Monoclonal gammopathy of undetermined significance (MGUS) can transform to multiple myeloma (MM). In myeloma, mutated VH genes with sequence homogeneity reveal a postfollicular origin. Previously, some MGUS cases showed mutated VH genes with intraclonal variation, indicating an earlier stage of arrest. We investigated progression from 2 of 2 MGUS to MM, in which VH genes confirmed clonal evolution. In one MGUS case, intraclonal heterogeneity was evident, and transformation to myeloma occurred rapidly with apparent homogeneity in the emergent clone. However, residual MGUS-derived sequences were detectable at this time. Heterogeneity in MGUS does not associate with benign disease, but it indicates an origin from a tumorigenic cell, most likely surface immunoglobulin*, undergoing somatic mutation. The remaining case displayed intraclonal homogeneity at the MGUS stage, conceivably resulting from a self-cloning outgrowth from MGUS with heterogeneity. Transformation can occur at either MGUS stage, but it involves a single cell in which somatic mutation is then silent. (Blood. 2003;101:4137-4139)

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Study design

Patients

Case 1 presented with advanced lung cancer with pulmonary involvement and a lesion in TH10. Immunofixation identified a trace IgA lambda band. Bone-marrow (BM) biopsy (sample 1/1) revealed normal composition, with plasma cells (PCs) lower than 5%. Magnetic resonance imaging revealed infiltration of most vertebrae 4 months later. Serum M-component was demonstrated (total IgA, 64 g/L; M level, not determined [ND]), with 80% PCs in BM (sample 1/2). A retrospective classification of TH10 could not rule out an isolated plasmacytoma at first biopsy; however, this had excluded any clinical marrow involvement. Further, postmortem examination revealed disseminated myeloma and confirmed concomitant squamous-cell cancer with multiple pulmonary lesions, suggesting a possible metastatic TH10 spread.

Case 2 initially had an M-gradient in her serum electrophoresis (total IgG, 15.7 g/L; M level, ND; PCs, 5% of nucleated BM cells; sample 2/1) that progressed over 67 months (IgG, 26.6 g/L; BM PCs, 30%; sample 2/2).

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Cell preparation and V<sub>H</sub> gene analysis

BM aspirates were taken for magnetic activated cell sorting of CD138<sup>+</sup> cells. Total RNA isolation from CD138<sup>+</sup> cells (1 × 10<sup>6</sup>-2.6 × 10<sup>6</sup> cells) and cDNA preparation using oligo-dT or constant region (C<sub>H</sub>) primer were as described. cDNA was amplified using V<sub>H</sub>-7 leader primers with 3' IgG or IgA primer. Cloning, sequence analysis, and V<sub>H</sub> gene alignments were as reported. For V<sub>H</sub> genes, between 16 to 30 clones/sample were sequenced from 2 separate polymerase chain reactions (PCRs).

For each MGUS and MM sample, 246 to 258 bp of the tumor C<sub>H</sub> were amplified and cloned to establish the Taq error rate using single S<sup>+</sup> complementary determining region 3 (CDR3) and C<sub>Y</sub> or C<sub>A</sub> primers (916-3690 bp analyzed/sample). Taq error depends primarily upon the initial amount of template available. We used the same cDNA preparation and identical amounts of template input for VH and C<sub>H</sub> amplification. This corrected for variations in amount of RNA isolated and efficiency of cDNA synthesis, reflected in the range of Taq error observed in C<sub>H</sub> (3.4 × 10<sup>-4</sup> to 1.6 × 10<sup>-3</sup> bp<sup>-1</sup>). Replicate analysis confirmed Taq error.

Results and discussion

Case 1 utilized V<sub>3</sub>,11 and case 2 V<sub>4</sub>,30,2 in sequential samples. These showed evidence for extensive somatic mutations (nucleotide sequences have been deposited in the European Molecular Biology Laboratory database; accession numbers AJ536045-AJ536051).

We observed intraclonal variation in sequence at the MGUS stage in case 1. Mutations in codons 107T>C and 120A>G were identified in 2 respective clones obtained from separate PCRs (Figure 1). Additional single nucleotide changes were also observed (not shown), with a mutational frequency (1.6 × 10<sup>-3</sup> bp<sup>-1</sup>) exceeding Taq errors in C<sub>H</sub> (3.4 × 10<sup>-4</sup> bp<sup>-1</sup>; 11 clones from 2 PCRs). This confirms heterogeneity in MGUS. It implicates a cell that is undergoing continual somatic mutation following tumorogenic arrest. This may be occurring in the GC environment, suggesting a less differentiated but slg<sup>+</sup> cell. This cell has acquired a proliferative potential, perhaps due to a chromosomal event 1 (Figure 2). It allows progeny with heterogeneous sequences to survive and migrate to the marrow to differentiate further. This heterogeneity also exists in clonally derived C<sub>H</sub> transcripts, demonstrated in only one MGUS case to date, and implicating the stage of isotype switch in event 1.

Following transformation to myeloma in this patient (sample 1/2, Figure 1), the observed ongoing mutational activity appeared to be silenced, as the frequency of single nonclonal mutations (1.5 × 10<sup>-3</sup> bp<sup>-1</sup>) was similar to Taq error (1.6 × 10<sup>-3</sup> bp<sup>-1</sup>). However, we observed 2 mutational changes at codons 68T>C and 107T>C in single clones at the MM stage that were identical to mutations at the early stage of disease (Figure 1). These 2 mutations were detected in samples taken at 2 different disease stages, negating random PCR error. Most likely, they represent residual MGUS clones as transformation was rapid. In the absence of paired samples, a very low level of nonshared mutations in some MM clones would have been assigned as Taq error in V gene analysis. Reports of stability of myeloma V gene sequence indicate the rarity of identifying such persisting residual clones by current technology. However, there have been sporadic reports that hint at this possibility. In 1 of 4 MM cases with more than 40% tumor cells, 2 clonally-related subclones with heterogeneity were observed, possibly reflecting a narrowing of a wider variation that existed previously. In a further MM case, significant intraclonal diversity was noted in the IgA variant transcripts but not the tumor isotype, suggesting emergence of a dominant clone but with residual variants. Given that MM is clonally homogeneous even in C<sub>H</sub> tumor-derived sequences, this suggests that the final transformation event (event 2, Figure 2) occurs in one cell of the population, at a site where somatic mutation is silent, possibly the bone marrow.

In case 2, we observed intraclonal homogeneity at both the MGUS and MM stage, confirming previous observations in 4 of 7 MGUS cases. A self-cloning phenomenon may underlie this observed sequence homogeneity in MGUS, being analyzed at a stage where hypermutation has ceased and heterogeneity has been lost because of clonal outgrowth. Transformation to myeloma then occurs at the invariant stage, involving event 2 (Figure 2).
**VH** gene analysis has revealed diversity in MGUS and has suggested that transformation may involve different pathways. MGUS may derive from a tumorigenic slg^+^ cell undergoing somatic mutation, which differentiates into Ig-secreting plasma cells. This population may persist and possibly self clone to homogeneity. However, it is susceptible to further transforming events that can occur in one cell at the heterogeneous or homogeneous stage, giving rise to homogeneous MM that is independent of the slg^+^ population. Progression to myeloma then involves factors intrinsic to this cell.

**Acknowledgments**

We wish to thank Dr. Judith Schuster and Irene Assmann for assistance in cell sorting.

**References**