Characterization of Plasma Cell Populations at Autopsy After Human Allogeneic Bone Marrow Transplantation

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Postmortem fixed tissue sections of the lymphoid and digestive systems of eight consecutive leukemic patients dying of various diseases after bone marrow transplantation (BMT) were analyzed for the presence of the heavy chains γ, α, μ, δ, and ε and light chains κ and λ, with the use of a standard immunoperoxidase method. Two distinct types of plasma cell populations were found. The first type was a widely distributed polyclonal plasma cell population, lacking IgD-positive plasma cells and germinal centers. The second type of plasma cell population, found in 6 of 8 patients, was a group of monoclonal plasma cell populations positive for the heavy chains γ, α, μ, or δ. Recent immunohistologic observations of the human lymph node suggest that the first type of polyclonal plasma cell population could arise from a nonspecific expansion of $sIgM^+$, $sIgD^-$ B lymphocytes. The lack of germinal centers, a structure closely involved in specific-antibody production, may correlate with the poor specific-antibody response documented in patients after BMT. The monoclonal plasma cell populations, found with an unexpectedly high frequency, are probably related to a functional T-cell defect. (Am J Pathol 1986, 124:74–81)

IMMUNOLOGIC studies in patients with bone marrow grafts have revealed a state of severe combined immunodeficiency which persists longer in patients suffering from chronic graft-versus-host disease (GVHD).[1] A salient feature of this immunodeficiency is the poor specific-antibody production upon exposure to various antigenic stimuli.[2–6] Although the underlying functional defects have been studied extensively,[1–6] there is as yet no available data on the *in situ* characterization of the immunoglobulin-producing cells.

In the present study, we performed an extensive *in situ* phenotyping of the plasma cell populations in 8 consecutive patients who died of various diseases after bone marrow transplantation (BMT).

Materials and Methods

Patient Population

The clinical and immunologic profiles of 8 consecutive patients who died after allogeneic BMT are summarized in Table 1. With one exception, the donors were HLA genotypically identical siblings. UPN_{39} was transplanted with his haploidentical mother. Prior to transplantation, and according to a previously described protocol,[7] all patients received cyclophosphamide, 120 mg/kg, and total body irradiation, 800–900 rads, except for UPN_{42}, who received high-dose cytosine arabinoside instead of cyclophosphamide. Clinical GVHD was scored according to Thomas et al.[8]

Autopsy Study

In each case, a complete autopsy was performed within 3–6 hours of death. Representative 3mm-thick tissue sections of the following sites were removed for immunoperoxidase study: tongue, tonsillar area, esophagus, stomach, jejunum, distal ileum, proximal colon, liver, thymus, spleen, and bone marrow from the right anterior iliac crest. Many lymph nodes were also sampled from the supraclavicular, mediastinal, peripancreatic, mesenteric, periaortic, and inguinal sites. Tissues were promptly fixed in B_5 for 5 hours and trans-

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Table 1—Patients' Characteristics

UPN	Age/Sex	Disease	Acute GVHD	Time and cause of death	Serum immunoglobulins* IgG	IgA	IgM	MG
23	27/M	ANLL (first remission)	IV	Day 87 (acute GVHD)	ND	ND	ND	ND
26	42/F	CML (accelerated phase)	II	Day 170 (leukemic relapse)	678	67	55	Neg
28	31/M	Refractory ANLL	none	Day 18 (histoplasmosis)	ND	ND	ND	ND
33	37/M	ANLL (first remission)	III—chronic	Day 219 (acute respiratory distress syndrome	351	36	35	Neg
34	23/F	CML (chronic phase)	IV	Day 78 (CMV interstitial pneumonia)	592	45	67	Neg
39	16/M	ALL (third remission)	IV	Day 37 (acute GVHD)	598	58	27	Neg
42	39/M	ANLL (first relapse)	IV—chronic	Day 126 (chronic GVHD)	ND	ND	ND	ND
43	32/F	CML (chronic phase)	IV	Day 69 (CMV interstitial pneumonia)	642	15	40	IgGK

ANLL, acute non lymphoblastic leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; GVHD, graft-versus-host disease; CMV, cytomegalovirus; MG, monoclonal gammopathy; ND, not done; Neg, negative.

* Normal range: IgG, 800–1400 mg/dl; IgA, 110–270 mg/dl; IgM, 100–245 mg/dl (immunoglobulin studies were done within 2 weeks of death).

ferred to 70% ethanol until conventional paraffin embedding and hematoxylin and eosin (H&E) staining. Bone marrow sections were decalcified in 10% EDTA.

Immunoperoxidase Staining

A three-step peroxidase-antiperoxidase (PAP) technique, as described by Taylor,[9] was performed on 4-μ-thick deparaffinized tissue sections. Rabbit anti-human antibodies (Behringwerke A.G., Marburg, West Germany) against the heavy chains γ, α, μ, δ, and ε and light chains κ and λ were used as primary antibodies. Optimum primary antibody dilutions, determined with normal human tonsils, were 1:500 for the anti-γ, -α and -μ heavy chains and anti-κ and -λ light chains and 1:200 for the anti-δ and -ε heavy chains. The specificity of these primary antisera was confirmed with human myeloma tissue, previously characterized. A goat anti-rabbit γ globulin (Behringwerke), diluted 1:30, was added as a second layer. A rabbit PAP complex (Bionetics, Kensington, Md), diluted 1:50, served as a third layer. All antibodies were diluted in 0.5 M Tris-HCl, pH 7.6, and incubated at room temperature in a humidity chamber for 30 minutes. Each incubation was followed by a 30-minute wash in 0.05 M Tris-saline buffer, pH 7.6. Peroxidase activity was visualized by adding diaminobenzidine (0.06% 3,3'-diaminobenzidine tetrahydrochloride) and hydrogen peroxide (0.03%) in 0.05 M Tris-saline buffer, pH 7.6, for 5 minutes at room temperature. From 1 to 6 samples of every autopsy site were stained.

Scoring System

Serial sections of the same specimen were scored for the presence of cells positive for the various heavy and light chains, with the use of a semiquantitative scale. Polyclonal and monoclonal patterns were initially interpreted as previously reported.[10]

Upon review, however, two very distinct patterns of plasma cell populations were defined: a monoclonal pattern (one light chain comprising at least 95% of the total plasma cell population) and a polyclonal pattern (equal distribution of κ and λ light chains).

Controls

Twenty tonsils and 18 lymph nodes with reactive follicular hyperplasia, obtained from patients under 35 years of age, were similarly processed and scored. Tonsils left at room temperature for up to 8 hours still showed an excellent immunohistologic preservation. Three patients in apparently good health, aged 17, 27, and 28 years, who died accidentally, underwent autopsy and served as morphologic controls.

Results

Morphologic Findings

Morphologic findings in the lymphoid tissues of all patients were remarkably similar, except for UPN_{26}, where massive leukemic infiltration occurred. The thymi were dysplastic, with a marked cortical involution, a loss of the corticomedullary junction, and a decrease of Hassall's corpuscles (Figure 1). Only a few small lymphocytes remained in the medulla and perivascular spaces, with variable numbers of mature plasma cells in the perivascular spaces and perilobular tissue. The white pulp of the spleen was deeply atrophied, with a few residual immunoblasts and plasma cells. All the lymph nodes were relatively hypocellular, with a population of small lymphocytes and larger transformed cells

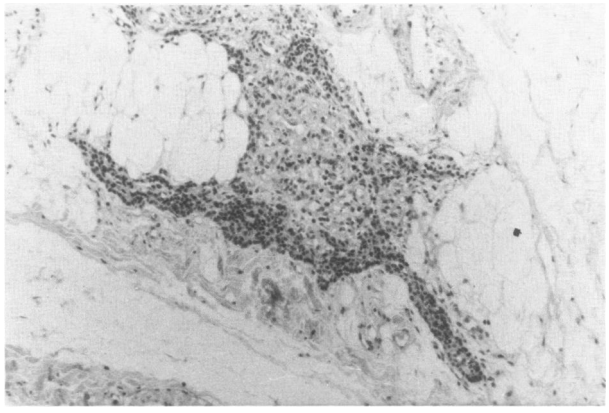

Figure 1—Thymus of UPN_{39} showing a marked cortical involution with a loss of the corticomedullary junction. Only a few small lymphocytes are present. (H&E, × 100)

(Figure 2). Their sinusoids were often filled with histiocytes showing erythrophagocytosis. Mature plasma cells were usually numerous, despite a total absence of germinal centers (Figure 3A). The bone marrows were generally hypocellular, except for UPN_{26}, as previously mentioned. The tonsils were also hypocellular, with a few remaining lymphocytes and rather frequent mature plasma cells. In the minor salivary glands of the tongue, mature plasma cells were distributed predominantly around the major excretory ducts and to a lesser extent in the stroma of the acini. The overall picture of the digestive tract varied according to the presence of GVHD and/or infectious processes. Variable numbers of plasma cells were usually found in the mucosa, at all levels. Mature plasma cells were also located in the portal areas of the liver.

Wherever found, these plasma cells looked morphologically mature and could not be distinguished from those seen in controls (Figure 3A). They sometimes varied slightly in size and shape and occasionally showed binucleation and trinucleation. Their nuclei frequently contained small, discrete nucleoli. No mitotic figures were observed. Even when abundant, these plasma cells were evenly dispersed, without formation of cohesive sheets or infiltration of the surrounding structures. They were often admixed with immunoblasts. Although not quantitated at autopsy, the plasma cells in the transplant group exceeded those found in the control autopsy group.

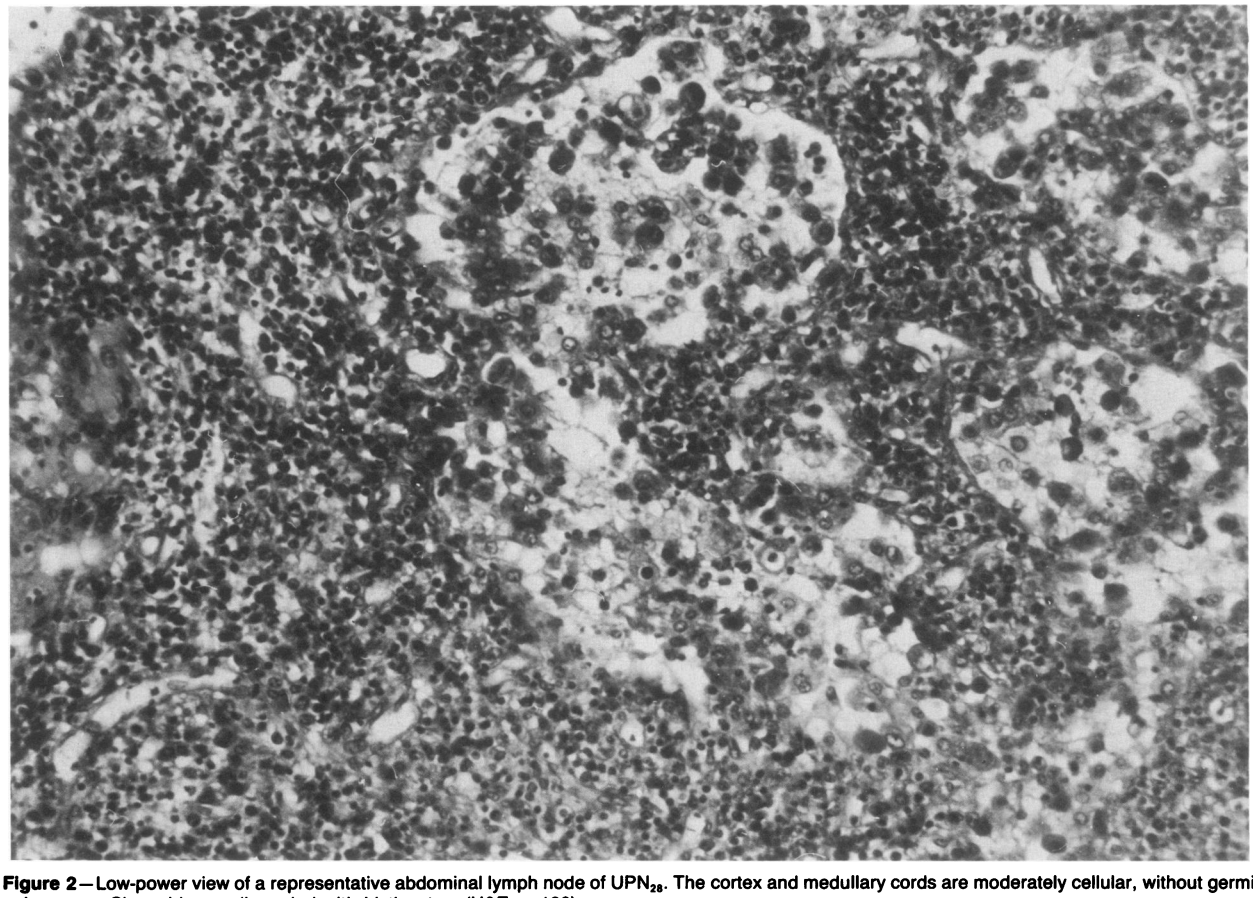

Figure 2—Low-power view of a representative abdominal lymph node of UPN_{28}. The cortex and medullary cords are moderately cellular, without germinal centers. Sinusoids are distended with histiocytes. (H&E, × 100)

Table 2—Immunoperoxidase Distribution of Light and Heavy Chains in the Lymphoid Tissue and Digestive Tract

Site	UPN_{23}	UPN_{26}	UPN_{28}	UPN_{33}	UPN_{34}	UPN_{39}	UPN_{42}	UPN_{43}
Tonsil	G≅A≫M	G>A>M	Mo A-λ	G>A≅M	G	NP	G>A≅M	G≫A≅M
Thymus	Mo A-λ (G)	G≫A	Mo A-G-λ	G≫A	ND	G	G≫A	G>M>A
Spleen	A>G	G≫A>M	G>A>M>E	G≅A>M	M>G>A	G≫A≅M≫E	G≅M≫A	G>M≫A
Bone marrow	NP	VRPC	G>A>M>E	NP	NP	NP	VRPC	VRPC
Cervical LN	A≅G≅M	G≫A>M	G>A>M>E	G>A≅M	G≅A>M≫E	G≫A≫M	G≅M≫A≫E	G≅M>A
Mediastinal LN	Mo A-λ D-κ (M) G-κ	G≫A≅M>E	G>A>M>E	Mo M-κ (AGE)	G≅M>A≫E	G>A>M≫E	G>M≫A	G>M>A
Pancreatic LN	Mo A-λ (M)	G≫A≅M>E	G>A>M	G>A≫M	M≅G>A≫E	G≅A≅M	G>M≫A	G≅M≫A
Mesenteric LN	Mo A-λ (M) G-λ	G>A>M	G≅A>M	Mo A-λ (MG)	G>M>A≫E	G≅A>M	G>M≫A≫E	G>M≅A
Aortic LN	Mo A-λ M-λ	G≫A≅M	G>A>M	G>A≅M	M>G>A≫E	G≅A>M≫E	G>M≫A≫E	G≅M>A
Inguinal LN	Mo A-λ	G≫A>M	G>A>M>E	M≫G	M>A≅G	Mo G-λ(A)	G≅M≫A	G≅M≫A
Minor salivary glands	A	A≫G≅M	Mo A-λ	A≫M	A>M	NP	Mo M-λ (AG)	A≫G
Oesophagus	A≅G	A	A	VRPC	A>G≅M	G≫A	G≫M≅A	Mo G-κ
Stomach	A≫M≅G	NP	A≫M	VRPC	M>G≅A	VRPC	A>M≅G	A≫M>G
Jejunum	A≫M≫G	A≫M	A>M>G>E	A>M≫G	A≅M	A>G≅M	A>M≫G≫E	A>M≅G
Ileum	NP	A≫M>G	A≫M>G>E	A>M≫G	M≅A>G	VRPC	Mo G-λ (AME)	A≅G>M>E
Ascending colon	A≫M>G	A≫M>G>E	A≫M>G>E	A>M	A≅M>G	A	A>M>G≫E	A>G>M>E
Liver	NP	G>A≅M	VRPC	G≅M	M>G	VRPC	Mo G-λ M-K (AE)	M>G

LN, lymph node; Mo, monoclonal (otherwise polyclonal); ( ), residual polyclonal plasma cell populations; VRPC, very rare plasma cells; NP, no plasma cells found; ND, not done.

Immunoperoxidase Study

Results are depicted in Table 2. UPN_{23} displayed a strikingly unusual picture. The digestive system contained a predominantly IgA-positive polyclonal plasma cell population. The lymphoid system was massively filled with an IgA-λ monoclonal plasma cell population with a few other clones of smaller size (one IgD-κ and four IgG-κ clones in six mediastinal lymph nodes sampled, one IgG-λ clone in a mesenteric lymph node, and one IgM-λ clone in a periaortic lymph node).

UPN_{26} showed a sharp delimitation between the IgA-predominant polyclonal plasma cell population of the digestive tract and the IgG-predominant polyclonal plasma cell population elsewhere in the lymphoid system. No monoclonal plasma cell population was recorded.

Two monoclonal plasma cell populations were present in UPN_{28}. An IgA-λ clone was located in the tonsils and surrounded the major excretory ducts of the minor salivary glands of the tongue, whereas an IgG-A-λ clone was found in the thymus. The digestive and lymphoid tissues otherwise contained polyclonal plasma cell populations of the IgA and IgG type (Figure 3B and C).

In UPN_{33}, the two predominant IgG and IgA polyclonal plasma cell populations of the lymphoid and digestive systems were still observed. A large IgM-κ monoclonal plasma cell population was seen in a mediastinal lymph node with a smaller IgA-λ monoclonal plasma cell population in a mesenteric lymph node.

There was no monoclonal plasma cell population in

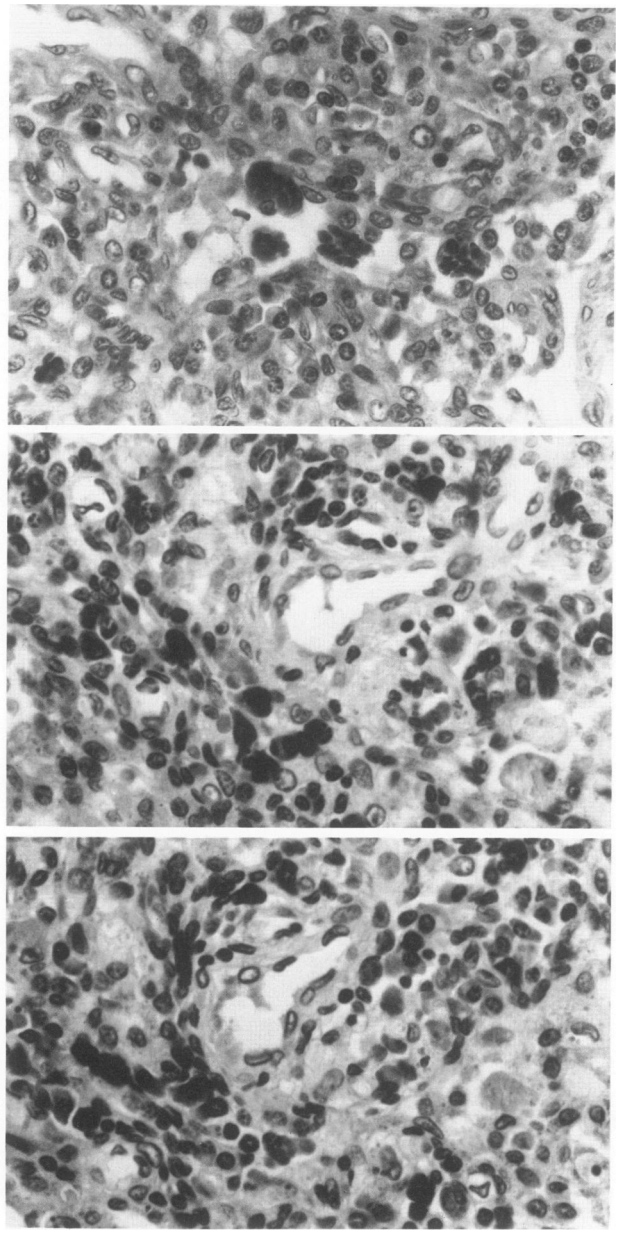

Figure 3—Representative inguinal lymph node of UPN_{28}. **A**—Medullary cords are hypocellular. Numerous mature plasma cells are present, sometimes with small nucleoli. Sinusoids contain histiocytes, often filled with red blood cells. (H&E, ×400) **B** and **C**—Immunoperoxidase staining with the light chains κ (**B**) and λ (**C**), illustrating a polyclonal distribution of plasma cells. (PAP, ×400)

UPN_{34}. The digestive tract contained a polyclonal plasma cell population with frequent IgA-positive cells. The lymphoid system was rich in polyclonal IgG plasma cells. All the tissues were heavily filled with intranuclear cytomegaloviruslike inclusions and rich in polyclonal IgM plasma cells.

In UPN_{39}, a single IgG-λ monoclonal plasma cell population was observed in an inguinal lymph node (Figure 4A and B), the lymphoid system otherwise

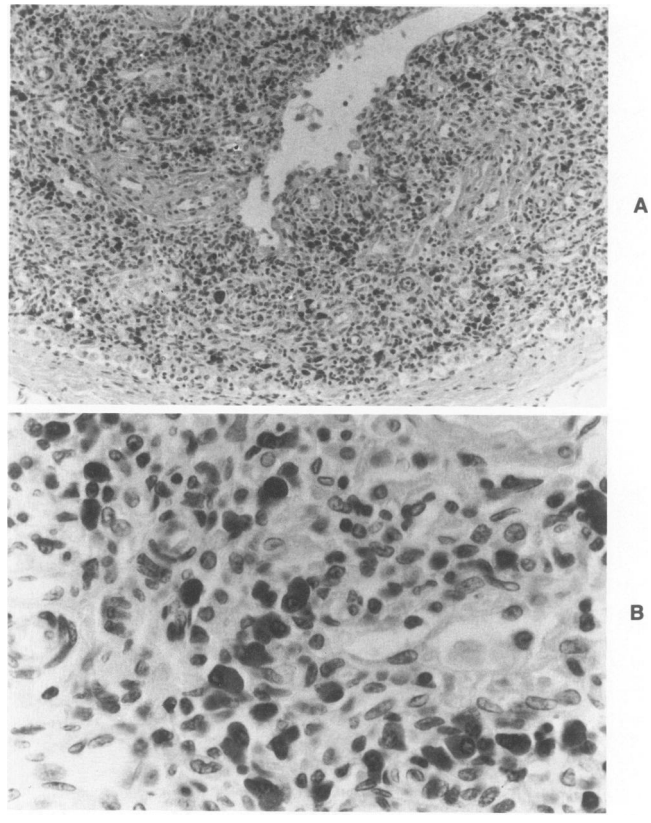

Figure 4—Immunoperoxidase staining of an inguinal lymph node of UPN_{39}. **A**—A small, localized clone of plasma cells positive exclusively for the light chain λ. (PAP, ×100) **B**—The plasma cells of this clone look essentially mature, with occasional small nucleoli. (PAP, ×400)

showing an IgG-predominant polyclonal plasma cell population and the digestive system, an IgA-predominant polyclonal plasma cell population.

UPN_{42} lymphoid and digestive systems contained, respectively, two distinct IgG- and IgA-predominant polyclonal plasma cell populations. The monoclonal plasma cell populations were located exclusively in the digestive system, with a small IgM-λ monoclonal plasma cell population surrounding a major excretory duct of a minor salivary gland, a well-circumscribed IgG-λ monoclonal plasma cell population in the ileal mucosa, a distinct IgG-λ monoclonal plasma cell popu-

Table 3—Distribution of Polyclonal Heavy Chains and Germinal Centers in Lymph Nodes and Tonsils of BMT Patients and Controls*

Group	Total number of samples	Germinal centers	Number of samples positive for the heavy chains				
			γ	α	μ	δ	ε
BMT patients	79	0	79	79	79	0	26
Controls	38	38	38	38	38	24	30

* Equal areas of tissue sections were compared.

lation spreading over four adjacent portal areas of the liver and finally a smaller IgM-κ monoclonal plasma cell population in a single portal space.

In UPN_{43}, the predominant IgG polyclonal plasma cell population of the lymphoid system contrasted with the predominant IgA polyclonal plasma cell population of the digestive tract. Numerous IgM plasma cells were also recorded. A single distinct IgG-κ monoclonal plasma cell population occupied the serosa of the esophagus.

Comparison With Controls

The polyclonal distribution of the heavy chains and the frequency of germinal centers in both tonsils and lymph nodes of all BMT patients (except for UPN_{26}, due to massive leukemic infiltration) and controls are compared in Table 3 (for BMT patients, samples with monoclonal plasma cell populations were excluded). No monoclonal plasma cell populations were found in the control group, in sharp contrast with the total of 18 monoclonal plasma cell populations observed in 6 of 8 BMT patients.

Discussion

The present study clearly defines two distinct types of plasma cell populations in a group of patients who died at various times following allogeneic BMT. The first type, found in every patient, consisted of widely distributed polyclonal proliferations that were positive for the heavy chains γ, α, μ, and ε. Plasma cells in the digestive tract were mainly positive for the α heavy chain, whereas the γ heavy chain predominated elsewhere in the lymphoid system. As opposed to the polyclonal plasma cell populations identified in the control group, the lymph nodes and tonsils of the transplant patients were characterized by the absence of germinal centers and IgD-positive plasma cells. These observations seem of interest to us, considering the immunohistology of the human lymph node and the proposed model of *in situ* B-cell maturation.[11-15] Thus, immunohistologic studies have revealed two types of B-lymphocytic populations: medium-sized $sIgM^+$, $sIgD^-$ B lymphocytes, so-called monocytoid B lymphocytes, or marginal zone B lymphocytes, form histologically distinct aggregates outside of the germinal center in the subsinusoidal and perifollicular areas; while the small $sIgM^+$, $sIgD^+$ B-cell subset forms the mantles of the germinal centers, which are composed of a mixture of dendritic reticulum cells, T lymphocytes (mostly of the helper T-cell phenotype), centrocytes, and centroblasts. The IgD-negative polyclonal plasma cell populations of our patients could conceivably derive from these $sIgM^+$, $sIgD^-$ B lymphocytes. Their lymph nodes and spleens, however, lacked the typical florid monocytoid B-cell areas described in reactive lymphadenitis.[11] Treatment of mice with large doses of cyclophosphamide (500 mg/sqm) eliminates marginal zone B lymphocytes.[16,17] Prior to transplantation, 7 of our 8 patients received cyclophosphamide (120 mg/kg) as part of their conditioning regimens. On the other hand, repopulating $sIgM^+$ B lymphocytes, presumably of donor origin, have been detected in the peripheral blood as early as 3 weeks after-grafting.[18] Also, bone marrow cells from normal rats are efficient at restoring marginal-zone cell numbers in cyclophosphamide-treated rats.[17] *In situ* immunohistologic demonstration of $sIgM^+$, $sIgD^-$ B lymphocytes in the lymph nodes of our patients could solve this dilemma. In accordance with studies in rodents,[19,20] van den Oord et al[11] have suggested that upon antigenic challenge, specific-antibody production in human lymph nodes follows a two-stage pathway; thus, in a first T-cell independant step, $sIgM^+$, $sIgD^-$ progenitors proliferate and differentiate nonspecifically into $sIgM^+$ $sIgD^+$ B lymphocytes, which then require a direct participation of the dendritic cells and helper T cells for further development into specific-antibody–producing cells.

Therefore, the absence of germinal centers and IgD-positive plasma cells in our patient group could be explained in several ways. First, a failure of differentiation of the $sIgM^+$, $sIgD^-$ B lymphocytes into $sIgM^+$, $sIgD^+$ B lymphocytes could result either from an intrinsic defect of the B cells or from a dysregulation of the T-cell lymphocytes either as an oversuppression or a lack of helper function.[1,4,6] Secondly, a severe deficit of the skin antigen-presenting cells, so-called Langherans cells, has been documented after BMT.[21] Although not tested in the current study, lymph nodes from these patients could also present a dendritic antigen-presenting cell defect. Finally, in both man and animals, germinal center development is highly dependent upon an adequate T-cell function.[22-24] The profound thymic dysplasia and the well-described impairment of the T-cell axis in transplant patients[1,4,6] could contribute to the failure of germinal center development. Moreover, previous studies in animals have stressed the crucial role of the germinal centers for specific-antibody production.[22,25] The *in situ* lack of germinal centers observed in our patients could correlate with the poor specific-antibody response after BMT.

The second type of plasma cell population, found in most patients (6 of 8), consisted of a group of monoclonal plasma cell populations positive for the heavy chains γ, α, μ, and δ. The absence of any invasive features, mitotic activity, and appreciable cellular atypia suggest their benign nature.[26] The relatively high

incidence of these monoclonal plasma cell populations was unexpected, considering their absence in our control group and the very rare occurrence of monoclonal gammopathies in patients following BMT.[27] In fact, we have detected only one monoclonal gammopathy (IgG-κ in UPN_{43}) among 50 consecutive patients undergoing transplants for various diseases (unpublished data). The frequency of these monoclonal plasma cell populations seems interesting in view of the increased frequency of oligoclonal B-cell proliferations[28] and monoclonal B-cell lymphomas[29] reported in BMT patients. However, the nature of these monoclonal plasma cell populations remains unknown. The presence of an IgD-positive monoclonal plasma cell population in UPN_{23} is perplexing, because in our control group IgD was related to germinal centers, a known site of specific-antibody production.[22,25] After human BMT monoclonal gammopathies were proposed as specific antibody reactions.[18] They were also shown to be specific antibodies against an administered antigen after BMT in rhesus monkeys.[30,31] In the rabbit spleen, specific-antibody-containing cells are clonally expanded.[32] We know from animal studies that a profound T-cell defect, as documented in nude mice and following thymectomy, favors the spontaneous occurrence of monoclonal proliferations.[33,34] A similar relationship could perhaps exist after BMT because these patients present a severe thymic dysplasia[35] and decreased T-cell functions.[1,4,6]

As a concluding remark, we cannot ascertain whether our observations represent the normal pattern of distribution of the plasma cell populations following allogeneic BMT because acute GVHD was documented in most of our patients (7 of 8). Further studies are needed to clarify this point.

Addendum

Since the submission of the manuscript, Schuurman et al[36] have reported IgE monoclonal plasma cell populations in a bone-marrow-grafted patient. Combined with our results, this suggests that all the heavy chains (γ, α, μ, δ, and ε) can be expanded in patients with bone marrow grafts.

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