Immunoglobulin Heavy Chain Variable Region Gene Usage in Bone Marrow Transplant Recipients: Lack of Somatic Mutation Indicates a Maturational Arrest

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Many recipients of bone marrow transplant (BMT) make normal amounts of serum immunoglobulin but are deficient in generating specific antibody responses to exogenous stimuli. To determine if abnormal usage of V_H genes contributes to this immunodeficiency, the usage of V_H genes was determined in peripheral blood B cells of four BMT recipients, two of whom had developed chronic graft versus host disease. The pattern of usage of V_H3 or V_H4 genes assessed at either 90 days or approximately 1 year after transplant was similar to that observed in healthy subjects and was marked by the over utilization of two elements, one V_H3 and one V_H4. However, the repertoires of each of the four BMT recipients appeared to be less complex than the repertoires of healthy subjects. The differences were a consequence of the accumulation of somatic mutations among rearrangements in the controls but not in the BMT recipients. The failure to accumulate somatic mutations in rearranged V_H genes is consistent with a defect in antigen driven B-cell responses. These results indicate that although the V_H gene content of the repertoire has normalized by 90 days posttransplant, a maturational arrest in B-cell differentiation associated with antigen activation persists for at least 1 year after BMT.

ALL MARROW recipients exhibit immunodeficiencies in the first 3 months after bone marrow transplantation (BMT). Both cellular and humoral immunity are affected, reflecting in part the recapitulation of ontogeny. B cells recover slowly in numbers, in frequency, and in function.[1-8] Impaired function of B cells isolated during the first 90 days after transplant is apparent in their reduced ability to proliferate or to secrete Ig in response to mitogens.[3,5,7-9] These in vitro functions usually approach normalcy by 1 year after transplant.[1-3,5-8,10,11] Coincident with recovering B-cell functions, serum Ig levels also return to normal.[5,10,12]

Despite this recovery, marrow recipients who survive this initial postgraft period do not always become fully immunocompetent. Early posttransplant B-lymphopoiesis is mono- or oligoclonal, and restricted clonality is more frequent in patients with chronic graft versus host disease (GVHD). Immune responses to the antigens φX174 and KLH are meager in patients with chronic GVHD.[6] As well, some long-term survivors suffer from opportunistic bacterial infections, especially with encapsulated bacteria such as *Streptococcus pneumoniae* and *Hemophilus influenzae* type b.[13,14] Susceptibility to infections is most severe in recipients with chronic GVHD; the mortality rate for infections in these patients is 15% to 40%.[15] Because immune responses to polysaccharides present in bacterial capsules are typically T-independent, at least a portion of the defect is likely intrinsic to B cells.

The nature of the B-cell defect(s) leading to this specific humoral immunodeficiency is uncertain. Normal levels of serum IgM and IgG[10,12] indicate that immunodeficiency is not because of a general failure to produce Ig or an overt lack of T-cell help in heavy chain class switching (although T-cell function may be impaired to some extent). One possibility is that abnormal restriction of the potential Ig repertoire in B cells from BMT patients, possibly because of effects of chronic GVHD, contributes to humoral immunodeficiency.

The Ig repertoire is established by the developmentally regulated usage of V genes[16,17] and the ensuing selection of B cells.[16-19] Variable regions of Ig heavy chains are generated during differentiation of a B cell by combinatorial association of three gene elements, V_H, D_H, and J_H. Approximately 100 V_H genes are organized into seven families based on nucleic acid hybridization and DNA sequence similarities.[20-24] The families range in size from one gene (V_H6 family)[23] to approximately 30 to 35 genes (V_H3 family).[20]

The usage of V_H families is not random during development[25-29] or in phenotypically defined subpopulations of B cells.[30,31] For example fetal B cells derived from 7 weeks of gestation use genes from the V_H5 and V_H6 families, exclusively. Although all V_H families are used by 15 to 18 weeks of gestation, V_H gene usage may still not be totally random. For example, at this time not all genes within the V_H3 family are used with equal frequency.[26,27,29] In adults, although the relative percentage of V_H family usage is approximately equivalent to the proportion of each family in the genome,[31-36] within V_H families certain elements predominate and some elements are underrepresented or absent.[37] During fetal development, unequal usage of the variable region gene families has been hypothesized to account in part for the immunodeficiency of human neonates,[16,27] which lack antibody responses to many antigens, including *H. influenzae*.[38] Concomitant then with the normalization of the repertoire would be the ability to respond to the wide variety of environmental antigens typically encountered. At least in adults, although the repertoire is not totally random within V gene families, immunodeficiencies are not apparent. However, it remains unknown if deviation from the normal representation of V_H

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Table 1. Patients Studied

Patient	Sex	Diagnosis	Onset of GVHD*	Age at Transplant	Age of Donor
UPN5012	F	Chronic myeloid leukemia	88	28	17
UPN5007	F	Acute myeloid leukemia	N	54	63
UPN4986	F	Chronic myeloid leukemia	N	37	33
UPN5403	M	Chronic myeloid leukemia	90	45	39

Abbreviation: N, no GVHD present.
* Number of days following BMT.

families or genes in B cells after BMT accounts, in part, for the observed immunodeficiencies.

The usage of V_H families following BMT appears to mimic usage during B-cell ontogeny.[39,40] Thus, to account for the observed immunodeficiencies, it might be expected that during the first year posttransplant, BMT patients would use a more limited set of V genes than would healthy adult subjects. This hypothesis is supported by the results of Storek et al[40] who found that the B-cell repertoire is restricted to fetal-type V_H genes early posttransplant. In this report, a direct assessment of the B-cell repertoire was made by analyzing the occurrence of specific V_H gene segments in rearrangements in peripheral blood (PB) B cells of BMT recipients at 90 days and 1 year following transplant. The results indicated that the pattern of usage of V_H genes was similar between BMT recipients and healthy subjects. However, BMT recipients exhibited a markedly reduced level of somatic mutation that is consistent with a maturational arrest at a fairly late stage of differentiation.

MATERIALS AND METHODS

BMT recipients and control subjects. Four patients (3 women, 1 man) and their marrow donors were recruited through the BMT program of the Fred Hutchinson Cancer Research Center under Institutional Review Board approval (Table 1). All were white. All received marrow from HLA-identical siblings. Two of the patients were diagnosed with GVHD within the first 100 days following transplant. Two healthy controls recruited from laboratory personnel under Virginia Mason Research Center (VMRC) Institutional Review Board approval were studied simultaneously. Analysis of the repertoire of these healthy subjects has been published.[37]

Cell isolations. All patient blood was obtained after approval by the Fred Hutchinson Cancer Research Center Institutional Review Board. Healthy subject blood was obtained after approval by the Virginia Mason Research Center Institutional Review Board. PB mononuclear cells (MC) were isolated from a Ficoll-Hypaque gradient (Pharmacia Biotech, Piscataway, NJ). B cells were stained with fluorescein-conjugated anti-IgM or anti-IgD (Coulter, Hialeah, FL), phycoerythrin (PE)-conjugated anti-CD19 or CD20 (Coulter), or appropriately conjugated mouse Ig isotype control antibodies. Viable lymphocytes were selected on the basis of forward and side-angle light scattering criteria. A Coulter Epics 750 or a Becton Dickinson Facstar flow cytometer (Becton Dickinson, Mountain View, CA) was used to sort live B cells into positively stained fractions. The non-B cell (negative) fraction of each sort was also collected. The purity of the sorted B cells ranged from 94% to 95%. Phenotypic analyses are shown (see Table 2).

Rearrangement library construction by V_H family-specific primer method. Sorted B cells were lysed in cell lysis buffer (50 mmol/L Tris, pH 8.0, 1 mmol/L Na EDTA, 0.5% NP-40, 0.5% Triton X-100 (JT Baker, Phillipsburg, NJ), 0.5% Tween 80, and 200 μg/mL proteinase K) and incubated at 50°C for 30 minutes. Cell lysates were sonicated for 30 seconds using a Sonifier 450 (Branson, Danbury, CT) fitted with a cup horn, at 50% duty cycle, 50% output to provide more uniform polymerase chain reaction (PCR) amplification. Proteinase K was heat-inactivated by incubation for 10 minutes at 95°C. The rearranged V_H genes were amplified using one of the family-specific 5′ primers, V_H3-L, 5′-CTGAATTCCATGGAGTTTGGGCTGAG-3′ or V_H4-L, 5′-CCGAATTCATGAAACACCTGTGGTTCTT-3′, corresponding to the 5′ ends of the leader sequences of V_H3 and V_H4 families, respectively, and the 3′ primer J_HAmp-7, 5′-GCTCTAGACT(T/C)ACCTGAGGAGACGGTGA-3′, complementary to the 3′ end of the 6 J_H gene sequences. Restriction sites (*Eco*RI for 5′ primers; *Xba* I for 3′ primers) included in the primers are underlined. An aliquot of lysate containing 15,000 to 50,000 cell equivalents was amplified by PCR using cycle conditions to obtain amplification in the linear range. The linearity of the PCR reactions has been confirmed by electrophoresing [α-^{32}P]dCTP-incorporated PCR products on a 2.0% agarose gel and quantifying the bands by phosphor imaging (Molecular Dynamics 400A PhosphorImager, Sunnyvale, CA).[37] The PCR products were cloned into *Eco*RI/*Xba*I digested pBS(M13+) phagemid vector and the recombinant plasmids were used to transform competent DH5aF′ (GIBCO-BRL, Gaithersburg, MD) or BSJ72.[41] The transformants were toothpicked into wells of a 96-well plate containing Luria Broth (10 mg/mL bacto-tryptone, 5 mg/mL bacto-yeast extract, 10 mg/mL NaCl, pH 7) with 100 μg/mL carbenicillin or ampicillin, 4 μg/mL kanamycin, and viruses containing single stranded DNA were rescued by the addition of K07 helper phage.

Germ-line library construction. V_H family-specific germ-line libraries were similarly generated as previously described from either the negative fraction collected from the cell sorts or unsorted PBMC. PCR reactions used for constructing V_H3 libraries were carried out using the 5′ primer VH3-L and either the 3′ primer VH3-RS, 5′-GACTCTAGACAATGACTTCCCCTACT-3′, which is complementary to the 3′ flanking recombination signal sequence of V_H3 genes, or VH3-FR3, 5′-GACTCTAGATCTCAGGCTGTTCATTTG-3′, which is complementary to a conserved V_H3 framework three (FR3) sequence. PCR reactions used for constructing V_H4 libraries were performed using the 5′ primer VH4-L and either the 3′ primer V_H4-RS, 5′-AATTCTAGACTGGGCTCACACTCACCTCC-3′, which is complementary to the 3′ flanking recombination signal sequence of V_H4 genes, or VH4-FR3, 5′-AATTCTAGACACAGAGCTCAGCTTCAG-3′, which is complementary to a conserved V_H4 FR3 sequence.

Library screening. Multiple replicate filters were prepared by dot blotting 10 μL of supernatants containing phage particles in a 96-well grid on Hybond N+ nylon filters (Amersham, Arlington Heights, IL). Filters were denatured in 0.5 mol/L NaOH, 1.5 mol/L NaCl, neutralized in 0.5 mol/L Tris-HCl, 1.5 mol/L NaCl, and UV cross-linked. Replicate filters prepared from the libraries were probed as previously described.[42,43] Hybridization with ^{32}P-labeled family-specific V_H probes allowed a determination of the total number of recombinant clones in each library. A panel of diagnostic ^{32}P-labeled oligonucleotide probes that identify individual V_H gene segments were also hybridized to replicate filters. The frequency of occurrence of each specific V_H gene segment was calculated by dividing the number of clones hybridizing with an oligo probe by the total number of clones hybridizing with the family-specific probe.

Table 2. Phenotype of Lymphocytes From BMT Recipients

	Donor			90 d Posttransplant			1 yr Posttransplant		
Patient	CD20	CD5 + CD20	IgM + IgD	CD19	CD5 + CD19	IgM + IgD	CD19	CD5 + CD19	IgM + IgD
	% of Total PBL			% of Total PBL			% of Total PBL		
UPN5012	8.22	3.64	ND*	7.91	4.36	2.17	13.6	6.09	11.7
UPN5007	15.1	5.3	ND	8.94	2.02	4.58	ND	ND	ND
UPN4986	5.05	2.58	9.6	13.13	4.74	10.39	14.54	6.73	10.24
UPN5403	9.68 (CD19)	4.0 (CD19)	4.06	3.70	2.43	1.09	16.62	6.04	12.4

Abbreviation: ND, not determined.

Oligonucleotide probes. The following oligonucleotide probes were used: M8, AGCAGCTATGCCATGAGCTGG[44]; M76, GCAGTTATATGGTATGATGGA[44,45]; M16, AGTAGCTATGGCATGCACTGG[46]; E36, AGTGGTAGTACCATATACTAC; H110, CGTATTAAAAGCAAAACTGAT[42]; M85, AGTAGCTACGACATGCACTGG[44]; M19, GGAAGCAATAAATACTACGCA[46]; M41, GGAAGTAATAAATACTACGCA[44,45]; E42, AGTATCTATTATAGTGGGAGC (reverse complement of M114[47]); M69, TGGTGGAGCTGGGTCCGCCAG[47]; E57, GAAATCTATCATAGTGGGAGC (reverse complement of M115[47]); M109, AGTGGTTACTACTGGAGCTGG[47]; M101, AGCAGTGGTAACTGGTGAATC[47]; E8, TCCATCAGCAGTGGTAGTTAC[47]; E44, CGTATCTATACCAGTGGGAGC (reverse complement of M121[47]); E58, TACATCTATTACAGTGGGAGC (reverse complement of M103[47]); M105, AGTGGTGATTACTACTGGAGT[47]; E13, TCCGTCAGCAGTGGTAGTTAC[47]; E7, TCCATCAGTAGTTACTACTGG[47]; M100, AGCAGTAGTAACTGGTGGGGC[47]; M86, GGTGGTTACTCCTGGAGCTGG.[47]

RESULTS

Regeneration of B cells following BMT. The number and percentage of B cells found in PB were determined at intervals after marrow transplant for the marrow recipients and before marrow donation for the marrow donors (Table 2). Mononuclear cells were stained with a fluorescein-conjugated anti-CD20 or anti-CD19, and in separate analysis, with fluorescein-conjugated anti-IgM and PE-conjugated anti-IgD, and analyzed by flow cytometry. For all patients at both timepoints, the percent of total lymphocytes that were B cells was within normal range.

Serum Ig levels were assayed at approximately 90 days and 1 year after transplant (Table 3). By 90 days, serum Ig levels were largely within the normal range. In patient UPN5012, IgM was below normal at 90 days and IgA was below normal at 1 year. In patient UPN4986, IgG was slightly low at 1 year, and in patient UPN5403, IgM was slightly low at 90 days after transplant.

Table 3. Serum Ig Levels of Marrow Recipients

Patient	IgM		IgG		IgA	
	90 d	1 yr	90 d	1 yr	90 d	1 yr
UPN5012	34*	154	846	1,060	90	28
UPN5007	190	ND	899	ND	93	ND
UPN4986	185	116	705	613	99	79
UPN5403	52	57	784	843	155	125
Normal	56-275		670-1,700		70-350	

Abbreviation: ND, not determined.
* mg/dL.

V_H gene-specific analysis. To assess the usage of individual genes within V_H3 and V_H4 families, libraries of rearrangements were generated using V_H family specific 5′ primers and the consensus J_H 3′ primer as described previously.[37] This system has been found to amplify approximately 25 V_H3 genes and 10 to 12 V_H4 genes,[37] which together account for more than 75% of the total expressed V_H repertoire.[48] Synthetic oligonucleotide probes that specifically identify both germ line and rearranged individual V_H elements directly in genomic DNA and in libraries of cloned V regions have been described.[37,42,44,46] For this report six V_H3 and 11 V_H4 gene segments were selected for analysis based on two criteria: (1) the gene could be amplified quantitatively from the germline in control experiments, and (2) specific, diagnostic oligonucleotide probes were available for the gene. The six V_H3 elements assessed here account for approximately 50% to 80% of the V_H3 component, and the V_H4 elements assessed account for virtually 100% of the V_H4 component of the expressed repertoire (discussed later). Therefore, we estimate that the 17 elements assessed comprise from 45% to 60% of the total expressed repertoire.

Rearrangements of the six V_H3 genes were assessed in PB B cells of four BMT recipients and compared to similarly obtained rearrangements from two healthy subjects. Heavy chain rearrangements were amplified quantitatively and the resulting PCR products were cloned into a phagemid vector. Identification of the V_H gene present in an individual clone was established by hybridization with a sequence-specific oligonucleotide probe. More than 700 independent rearrangements from each individual were analyzed. For controls, amplifications, and subsequent identification of nonrearranged V_H3 and V_H4 genes from the same individuals were also performed.

The occurrence of V_H3 genes in rearrangements for all subjects is shown in Fig 1. The occurrence of rearranged V_H3 genes assessed at 90 days and approximately 1 year after transplant is similar to that observed in the healthy controls. The variation between individuals is similar to that seen previously in a healthy population.[49] One of the healthy subjects (Nor4882) and one of the BMT recipients (UPN5403) have a deletion of the V3-11 gene and one of the BMT recipients (UPN5007) has a deletion of the V3-33

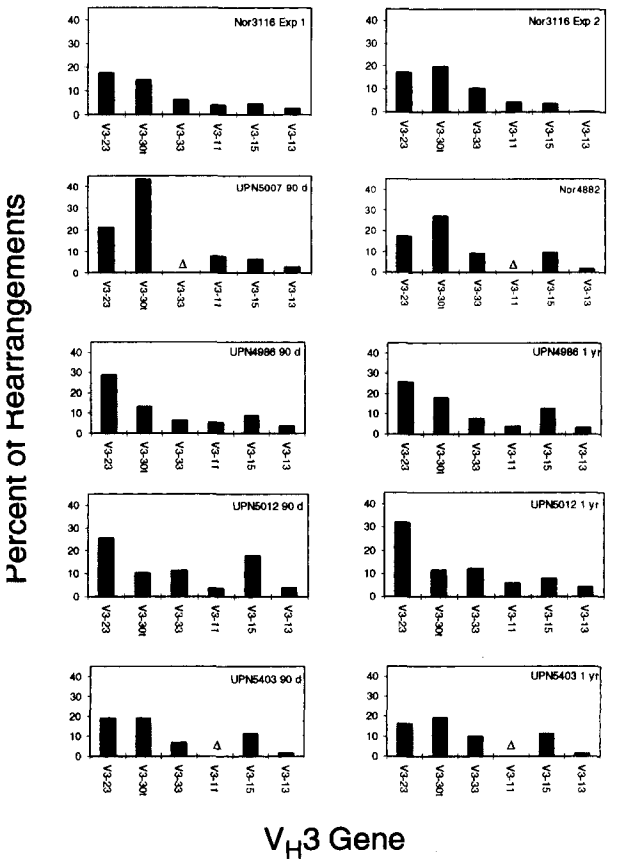

Fig 1. V_H3 repertoire in BMT recipients and normal subjects. The frequency of representation of six V_H3 genes in phagemid clones of quantitatively amplified rearranged V_H3 genes is shown. Data in each panel represent analysis of at least 700 rearrangements. The sample used in experiment 2 of subject Nor3116 was obtained 8 months after the sample used in experiment 1. (Δ) indicates that subject has a germ-line deletion of this V_H element. V_H nomenclature is that of Matsuda et al,[55] except V3-30t which encompasses a complex allelic region and may include V3-30, V3-30b, and/or V3-30.4. Data for Nor3116 and Nor4885 are from Suzuki et al.[37]

gene (data not shown). These deletions account for the failure to detect rearrangement of these genes in these subjects (Fig 1).

In addition, rearrangements of individual V_H4 genes were assessed in PB B cells of one of the BMT recipients and from one of the healthy subjects (Fig 2). As was the case for V_H3 rearrangements, no significant difference was observed between the patient and the control. To the extent that these results are representative of the entire B-cell repertoire, they indicate that the repertoire following BMT contains substantially the same assortment of V_H genes as that found in nontransplanted controls.

The repertoires of the BMT recipients are less complex than are the repertoires of healthy subjects. Figure 3 shows the percent of rearrangements in which the germline V_H gene of origin could be assigned by hybridization. Among the V_H3-containing rearrangements (A), there was a slight trend toward increased identification in BMT recipients compared to the two healthy subjects ($p < .005$ for patients v controls). This trend was more pronounced among V_H4-containing rearrangements (B). Sequence-specific oligonucleotide probes to 9 V_H4 loci (11 distinct gene segments) identified more than 99% of the V_H4 rearrangements in the BMT recipient, but only 80% of the V_H4 rearrangements in the healthy subject ($p < 10^{-5}$ for patients v control). This observed difference in the percentage of identified rearrangements between healthy subjects and the BMT patients could be because the BMT recipients used fewer V_H genes. However, extensive hybridization and sequence analysis have not revealed the presence of additional V_H genes rearranged in healthy subjects but not rearranged in BMT recipients (A.M. Glas and E.C.B. Milner, unpublished observations, 1994). Alternatively, and more likely, these results suggest that the abrogation of hybridization resulted from the accumulation of somatic mutations in the target regions of the probes in healthy subjects but not BMT recipients. Therefore the accumulation of somatic mutations was assessed in the two groups.

Detection of somatic mutations by sequence-specific hybridization. The accumulation of somatic mutations in BMT recipients and healthy subjects was addressed directly in the following manner. Somatic mutations in one V_H3 gene, V3-23, can be detected by sequential hybridization with multiple probes. The germ line sequence of V3-23 can be detected by either a CDR1 probe or a FR3 probe. Hybridization of both of these probes on rearranged V3-23 genes indicate which have retained the germ-line sequence through the target regions. However, rearranged V3-23 genes that have accumulated one or more mutations in the target site of one or the other probe will display a loss of concordance when hybridized. Figure 4 shows the percent of V3-23 rearrangements that have lost concordance for one of the probes in the four BMT patients and the two healthy subjects. Among the BMT recipients, the percent of V3-23 rearrangements isolated from $CD19^+$ or $CD20^+$ B cells that have acquired mutations ranged from <1% to approximately 10%. For patients UPN5403 and UPN4986 the percentage was similar between samples taken 90 days and 1 year posttransplant. The distribution of somatic mutations in the healthy subjects was assessed in different B-cell populations. In one of the healthy subjects, rearrangement libraries were constructed from three B cell populations: (1) $CD19^+$ B cells (all B cells), (2) $CD19^+$, IgD^+ B cells (preimmune B cells), and (3) $CD19^+$, IgD^- B cells (antigen-driven B cells). Among $CD19^+$ B cells, approximately 30% had acquired mutations in CDR1 of V3-23 (Fig 4). When the $CD19^+$ B cell population was further fractionated on the basis of IgD expression, it was found, as expected, that the vast majority of mutations could be attributed to the IgD^- population. More than 70% of V3-23-containing rearrangements from IgD^- cells had detectable mutations. In contrast, approximately 10% of rearrangements from IgD^+ cells had detectable mutations, a value that is comparable to that observed among the BMT recipients. In a second experiment, B cells from another healthy subject were sorted for IgM. Approximately 20% of V3-23-containing rearrangements from IgM^+ cells in this subject had acquired mutations. This value is approximately

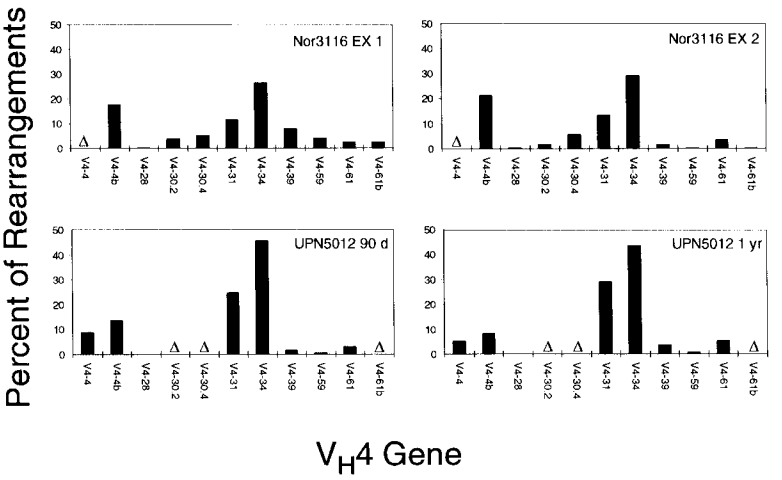

Fig 2. V_H4 repertoire in a BMT recipient and a normal subject. The frequency of representation of nine V_H4 loci (11 elements) in phagemid clones of quantitatively amplified rearranged V_H4 genes is shown. Data in each panel represent analysis of at least 700 rearrangements. The sample used in Nor3116 experiment 2 was obtained 8 months after the sample used in experiment 1. (Δ) indicates that subject has a germline deletion of this V_H element. Data for Nor3116 are from Suzuki et al.[37]

twice the frequency of occurrence of mutations among the BMT recipients. Together, these results indicate that utilization and diversification of V_H genes in the peripheral B-cell repertoire of BMT recipients is similar to the utilization and diversification of V_H genes in the preimmune component of the peripheral B-cell repertoire of healthy subjects.

DISCUSSION

In this report, the usage of V_H3 and V_H4 genes in four BMT recipients was assessed. In this regard, the frequency of rearrangements that contained each of 6 distinct V_H3 loci, and 9 V_H4 loci (11 gene segments) was determined among PB B cells of these subjects. We have previously found that healthy adult subjects exhibit a biased but highly reproducible pattern of V_H gene utilization in rearrangements.[37,49]

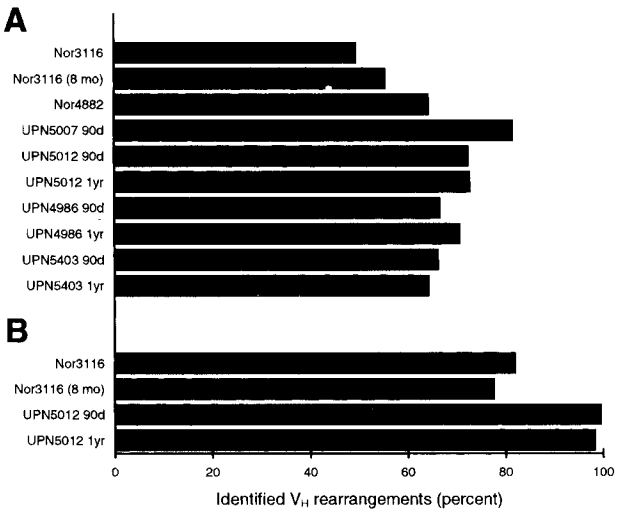

Fig 3. Germ-line complexity of the amplifiable V_H3 (A) or V_H4 (B) repertoires. Bars represent the percent of rearrangements in each library for which the germ-line gene of origin could be assigned by sequence-specific hybridization.

The results reported here indicate that, compared to healthy subjects, there were no apparent differences in the spectrum of genes used at either 90 days or approximately 1 year after transplant among the BMT recipients. We interpret these observations to mean that the processes involved in generating the antibody repertoire are largely functional within the first few months following BMT. Furthermore, these results indicate that the immunodeficiencies common among BMT recipients are not likely because of the failure to use appropriate V region genes in generating the preimmune antibody repertoire.

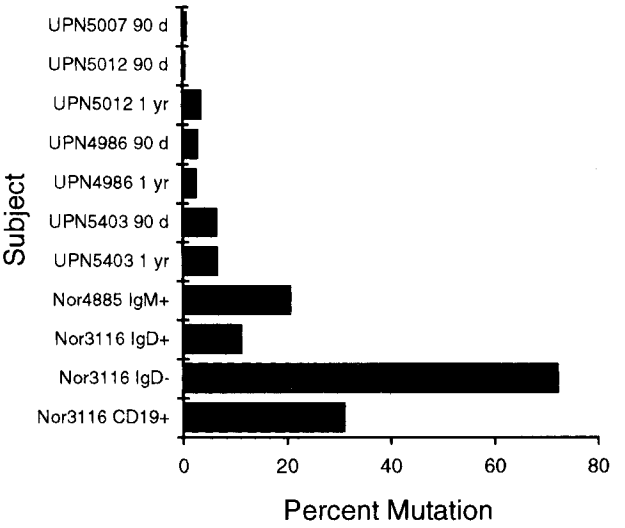

Fig 4. Analysis of somatic mutation in rearranged V_H3 genes from BMT recipients and normal subjects. The accumulation of somatic mutations in one V_H3 gene, V3-23, was assessed by sequential hybridization with the probes, M8 and M18, as described in Materials and Methods. Clones that have accumulated one or more mutations in the target site of one or the other probe will display a loss of concordance when hybridized. Each instance of discordant hybridization is recorded as a single mutation. The results are presented as the percent of V3-23 rearrangements that have acquired a mutation (ie, lost concordance for one of the probes).

As for the specific immunodeficiency of BMT recipients, our results provide indications of possible mechanisms contributing to poor antibody responses. Strikingly, rearranged V_H genes exhibited much less somatic mutation in BMT recipients than did similar rearrangements obtained from healthy subjects. In contrast to healthy subjects, more than 90% of peripheral B cells from BMT recipients are phenotypically $CD19^+$, IgM^+, IgD^+. This phenotype is characteristic of preimmune B cells. When we stratified the peripheral B cell compartment from the healthy subjects, as expected, somatic mutations were found preferentially among the IgD^- population, a population that is all but nonexistent among BMT recipients. The frequency of mutations among the IgD^+ population, and, to a lesser extent, the IgM^+ population, was similar to that seen in the BMT patients (Fig 3). Thus, by both cell-surface phenotype and extent of somatic diversification, the B-cell repertoire of BMT recipients resembles the preimmune component of the B-cell repertoire of a healthy adult, but lacks features of a mature B-cell repertoire.

The accumulation of somatic mutations is a characteristic of T cell-dependent antigen-driven responses.[50] Conversely, the absence of a somatically diversified B-cell population suggests an absence of antigen-driven processes. The failure in the BMT recipients to accumulate somatic mutations in rearranged V_H genes is consistent with a defect in antigen-driven B-cell responses. However, not all aspects of antigen-driven responses are defective in BMT recipients. For example, BMT recipients are able to make high titers of antibodies and to exhibit class switching, processes that are also dependent on the presence of functional $CD4^+$ T cells. As such, the data are most consistent with a maturational arrest, which may be limited to the stage of B-cell differentiation during which somatic mutation occurs.

As somatic mutation and affinity maturation are thought to occur primarily in lymph node germinal centers, one attractive hypothesis is that a failure of germinal center processes prevents the normal accumulation of somatic mutations following immunization in BMT recipients. Consistent with this hypothesis are the observations that germinal centers are generally absent from lymph nodes on histologic analysis for months to years in BMT recipients,[51] and that the reconstitution of $CD4^+CD8^-$ T cells (but not $CD4^-CD8^+$ T cells) is similarly delayed.[52] In contrast to BMT recipients, germinal centers are present in neonates from about 1 month of age.[53]

Although GVHD is a potent suppressor of immune function, the presence of GVHD cannot be the direct cause of the apparent maturational arrest observed in these studies because two of the patients were free of GVHD. Significantly, there were no observable differences in the diversification of the antibody repertoire between patients with or without GVHD.

Although the data presented here provide an explanation for the specific immunodeficiencies after BMT, further study is needed to determine the parameters of recovery of the capacity to mount an effective antibody response. In this regard, analysis of the cause and effect relationship between ineffective antibody responses and germinal center formation in BMT recipients is likely to be especially illuminating. It may be, for example, that the pretransplant conditioning regimen disrupts a critical cellular function, or destroys a critical population of cells, that is not restored by marrow transplant. In addition, a more comprehensive analysis of recovery of T-cell function might provide insights. Neither T-cell phenotype nor functional analysis was available for the patients studied here. In general, recovery of normal numbers of $CD4^+$ T cells is slow and may not be achieved for more than 1 year posttransplant.[54]

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