To the editor:

Clonotypic B cells in the peripheral blood of patients with multiple myeloma

The frequency and the biologic significance of circulating clonal cells expressing CD19 in patients with multiple myeloma (MM) remain an issue of controversy. So far, in a small number of patients, the proportion of circulating clonotypic cells has been determined using different methods based on IgH-specific primers, and highly divergent results were reported.1,2

Recently, a brief report from Rasmussen et al also addressed this question.3 In this paper, the numbers of clonal cells in the peripheral blood (PB) from patients with MM were investigated by quantitative real-time polymerase chain reaction (PCR). The levels of CD19+ cells in PB were assessed by flow cytometry, and the CD19 mRNA was quantitated by real-time RT-PCR.

With this approach, the frequency of clonotypic B cells cannot be determined. Thus, it remains unclear whether—and if so, in what proportion—the CD19+ or the CD19+ CD19-mRNA–positive cells described by Rasmussen et al belong to the malignant clone. To address this question, an assessment of clonality in sorted CD19+ and CD19+ cell fractions is necessary.

Using a quantitative PCR assay with allele-specific oligonucleotides (ASOs) based on the method of limiting dilutions,4 we investigated the kinetics of clonotypic cells in the PB in the course of a sequential high-dose therapy (HDT) regimen. The absolute numbers of clonotypic cells were found to be very low prior to HDT (median, 0.004%; n = 20). But we observed a significant decrease in the proportion of circulating clonotypic cells after the first cycle of HDT (median, from 65 to 2.7 cells/mL PB; n = 20). The second cycle did not lead to a further reduction (median, 3.5/mL; n = 20).5 Upon analyzing highly purified CD19+ and CD19+ fractions of PB, a significant reduction of clonotypic cells was observed after one cycle of HDT only in the CD19+ cell fractions (53/mL, n = 8, before, compared with numbers below the detection limit, n = 10, afterward), while the median number of CD19+ clonotypic cells remained stable (0.92/mL before, compared with 1.05/mL afterward). With disease progression, an expansion of CD19+ and CD19+ cells occurred (63/mL and 21/mL, respectively; n = 7).6 Because different properties of the antibodies used against CD19 are discussed to explain the heterogenous findings regarding the frequency of circulating clonotypic B cells, highly purified B-cell fractions were obtained using CD20 as a second pan-B-cell marker. The proportions of clonotypic cells found did not differ significantly from those found in CD19-sorted cell fractions.7

Taking these results together, we conclude that the absolute numbers of circulating clonotypic cells in MM are small and that, prior to HDT, most of these cells are CD19+. Our results reveal direct evidence that circulating CD19+/CD20+ clonotypic B cells seem to be resistant even to sequential HDT and comprise the vast majority of clonotypic cells in the PB of patients in remission after HDT. An expansion of CD19+ clonotypic cells and an even more pronounced expansion of CD19+ clonotypic cells occur with disease progression. These findings were the rationale for a B-cell–directed consolidation therapy for patients in remission after autologous transplantation.8

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Response:

Controversies surrounding the circulating clonotypic B lymphocytes in multiple myeloma

Since the article from Pilarski’s laboratory in 1995 reporting the presence of high numbers of clonotypic CD19+ B lymphocytes in the peripheral blood of patients with myeloma,1 numerous groups have reported conflicting data on the frequency and characteristics of circulating clonotypic B lymphocytes.2,3 Recently, we published a paper dealing with the frequency and response to treatment of circulating clonotypic cells in myeloma.4 In their letter, Cremer et al question the rationale for this study, since no data were reported on the frequency of clonotypic cells within a purified CD19+ population of B lymphocytes.

We fully agree with Cremer et al that, in order to determine the frequency of CD19+ clonotypic cells, it is necessary to perform allele-specific oligonucleotide (ASO) polymerase chain reaction (PCR) on purified CD19+ cells. Actually, we did determine the frequency of

References

clonal cells in the CD19 compartment in a cohort of patients with myeloma, including all patients in the reported study, by combining single-cell ASO IgH RT-PCR with flow sorting and Poisson statistics. These data were obtained to verify a rationale for CD19 depletion of stem cell products from patients with myeloma. But the data were not included in the paper since they did not contain novel information concerning the controversial aspects addressed in the paper.

We determined the proportion of clonal CD19+ cells after peripheral blood stem cell (PBSC) transplantation in 10 patients and found variable levels of clonal cells in the CD19 compartment after transplantation, ranging from 0.101% to 6.103% (mean, 1.019%) of CD19+ cells. This is comparable to the clonal cell levels reported by Chen and Epstein,2 who found levels from 0.04% to 5.00% (mean, 1.60%). Rottenburger et al3 reported PCR negativity in CD19+–enriched fractions in approximately 50% of patients with multiple myeloma (MM) in remission. In contrast, we found clonal cells in the CD19+ compartment in all 10 patients with MM observed in continuous remission for at least 3 months. Pilarski’s group reported that cells originating from the lymphocyte gate contained a polyclonal B-lymphocyte population with a minor fraction of clonotypic CD19+ cells.1 In general, nearly all CD19+ cells that we have identified in patients with myeloma originate from the lymphocyte gate. The frequency of clonotypic cells that we identified within this CD19+ lymphocyte gate was comparable to that found both by Pilarski’s group1–3 and Chen and Epstein2; thus, on this point, there is no controversy.

Pilarski’s group reported the controversial finding that most cells originating from the monocyte gate were clonotypic, expressing CD19 surface expression, obscuring their detection by flow cytometry, as reported.4 In the published study, we showed that in general, there is a low frequency of circulating clonal cells in patients with myeloma, and most importantly, we showed that circulating clonal cells respond well to induction therapy, even in situations where high numbers of clonal cells were present. Although most circulating clonal cells responded to chemotherapy, we have (like Cremer et al) also found a small proportion of clonal CD19+ cells that are resistant to high-dose chemotherapy. But whether there is a rationale for consolidation therapy using anti-CD20 antibodies is questionable, since there is no proof that the clonotypic B lymphocytes are involved in the pathogenesis of the disease. Additionally, our ongoing studies on the nature of the circulating CD19+ clonotypic B lymphocytes have resulted in identification of a CD19+/CD20− subset (Figure 1).

To the editor:

**Dalteparin-induced alopecia in hemodialysis patients: reversal by regional citrate anticoagulation**

Chronically intermittent hemodialysis for end-stage renal failure requires anticoagulation to prevent clotting in the extracorporeal circuit. Anticoagulation usually is performed by continuous infusion of unfractionated heparin or by bolus administration of low-molecular-weight heparin.1 At our hemodialysis unit, we use an intravenous bolus injection of an average of 80 IU dalteparin (Fragmin; Pharmacia, Stockholm, Sweden) per kilogram of body weight. In 1998, regional citrate anticoagulation was introduced for patients at high risk for hemorrhage, for patients suffering from heparin-induced thrombocytopenia type II, or for patients in which the extracorporeal circuit clotted despite an extremely high dalteparin dose.2,3 During the last 3 years, 5 of our female patients on long-term anticoagulation with dalteparin complained about excessive hair loss, which developed in the course of anticoagulation during the first month of treatment. This effect is commonly seen when anticoagulation is performed using unfractionated heparin or digoxin.4 We postulated that anticoagulation using regional citrate anticoagulation might be the cause of the alopecia because this strategy is known to induce less arteriolosclerosis and thrombocytopenia than unfractionated heparin.5 Later, we noticed that the alopecia was frequently associated with significant weight loss of the patients. Regional citrate anticoagulation and heparin-induced thrombocytopenia type II are known to be associated with a significant increase in body weight.6–8 In contrast, we found clonal cells in the CD19 compartment in all 10 patients with MM observed in continuous remission for at least 3 months. Pilarski’s group reported that cells originating from the lymphocyte gate contained a polyclonal B-lymphocyte population with a minor fraction of clonotypic CD19+ cells.1 In general, nearly all CD19+ cells that we have identified in patients with myeloma originate from the lymphocyte gate. The frequency of clonotypic cells that we identified within this CD19+ lymphocyte gate was comparable to that found both by Pilarski’s group1–3 and Chen and Epstein2; thus, on this point, there is no controversy.

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References

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