Intramedullary Influences on in Vitro Granulopoiesis in Human Acute Myeloid Leukemia*

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Summary

Provision of granulocyte-monocyte colony stimulating activity by human bone marrow (CSA_{BM}) was determined in 21 patients with acute myeloid leukemia (AML) utilizing in vitro culture techniques to assess intramedullary cellular interactions on human granulopoiesis. CSA_{BM} production of these patients was compared to that from normal marrow by testing the capacity of conditioned mediums from adherent marrow cells to promote granulocyte-monocyte colony formation in agar of relatively light density nonadherent human marrow target cells.

Morphologic, cytochemical, density and phagocytic characteristics of normal marrow cells suggested that CSA_{BM} production was provided by mid-density adherent cells including those of the monocyte-macrophage series. Significantly decreased CSA_{BM} provision was found in 62% of patients with AML at diagnosis or relapse. Only 33% of these patients entered chemotherapy-induced complete remission, in contrast to an 88% remission rate in the patients with normal CSA_{BM}. Sequential studies in 9 patients during complete remission showed normal or increased CSA_{BM}, which generally decreased concomitant with relapse. These findings suggest that adequate CSA_{BM} provision may be essential for sustaining normal granulopoiesis in AML and may reflect persistence of a normal marrow monocyte-macrophage population. Monitoring this parameter appears useful for evaluating microenvironmental influences on granulopoiesis and assessing prognosis in AML.

Microenvironmental influences within bone marrow and spleen have been shown to be critical for hemopoietic stem cell proliferation and differentiation in experimental animals (McCulloch, Siminovich et al., 1965; Trentin, 1971; Gallagher, McGarry et al., 1971; Knospe and Crosby, 1971; Chamberlin, Barone et al., 1974; Matioli and Rife, 1976; Cline, LeFevre et al., 1977). Histologic and functional studies by these investigators have demonstrated that locally active cell-derived factors provide stromal influences contributing to the support of hemopoiesis.

In vitro marrow culture techniques have permitted analysis of factors involved in the regulation of granulopoiesis by evaluating the ability of

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granulocytic progenitor cells (CFU-C) to form granulocyte-macrophage colonies in agar under the necessary influence of the humoral stimulatory substance termed colony stimulating activity (CSA) (Rickard, Shadduck et al., 1970; Metcalf, 1973). Human marrow cells require cellular sources of CSA for their in vitro proliferation, whereas murine marrow is stimulated as well by CSA present in serum and urine (Foster, Metcalf et al., 1968; Metcalf and Stanley, 1969; Pike and Robinson, 1970; Metcalf and Moore, 1975). Recent studies in mice have shown that marrow CFU-C proliferation is related predominantly to intramedullary CSA elaboration by cells firmly adherent to the inner surfact of hemopoietic bone (Chan and Metcalf, 1972; Chan and Metcalf, 1973). Thus, local production of CSA within the marrow plays a major role in influencing granulopoiesis.

Cellular sources of CSA are also present within human marrow and can be selectively harvested by their adherence and density characteristics (Haskill, McKnight et al., 1972; Messner, Till et al., 1973; Moore, Williams et al., 1973; Senn, Messner et al., 1974). We have employed these physical separation techniques to evaluate marrow cell-derived CSA levels in normal subjects and patients with acute myeloid leukemia (AML) in order to determine the possible role of human marrow CSA provision as a microenvironmental stimulus for granulopoiesis.

Methods

The methodology for these studies has recently been described in detail (Greenberg, Mara et al., 1978). The buoyant component of aspirated human marrow cells were obtained by Hypaque-ficoll density centrifugation. These cells were then permitted to adhere to plastic tissue culture dishes (Messner, Till et al., 1973). The nonadherent cells were rinsed off and the remaining adherent cells were incubated in modified McCoy's medium containing 15% fetal calf serum and 0.5 mM 2-mercaptoethanol for 7 days at 37°C. With regard to the kinetics of the CSA production, an initial rise within 1–2 days occurred, followed by a fall and then a more marked sustained rise by 5–7 days of cellular incubation. After this incubation, the conditioned medium was harvested and stored at −20°C until use. Target normal human marrow cells were provided by obtaining buoyant cells less dense than 1,068 g/cm^3, utilizing the bovine serum albumin neutral density (density cut) procedure (Greenberg, Mara et al., 1976; Heller and Greenberg, 1977). These cells were then permitted to adhere to tissue culture dishes and the nonadherent buoyant cell population was harvested and used as target cells. In selected experiments continuous albumin density gradients were performed. Cells and test conditioned mediums were incubated for 7–10 days in agar culture at 37°C and colonies were counted, as previously described (Greenberg, Nichols et al., 1971; Greenberg, Mara et al., 1976). Colonies consisted of more than 50 cells, with granulocytic-monocytic differentiation. Quantitative estimates of effective CSA concentrations were obtained by performing titration curves of conditioned mediums. For standardization, the number of colonies produced

by these concentrations were compared to those stimulated by a stable leukocyte conditioned medium CSA source. The data were analyzed by curve-fitting computer programs (Greenberg, Bax et al., 1974).

Results

A sigmoid-shaped dose response curve of CSA values was obtained with increasing numbers of adherent and total marrow cells. Plateau levels of CSA occurred at approximately $3–15 \times 10^5$ adherent cells. Most normal specimens provided this number of adherent cells, with approximately 9% of the marrow cells being adherent. All of the CSA was provided by the adherent cell population, and specifically no CSA was provided by the nonadherent target marrow cells. Plasma from normal marrow or peripheral blood had no demonstrable CSA when nonadherent target cells were used, whereas the presence of the adherent cells permitted colony formation to occur. This indicated that substances present in normal serum enhance CSA production by endogenous CSA-producing cells rather than providing CSA itself. Control plates lacking a CSA source had no colony formation. Density distribution profiles showed that the CSA-producing cells represented a subpopulation of the adherent cells, with a peak density of 1.066 g/cm^3.

The morphologic, cytochemical and phagocytic characteristics of the adherent marrow CSA-producing cells were assessed, and showed 84–87% of these cells to be α-naphthyl acetate esterase positive, to morphologically resemble monocytes, and to be capable of phagocytosing latex particles. These data suggest that mid-density monocytes and macrophages contribute a major portion of the marrow CSA.

With these studies providing a background for quantitating and characterizing the normal marrow CSA-producing cells, we turned our attention to patients with AML. All AML patients received the same chemotherapeutic induction regimen (daunomycin, cytosine arabinoside, and 6-thioguanine) and maintenance program (monthly cytosine arabinoside and 6-thioguanine), as previously reported (Embury, Elias et al., 1977). In comparison with 16 control subjects, significantly low marrow CSA levels were found in 13 of 21 AML patients at diagnosis or relapse. Only 4 of the 13 patients (33%) with low marrow CSA entered complete remission, whereas 7 of 8 patients (88%) with normal CSA did achieve complete remission ($p < 0.01$). These data have recently been more completely described (Greenberg, Mara et al., 1978). All 4 patients in partial remission and 42 of 46 in complete remission had normal marrow CSA values. In comparison with control subjects, low CSA values were particularly found in patients with acute myeloblastic as opposed to myelomonocytic leukemia. These two entities were distinguished by previously defined morphologic criteria (Hayhoe and Cawley, 1972). It should be emphasized, however, that this method of categorization is less sensitive and specific than current cytochemical techniques (Bennett, Catovsky et al., 1976). Other clinical parameters and patterns of marrow colony formation were evaluated, and showed no significant correlation with complete remission

rates or marrow CSA levels. Further experiments showed the absence of inhibitors of CSA production or dilution of CSA-producing cells in the AML marrow. Sequential studies of 3 patients with AML in stable remission demonstrated persisting normal marrow CSA and CFU-C values (obtained monthly, just prior to the chemotherapy pulses). In contrast, in 6 patients in remission when these studies were begun who subsequently relapsed, marrow CSA values decreased within 2 to 3 months of relapse. Marrow CSA values paralleled marrow CFU-C during remission until relapse, with normal CSA values persisting longer than CFU-C.

Discussion

In these studies we have quantitated marrow cell CSA provision, characterized the marrow CSA-producing cells, and assessed alterations of marrow cell CSA levels in normal subjects and patients with AML. Alteration of marrow CSA levels in AML correlated well with the patients' clinical status and prognoses. Patients at diagnosis or relapse had significantly decreased marrow CSA values, particularly patients with acute myeloblastic leukemia and those failing remission induction. Low marrow CSA was a significant negative prognostic indicator, since only 33% of patients with this finding entered drug-induced complete remission, whereas complete remission occurred in 88% of patients with normal CSA.

Sequential investigations showed that marrow CSA provision was generally normal in patients during stable remission. In contrast, low or progressively decreasing marrow CSA values occurred in patients with impending relapse. Marrow CFU-C and CSA correlated well in these patients during remission, with a decrease in marrow CFU-C occurring earlier than low marrow CSA in impending relapse. Prior studies (Greenberg, Bax et al., 1974) have shown that the relatively nontoxic maintenance chemotherapy regimen utilized was not associated with decrements of CFU-C one month post therapy. The prolonged persistence of marrow CSA may relate to relatively longer life span of monocyte-macrophages in comparison with granulocytic progenitor cells (Van Furth, 1970). The sequential marrow CFU-C patterns found during remission of AML are similar to those previously reported from this and other laboratories (Greenberg, Nichols et al., 1971; Bull, Duttera et al., 1973; Heller and Greenberg, 1977).

These findings that normal marrow CSA values were associated with both achievement and persistance of complete remission suggest that adequate marrow CSA provision may be essential for sustaining normal granulopoiesis in AML following induction chemotherapy. The absence of CSA in plasma from marrow or peripheral blood further implicated local cellular sources as being important providers of CSA. Thus, marrow CSA production may provide a measure of intramedullary influences involved in regulating granulopoiesis. Histologic examination of human marrow has indicated that granulopoiesis occurs within the marrow parenchyma (Weiss, 1970), where granulocytic precursors and cells of the monocyte-macrophage series are in

proximity. Thus, it is possible that short range interactions could occur in this microenvironment between adherent-cell elaborated stimulatory substances and granulocytic precursors. In addition to CSA production by these aspirable marrow cells, recent studies from our laboratory have shown that stromal cells firmly adherent to endosteal human bone also produce CSA. The relative contribution of those two intramedullary sources for CSA production is currently being assessed. In this model, long range influences could also participate in the response to major perturbations such as infection or antigenic challenge.

Monitoring marrow CSA provision appears useful for evaluating microenvironmental influences and intramedullary cellular interactions on granulopoiesis, and assessing prognosis and clinical status in AML.

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