles. (H&E, ×64) **b**—UPN 554, Day 19 after BMT, acute leukemia. Hassall's corpuscles are visible as small concentrically arranged epithelial cells. The epithelial part of the thymus is more atrophic than in **a**. Note difference in magnification. (H&E, ×160)

thelial cells in the area beneath subcapsular epithelial cells. Lymphocytes are also found in the thymic capsule and around the dilated subcapsular capillaries. The infiltration in some cases is very intense, partially obscuring the limits of the epithelial space. Histologic evidence of thymic epithelial cell damage is especially evident when generalized GVHD is present. In the absence of generalized GVHD lymphoid cells within the thymus are less numerous. Necrotic changes of individual epithelial cells may, however, also be found. At 2 months after BMT, the thymus remnant is very atrophic. By comparison, it appears that the epithelial cell damage found at earlier times had led to a more pronounced loss of the epithelial tissue of the thymus. In contrast, the lymphoid infiltration within the epithelial space is less marked than earlier. Individual cell necrosis is only rarely found. The connective tissue of the thymic capsule, however, may contain numerous lymphoid cells, sometimes arranged as small nodular areas around dilated capillaries. In silver-stained slides the atrophy of the thymic epithelial space is especially evident. An increase of reticulin fibers around capillaries is found at places that formerly must have been cortical thymic tissue. Some plasma cells may be found here as well. The border of the epithelial remnant is usually not distinct. With the help of the silver stain and particularly the immunohistochemical stains for keratin, "open" connections between the surrounding connective tissue and interepithelial areas become evident.

Changes of Thymic Remnant in the Third and Fourth Month After BMT—Early Restitution or Persistent Atrophy

In the third month, characteristically, the lymphoid infiltration around subcapsular capillaries is pronounced and often focally increased. Cytologically,

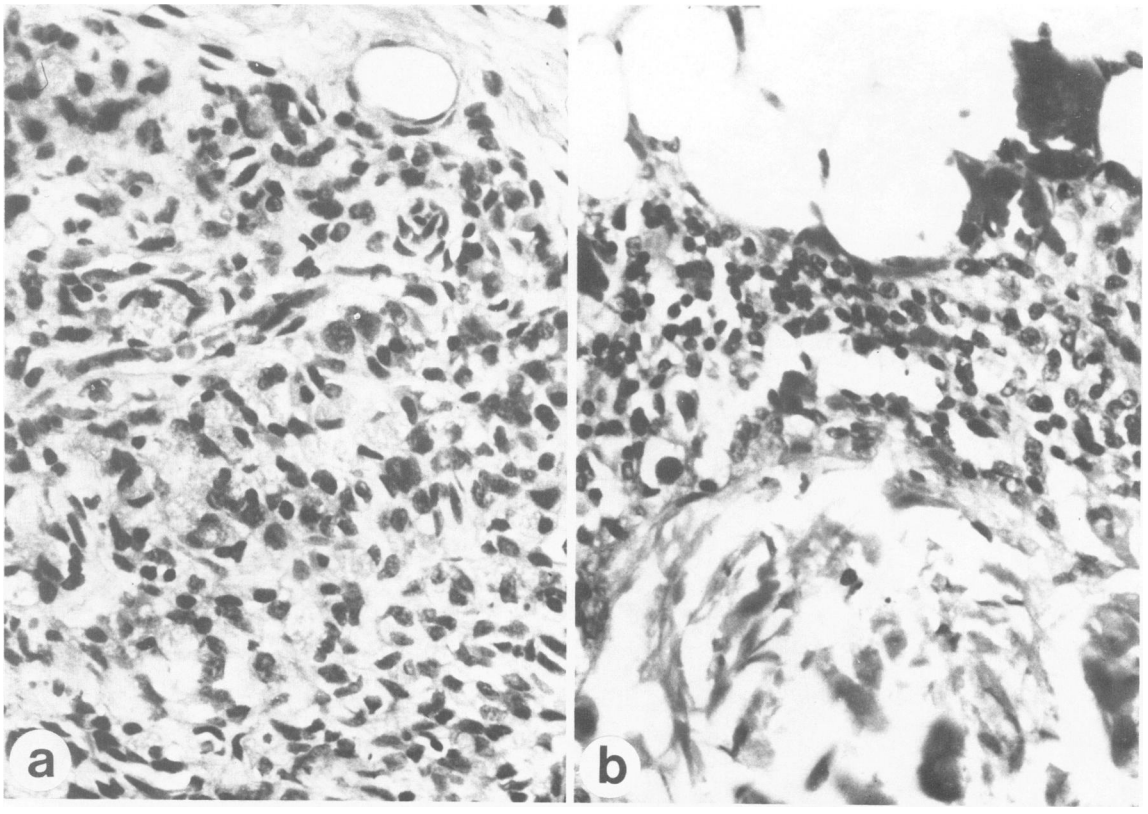

Figure 2—Histologic view of changes suggestive of intrathymic GVHR. UPN 1212, Day 30 after BMT after second BMT in aplastic anemia. (H&E, ×400) **a**–Foamy macrophages in between epithelial remnant. **b**—Apoptotic single cell necrosis and lymphoid infiltration at the margin of a Hassall's corpuscle.

not only lymphocytes but also plasma cells and sometimes hematopoietic cells are found.

Around Day 90 after transplantation, signs of restitution are observed. Among the epithelial cells of the subcapsular layer, as far as it is preserved, putatively immature lymphoid cells are found, with pleomorphic open nuclei and a small rim of basophilic cytoplasm (Figure 3a). One specimen (UPN 1097) shows small nodular accumulations of lymphoid cells interpreted as a sign of returning cortical lymphopoietic activity (Figure 3b). Other specimens, however, at the same time after transplantation, with similar age and pretreatment, show persistent atrophy with no sign of lymphoid restitution. Epithelial cells are pleomorphic, with oxyphilic, rather broad cytoplasm. The epithelial cell nuclei in some cases show marked anisokaryosis and also the formation of giant cells.

Late Changes of Thymus After BMT

Most autopsy thymi investigated 6 months and later after transplantation show atrophy without signs of active cortical lymphopoiesis (Figure 4a). The Hassall's corpuscles are absent or extremely small even in cases without irradiation (AA). Silver staining reveals almost no epithelial cells but largely fibrotic tissue containing blood vessels and mature small lymphocytes and plasma cells. Remaining foci of epithelial cells consist of uniform oxyphilic cells with marked anisokaryosis lacking lymphocytic infiltration. Two cases in the series, however, showed distinctive signs of reconstituting thymic function. The first case (UPN 644) was a boy who died at the age of 13 from an automobile accident who had received a BMT from his MLC histocompatible sister at the age of 8. Localized chronic GVHD of the skin developed within the first year after transplantation, but resolved. Two years and 8 months after transplantation, immunologic findings were largely normal. Infection with measles 6 months before his death showed a normal course. The thymus of this boy grossly shows normal structure (Figure 4b). Cortical and medullary areas are well delineated. The cortical thymocytes consisted of densely packed lymphoid cells. The relationship of cortical to medullary areas is normal. The whole organ appears, however, to be smaller than average. The number of Hassall's corpuscles is diminished. Some foci of epithelial cells showed the oxyphilic change as described.

Histologic investigation of the peripheral lymphoid

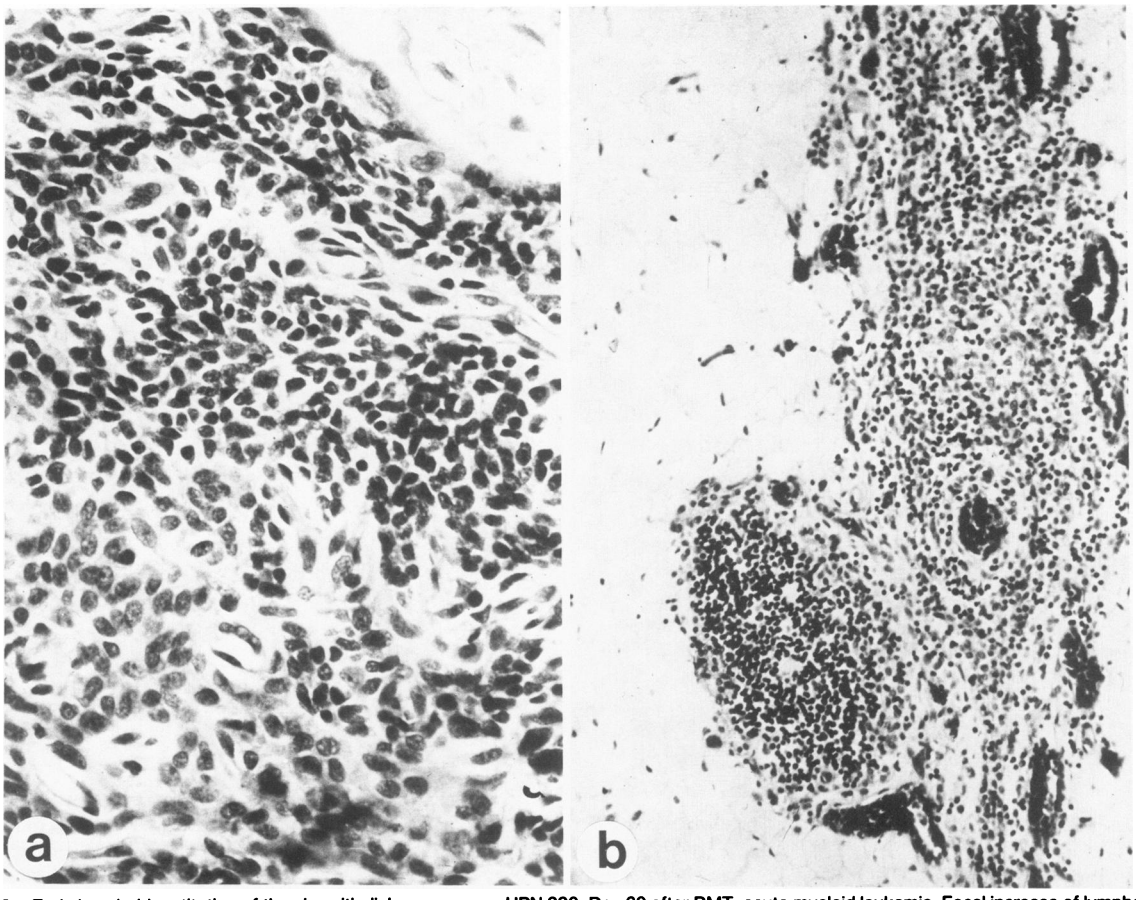

Figure 3—Early lymphoid restitution of thymic epithelial area. **a**—UPN 990, Day 60 after BMT, acute myeloid leukemia. Focal increase of lymphoid cells among epithelial cells. The cytologic identity of these cells is not yet clear. (H&E, ×400) **b**—UPN 1079, Day 88 after BMT, acute myeloid leukemia. Focal cortical nodule of putatively immature cells within the limits of the epithelial part of the thymus (see Discussion). The capsule is not fibrotic and not infiltrated by lymphoid cells. (H&E, ×64)

organs in this case (UPN 644) revealed normalization of the B-cell areas with appearance of germinal centers in the spleen and lymph nodes, which was not found in the other cases that showed thymic atrophy.

The second patient (UPN 510) underwent BMT for acute lymphocytic leukemia in third remission. No GVHR was clinically observed. At Day 1725 after transplantation (4.5 years) he died of acute gastrointestinal hemorrhage due to portal vein thrombosis. The thymus gland was atrophic, but multiple foci of lymphopoiesis were present. These lymphoid foci were localized partially within the connective and vascular tissue surrounding the epithelial strands, partially within the epithelial space.

Immunohistologic Findings of Thymi After BMT

The results of the immunohistologic investigation of autopsy thymi at different time intervals after BMT are summarized in Table 3. The different epithelial components of normal thymuses may be characterized by antibodies to different keratins as well as by the HLA-DR reactivity. Antibody 35 beta H 11 usually stains all epithelial cells of cortex and medulla, whereas antibodies 34 beta H 12 and 482 react only with the subcapsular epithelium and the medullary epithelial cells.[30] The epithelial remnant of the thymus after BMT shows a uniform keratin positivity where no distinction between medullary or cortical epithelial cells can be made (Figure 5d). The normal Leu-7^+ subcapsular epithelial layer[34,35] is lost very early after BMT (Figure 5c). At Day 4 a weak indistinct reactivity is seen in the outer margin of the epithelial space, whereas in later thymi no Leu-7^+ epithelial cell reactivity can be seen. With time after BMT the atrophy of the epithelial remnant increases. Also, at the later stages the outer borders are irregular and show interruptions of the basement membrane.

Before Days 14 and 19 epithelial cells are HLA-DR^-. For example, by Day 4 HLA-DR reactivity is largely lost. If the reaction pattern of HLA-DR is compared with that of macrophages (KiM3), the in-

traepithelial HLA-DR$^+$ cells correspond in distribution and frequency that of KiMp$^+$ cells (not shown). The diffuse HLA-DR positivity of the thymus at Day 14 and 19 coincides with the infiltration of activated T-lymphocytes within the epithelial space (Figure 6b and c) and is interpreted as a sign of intrathymic GVHD. Until Day 177 in these cases, no significant lymphoid restitution of the epithelial space is found. All lymphoid cells within the capsule and in the epithelial space show a mature phenotype (Leu-1$^+$, Leu-4$^+$, Leu-3a$^+$, or Leu-2a$^+$). The thymus cortex antigen T6 is not found in any of these cases.

The amount of mononuclear lymphoid infiltration of the thymus capsule, however, increases with time. Surrounding the dilated capillaries of the thymic capsule, nodular accumulations of lymphoid cells are found. Almost all lymphoid cells have a phenotype of T lymphocytes. By Day 4 after BMT, T lymphocytes with a predominance of T-helper subtype are found in the thymus capsule and the epithelial space (Figure 5a and b). At later times T-helper-subtype cells decrease significantly, whereas the numbers of T lymphocytes of suppressor cytotoxic cell phenotype increase (Figure 7). These cells occasionally accumulate within the thymus capsule and, in particular, also invade the epithelial area at later times (Figure 7a–d). However, no cells with precursor phenotype are found in these foci (Figure 7a). In addition to the lymphoid cells, less than 5% HNK 1$^+$ lymphoid cells and about 30% macrophages (KiM6$^+$, KiM8$^+$) are found within the infiltrates. No B lymphocytes are found. It should be mentioned, however, that pan-B-cell antibody does not stain plasma cells.

Peculiar thymic alterations in the thymus after 14 days after BMT (OA84-26 and to a lesser extent in OA84-48) are interpreted as a sign of a thymic GVHR (Figure 6). The thymic epithelial space is heavily infiltrated by lymphoid cells of mature phenotype with a slight predominance of T-helper phenotype. The infiltration is especially dense in the immediate sur-

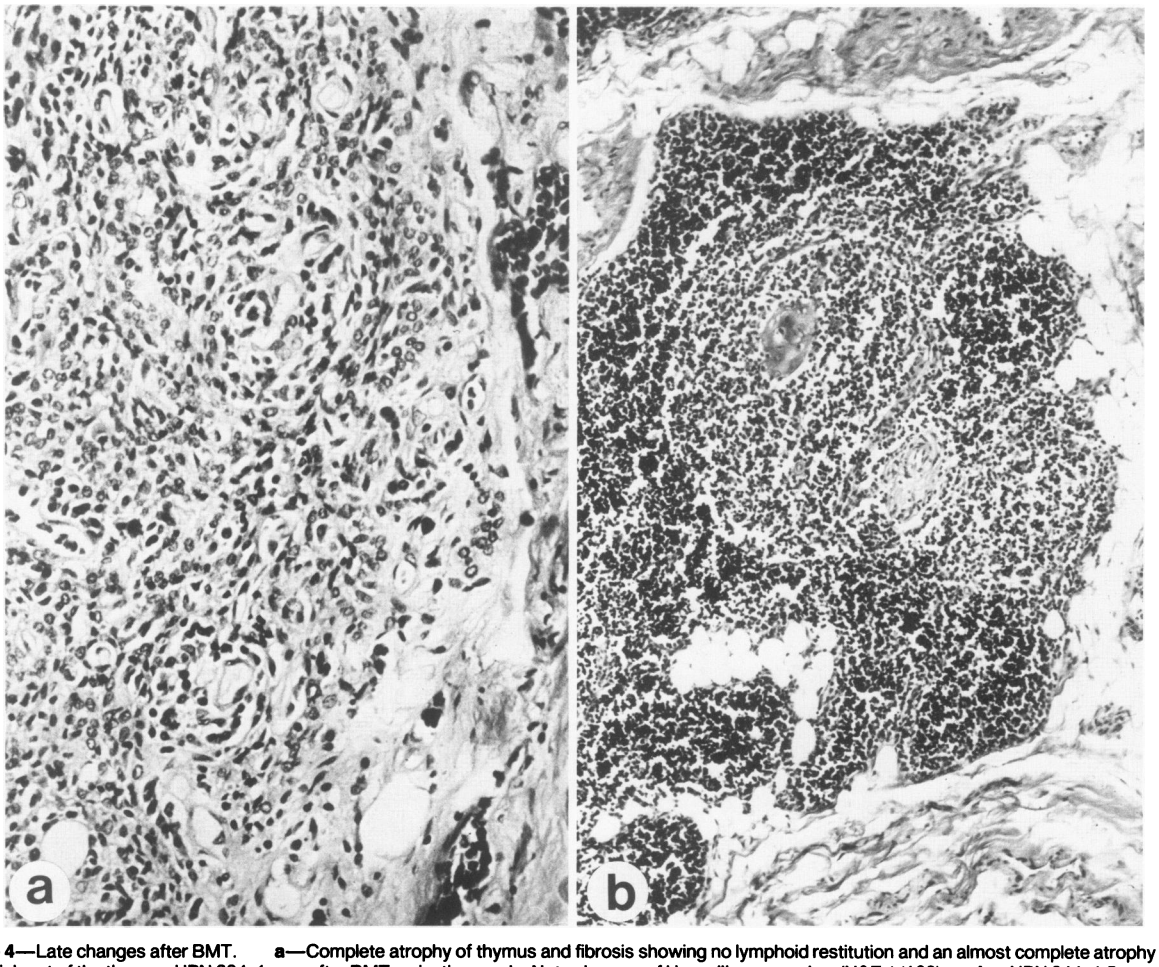

Figure 4—Late changes after BMT. **a**—Complete atrophy of thymus and fibrosis showing no lymphoid restitution and an almost complete atrophy of the epithelial part of the thymus. UPN 394, 1 year after BMT, aplastic anemia. Note absence of Hassall's corpuscles. (H&E, ×160) **b**—UPN 644, 4.5 years after BMT, acute leukemia, death in automobile accident. Showing good lymphoid restitution of the thymus, including the demarcation of cortical and medullary areas and rather broad lymphopoietic structures of thymus cortex. Small Hassall's corpuscles are visible. (H&E, ×64)

Table 3—Immunohistologic Findings in Thymus After BMT

UPN	Autopsy	Days after transplant	Mononuclear infiltration	T lymphocyte infiltrate of thymic capsule + epithelial space†		Macrophages	Helper/supressor Leu-3a/Leu-2a subtype ratio	HLA-DR reactivity of ECs	OKT 6 reactivity of ECs	HNK1+ cells
				TC	TES					
2766	OA85-13	4	(+)				5	(+)	0	<5%
2428	OA84-26	14	++	25%	45%	30%	2,5‡	++	0	<5%
2529	OA85-38	20	+	50%	20%	30%	4	–	0	<5%
2551	OA84-48	19	+	40%	15%	30%	1,6	(+)	0	<5%
2963	OA85-68	54	++	50%	20%	25%	1,0	–	0	<5%
2470	OA84-47	84	+++§	50%	20%	25%	0,4	–	0	<5%
2343	OA84-43	177	+++§	60%	10%	30%	0,3	–	0	<5%

*+, <50 positive cells/high-power field (25×); ++, 50–100 positive cells/high-power field (25×); +++, >100 positive cells/high-power field (25×).
†TC, thymic capsule; TES, thymic epithelial space.
‡Many activated cells.
§Nodular accumulation of lymphoid cells.

roundings and within the margins of Hassall's corpuscles, where the numbers of Leu-2a$^+$ and Leu-3a$^+$ cells are equal. Many lymphoid cells are larger and appear to be activated. The lymphoid infiltration is accompanied by a significant increase of macrophages within the epithelial space. Furthermore, there is a diffuse "dirty" positivity for HLA-DR in epithelial cells (Figure 6c).

Discussion

Thymic recovery would appear to be a logical consequence of BMT and a necessary prerequisite for immunologic restitution of the host. Unexpectedly, however, earlier studies did not show any significant thymic lymphopoiesis in allogeneic BMT in man.[21] It should be cautioned that in both the above and the present series, the thymuses studied were all from patients who died, usually with severe immunodeficiency and infection, and often with severe GVHD. The only exception was the young boy (UPN 644) who was killed in an auto accident while clinically well. Therefore, the data are skewed to reflect the morphology of immunodeficient and highly stressed patients. The status of thymus glands from clinically well patients, especially those who have recovered from GVHD, remains largely unstudied. In experimental BMT thymus restitution has usually only been observed in syngeneic donor–host combinations or if all lymphoid cells of bone marrow, but not the pluripotent hematopoietic stem cells, were eliminated from the graft by *in vitro* preincubation with highly specific anti-lymphocyte sera.[36,37] This treatment, as recently shown, also eliminates prethymic precursor cells from the bone marrow cell suspension without damaging the pluripotent hematopoietic stem cells. It appears from these experimental data in rats and mice that differentiation of prothymocytes within the bone marrow "educated" to their new MHC environment is necessary for restitution of thymus function. Furthermore, BMT in dogs in allogeneic and haploidentical strain combinations does not lead to significant restitution of lymphopoeitic activity of the thymus except in better matched combinations.[38] Because Class 1 MHC antigens usually are matched in human BMT, one may conclude that antigenic differences between donor and recipient are of extreme importance for the immunologic restitution of patients after BMT, even more than for the restitution of hemopoietic activity. A second conclusion would be that lymphoid restitution to normal is possible even in strong allogeneic donor–host combinations, at least in mice and rats, if all cells with

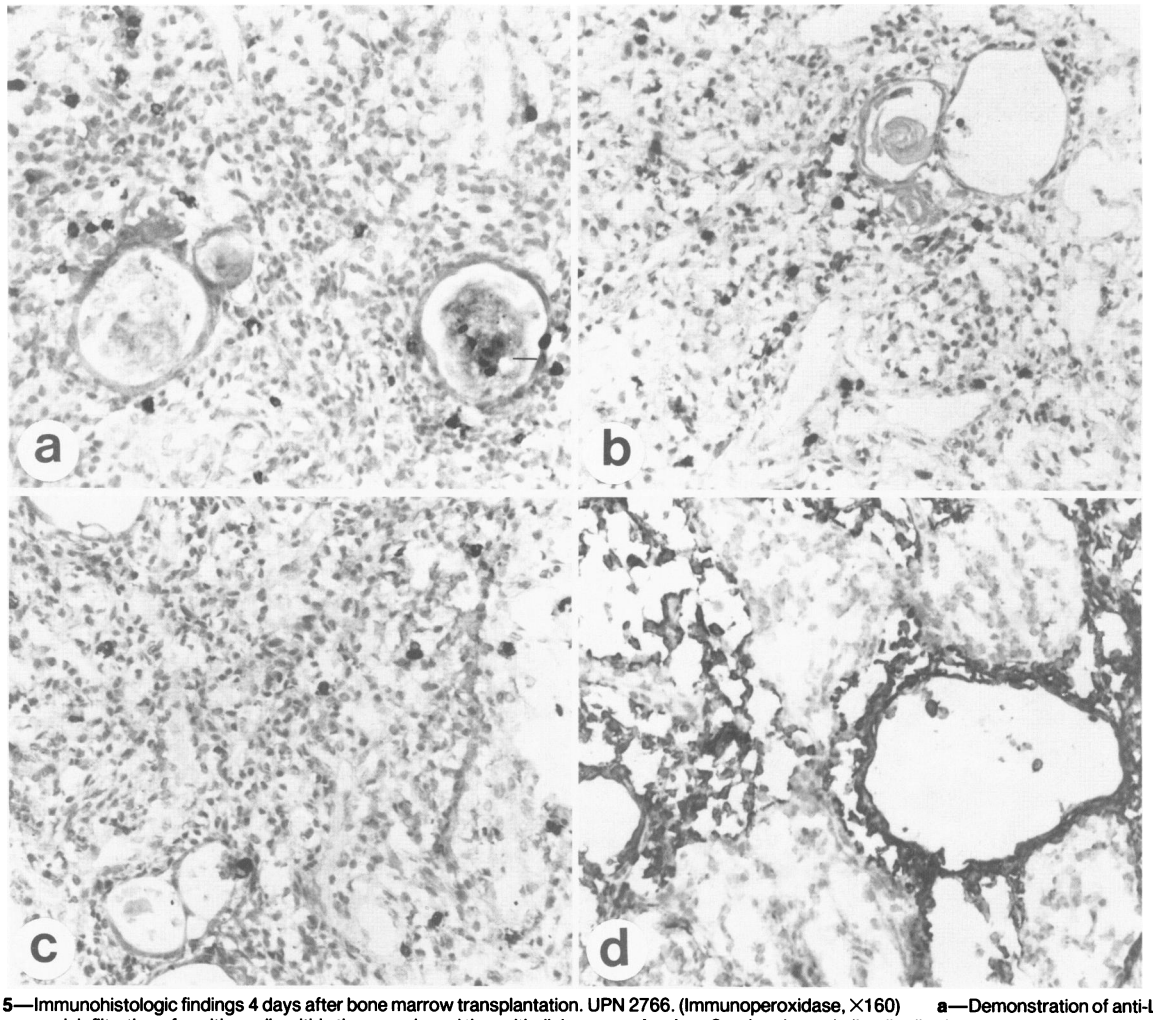

Figure 5—Immunohistologic findings 4 days after bone marrow transplantation. UPN 2766. (Immunoperoxidase, ×160) **a**—Demonstration of anti-Leu-3a showing a weak infiltration of positive cells within the capsule and the epithelial part. **b**—Leu-2a, showing a similar distribution. **c**—Anti-Leu-7, showing some positive cells within the epithelial part as well as in the capsule. The margin of the epithelial part sometimes shows a weak "blurred" appearance of anti-Leu-7 reactivity. **d**—Anti-keratin staining of the epithelial part, showing a very isomorphic keratin-positive pattern of epithelial cells.

lymphoid differentiation are eliminated from the donor bone marrow.

In man, the barriers to lymphoid recovery are even more complex than in animals. In addition to the genetic differences between donor and host, there may be further procedures in the pretransplant and posttransplant period leading to additional damage of thymic tissue which decrease the probability of lymphoid recovery. The present study showed that the lethal total body irradiation usually included in the conditioning of leukemic patients caused a higher degree of thymic atrophy in the early posttransplant period than was seen in the thymus of aplastic anemia patients, who do not usually receive total body irradiation.

In view of these barriers to thymic restitution, one may ask whether restitution is possible at all in the commonly used protocols of BMT in man. This histologic evaluation of 36 cases collected over many years, including patients up to 5 years after BMT, shows that single cases reveal unequivocal restitution of the lymphoepithelial structure of the thymus. The first signs are seen at 2–3 months after BMT with the formation of lymphoid nodules within the epithelial space of the thymic remnant. An almost complete recovery was documented in a boy killed in an automobile accident 4.5 years after BMT.

The morphologic sequences of thymic restitution are very similar to findings in human ontogenesis[39] and in experimental BMT,[19] showing an early focal infiltration with immature lymphoid cells, leading to focal, nodular accumulations of precursor cells and to a confluent cortical structure, so that thymic recovery may be effected by single precursor cells meeting their

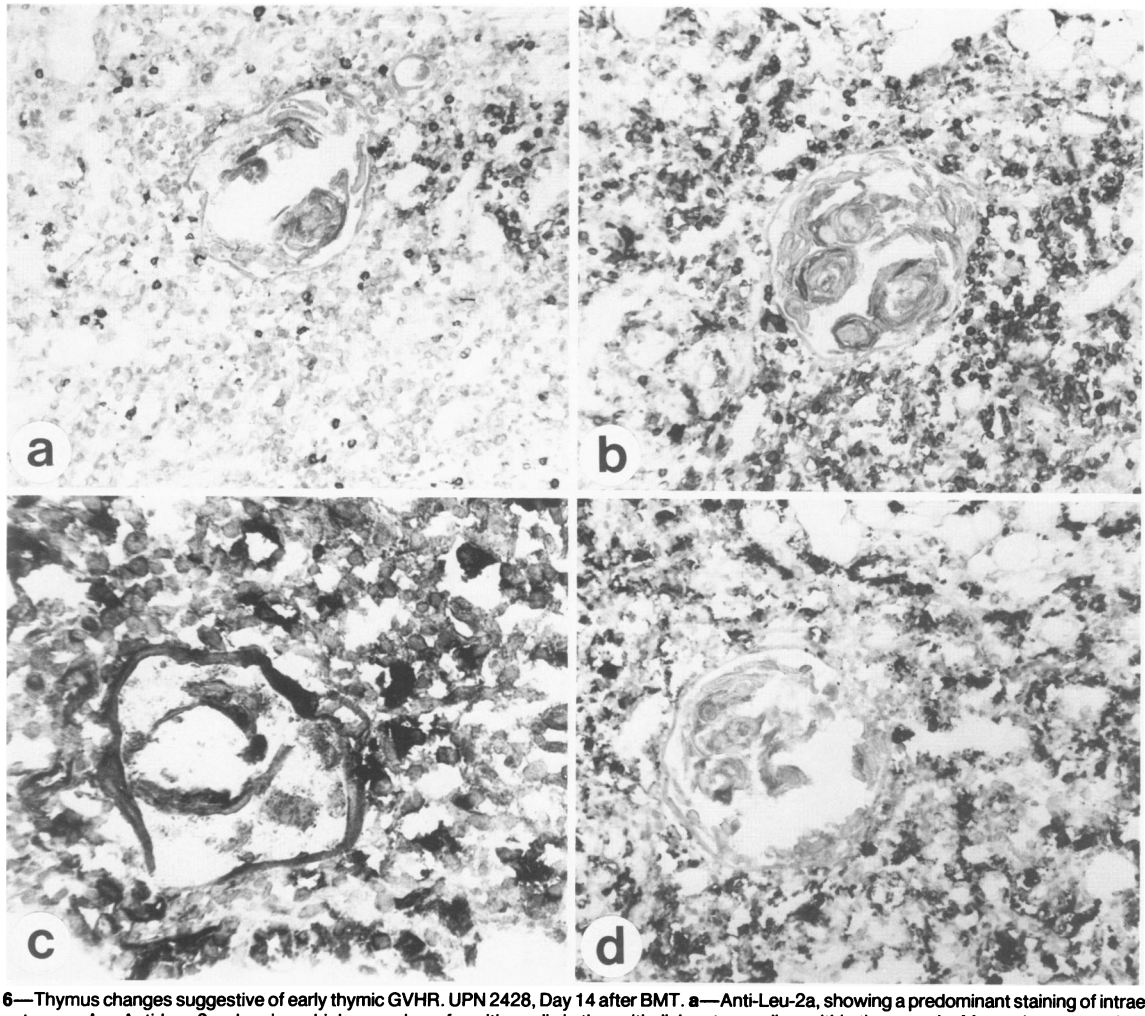

Figure 6—Thymus changes suggestive of early thymic GVHR. UPN 2428, Day 14 after BMT. **a**—Anti-Leu-2a, showing a predominant staining of intraepithelial lymphocytes. **b**—Anti-Leu-3a, showing a higher number of positive cells in the epithelial part as well as within the capsule. Macrophages are also positive with this antigen. **c**—Anti-HLA-DR, showing a diffuse HLA-DR positive staining of epithelial cells especially at the margin of Hassall's corpuscles. **d**—KiM8, showing the distribution of activated, rather large macrophages within the epithelial part of this thymus (**a–d**, the same area). (Immunoperoxidase, **a, b,** and **d**, ×160; **c**, ×400)

proper environment.[40] The focal recovery shown in these thymi is in good agreement with these experimental data. Most cases, however, confirming very recent data of Thomas et al,[27] and all our cases investigated by immunohistochemistry thus far have not shown the appearance of immature thymocytes carrying the T6 antigen or tdT in the thymus after BMT. The time course of thymus restitution in these selected cases is in good agreement with peripheral blood lymphocyte values of those mostly juvenile patients without GVHD who after BMT show increasing numbers of T-helper cells with eventual normalization of T-helper/T-suppressor subtype ratio starting at about 3–6 months after BMT.[12,41]

Seemayer and colleagues[29] showed that GVHD leads to significant thymus damage that may cause a long-lasting state of immunodeficiency. We were able to show alterations of thymic remnants after BMT which may be interpreted as thymic GVHD in man as well, although more definitive evidence will require matched control tissues from recipients of syngeneic bone marrow grafts. These changes consisted of a predominantly lymphoid infiltration of epithelial and capsular areas of the thymic remnant with many activated lymphoid cells, apoptotic single cell necrosis, especially prominent in the outer shell of Hassall's corpuscles, and aggregation of macrophages and foam cells within the epithelial part of the thymus. The immunohistologic investigation showed that this infiltration concords with a diffuse expression of Class 2 histocompatibility antigens by epithelial cells that in all other cases did not express Class 2 antigens and an infiltration by T lymphocytes in the same locales as the Hassall's corpuscles showing single-cell necrosis.

These changes are found as early as 14 days after BMT, thus being even earlier than manifestations in other organs (skin, liver, and intestinal tract).[37] At this time, T cells of helper subtypes predominate, whereas lymphoid infiltrates of the skin in GVHD usually show more T lymphocytes of cytotoxic/suppressor type. However, the larger studies of skin GVHD showed that TH- and TS-subtypes may be present in variable numbers and that the predominance of TS cells may reflect the generalized deficit of TH cells after BMT. Therefore, the relatively high numbers of TH cells in our early cases of thymic GVH may reflect the early accumulation of these cells within the thymus after bone marrow grafting. Experimental

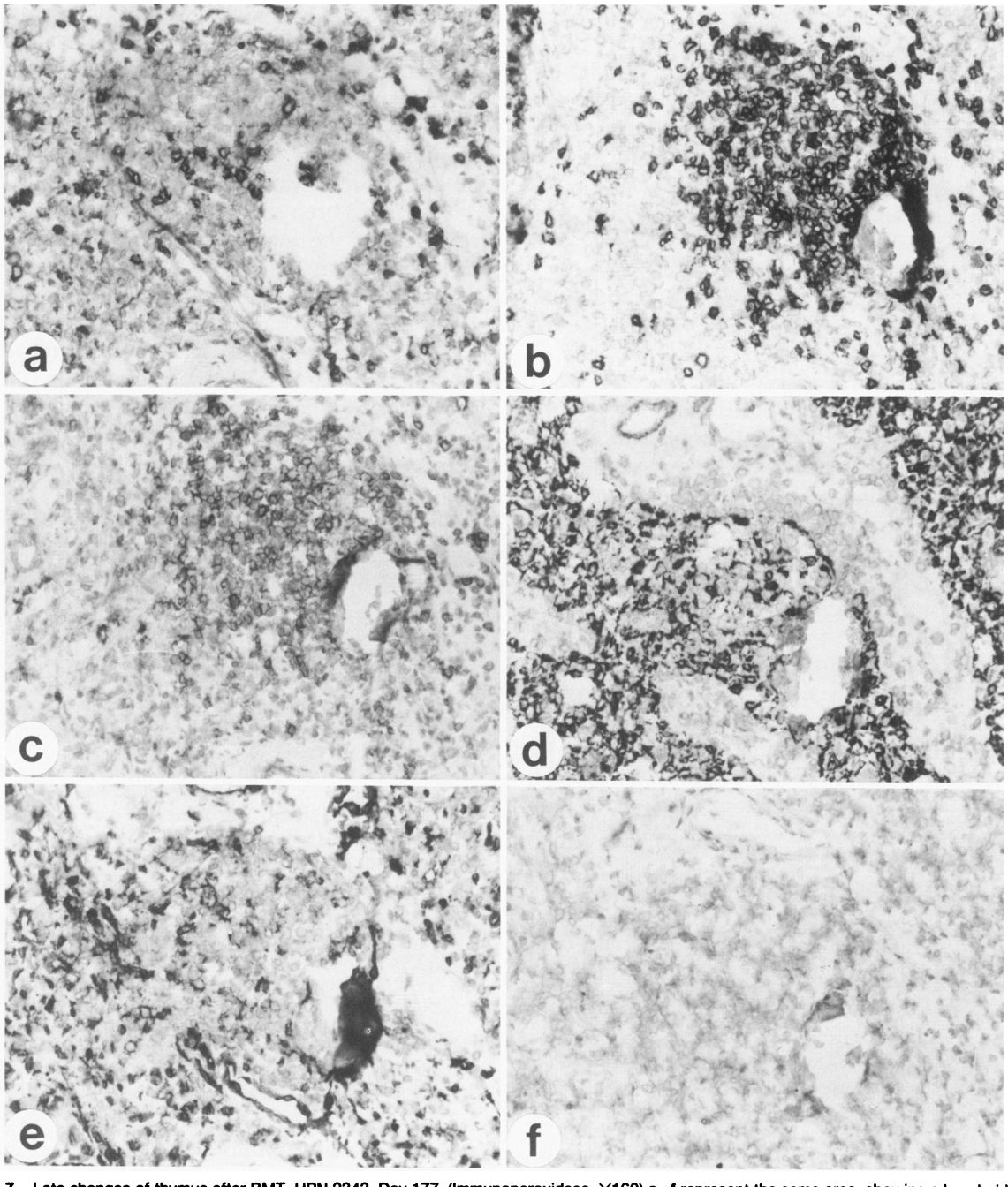

Figure 7—Late changes of thymus after BMT. UPN 2343, Day 177. (Immunoperoxidase, ×160) **a**–**f** represent the same area, showing a lymphoid nodule partially localized in the capsule and perivascular space partially within the atrophic epithelial remnant, which is hypothesized to be an immature focus (see Discussion). **a**—Anti-Leu-3a, showing only a few positive lymphoid cells in addition to positive macrophages. **b**—Anti-Leu-2a, representing the bulk of positive lymphoid cells. **c**—Anti-Leu-1, showing only relatively weak activity suggestive of immature (or activated) lymphocytes. **d**—Keratin staining. Lymphoid nodule is localized partially within the capsule, partially within the epithelial space. **e**—Anti-HLA-DR, showing only macrophages and endothelial cells positive, whereas epithelial cells are negative. **f**—Anti-T6, showing complete negativity.

GVHD in transplanted mice also causes significant thymic atrophy. Immunohistologic analyses of intrathymic events show that precursor cells (THY1$^+$, Lyt1,2,3$^-$) get access to the thymus. In the presence of mature GVHD inducing T lymphocytes, however, no further differentiation of these precursor cells could be demonstrated.[33]

Histologic signs of active thymic GVHR are seen only in thymi between Day 14 and 2 months after BMT. Later the thymi show severe atrophy of the epithelial compartments and fibrosis of the capsule, even if GVHD changes are prominent in peripheral tissues (skin, liver, and intestinal tract). Furthermore, the disappearance of Hassall's corpuscles in cases of aplastic anemia, where early after BMT large cystic Hassall's corpuscles are found, may argue in favor of an atrophic process caused by GVHR, which has been also confirmed experimentally in mice.[42]

Investigations of peripheral blood lymphocytes with monoclonal antibodies after BMT have shown that even in the absence of significant GVHD there is a selective deficit of T4$^+$ cells with reversed T4/T8 ratio. Recent investigations clearly show that Class 2 major histocompatibility complex molecules are specifically involved in the development of human T^{4+}, T8$^-$ inducer phenotypes. Therefore, in addition to GVHD, lack of specific epithelial cells carrying Class 2 MHC determinants[27] may play a significant role in deficient lymphopoiesis in marrow transplanted patients. Our studies do not allow us to judge whether the lack of HLA-DR$^+$ cortical and medullary epithelial cells is a consequence of the conditioning or whether the cells are the first targets to be destroyed by GVHR. However, even very early after BMT (Day 4) the epithelial HLA-DR reactivity is largely lost. After Day 4, diffuse Class II epithelial positivity was only seen in GVHD, suggesting new expression due to an intrathymic immune process. The lack of lymphopoietic activity within the thymus may thus contribute more to the deficit of CD4$^+$ cells than to CD8$^+$ cells after BMT. In fact, dense lymphoid aggregates appearing within the thymic capsule and perivascular spaces 3 months and later after BMT contain predominantly Leu-2a$^+$ (CD8$^+$) lymphoid cells. Perivascular accumulation and cytologic features as well as mitotic activity are taken as indicative of lymphopoietic activity in close connection with the atrophic thymic remnant (Figures 3b and 7). The lack of frozen material in these specific cases forbade definitive phenotypic marking to prove the immaturity of these cells. We hypothesize that they are immature and predict that phenotypic study of such late cases will, when available, confirm this prediction.

We propose that pretreatment, on the one hand, and GVHD, on the other, may contribute to irreversible damage of the thymic microenvironment in some cases. Because, however, focal restitution may take place in the absence of severe GVHD damage, the damage due to preconditioning alone is not sufficient to prevent thymic recovery. Even if the thymus restitution is not complete, it may be effective enough to generate postthymic precursor cells and mature lymphocytes, leading to normalization of immunologic reactivity. So far, there is no convincing evidence that any extrathymic site is capable of substituting for the thymic function after lethal irradiation and BMT.

The structure of a purely epithelial remnant of a thymus after BMT is very similar to thymus findings in the acquired immunodeficiency syndrome. In this disease it has been speculated that the deficit of T4$^+$ cells reflects the inability to repopulate these cells from precursors within the thymus.[43–45] Although lymphoid recovery of the thymus is demonstrated in some of our cases, most patients dying after BMT do not show thymic recovery or lymphopoietic activity. Although this may reflect a selection bias, the data suggest that different conditioning regimens may have different effects on the thymus, eventually destroying important parts of the thymic microenvironment. Further immunohistologic and molecular studies focused on the later stages after BMT and functional characterization of lymphoid cells and their activities by *in vitro* cloning are needed to clarify the role of thymic lymphopoiesis after BMT.

References

1. Thomas ED, Storb R, Clift RA, et al: Bone-marrow transplantation. N Engl J Med 1975, 292:832–843, 895–902
2. Santos GE, Tutschka PJ, Brookmeyer R, et al: Marrow transplantation for acute nonlymphocytic leukemia after treatment with busulfan and cyclophosphamide. N Engl J Med 1983, 309:1347–1357
3. Sale GE, Shulman HM, eds: The Pathology of Bone Marrow Transplantation. New York, Masson Publishing, 1984
4. O'Reilly RJ: Allogeneic bone marrow transplantation: Current status and future directions. Blood 1983, 62:941–964
5. Storb R, Deeg HJ, Thomas ED, et al: Marrow transplantation for chronic myelocytic leukemia: A controlled trial of cyclosporine versus methotrexate for prophylaxis of graft-versus-host disease. Blood 1985, 66:698–702
6. Neiman PR, Reeves W, Ray G, Fluornoy 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, 136:136–154
7. Atkinson K, Shulman HM, Deeg HJ, Weiden PL, Graham TC, Thomas ED, Storb R: Acute and chronic graft-versus-host disease in dogs given hemopoietic grafts from DLA-nonidentical littermates. Two distinct syndromes. Am J Pathol 1982, 108:196–205

8. Friedrich W, O'Reilly RJ, Koziner B, Gebhard DF, Good RA, Evans RL: T-lymphocyte reconstitution in recipients of bone marrow transplants with and without GVHD: Imbalance of T-cell subpopulations having unique regulatory and cognitive functions. Blood 1981, 59:696–791
9. Lynch DC, Knott LJ, Thomas RM, Harper P, Goldstone AH, Davis EG, Levinski RJ: T cell regeneration after allogeneic and autologous bone marrow transplantation. Br J Haematol 1983, 53:451–458
10. Favrot M, Janossy G, Tidman N, Blacklock H, Lopez E, Bofill M, Lampert I, Morgenstein G, Powles R, Prentice HG, Hoffbrand AV: T cell regeneration after allogeneic bone marrow transplantation. Clin Exp Immunol 1983, 54:59–72
11. Ashkenazi J, Berth C, Elfenbein J: T cell phenotypic profile and colony formation during recovery from cytotoxic therapy-induced marrow aplasia. Cancer Res 1985, 45:6513–6518
12. Zander A, Reuben J, Johnston D, Vellekoop L, Dicke K, Yau J, Hersh E: Immune recovery following allogeneic bone marrow transplantation. Transplantation 1985, 40:177–183
13. Paulin T, Ringden O, Lonngvist B: Faster immunological recovery after bone marrow transplantation in patients without cytomegalovirus infection. Transplantation 1985, 39:377–384
14. Witherspoon RP, Storb R, Ochs HD, Flournoy N, Kopecky KJ, Sullivan KM, Deeg HJ, Sosa R, Noel DR, Atkinson K, Thomas ED: Recovery of antibody production in human allogeneic marrow graft recipients: Influence of time posttransplantation, the presence or absence of chronic graft-versus-host disease, and antithymocyte globulin treatment. Blood 1981, 58:360–368
15. Atkinson K, Incefy GS, Storb R, Sullivan KM, Iwata T, Dardenne M, Ochs HD, Good RA, Thomas ED: Low serum thymic hormone levels in patients with chronic graft-versus-host disease. Blood 1982, 59:1073–1077
16. Witherspoon RP, Matthews D, Storb R, Atkinson K, Cheever M, Deeg HJ, Doney K, Kalbfleisch J, Noel D, Prentice R, Sullivan KM, and Thomas ED: Recovery of in vivo cellular immunity after human marrow grafting. Transplantation 1984, 37:145–150
17. Steinmann GG: Changes in the human thymus during aging. Curr Top Pathol, 1986, 75:43–88
18. Haynes BF, Warren RW, Buckley RH, McClure JE, Goldstein AL, Henderson FW, Hensley LL and Eisenbarth GS: Demonstration of abnormalities in expression of thymic epithelial surface antigens in several cellular immunodeficiency diseases. J Immunol 1983, 130:1182–1188
19. Muller-Hermelink HK, Gulden M, and Bathmann R: Restitution of the thymus in lethally irradiated mice after transplantation of syngeneic or allogeneic bone marrow. Immunobiology 1984, 167:462–482
20. Von Gaudecker B: The development of the human thymus microenvironment. Curr Top Pathol 1986, 75:1–42
21. Muller-Hermelink HK, von Gaudecker B: Ontogenese des lymphatischen Systems beim Menschen. Verh Anat Ges 1980, 74:235–259
22. Cordier AC: Ultrastructure of the thymus in "nude" mice. J Ultrastruct Res 1974, 47:26–40
23. Cordier AC, Haumont SA: Development of the thymus, parathyroids and ultimo-bronchial bodies in NMRI and nude mice. Am J Anat 1980, 157:227–263
24. Beschorner WE, Hutchins GM, Elfenbein GJ, Santos GW: The thymus in patients with allogeneic bone marrow transplants. Am J Pathol 1978, 92:173–186
25. Shulman HM, Sullivan KM, Weiden PL, et al: Chronic graft-versus-host syndrome in man: A long-term clinopathological study of 20 Seattle patients. Am J Med 1980, 69:204–217
26. Sale GE: Pathology of the lymphoreticular system. The Pathology of Bone Marrow Transplantation. Edited by GE Sale, HM Shulman. New York, Masson Publishing, 1984, pp 171–191
27. Thomas J, Sloane P, Imrie F, Ritter M, Schuurman H-J, Huber J: Immunohistology of the thymus in bone marrow transplant recipients. Am J Pathol 1986, 122:531–540
28. Witherspoon R, Storb R, Ochs D, Flournoy N, Kopecky K, Sullivan M, Deeg HJ, Sosa R, Noel R, Atkinson K, Thomas ED: Recovery of antibody production in human allogeneic marrow graft recipients: Influence of time post transplantation, the presence or absence of chronic graft-versus-host disease, and antithymocyte globulin treatment. Blood 1981, 58:360–368
29. Seemayer T, Lapp W, Bolande R: Thymic involution In murine graft-versus-host reaction: Epithelial injury mimicking human thymic dysplasia. Am J Pathol 1977, 88:119–127
30. Seddik M, Seemayer T, Lapp W: The graft-versus-host reaction and immune function. Transplantation 1984, 37:286–290
31. Lapp WS, Ghayur T, Mendes M, Seddik M, Seemayer T: The functional and histological basis for graft-versus-host-induced immunosuppression. Immunol Rev (In press)
32. Seemayer T: The graft-versus-host reaction: A pathogenetic mechanism of experimental and human disease. Perspect Pediat Pathol 1979, 5:93
33. Muller-Hermelink HJ, von Gaudecker B, Hansman M-L: The Human Thymus Microenvironment: Immune histochemical characterization on a light and electron microscopic level. (manuscript in preparation)
34. Chan WC, Zaatari GS, Tabei S, Bibb M, Byrnes RK: Thymoma: An immunohistochemical study. Am J Clin Pathol 1984, 82:160–166
35. Janossy G, Montano L, Selby WS, Duke O, Panayi G, Lampert I, Thomas JA, Granger S, Bofill M, Tidman N, Thomas HC, Goldstein G: T cell subset abnormalities in tissue developing during autoimmune disorders, viral infection, and graft-versus-host disease. J Clin Immunol 1982, 2:42
36. Muller-Ruchholtz W, Blank M, Wottge H-H, Ulrichs K, Muller-Hermelink HK: Relevance of prethymic precursor cells for GVHR prevention in rat and mouse fully allogeneic combinations. Transpl Proc 1983, 15:1463–1468
37. Muller-Hermelink HK, Marino M, Palestro G, Schumacher U, Kirchner Th: Immunohistological evidences of cortical and medullary differentiation in thymoma. Virchows Arch [Pathol Anat] 1983, 408:143–161
38. Deeg HJ, Severns E, Raff RF, Sale GE, Storb R: Specific tolerance and immunocompetence in haploidentical but not in completely allogeneic canine chimeras treated with methotrexate and cyclosporine. Transplantation (In press)
39. Muller-Hermelink HK, Marino M, Palestro G: Pathology of thymic epithelial tumors. Curr Top Pathol 1986, 75:207–268
40. Kingston R, Jenkinson EJ, Owen JT: A single stem cell can recolonize an embryonic thymus producing phenotypically distinct T cell populations. Nature 1985, 317:811–813
41. Witherspoon RP, Lum LH, Storb R: Immunologic reconstitution after human marrow grafting. Semin Hematol 1984, 21:2–10
42. Seddik M, Seemayer T, Lapp W: The graft-versus-host reaction and immune function: III. Functional pre-T-cells in the bone marrow of graft-versus-host reactive

mice displaying T-cell immunodeficiency. Transplantation 1986, 41:238–242
43. Joshi VV, Oleske JM: Pathologic appraisal of the thymus gland in acquired immunodeficiency syndrome in children: A study of four cases and a review of the literature. Arch Pathol Lab Med 1985, 109:142–146
44. Davis AE: The histopathological changes in the thymus gland in the acquired immune deficiency syndrome. Ann NY Acad Sci 1984, 7:493–502
45. Savino W, Dardenne M, Marche C, Trophilme D, Dupuy J-M, Pekovic D, Lapointe N, Bach J-F: Thymic epithelium in AIDS: An immunologic study. Am J Pathol 1986, 122:302–307

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