duration of IFN-α treatment prior to study entry was 30 months (range 12-54), and the daily dose administered varied from 3 to 9 MIU (Table 1). Interferon-α was discontinued 15 days before. Filgrastim was administered (s.c) at a dose of 15 mg/kg/day for 5 days. Leukaphereses were then started and performed daily, using a Cobe Spectra blood separator, until the target cell yield was obtained (>1x10^6 CD34+ cells/kg b.w.). Apheresed cells were then cryopreserved. The Ph+ cell contamination both in the patients' bone marrow and in the leukapheresis products was evaluated with a quantitative competitive PCR technique (QC-PCR),8 The priming treatment with filgrastim was well tolerated. Bone pain (WHO grade I-II) occurred in three cases. Seven out of 9 patients (78%) yielded more than 1x10^6 CD34+ cells/kg in one (4 cases) or 2 to 4 (3 cases) collections (Table 2). In 5 of the 9 mobilized cases the levels of BCR/ABL transcript in the first apheresis product were 10 to 700 fold higher than the levels of BCR/ABL transcript measured in the pre-apheresis bone marrow samples (Table 2). In 3 of these 5 patients the amount of BCR/ABL transcript decreased significantly in the subsequent aphereses reaching pre-G-CSF mobilization values (Table 2). Using the QC-PCR to assess the Ph+ minimal residual disease in the leukapheresis products we found that priming treatment with filgrastim could induce an earlier mobilization of Ph+cells. This could be due to functional impairment of adhesion molecules necessary to retain progenitors in the bone marrow microenvironment, or alternatively, to the induction of differentiation of CM.10 cells or their partial elimination by earlier aphereses. No significant correlation (r = 0.0069; p = 0.78) was found between the level of BCR/ABL transcript and the number of CD34+ cells collected. No patient has been autografted as yet, because all patients remain in complete or major cytogenetic remission after collection. We therefore cannot exclude genetic remission shows that Ph+ cells present in autologous transplants of chronic myelogenous leukemia (CML) contribute to relapse after autologous bone marrow transplantation. Blood 1994; 83:3068-76.


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Key words
CM L, IFN α, G-CSF, autografting

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References


All-trans retinoic acid potentiates the in vitro inhibitory effects of IFN-α in parental and p210-bcr/abl transducted murine myeloid cell lines

Sir,

Recently, a great deal of interest has been focused on the use of ATRA in the treatment of Ph+ CM L.13 Preclinical observations showed that all-trans retinoic acid (ATRA) synergizes with IFN-α to induce suppressive effects on Ph+CML progenitor cells.15 As
the same combination did not show similar effects on progenitors from patients with Ph-negative myelo-proliferative diseases it has been suggested that the suppressive effect of ATRA, either alone or in combination with IFNα, could be related to the expression of p210 bcr/abl oncoprotein. To dissect the role of p210 bcr/abl expression regarding the cell sensitivity to ATRA, in this study, we evaluated the effects of ATRA with and without IFNα on parental (32D) and p210 transfected (LG7) murine myeloid cell lines. Proliferation of 32D and LG7 cell lines was assayed by the 3-(4,5-dimethylthiazol,2-yl)-2,5-diphenyltetrazoliumbromide MTT method. The detection and quantification of p210 bcr/abl transcript in LG7 cells was made by using a quantitative, competitive polymerase chain reaction (PCR). The results were expressed as mean values ± SD of the data obtained from three experiments performed in triplicate. Data were analyzed by the Student’s t test and differences with p < 0.05 were considered statistically significant.

We observed that the incubation of 32D and LG7 cell lines with scalar concentrations of ATRA or IFNα inhibited the cell growth in a dose-dependent manner. However, no significant difference in growth inhibition was found between the parental (32D) and the p210 transfected (LG7) cell line. In both cell lines the ATRA inhibition dose 50 (ID50) was 0.01 mM, while the IFNα ID50 was 2,000U/mL. When suboptimal concentrations of ATRA (0.01 mM) were added (2 hours before) to scalar doses of IFNα a significant increase (p < 0.05) of cell growth inhibition compared to that produced by IFNα alone was observed both on 32D and LG7 cell lines (Figure 1). In contrast, when suboptimal concentrations of ATRA (0.01 mM) were combined (2 hours before) to escalating doses of ATRA we did not observe any additive or synergistic effects in comparison to the growth inhibition induced by ATRA alone (Figure 2). To verify that the effects of ATRA, either alone or in combination with IFNα, were not due to the absence
of p210 bcr/abl fusion protein in LG7 cells, detection and quantification of p210 bcr/abl transcript in these cells was done using a quantitative, competitive PCR. The presence of bcr-abl transcripts in the LG7 cell line was further demonstrated by western blot analysis (data not shown). Our data suggest that ATRA can potentiate the inhibitory effects of IFNα both on Ph-negative and Ph+ leukemic cells. The mechanism(s) of synergism is unknown. It does not seem to be related to p210 expression, but appears to be influenced by preincubation of target cells with ATRA. These findings suggest that pretreatment with ATRA could induce activation of IFNα-induced genes which in turn could favor the clinical response to IFNα.

Key words
ATRA, IFNα, Ph+ CM L, p210 bcr/abl, cell lines

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