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Letters

Problems Associated with the Study of Cytokines in Patients with Leukaemia

H.D. Preisler and A. Raza

THE CLINICAL USE of cytokines may favourably alter the proliferative behaviour of leukaemia cells. The lack of a consistent relation between the cell cycle characteristics of leukaemia cells and the course of the disease or treatment outcome results from two problems inherent to cell cycle studies. First is the inaccuracy of the assessments of the per cent cells in S-phase due to variable dilution of marrow aspirate cells by peripheral blood cells 2–4. Then, even if an accurate labelling index (Li) were available, the data would still be of marginal interest since the most important characteristic is length of the cell cycle [4].

One attempt to overcome the problem of dilution by peripheral blood is to estimate the extent of dilution [4]. Other approaches include the release of cells from marrow biopsy samples by shaking or enzymatically [1,2]. While these approaches demonstrated that many more cells were in S-phase than had been observed when aspirates were used, there has been no demonstration that all marrow cells are released or that the released cells are representative. To avoid these problems, we administer halogenated pyrimidines embed marrow biopsy specimens in plastic and use monoclonal antibodies to identify S-phase cells [4]. Our studies demonstrate that use of cells "released" from biopsy samples underestimates the proportion of leukaemia cells in S-phase [5]. We use Li with measurement of the duration of S-phase (Ts) to calculate cell cycle time (Tc).

Patient 1 (Table 1) did not receive any cytokine. The biopsy Li, Ts and Tc were unchanged over the 3 days of study. But if the aspirate Li is used, there would appear to be a 235% increase in Li. If this Li is used to calculate Tc, the proliferative rate of the leukaemia cells would seem to have dramatically increased. Also if the spuriously low aspirate Li is used, Tc is extremely long. Patient 2 received a 3 day course of retinoic acid and interferon [5]. Li fell in both the aspirate and biopsy samples. Unexpectedly, however, Ts also fell, i.e.an acceleration of the proliferative rate rather than a slowing as would have been concluded if only Li was used. Patient 3 received recombinant human granulocyte–macrophage colony-stimulating factor (rhGM-CSF). While the aspirate Li doubled, that of the biopsy specimen fell. Ts was slightly prolonged. Thus, rhGM-CSF was associated with a slowing of the leukaemia cell proliferative rate and not with acceleration, as would have been concluded if marrow aspirate data had been used. These paradoxical effects are not rare.

Thus, it is impossible to measure accurately the effects of cytokines on leukaemia cell proliferation *in vivo* unless intact marrow biopsy specimens are used and unless Ts and Li are used to calculate Tc. The use of peripheral blood in these studies[6] further confounds the issue since apparent alterations in the kinetics of the cells in this compartment can be the result of the mobilisation of immature cells from the marrow and other sites of proliferation rather than an effect on cell proliferation.

The failure to use appropriate methods in studies of cytokines will have a negative impact since inaccurate and/or incomplete cell cycle data will lead to erroneous conclusions about the effects of these agents and their clinical usefulness. Unfortunately, none of the reported studies of the effects of cytokines on leukaemia cell proliferation used methods capable of providing accurate and complete data [6–9].

1. Preisler HD, Raza A, Larson R, *et al.* Some reasons for the lack of progress in the treatment of acute myelogenous leukaemia. *Leuk Res* 1991, **15**, 773–780.
2. Dosik GM, Barlogie B, Goldie W, Johnston D, Tekell JL, Drewinko B. Flow cytometry of DNA content in human bone marrow: a critical reappraisal. *Blood* 1980, **55**, 734–740.
3. Hiddemann W, Buchner W, Andreef M, Wormasen B, Melamed MR, Clarkson BO. Bone marrow biopsy instead of "marrow juice" for cell kinetic analysis: comparison of bone marrow biopsy and aspiration material. *Leuk Res* 1982, **6**, 601–612.
4. Raza A, Maheshwari Y, Preisler HD. Differences in cell cycle characteristics amongst patients with acute nonlymphocytic leukaemia. *Blood* 1987, **69**, 1647–1653.
5. Preisler HD, Raza A, Hulette B, *et al.* The use of biologically active agents in the treatment of AML and other neoplastic diseases. *Status of Differentiation Therapy*, Volume 2, 1991. In press.
6. Bettelheim P, Valent M, Andreef A, *et al.* Recombinant human granulocyte macrophage colony-stimulating factor in combination with standard induction chemotherapy in *de novo* acute myeloid leukaemia. *Blood* 1991, **77**, 700–711.
7. Tafuri B, DeFelice MC, Petti A, *et al. In vivo* and *in vitro* cell kinetic effects of rhGM-CSF in the combined cytokine-chemotherapy treatment of acute myeloblastic leukaemia. *Haematologica* **76**, suppl 4, 10/91, abstract 128.
8. LaCombe F, Dumain P, Puntous M, *et al.* Clinical and biological results in acute myeloid leukaemia patients treated with GM-CSF and intensive therapy. *Haematologica* **76**, suppl 4, 10/91, abstract 139.
9. Buchner T, Hiddemann W, Wormann B, *et al.* Recombinant human GM-CSF priming and long term administration together with multiple course chemotherapy vs CT alone in newly diagnosed acute myeloid leukemia. *Blood* **78**, 10, suppl 1, 11/91, abstract 641.

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Correspondence to H.D. Preisler. The authors are at the Department of Internal Medicine, Division of Hematology–Oncology, College of Medicine, 231 Bethesda Avenue, K Pavillion (ML508) Cincinnati, Ohio 45267–0508, U.S.A.
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Handedness and Breast Cancer

Sidney Lowry

THE REPORT by Olsson and Ingvar [1] that left handedness is uncommon in breast cancer patients fails to mention a possible

Correspondence to S. Lowry at the Department of Oncology, Whitla Medical Building, 97 Lisburn Road, Belfast BT9 7BL, U.K.
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