To the Editor:

We read with interest the recent article by Schiro et al describing the suppressive effects of interleukin-1 receptor antagonist (IL-1Ra) on colony growth in juvenile chronic myelogenous leukemia (JCML). However, we differ in opinion on the conclusion reached that their data implicate a central role for IL-1β in JCML. We have published data which shows that a unifying, characteristic feature in JCML is a selective, 10-fold hypersensitivity by the JCML hematopoietic progenitor cells to granulocyte-macrophage colony-stimulating factor (GM-CSF).² Our original report described this effect in 8/8 JCML patients studied. Our observations of selective GM-CSF hypersensitivity have now been extended to a total 38 JCML patients and all 38 have exhibited a remarkably similar degree of hypersensi-
tivity despite widely heterogeneous clinical courses. We believe that this hypersensitivity to GM-CSF is a characteristic that is reflective of the pathogenetic defect within the GM-CSF signal transduction pathway in JCML hematopoietic progenitor cells.

Our conclusions were based on a series of experiments which we have published previously. We showed in 13 patients we investigated that JCML monocytes, in our 1991 report, measured the cytokine production of adherent cells (mostly monocytes) can completely abrogate the characteristic 'spontaneous' colony growth. This abrogation of growth by adherent cell depletion has also been observed by others, as well as by Schiro et al in their report. Therefore, because most reports agree that the monocytes drive the system, we feel that Schiro et al contradict themselves when they state that "no definitive evidence has been provided on the cell source of these growth factor molecules." Because the nonadherent hematopoietic progenitor cells could not support their own growth, effectively ruling out an autocrine definition, we and others searched for evidence in JCML for a paracrine-driven stimulus being produced by the adherent monocytes. In our 1991 report, we measured the cytokine production from highly purified JCML monocytes. Using enzyme-linked immunosorbent assays (ELISAs), we were unable to show consistently excessive cytokine production in JCML monocytes for GM-CSF, G-CSF, IL-1α, IL-1β, IL-6, tumor necrosis factor α (TNFα), or interferon γ (IFNγ). We were measuring secreted protein and did not measure mRNA levels within the cells as in the report by Schiro et al. Our hypothesis was that because the nonadherent progenitor cells could not grow in an autocrine manner, but rather required the monocytes for growth, that the stimulus would occur in the form of a secreted cytokine. Although we did not measure IL-1Ra in these experiments, in another set of experiments in the same report we did use neutralizing antibodies against IL-1α and IL-1β. These neutralizing antibodies produced inhibition of 'spontaneous' JCML colony growth by only 4% ± 7% (Mean ± SEM) and 11% ± 10%, respectively, in eight JCML patients. Likewise, Freedman et al observed 0% inhibition of colony growth with anti–IL-1α in two patients, whereas Bagby et al observed 58% inhibition in the only patient tested in their report. We have not yet assessed the ability of IL-1Ra to inhibit 'spontaneous' colony growth. However, in contrast with all of this data, neutralizing antibodies against GM-CSF in the same eight JCML patients in our report, inhibited 'spontaneous' colony growth by 87% ± 9%. This range of colony growth inhibition continues to hold true in all JCML patients we have tested since that report, now numbering over 50. Therefore, because of the consistent, significant inhibition by anti–GM-CSF and the lack of consistent excessive production of GM-CSF by the JCML monocytes or any other cytokine for that matter, we felt that a true paracrine-type mechanism had also been effectively ruled out. Hence began our investigations which led us to the discovery that JCML nonadherent progenitor cells are selectively hypersensitive to GM-CSF.

In examining the hypersensitivity dose-response assays, one could hypothesize that the addition of increasing amounts of GM-CSF to the culture system potentially only provided an indirect stimulus for the subsequent observed colony growth. It is potentially possible that the GM-CSF was acting by stimulating some of the nonadherent myeloid progenitor cells and/or the lymphocytes in the culture system to produce another cytokine, such as IL-1, which was the actual direct stimulus. In this report, we repeated the dose-response assays on three JCML patients to investigate this possibility. Nonadherent JCML peripheral blood progenitor cells were obtained after three consecutive rigorous adherence depletion steps as previously outlined. Cells were plated into 1 mL agarose with media and nutrients and increasing concentrations of GM-CSF (R & D, Minneapolis, MN). In addition, we also placed the following additives into each dish: 10 μg neutralizing polyclonal anti–G-CSF (Genzyme, Cambridge, MA), 10 μg neutralizing polyclonal anti–M-CSF (Genzyme), 10 μg neutralizing monoclonal IL-3 (Genzyme), 10 μg neutralizing monoclonal IL-6, 10 μg neutralizing polyclonal anti-IFNγ (R & D), 10 μg neutralizing polyclonal anti-TNFα (R & D), 10 μg neutralizing polyclonal anti–stem cell factor (R & D), 10 μg neutralizing polyclonal anti–IL-1α, 10 μg neutralizing polyclonal anti–IL-1β (R & D), and 100 ng IL-1Ra (R & D). Cultures were maintained at 37°C in 5% CO2, for 14 days, and then colony-forming unit granulocyte/macrophage (CFU-GM) were counted as previously described. The results shown in Fig 1 are displayed as the percentage of maximal growth. Results of simultaneous cultures without the antibody additives are also plotted. The zero point (no GM-CSF added) again shows that, in two of three JCML patients, monocyte depletion abrogates growth, and therefore, this is not an autocrine-driven disease. These data further show that none of the above additives played any significant role in the hypersensitive growth obtained by GM-CSF. This includes IL-1Ra, which was used at a concentration of 100 ng/mL, identical to that in the report by Schiro.

Fig 1. Hematopoietic growth factor dose-response curves for CFU-GM with increasing concentrations of GM-CSF. The data are expressed as percent of maximal numbers of CFU-GM to more accurately reflect changes in sensitivity. The shaded area represents the range of CFU-GM responsiveness of bone marrow progenitors from normal volunteers. The curve marked by (○) represents JCML CFU-GM responsiveness to GM-CSF in the presence of multiple anticytokine neutralizing antibodies as well as IL-1Ra as outlined in the text. The curve marked by (●) represents identical JCML CFU-GM cultured at the same time, but without the other antibody additives. A, B, and C represent the results obtained from three different JCML patients, respectively.
et al, and moreover showed no effect for addition of anti-IL-1α nor anti-IL-1β. Further, these data show no effect for TNFα or stem cell factor (Steel factor) in this hypersensitive colony growth. However, these IL-1α experiments do not address the potential suppressive effects of TNFα on normal hematopoiesis in JCML as reported by Freedman et al. Dose-response assays for IL-3 (R & D) were simultaneously performed in a similar fashion except that the anti-IL-3 was omitted. These results showed normal dose-response curves for the three JCML patients, both with and without the additives (data not shown), showing that this was a selective hypersensitivity to GM-CSF.

On the basis of these results as well as previous data, we contend that the primary, most direct problem for myeloproliferation in JCML still appears to be the pathogenetic defect leading to 10-fold selective hypersensitivity to GM-CSF. These experiments indicate it is a direct effect by GM-CSF itself, not by other cytokines, and suggests that mutations may be present within the GM-CSF signal transduction pathway in JCML myeloid progenitor cells. Our results regarding GM-CSF hypersensitivity have now been confirmed preliminarily by Lapidot et al. Schiro et al do show 40% inhibition of colony growth in the one JCML patient they tested with anti-GM-CSF. This 40% inhibition does fall into the range they observed with inhibition by IL-1Ra (35% to 82%). Their data, which shows absence of GM-CSF mRNA in single JCML colony preparations, but then presence of GM-CSF secreted protein in JCML cell culture supernatants, is quite confusing. Whereas their data for RNA production of IL-1β and IL-1Ra within JCML cells appears solid, this does not prove a central role for IL-1 in JCML nor does it disprove the GM-CSF hypersensitivity. Further, the specificity of this finding for JCML becomes questionable because the authors state, "a similar pattern of IL-1β gene expression was observed in CFU-C obtained from BM cells of normal individuals but in the presence of colony-stimulating factors."

In summary, we view both TNFα and IL-1 as contributing to the pathology of JCML, but both in an indirect manner. Due to the GM-CSF hypersensitivity in all granulocytic, monocytic, and erythroid progenitors, the monocytes are likely inherently activated. Therefore, the JCML monocytes are producing variably increased levels of several cytokines such as GM-CSF, IL-1α, IL-1β, TNFα, IL-6, or others. This is supported by the wide range of levels observed for these cytokines as measured by several investigators in various systems (ie, ELISAs by us, RNA levels by Schiro et al). The IL-1 and TNFα may in turn stimulate further GM-CSF production, and thus, drive the system in an indirect way. The TNFα very well may also suppress normal hematopoiesis. Nevertheless, we have shown that it does not matter if you have low, normal, or high levels of GM-CSF, IL-1, or whatever; all JCML cells are selectively hypersensitive to even low, basal levels of GM-CSF. Furthermore, the present graphs show that these JCML cells will proliferate and form colonies in a hypersensitive response to GM-CSF, even in the presence of neutralizing concentrations of antibodies to stem cell factor, G-CSF, M-CSF, IL-3, IL-6, IFNγ, TNFα, IL-1α, IL-1β, and additionally in the presence of IL-1Ra. Although we cannot dispute that treatment of JCML patients with IL-1Ra might, albeit quite indirectly, provide some therapeutic benefit, we believe the efforts in JCML should be concentrated on identifying the aberrancies within the GM-CSF signal transduction pathway as well as therapeutic strategies to block this hypersensitive loop.

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Peter D. Emanuel
Jitka M. Sokol
Department of Medicine
Comprehensive Cancer Center
University of Alabama at Birmingham
Birmingham, AL

REFERENCES


Response

To the Editor:

In our report, we have shown that the addition of human recombinant IL-1Ra to the clonogenic assay of bone marrow or peripheral blood mononuclear cells isolated from JCML patients, was able to inhibit in all cases the spontaneous proliferation. We also performed a molecular evaluation to show that a significant transcription of the IL-1β gene is present in unfractionated and uncultured peripheral blood cells. Moreover, we have showed that JCML leukemic clonalogenic cells (or more likely their monocytic progeny) were similarly able to accumulate significant amounts of the IL-1β-specific mRNA. Finally, we did not find an abnormal GM-CSF production by the JCML clonalogenic cells. These findings do not contradict Emanuel and Sokol's hypothesis on selective GM-CSF hypersensi-
tivity of JCML hematopoietic progenitors. Although it may be matter of discussion whether IL-1 has a central role in the regulation of JCML proliferation, the authors do agree that IL-1 and TNFα, albeit indirectly, may contribute in driving the proliferation of JCML progenitors. They reported that the addition of several neutralizing antibodies against many different cytokines (including IL-1β) to the JCML peripheral blood progenitors, were not able to modify the GM-CSF driven proliferation of nonadherent purified populations. Because most groups have reproducible data showing that the removal of adherent cells can completely abrogate the spontaneous colony growth, it is very difficult to question our findings when a different experiment was performed. Whether IL-1β is not the only accessory cytokine that might play a central role in the JCML proliferation, this remains a distinct possibility. Indeed we agree that TNFα can play a similar role. Finally we would like to comment on the concept of GM-CSF hypersensitivity of JCML progenitor cells. Whether this phenomenon has a molecular counterpart in the GM-CSF signal transduction pathway or, more simply, reflects a priming effect of some accessory cytokines (ie, IL-1 and TNFα could upregulate the expression of the GM-CSF receptor) still remains a matter of investigation. However, it is our opinion that in the absence of clear-cut experimental evidence supporting either this or another hypothesis, more caution should be recommended in considering the results of the hypersensitivity dose-response assay as the demonstration of the JCML pathogenetic defect. In this respect, it would be of interest to know whether the results shown in Fig 1 were normalized for the number of (normal or leukemic) CD34-positive cells plated in the assays.

Andrea Biondi
Raffaella Schirò
Alessandro Rambaldi
Clinica Pediatrica Università di Milano
Divisione di Ematologia Ospedali Riuniti di Bergamo
Istituto “M. Negri”, Bergamo, Italy

REFERENCES
Interleukin-1 beta does not play a central role in juvenile chronic myelogenous leukemia [letter; comment]

PD Emanuel and JM Sokol