Ig V_H Gene Mutational Patterns Indicate Different Tumor Cell Status in Human Myeloma and Monoclonal Gammopathy of Undetermined Significance

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Plasma cell tumors display a wide spectrum of clinical progression, ranging from aggressive multiple myeloma to a benign form known as monoclonal gammopathy of undetermined significance (MGUS), which requires no treatment. Because both diseases involve mature Ig-secreting plasma cells, the reason for this variation in malignant behavior is unclear. However, assessment of malignant potential is desirable for choice of treatment protocols. Ig variable (V_H) gene sequence analysis has previously shown the tumor cell of multiple myeloma to be postfollicular, with mutated homogeneous clonal sequences indicating no continuing exposure to the somatic hypermutation mechanism, and this was confirmed in 7 of 7 patients. Comparison of the V_H gene sequences in the monoclonal cells in MGUS yielded a different result, with 3 of 7 patients demonstrating mutated heterogeneous sequences consistent with the tumor cells remaining under the influence of the mutator. In 1 of 3 of these patients, an IgM-positive precursor cell was identified that expressed heterogeneous V_H sequences similar to those of the isotype-switched plasma cell. These results indicate that the clonal cells in MGUS differ from those in myeloma and suggest that the difference may reflect malignant potential.

MULTIPLE MYELOMA is a malignant tumor involving plasma cells that accumulate in the bone marrow (BM). Clinical manifestations are diverse but often include osteolytic lesions and renal impairment. The tumor cells generally secrete a monoclonal Ig paraprotein that can be identified in serum, and free Ig light chains (Bence-Jones proteins) may be present in urine. However, levels of normal Ig tend to be low. Prognosis remains poor despite modern chemotherapy, with a median survival of about 30 months.[1] Recent protocols of intensive chemotherapy, sometimes with an autologous BM transplant, have improved initial response, although long-term survival remains to be assessed.[2]

Although monoclonal gammopathy of undetermined significance (MGUS) also involves monoclonal plasma cells, the clinical manifestations are different.[3] In a study at the Mayo Clinic in 1988, MGUS was distinguished from myeloma by having a lower level of serum paraprotein; little or no monoclonal protein in urine; the absence of lytic lesions, anemia, hypercalcemia, and renal insufficiency; and, most importantly, stability of the level of paraprotein and failure to develop other abnormalities.[3] A further distinction is that patients with MGUS tend to maintain normal levels of polyclonal Ig in serum.[3] With regard to the monoclonal cell populations in the two diseases, patients with myeloma characteristically have greater than 10% plasma cells in the BM, and those cells are more often in division, as judged by the plasma cell labeling index.[3]

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The relationship between myeloma and MGUS remains uncertain despite many clinical studies. In fact, the term MGUS was introduced by Kyle and Lust[3] to replace benign monoclonal gammopathy on the grounds that only time will show whether a monoclonal protein is truly benign or the first manifestation of myeloma or another lymphoproliferative disease. The Mayo Clinic observed 241 patients with a known serum paraprotein and found that 36 (15%) developed myeloma within a median time of 9.6 years.[3] Other studies have produced similar figures and have indicated that prediction of patients who may undergo transformation to myeloma is not currently feasible.[4] A particular frustration is in the problem of discriminating between stage I myeloma and MGUS, because the ability to do this is important to both patient and physician, given that prevalence of MGUS is considerable, especially in the older age group.[5] In addition, early prognostic discrimination may influence the entry of patients into protocols involving treatment with cytokines. The limits of current information concerning the monoclonal plasma cell of MGUS also affect nomenclature of the cell of origin. Although a clonal population clearly exists, there are arguments for not referring to it as a tumor.[3] However, in the absence of a suitable alternative, the term tumor will be used to describe the monoclonal cell population of MGUS in this report.

Analysis of rearranged functional Ig variable region genes in B-cell tumors is providing information that relates to the clonal history of the cell of origin.[6,7] In particular, accumulation of somatic mutations in the V-genes indicates that the cell has been exposed to the hypermutation mechanism and has therefore traversed the germinal center (reviewed by Berek[8]). For myeloma, numerous studies have shown that the tumor cell has undergone extensive somatic hypermutation, but, in contrast to follicular lymphoma,[6,7] homogeneity of tumor V_H sequence indicates that it is no longer being exposed to the mutator and can be considered as a postfollicular cell.[9,10] This report describes a parallel analysis of the tumor cells of MGUS and shows in some patients a heterogeneity of clonal sequences, suggesting a clonal history closer to follicular lymphoma than to myeloma.

MATERIALS AND METHODS

Patients. Unselected patients from the Haematology (UK) or Immunology (Germany) Clinics were diagnosed as having multiple

Table 1. Characteristics of MM/MGUS Patients

Patient No.	Age (yr)	Diagnosis/Stage*	Months From Diagnosis	Paraprotein		% Plasma Cells	Polyclonal Ig	Osteolytic Lesions	Bence-Jones Proteinuria
				Class	Level (g/L)				
1	55	MM/II	67	IgAλ	3	1.8	–	+	+
2	80	MM/IIA	0	IgGκ	59	14.0	–	+	+
3	52	MM/IIIB	0	IgGλ	79	15.0	–	+	–
4	86	MM/I	0	IgGκ	18	5.0	–	+	ND
5	73	MM/I	7	IgGλ	0†	8.0	–	+	–
6	74	MM/II	15	IgGκ	43	5.0	–	+	+
7	78	MM/IIIA	19	IgGκ	24	11.0	–	+	+
8	63	MGUS	11	IgAλ	18	8.6	+	–	+
9	66	MGUS	19	IgGκ	13	0.5	+	–	–
10	41	MGUS	13	IgGκ	3	6.2	+	–	–
11	71	MGUS	125	IgGλ	5	8.0	+	–	–
12	78	MGUS	0	IgGλ	8	5.0	+	–	–
13	67	MGUS	3	IgGκ	10	1.3	+	–	–
14	87	MGUS	1	IgGκ	13	7.0	+	–	–

Abbreviation: ND, not done.

* Durie and Salmon.[11]

† Nonsecretory myeloma.

myeloma (7 patients) or MGUS (7 patients) using clinical and laboratory criteria (Table 1).[11] Among those with myeloma, patients no. 1, 6, and 7 had recently completed a course of chemotherapy at the time of investigation, and the tumor load was reduced, particularly for patient no. 1. Assignment to the myeloma category was performed by the clinician and, for patients no. 4 and 5 with stage 1 disease, was influenced by the presence of osteolytic lesions. This feature was present in all the patients with myeloma, none of whom had metastatic carcinoma. Six of seven of the group had an identifiable monoclonal Ig in the serum that was of the same Ig class and light chain type as found in the plasma cells in the BM. Patient no. 5 was unusual in having no detectable monoclonal Ig in serum, but IgGλ was detectable in plasma cells in the marrow, consistent with nonsecretory myeloma. Four of six evaluable patients in the group had detectable urinary Bence-Jones protein, and all had reduced levels of normal polyclonal Ig in serum.

Patients in the MGUS category were all untreated and had monoclonal Ig in serum at less than 30 g/L, less than 10% plasma cells in BM, less frequent urinary Bence-Jones protein (1/7), absence of lytic lesions, and normal levels of polyclonal Ig in serum. Although none of these features is sufficient to assign the patient to the MGUS group, the total profile would be consistent with MGUS.[3] Lack of progression of disease and stability of the serum monoclonal Ig levels were seen in patients no. 8 through 11, but patients no. 12 through 14 are too recently diagnosed for this information.

Cell preparation and phenotypic analysis. Heparinized BM aspirates were taken and mononuclear cells (MNCs) were separated by centrifugation on Ficoll-Hypaque. The degree of infiltration of the BM by monotypic plasma cells was determined by direct immunofluorescent staining for surface CD38 and cytoplasmic κ or λ light chains, as described previously, using the Facstar Plus system.[12] In some cases, plasma cell involvement was assessed by staining cytocentrifuged MNC preparations, followed by fluorescence microscopy.

Preparation of cDNA. For tumor cells, the V_H gene analysis was performed using RNA as a source material. The fact that plasma cells are likely to contain more RNA than contaminating normal B cells, together with the reduced likelihood of amplifying an aberrantly rearranged V_H gene on the allelic chromosome, made this a preferred approach. Total RNA (2 to 8 μg) was isolated from the MNC fraction (1 to 5 × 10^6 cells) of the BM aspirates using RNAzol B (Cinna Biotecx Labs, Inc, Houston, TX), with added t-RNA (2 μg) as a carrier. Reverse transcription was performed using an appropriate constant region primer to match the identified tumor-derived Ig. For IgG or IgA, a Cγ primer (5′-CACCGTCACCGGTTCGG) or a Cα primer (5′-CTGGGTGCTGCAGAGGCT) was used, respectively, with a first-strand cDNA synthesis kit (Pharmacia, Uppsala, Sweden).

Amplification and sequencing of V_H genes. For analysis of the V_H of tumor cells, one-fifth to one-third of a sample of cDNA, was amplified by PCR using a mixture of 5′ oligonucleotide primers specific for each of the V_H leader sequences of V_H1-6 families (V_H1: 5′-CTCACCATGGACTGGACCTGGAG; V_H2:5′-ATGGACATACTTTGTTCCACGCTC; V_H3: 5′-CCATGGAGTTTGGGCTGAGCTGG; V_H4: 5′-ACATGAAACAYCTGTGGTTCTTCC; V_H5: 5′-ATGGGGTCAACCGCCATCCTCCG; V_H6: 5′-ATGTCTGTCTCCTTCCTCATCTTC) together with 3′ primers specific for the constant region (see above). In all cases, polymerase chain reaction (PCR) conditions were as described.[10]

Gel-purified products of predicted size were blunt-end ligated into pGEM-TA vector (Promega, Madison, WI) and used to transform JM109 competent cells (Promega). Clones found to contain an insert of appropriate size by restriction analysis of plasmid DNA were sequenced by the dideoxy chain termination method, with alignment being made to current EMBL/GenBank and V-BASE sequence directories,[13] using MacVector 4.0 sequence analysis software (International Biotechnologies, Inc, New Haven, CT). At least two independent PCR amplifications were performed from each sample.

Investigation of tumor-related V_H-Cμ transcripts. For patient no. 13, precursor V_H-Cμ transcripts with the tumor-related clonal signature in CDR3 were sought using a three-step seminested PCR approach. Total RNA (5 μg) was reverse transcribed using an outer Cμ1 primer (5′-GACGGAATTCTCACAGGAGAC). In step 1, one-fourth of the cDNA (18 μL) was amplified using the 5′-V_H3 leader primer together with an inner 3′-Cμ2 primer (5′-CGAGGGGGAAAAGGGT). The product of predicted size was cloned and 15 randomly selected colonies were sequenced to confirm the presence of Cμ. In step 2, 1/25 of the PCR product of step 1 was amplified with a 5′-CDR3–specific primer (5′-GGATATTAYTATGATMGT) together with the 3′-Cμ2 primer. Amplification conditions were modified to include an annealing temperature of 42°C for 1 minute. In step 3, to obtain a full V_H sequence, 1/25 of step 1 product was

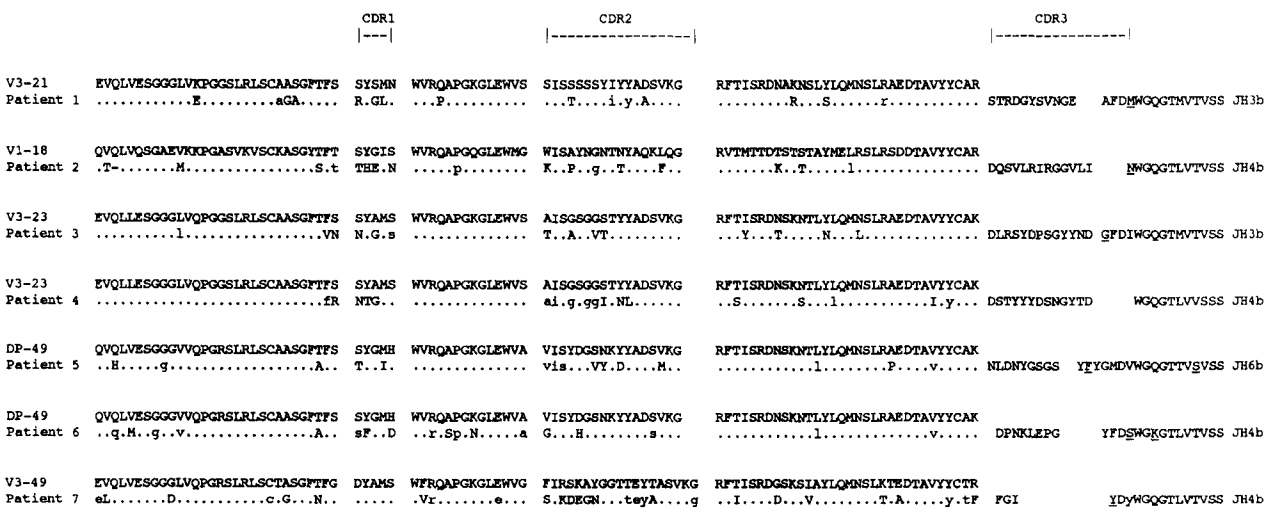

Fig 1. Deduced amino acid sequences of the V_H regions of the tumor-related clones from patients with myeloma. Comparisons are made with the closest germline V_H genes. Upper case letters, replacement mutations; lower case letters, silent mutations. Replacement mutations in the J_H regions are underlined.

amplified with the 5′-V_H3 leader primer together with a 3′-CDR3-specific primer (5′-GYAGTTACCACCACKATCATA) and an annealing temperature of 52°C for 1 minute. PCR products of predicted size were cloned and sequenced.

RESULTS

V_H sequences from tumor cells of patients with myeloma. Preparations of cDNA obtained from BM MNC populations of 7 patients with myeloma were amplified with mixed 5′-V_H leader-specific primers together with a 3′-constant region primer chosen from the Ig class of the tumor-derived paraprotein (Table 1). The PCR products were cloned and sequenced; in all cases, a predominant V_H sequence with an identical CDR3 clonal signature was evident and was confirmed by repeated PCR. Remaining clones contained individually distinct V_H sequences, presumably derived from normal plasma cells or B cells. Deduced amino acid sequences of the predominant clones are shown in Fig 1; nucleotide sequences have been submitted to the EMBL database (accession nos. X88806-12).

Sequence analysis of the tumor-related clones (Fig 1 and Table 2) indicated that 6 of 7 were derived from the V_H3 family and 1 of 7 from the V_H1 family. For all sequences, homology with germline sequence was quite low, suggesting that a significant degree of somatic mutation has occurred. Deviations from germline sequence were also evident in J_H, again consistent with somatic mutation events. However, although the V_H repertoire now includes a large content of allelic variation, assignment of each nucleotide change to a somatic event, particularly for polymorphic genes such as the V_H3 family, may still require identification of the corresponding germline gene in the individual patient and this has not been performed. In Table 2, each nucleotide change has been treated as a separate event and is listed as a replacement (R) or silent (S) mutation. The distribution of the R and S mutations indicates a high R:S ratio in the CDRs of patients no. 2 (10:1) and 3 (6:1), which could suggest a role for antigen selection.[14] However, these ratios did not exceed 2.5:1 for the remaining 5 patients. This inconsistency in distribution of replacement mutations is similar to that reported for other cases of myeloma[9,10] and leaves open the role of antigen selection.

V_H sequences from tumor cells of patients with MGUS. A similar approach was used to identify the tumor-related sequences from patients with MGUS. Repeated sequences were again identified after PCR and cloning, although the

Table 2. Analysis of V_H Genes From Cases of Myeloma

Patient No.	Ig Class	V_H Family	GL Donor	% Homology	R/S Mutations FWR	R/S Mutations CDR	J_H	Tumor-Derived Sequences/ Clones Sequenced
1	IgA	V_H3	V3-21	94.2	6/3	5/2	J_H3b	8/8
2	IgG	V_H1	V1-18	91.5	5/3	10/1	J_H4b	10/10
3	IgG	V_H3	V3-23	94.2	8/2	6/1	J_H3b	8/8
4	IgG	V_H3	V3-23	93.2	4/3	8/5	J_H4b	10/10
5	IgG	V_H3	DP-49	94.2	3/3	6/5	J_H6b	10/12
6	IgG	V_H3	DP-49	92.2	4/9	6/3	J_H4b	12/13
7	IgG	V_H3	V3-49	88.7	13/8	8/5	J_H4b	7/12

Table 3. Analysis of V_H Genes From Cases of MGUS

Patient No.	Ig Class	V_H Family	GL Donor	% Homology	R/S Mutations FWR	R/S Mutations CDR	J_H	Tumor-Derived Sequences/ Clones Sequenced
8	IgA	V_H3	DP-49	91.5	3/8	12/2	J_H4b	10/10
9	IgG	V_H1	V1-24P	93.9	6/5	4/2	J_H4b	15/24
10	IgG	V_H2	S12-4	95.3	5/2	5/1	J_H5b	9/9
11	IgG	V_H3	DP-53	90.5	9/7	8/4	J_H6c	11/11
12	IgG	V_H3	VH3-8	93.2	8/5	5/2	J_H4b	6/8
13	IgG	V_H3	DP-58	94.6	6/4	5/1	J_H4b	16/20
14	IgG	V_H4	DP-67	93.9	6/4	5/3	J_H4b	4/12

number of sequences derived from contaminating B cells tended to be slightly higher (Table 3). Deduced amino acid sequences are shown in Fig 2 with nucleotide sequences again available from the EMBL data base (accession nos. X88813-15, X88824-26, and X88842). Sequence analysis showed that, as for the cases of myeloma, the most common V_H family used was again V_H3 (4/7), with the remaining 3 from the V_H1, V_H2, and V_H4 families. Deviations from corresponding germline sequences in the database suggested a high degree of somatic mutation, with the percentage of homologies being similar to those found for the myeloma sequences. Somatic events were confirmed by nucleotide changes in the relatively nonpolymorphic V_H4 gene (DP-67) used by patient no. 14 and by changes in J_H sequences (Fig 2 and Table 3). Analysis of the distribution of somatic mutations indicated an increased R:S ratio in the CDRs for some patients but not all, giving a heterogeneous picture similar to that seen in myeloma. The sequence derived from V_H4 did not show clustering of replacement mutations in the CDRs (R:S = 5:3), and the question of the role of antigen selection in myeloma or MGUS remains to be decided.

Analysis of intraclonal sequence heterogeneity. The tumor-derived V_H sequences from each patient could be identified from the CDR3 clonal signature, and, by comparing the mutational patterns of the individual clones at the nucleotide level, it was possible to investigate intraclonal sequence variation. For the cases of myeloma, all the clones obtained from each patient were sequenced (Table 2) and all had identical sequences with no nucleotide differences, consistent with an absence of intraclonal variation. In contrast, for the cases of MGUS, there was evidence for intraclonal variation among tumor-derived V_H sequences from 3 of 7 patients, indicative of ongoing mutational events in the neoplastic cell. This intraclonal heterogeneity is illustrated by the nucleotide sequences of the V_H genes of the three patients' tumor cells shown in Figs 3, 4, and 5. The heterogeneous mutations appear to vary in incidence among the 3 patients and to be scattered throughout the V_H sequences. However, replacement mutations occur in CDR3 in all cases. For patients no. 10 and 13, many of the nucleotide changes were identified in more than one sequence and indicate that several clonal members were undergoing further mutational events. For patient no. 14 and for some of the changes in patients no. 10 and 13, nucleotide changes occurred in only one sequence, and there is a theoretical possibility that such differences could have arisen due to Taq polymerase error. However, application of the

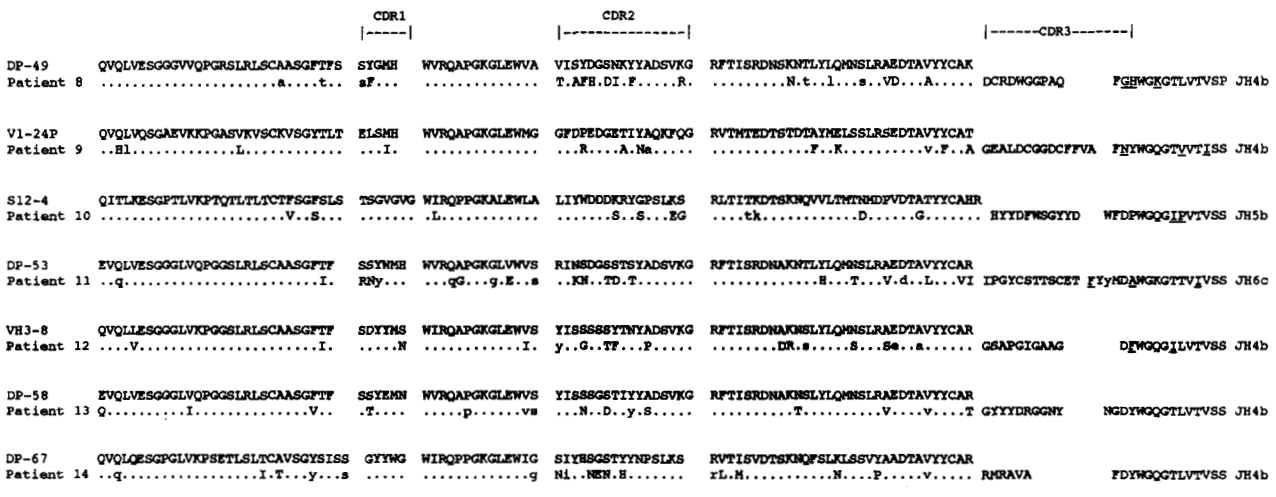

Fig 2. Deduced amino acid sequences of the V_H regions of the tumor-related clones from patients with MGUS. Comparisons are made with the closest germline V_H genes. Upper case letters, replacement mutations; lower case letters, silent mutations. Replacement mutations in the J_H regions are underlined.

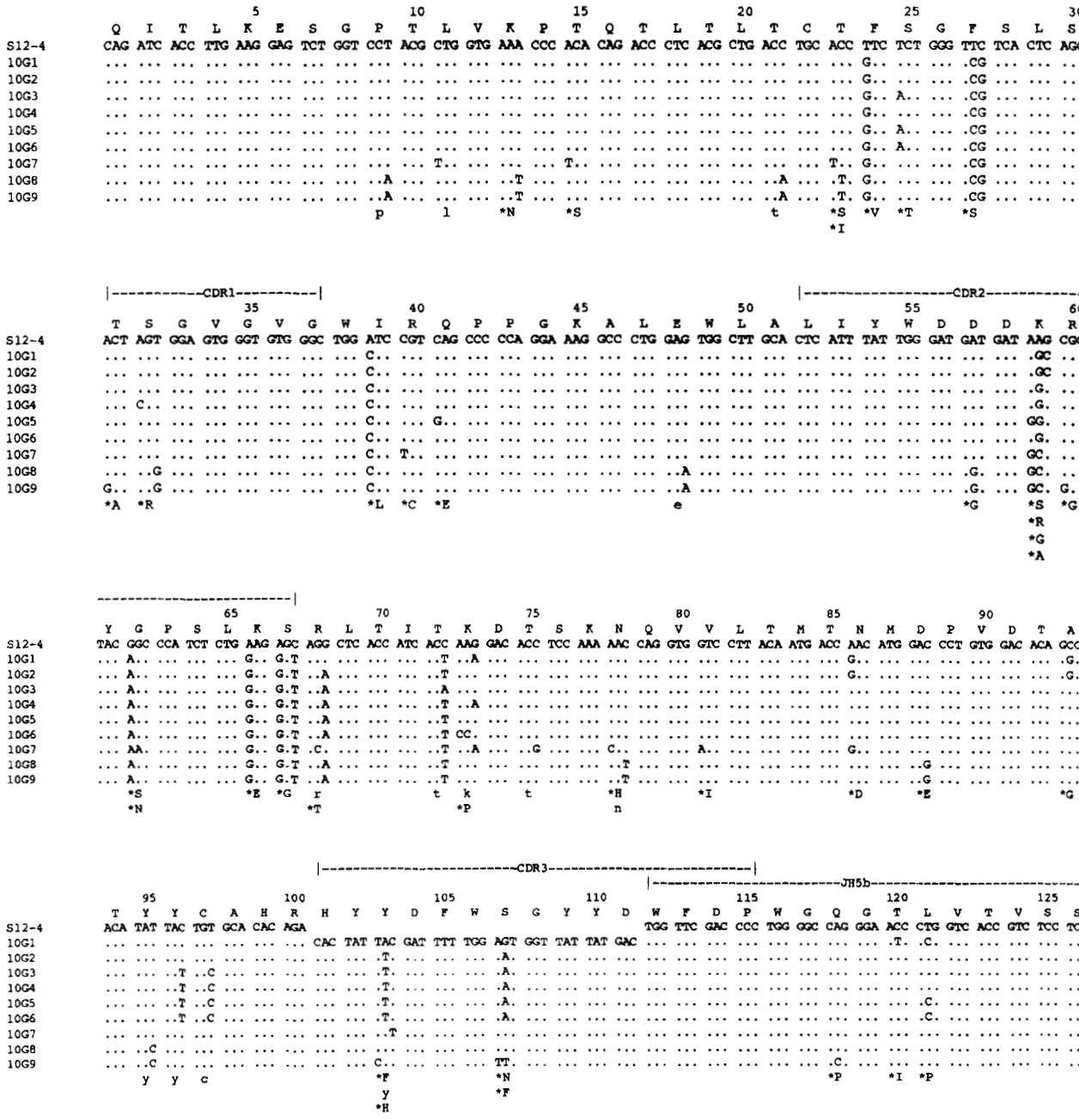

Fig 3. Intraclonal heterogeneity of the nucleotide sequences of the V_H gene used by IgG-positive tumor cells from patient no. 10 with MGUS. 10G1 through 10G9 represent individual cloned sequences that are compared with the closest germline gene, S12-4. Silent or replacement mutations are indicated below the sequences, in lower case or starred upper case letters, respectively.

same approach to the myeloma cases showed no intraclonal nucleotide changes, which would support the conclusion that these single changes are also the result of ongoing somatic mutation. The highest degree of heterogeneity was seen in patient no. 10, in whom 9 of 9 distinct tumor sequences were obtained (Fig 3). The pattern was consistent with the existence of two related parental clones that were not identified, but which gave rise to sequences 10G1-7 or 10G8-9 respectively (Fig 3). Sequences from patient no. 13 were more consistent with existence of a major clone (13G1) that has accumulated different individual single mutations or double mutations (Figs 4 and 6). The pattern of intraclonal variation may be explained by assuming the existence of a parent clone (13G0) that acquired an additional mutation to become 13G1, but that also diverged to accumulate different mutations to generate the minor clone 13G2, together with additional mutated progeny (Fig 6). Because of the low

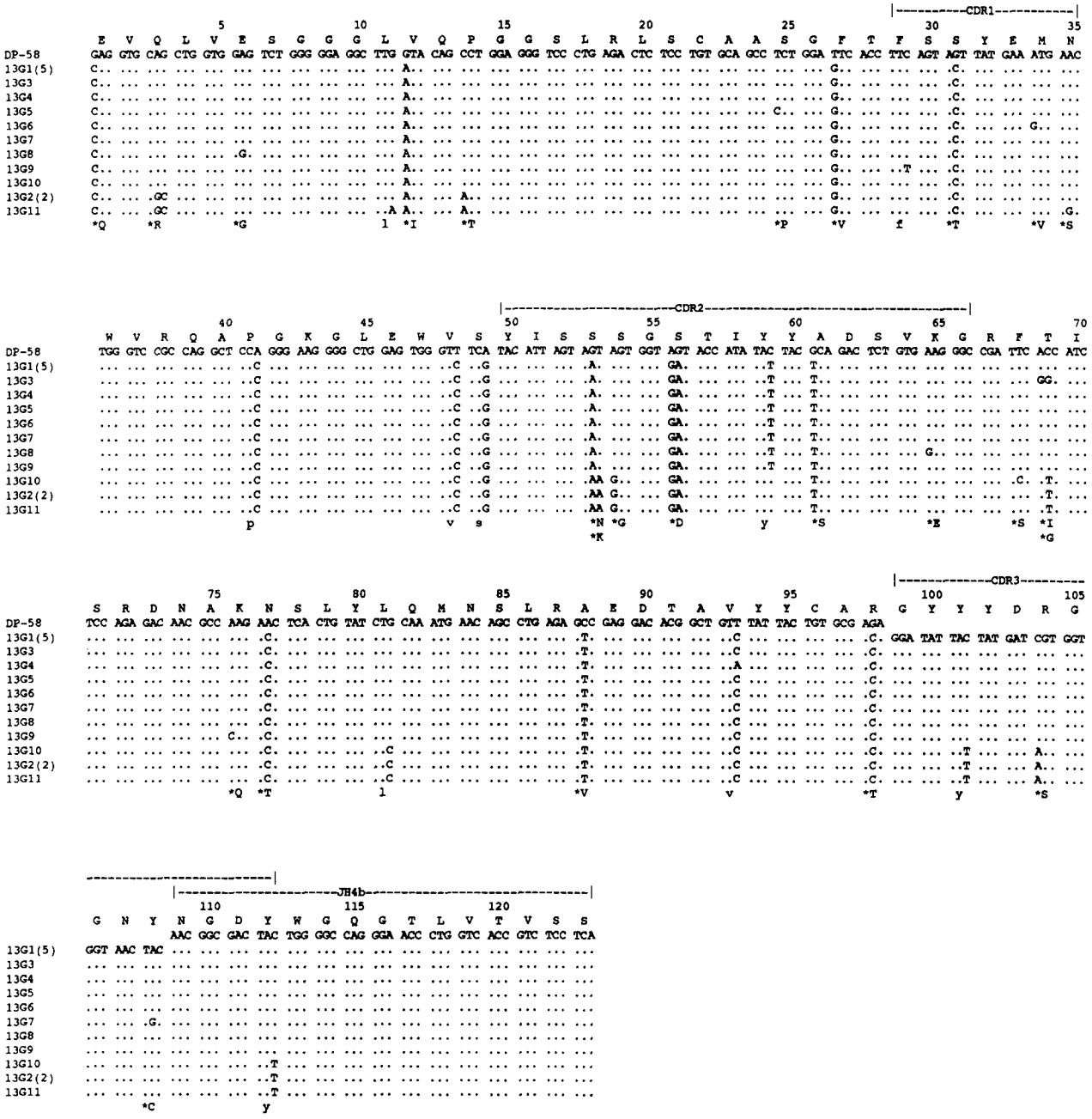

Fig 4. Intraclonal heterogeneity of the nucleotide sequences of the V_H gene used by IgG-positive tumor cells from patient no. 13 with MGUS. 13G1 through 13G11 represent individual cloned sequences that are compared with the closest germline gene, DP-58. Numbers in brackets indicate that some sequences were obtained in more than one clone. Silent or replacement mutations are indicated below the sequences, in lower case or starred upper case letters, respectively.

percentage of tumor sequences obtained from patient no. 14, only four clones were available for investigation, but heterogeneity was detected, with all the changes generating replacement amino acids.

V_H mutational pattern of an IgM-positive tumor precursor cell. In 1 of the cases of MGUS (patient no. 13) that had shown intraclonal V_H sequence variation in the IgG-positive tumor cell, it was possible to detect a preswitched IgM-positive cell that belonged to the tumor cell clone. Identification of the precursor was made by using a 5′-CDR3 primer together with a 3′-Cμ primer (step 2 of the seminested PCR in the Materials and Methods). Sequencing of 10 clones of the product showed a single sequence homologous to 13G1 (Fig 7), which did not contain the additional mutations seen in the CDR3/J_H regions of the IgG clones 13G7 or 13G10 (Fig 4). To obtain the full V_H-Cμ sequence, the third step of the seminested PCR was performed, and the sequences are shown in Fig 8. Clonal relationship with the isotype-

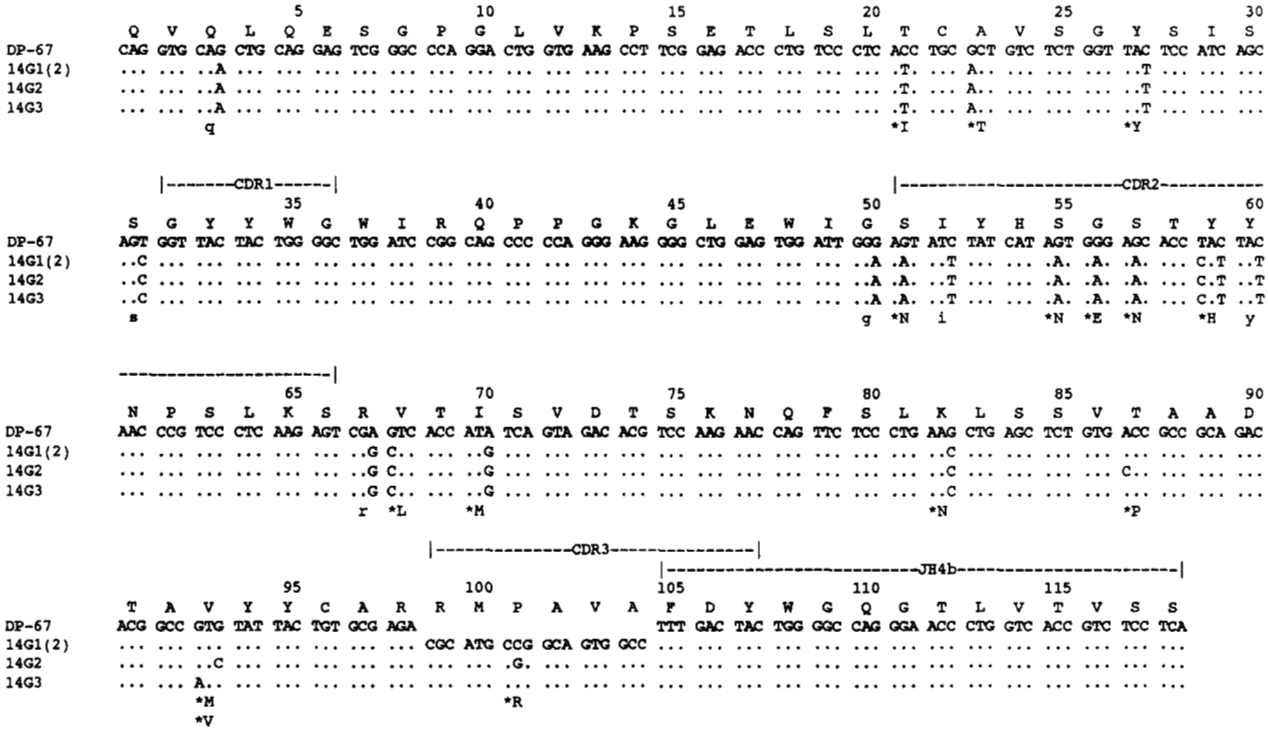

Fig 5. Intraclonal heterogeneity of the nucleotide sequences of the V_H gene used by IgG-positive tumor cells from patient no. 14 with MGUS. 14G1 through 14G3 represent individual cloned sequences that are compared with the closest germline gene, DP-67; the 14G1 sequence was found in two clones. Silent or replacement mutations are indicated below the sequences, in lower case or starred upper case letters, respectively.

switched tumor cells was indicated both by the CDR3 sequence and by the match to the DP-58 germline gene. There were also common deviations from germline in the V_H sequences of both the IgG clones and the IgM clones (Figs 4 and 8).

These data allowed a parallel analysis of intraclonal heterogeneity of the V_H sequences of the IgM-positive tumor cells (Fig 8). Overall, the degree of heterogeneity in the IgM-positive cells appeared less than in the IgG-positive cells. None of the IgM-derived sequences was identical to the isotype-switched sequences; however, the predominant IgM sequence (13M1) closely resembled that of the predominant IgG clone 13G1, with only a single nucleotide difference in codon 69 (Figs 4 and 8). The other IgM clones were similar to 13M1 but had other scattered single mutations, one of which (G to A in codon 11 of 13M4) was also present in

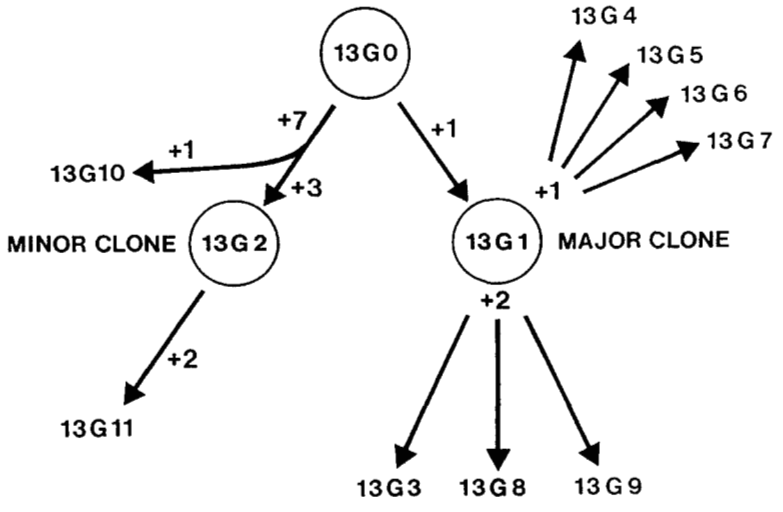

Fig 6. Intraclonal heterogeneity in tumor cells from patient no. 13. The V_H sequences from Fig 4 have been arranged in a genealogical tree according to mutational patterns. Clone 13G0 represents a hypothetical parental clone that acquired independent mutations to generate clones 13G1 and 13G2. Numbers prefixed by + indicate the number of mutations that have occurred between clonal members.

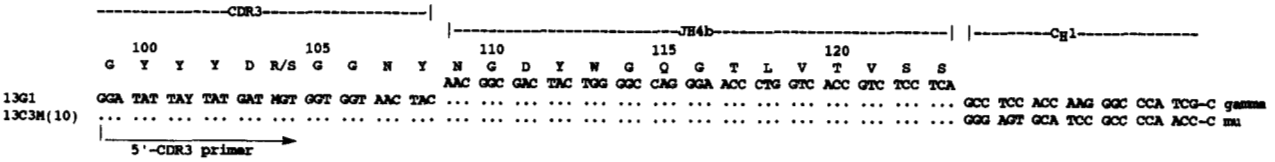

Fig 7. Nucleotide and deduced amino acid sequences of the CDR3-Cμ transcript from patient no. 13 with MGUS. Ten clones of identical sequence were obtained (13C3M) and comparison has been made with the sequence of the CDR3-Cγ transcript, 13G1. The position of the 5'-CDR3 primer is indicated.

an IgG clone (13G11). Other mutations present in the IgG clones related to the divergent 13G2 group (Fig 6) were not seen in IgM, perhaps indicating that at least some of the additional mutations occurred after isotype-switching.

DISCUSSION

During maturation of a B lymphocyte, Ig V_H genes undergo a process of recombination to generate a V_H-D_H-J_H–constant region unit. Heterogeneity of usage of the individual genetic components, together with gain and loss of nucleotides at the joints, leads to a unique amino acid sequence particularly in CDR3.[15] In a normal B cell, this process produces a wide range of available sequences for antigen recognition, with the unique CDR3 present at the center of the antigen-binding site.[16] In neoplastic B cells, the CDR3 presents a useful clonal marker for detection and tracking of the tumor clone. When a normal B cell enters the germinal center, the V_H genes are exposed to the somatic hypermutation mechanism, thereby generating nucleotide substitutions throughout the sequence.[8] Antigen selection can then lead to a concentration of replacement amino acids in the CDR sequences, which are known contact points for antigen.[8,14]

Because neoplastic B cells are considered to be frozen at a point in differentiation, analysis of V_H gene sequences can show aspects of the clonal history of the tumor cell. In the case of chronic lymphocytic leukemia, the V_H genes appear

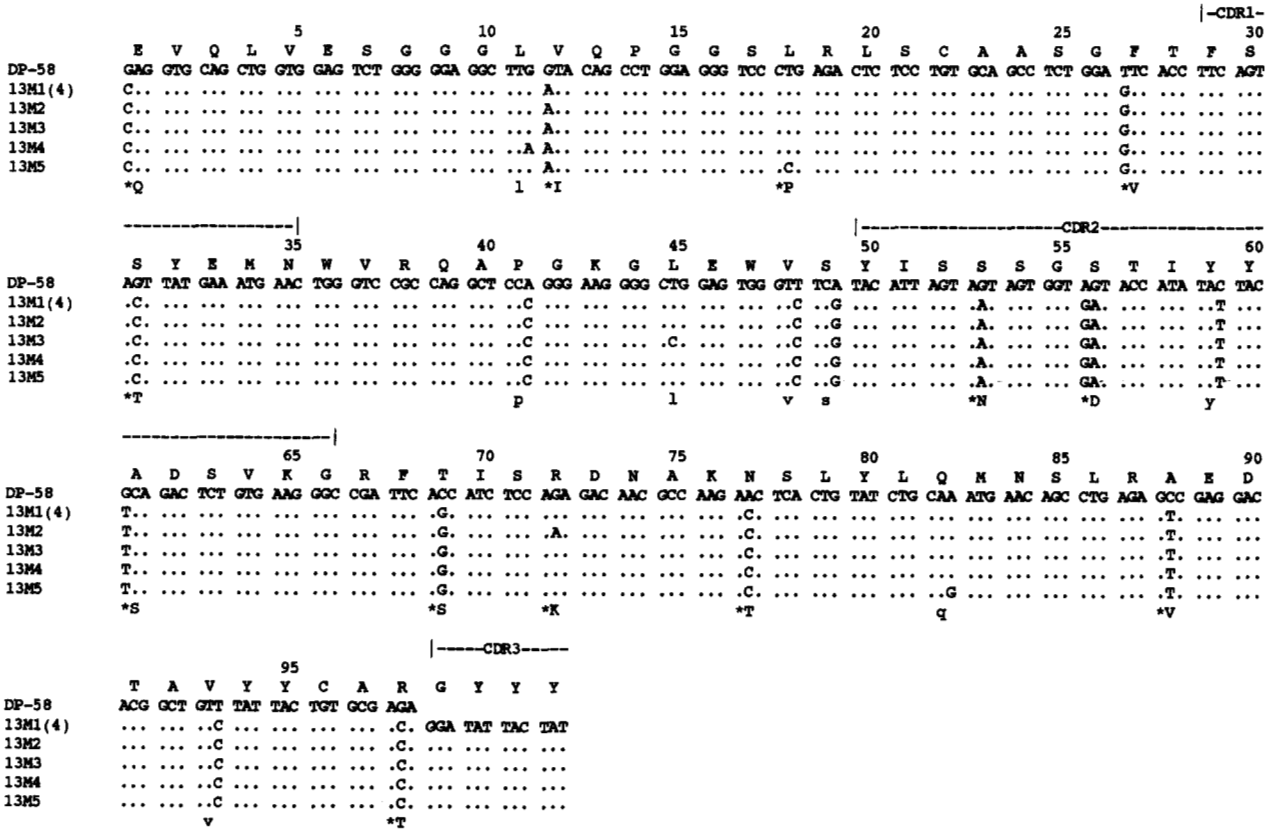

Fig 8. Intraclonal heterogeneity of the nucleotide sequences of the V_H gene used by IgM-positive clonal precursor tumor cells from patient no. 13 with MGUS. 13M1 through 13M5 represent individual cloned sequences that are compared with the closest germline gene, DP-58; the 13M1 sequence was found in four clones. Silent or replacement mutations are indicated below the sequences, in lower case or starred upper case letters, respectively.

to have a low number of somatic mutations, indicating that they may not have been in contact with the mutator that is likely to be localized in the germinal center.[8] In contrast, cases of follicular lymphoma (FL) tend to harbor more mutations, as would be expected from their residence in the germinal center.[17] Interestingly, tumor cells from patients with FL often display heterogeneous clonal sequences, with the same clonal signature in CDR3, but with additional mutations occurring in V_H.[6,7] This picture is consistent with continuing exposure of the tumor cells to the mutator subsequent to neoplastic transformation. A similar phenomenon has also been observed in cases of endemic Burkitt's lymphoma.[18] Although the role of antigen in driving tumor cell growth remains unknown, it is likely that the neoplastic event releases tumor cells from the apoptotic death that awaits normal unselected B cells.[19]

Several groups have analyzed V_H gene profiles in cases of multiple myeloma, and a consistent pattern has emerged.[9,10,20] It appears that the tumor cells are extensively somatically mutated and that the sequences are homogeneous. These findings suggest that the tumor cell has traversed the germinal center, but that it is no longer exposed to the mutator. Absence of intraclonal variation in myeloma has been observed at different stages of disease from presentation to plateau, indicating that it is a feature of the tumor cell population rather than a consequence of outgrowth of a member of the clone.[21] It could be concluded from this that the neoplastic event has occurred at a postfollicular stage, but this conclusion is questioned by the finding of IgM-positive B cells in the BM of identical V_H sequence to the isotype-switched cells.[22,23] The malignant potential of the IgM-positive cells, which tend to be present in low numbers, is unknown. One possibility is that they represent a population that has undergone a neoplastic event and that is still capable of isotype-switching. To reach full malignant status, a second genetic change may be necessary, and this may occur in a single isotype-switched cell, giving rise to the homogeneous V_H sequence characteristic of myeloma.[10]

Despite extensive investigations of serologic and cellular markers, the relationship between the plasma cell tumors myeloma and MGUS remains speculative.[3] However, clinical progression of the two tumors differs considerably, suggesting that there are major differences between either the nature of the tumor cell or its environment. In a minority of cases, MGUS can convert to myeloma,[3] although this process is usually slow and it has not always been verified that the same clone is involved.

V_H gene analysis has shown that, in 3 of 7 of our MGUS patients, there is intraclonal heterogeneity within the tumor population. This is reminiscent of follicular lymphoma and indicates that the tumor cell in MGUS continues to be exposed to the mutator after transformation. It also suggests that the BM tumor cells in MGUS are being continuously repopulated from the germinal center, rather than replicating in situ, a situation that may differ in myeloma. This is consistent with the fact that the plasma cell labeling index tends to be higher in myeloma.[24] For MGUS patient no. 13, we were able to identify the IgM-positive precursor cell, and this also showed mutational heterogeneity. This raises two possibilities for MGUS: the IgM population may harbor some genetic change, but, as for myeloma, it is still able to undergo isotype-switching. This capacity lies in several clonal members, so that the isotype-switched population is also heterogeneous. The isotype-switched cells may also be exposed to the mutator, accumulating additional changes. In contrast to myeloma, a subsequent chromosomal event has not occurred in the isotype-switched cells to generate a malignant cell with a single V_H sequence, and the tumor is benign. The fact that heterogeneity is not found in all cases of MGUS may be due to slight differences in relative growth rates of the members of the clone, which can lead to self-cloning. To assess this possibility, it is necessary to investigate more patients at an early stage of disease; such an investigation is in progress. An alternative explanation is that there is heterogeneity within the category of MGUS, with some cases involving cells that are no longer exposed to the mutator. In either case, a secondary genetic event could occur in the benign population, accounting for the cases that do transform from MGUS to myeloma. A similar emergence of a highly malignant subclone with a single V_H gene sequence from a tumor population containing intraclonal variants was documented in a case of follicular lymphoma that transformed into lymphoblastic lymphoma after chemotherapy.[7]

Whether this type of analysis is useful for distinguishing between early stage myeloma and MGUS is not yet evident; investigation of larger numbers of patients and a longer period of observation will be required. Heterogeneity was observed only in 3 of 7 patients, and, although that can be compared with a lack of heterogeneity in more than 60 patients with myeloma,[20] it is not diagnostic for MGUS. More patients will need to be investigated to see if heterogeneity delineates a subset of patients and if chromosomal changes correlate with the V_H gene profiles. Clearly, availability of V_H gene tags is providing new tools for old questions.

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