transplantation (ABMT) was performed. Two months later karyotype analysis showed 70% Ph· cells, and IFN was resumed at 9 MU/day; 16 months later (April 1992) major cytogenetic response (73% Ph·) was achieved. The status was maintained (last karyotype analysis performed in January 2000), with a complete cytogenetic response (0% Ph· metaphases) since January 1997. The daily IFN dose was progressively reduced, down to the current dose of 3 MU/week.

Evaluation of BCR-ABL transcript was firstly performed in June 1996 (83 months after diagnosis), in major cytogenetic response: following the same technique described above, presence of a b3/a3 junction was revealed. Three more samples, collected between January 1997 (first evidence of complete cytogenetic response) and June 1999, were negative for BCR-ABL rearrangement, both at first and second step of the “nested” RT-PCR. The last sample (January 2000) is weakly positive, indicating the recurrence of the same b3/a3 transcript.

ABL exon 2 encodes for part of the SH3 domain, which is generally considered to play a negative regulatory role for the tyrosine kinase activity (SH1 region). But recent data suggest that, although “in vitro” SH3 is not required in the BCR-ABL protein for growth factor–dependent proliferation, inhibition of differentiation, and protection from apoptosis, “in vivo” lack of SH3 reduces the leukemogenic potential. In addition, proliferation rate and binding capability to extracellular matrices is lower in SH3-deleted BCR-ABL expressing hematopoietic cells than in those with wild-type BCR-ABL.

The long-lasting and quite indolent disease history of these 2 patients, supported by the evidence of a complete cytogenetic and molecular response obtained with IFN therapy in patient 2, are in agreement with the data of this study and do not confirm the hypothesis of a possible correlation between the lack of ABL exon 2 in BCR-ABL rearrangement and a more aggressive form of CML, as described by others.2,3,5,7

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References

To the editor:

Levels of circulating CD19+ cells in patients with multiple myeloma

Clonotypic B cells at different stages of differentiation are present in patients with multiple myeloma (MM). But the nature and frequency of the less mature clonotypic B cells expressing CD19 is a standing controversy in myeloma research.1,2

Currently, the numbers of CD19+ cells are determined by flow cytometry using monoclonal antibodies (MoAbs) specific for the CD19 surface molecule. It has been suggested that the controversy concerning the levels of CD19+ cells in the peripheral blood (PB) of MM patients stems from the use of different CD19 MoAbs that recognize different epitopes with a heterogeneous pattern for clonal and polyclonal B-cells.2,3 In addition, it has been reported that the CD19 epitope defined by the Leu-12 MoAb (Becton Dickinson Immunocytometry Systems, San Jose, CA), not normally recognized on these clonal cells, is exposed when they are treated with neuraminidase.3

The frequency of CD19+ cells in MM patients have become clinically important because clonal CD19+ cells may contain the drug-resistant compartment in myeloma,4 and the CD19+ cell levels at diagnosis have been shown to be a prognostic factor.5 We therefore investigated whether the CD19 MoAbs B4 (Coulter Clone, Miami, FL), Leu-12, and FMC63 (Serotech, Oxford, England) recognized different epitopes and whether these epitopes displayed a different pattern on B cells from healthy donors when compared to MM patients.

In brief, 3 different CD19 double stainings were used. The 2 MoAbs were added together; cells were incubated for 30 minutes with MoAb-1, followed by the addition of MoAb-2; and cells were incubated for 30 minutes with MoAb-2, followed by the addition of MoAb-1. With each experiment, single staining of the actual MoAbs and an isotype-matched control were performed. All combinations of MoAbs including the FITC and the PE-conjugated MoAbs were tested in this setting. For all combinations maximum fluorescence intensity was obtained for both CD19 MoAbs in at
least one staining procedure, showing that the MoAbs were not competing for the same binding site (epitope). In addition, the B4, Leu-12, and FMC63 epitopes were tested for sensitivity to neuraminidase treatment. No effects were seen on the single staining or when MoAbs were used in combinations. As a positive control for neuraminidase treatment, the CD34 molecule expressed on KG1 cells was used. The median channel value of the neuraminidase-sensitive class I epitope was significantly lowered in all experiments, whereas the neuraminidase-insensitive class III epitope was unaltered.

In contrast to the Leu-12 FITC/PE, B4 PE, and FMC63 PE MoAbs, the B4 FITC and FMC63 FITC MoAbs showed an additional stained population originating from the monocyte gate when serum levels in the PBS staining buffer were 0%. But this population was reduced with increasing serum concentration. The effect of using PBS with different serum concentrations was identical on B cells from healthy donors and MM patients. To test the possibility that the identification of CD19 stained cells originating from the monocyte gate was due to unspecific binding of the CD19 MoAbs, these CD19 stained cells were flow-sorted as single cells and CD19 RT-PCR was performed. For both healthy donors and MM patients, the frequency of CD19+ (mRNA+) cells within this population was between 1:100 and 1:1000, documenting the unspecific binding of the B4 and FMC63 FITC MoAbs. When 10% serum was included in the PBS incubation and washing buffer to avoid unspecific binding of the B4 FITC MoAb, a good correlation (R = 0.977) between the numbers of CD19+ cells detected by B4 and Leu-12 was found in 59 MM patients, thus illustrating that under optimal conditions a reliable quantitation is achieved with both MoAbs. When CD19+ cell levels in untreated MM patients (n = 9) with healthy donors (n = 20) were compared, there was no significant difference in the mean levels (P = .803), but a pronounced heterogeneity in MM patients was observed, a result similar to that reported by Kay et al.1.

The CD19 epitopes defined by the B4, Leu-12, and FMC63 MoAbs were similarly expressed on B cells from healthy donors and MM patients, and neuraminidase treatment had no effect on these CD19 epitopes. In addition, the level of circulating B cells in MM patients was not significantly different from healthy controls. Thus unspecific binding of the B4 and FMC63 FITC-conjugated MoAbs to monocytes creating false high CD19+ cell levels may explain the controversy regarding the frequency of PB-localized CD19+ cells.

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