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The t(11;14) (q13;q32) in Multiple Myeloma Cell Line KMS12 has Its 11q13 Breakpoint 330 kb Centromeric From the Cyclin D1 Gene

To the Editor:

Recently Chesi et al[1] presented the characterization of 14q32 breakpoints in two t(11;14)-bearing multiple myeloma cell lines, KMS12 and SK-MM2. In KMS12 the position of the 11q13 breakpoint could not be determined by polymerase chain reaction (PCR) screening of a YAC-contig extending 500 kb centromeric and 200 kb telomeric from the cyclin D1 gene. In the *Blood* issue of August 15, 1996 we reported the accurate mapping of breakpoints within the 11q13-/BCL1 region in mantle cell lymphoma by multicolor barcode fiber fluorescence in situ hybridization (FISH).[2] Using this technique we have analyzed the KMS12 cell line with cosmid and P1 clones from 11q13/BCL1 and a contig of 14q32 cosmid and plasmid probes covering J_H and the entire C_H region. The results (Fig 1) show that the breakpoint on 11q13 is approximately 215 kb centromeric from the BCL1-MTC, or 330 kb centromeric from the cyclin D1 gene. Thus, the BCL1 breakpoint region is extended to at least 350 kb. The fact that the YAC contig screened by Chesi et al did not contain the breakpoint might be due to unstability of YACs cloned from the region centromeric from the BCL1-MTC.[3]

To characterize the IgH constant region genes complex involved in the translocation, separate hybridizations were performed with γ- and α-specific plasmid probes and cosmids from J_H-S_μ, in combination with 11q13 probes. The 14q+ chromosome contains, from breakpoint to centromere, two γ genes and one α gene, confirming the localization of the breakpoint in the switch-region of γ2. The 11q− chromosome contains only a few kilobases of sequence representing the J_H-S_μ region (Fig 1). This suggests that γ2 class switch deletion has actually occurred. In conclusion, these data suggest that the observed overexpression of cyclin D1 in KMS12 may be caused

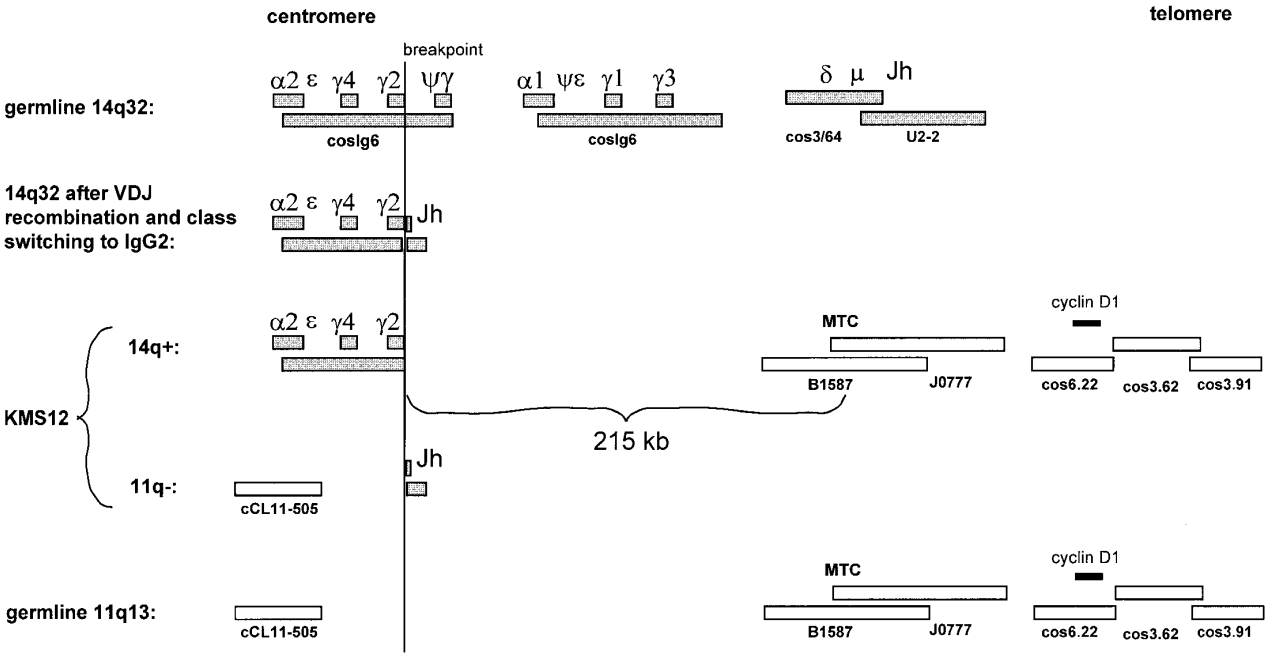

Fig 1. The top panel shows the germline configuration of the 14q32 plasmid and cosmid probes. The second panel shows how 14q32 would look after VDJ recombination and class switching. The third and fourth panels show the reciprocal translocation products observed in KMS12 cells, while the bottom panel represents the germline configuration of the 11q13 cosmid and P1 probes. All probes are described in Vaandrager et al,[2] except cosIg6,[4] cCL11-505 (JCRF, Tokyo, Japan), the γ plasmid,[5] and the α plasmid.[6]

by the presence of immunoglobulin sequences 330 kb centromeric from the cyclin D1 gene.

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Reconstitution of Membrane IgD$^-$ (mIgD$^-$) B Cells After Marrow Transplantation Lags Behind the Reconstitution of mIgD$^+$ B Cells

To the Editor:

Despite B-cell counts usually normalized by 1 year, humoral immunity and the incidence of bacterial infections continue to be abnormal even after 1 year following bone marrow transplantation (BMT). In a recent issue of *Blood,* Suzuki et al[1] reported a possible explanation: even at 1 year after grafting, the bulk of the B cells are naive (without somatically mutated Ig variable chain genes), whereas memory (somatically mutated) B cells are lacking. Compared to naive B cells, memory B cells are capable of brisker differentiation into plasma cells that produce higher-affinity antibodies, which should result in more efficient opsonization of phagocytes and neutralization of pathogens.[2] Because the DNA method used by Suzuki et al is laborious, only 4 patients were studied. Here we report a confirmatory study using a simple flow cytometric technique and a sample size of 59 patients. Naive B cells were defined as mIgD$^+$ and memory B cells as mIgD$^-$ since B cells without somatic mutations are primarily found in the mIgD$^+$ subset and those with somatic mutations in the mIgD$^-$ subset of circulating B cells.[1,3]

Blood from 59 marrow transplant recipients, 14 normal neonates, 2 normal 4-year-old children, and 24 normal adults was examined. Demographic and clinical characteristics of 53 of the 59 patients were reported in our corresponding study on T-cell reconstitution[4] and characteristics of the remaining 6 patients were similar. B cells were defined as mononuclear cells expressing CD19 and/or CD20 and not expressing markers of T cells, natural killer cells, or monocytes; details of the three-color flow cytometric method were described.[5] The difference in B-cell subset counts between subject groups was tested by Mann-Whitney-Wilcoxon rank-sum test.

As shown in Fig 1, both mIgD$^+$ and mIgD$^-$ B cells were barely detectable at 2 months after allogeneic BMT. Although the median mIgD$^+$ B-cell count became normal by 1 year and supranormal by 5 years after allogeneic BMT, the median mIgD$^-$ B-cell count lagged behind: it was still subnormal at 1 year and became normal by 5 years after allogeneic BMT. (Subnormal/supranormal denotes higher/lower cell numbers than those of the normal adults, $P \leq .05$). After autologous BMT, the reconstitution of both naive and memory B cells appeared to proceed faster than after allogeneic BMT; nevertheless, the lagging of mIgD$^-$ B cells was also apparent: at 1 year after grafting the median mIgD$^-$ B-cell count was subnormal whereas the median mIgD$^+$ B-cell count was supranormal.

Both the tendency to develop supranormal B-cell counts as well as the lag of mIgD$^-$ behind mIgD$^+$ B-cell reconstitution posttransplant were reminiscent of B-cell development in early life: children had supranormal median mIgD$^+$ B cell counts both at birth and at the age of 4 years; their median mIgD$^-$ B cell counts were subnormal at birth and supranormal at the age of 4 years.

Together with the results of Suzuki et al our findings suggest that the prolonged deficiency of humoral immunity after transplant may be due in part to the very slow reconstitution of memory B cells.

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ACKNOWLEDGMENT

Supported by National Institutes of Health Grants No. CA 18221 and CA68496.

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