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The Secretion of Plasminogen Activators by Human Bone Marrow Progenitor Cells

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A. Introduction

Leukaemic cells from patients with acute myeloid leukaemia (AML) and chronic myeloid leukaemia (CML) secrete plasminogen activators either of the urokinase (u-PA) or of the tissue plasminogen activator (t-PA) type. The enzyme has prognostic significance in that those individuals with AML whose cells secreted only t-PA failed to respond to combination chemotherapy, whereas those whose cells released u-PA alone or a combination of u-PA and t-PA could be induced to remission (Wilson et al. 1983).

Poor responses to chemotherapy are also seen when leukaemic cells display features of the early progenitor phenotype (Francis et al. 1979, 1981 a, b). It seemed likely, therefore, that secretion of the two species of plasminogen activator by haemopoietic cells might be differentiation-linked and that the association between u-PA secretion and favourable therapeutic outcome would reflect the tendency of early cells to release t-PA, whereas later cells would release u-PA.

B. Materials and Methods

Bone marrow cells were separated on the basis of differences in buoyant density in continuous Ficoll-Isopaque gradients (Loos and Roos 1974). Granulocyte-macrophage colony-forming cells (CFU-GM) were cultured using the method of Pike and Robinson (1970). The culture method for CFU-GM was that of Fauser and Messner (1978). Plasminogen activator and caseinolytic plaque assays have previously been described in detail (Wilson et al. 1980, 1983; Wilson and Francis 1987).

C. Results

Marrow samples from 11 normal subjects were fractionated by equilibrium density centrifugation, and cells from each fraction were examined to determine the type and rate of plasminogen activator that they produced. Representative results from two experiments are presented in Fig. 1 in two ways. In the first (Fig. 1 a, b), the rate of enzyme synthesis by all the cells in each gradient fraction is plotted as a function of gradient density. In the second (Fig. 1 c, d), the rate of synthesis has been corrected for the number of cells in each fraction and is expressed in terms of milliunits of enzyme/10^7 cells for 24 h. It is evident from both graphic presentations that the low-density cells (approximately 1.045–1.065^{-3} g cm^{-3}) synthesized exclusively t-PA. More mature, higher density cells (approximately 1.07–1.085 g cm^{-3}) released a mixture of t-PA and u-PA. It can also be noted from the profiles plotted in Fig. 1 a and b that the cells which produced t-PA comprised two populations. When they were corrected for cell number, only one peak of t-PA production (density

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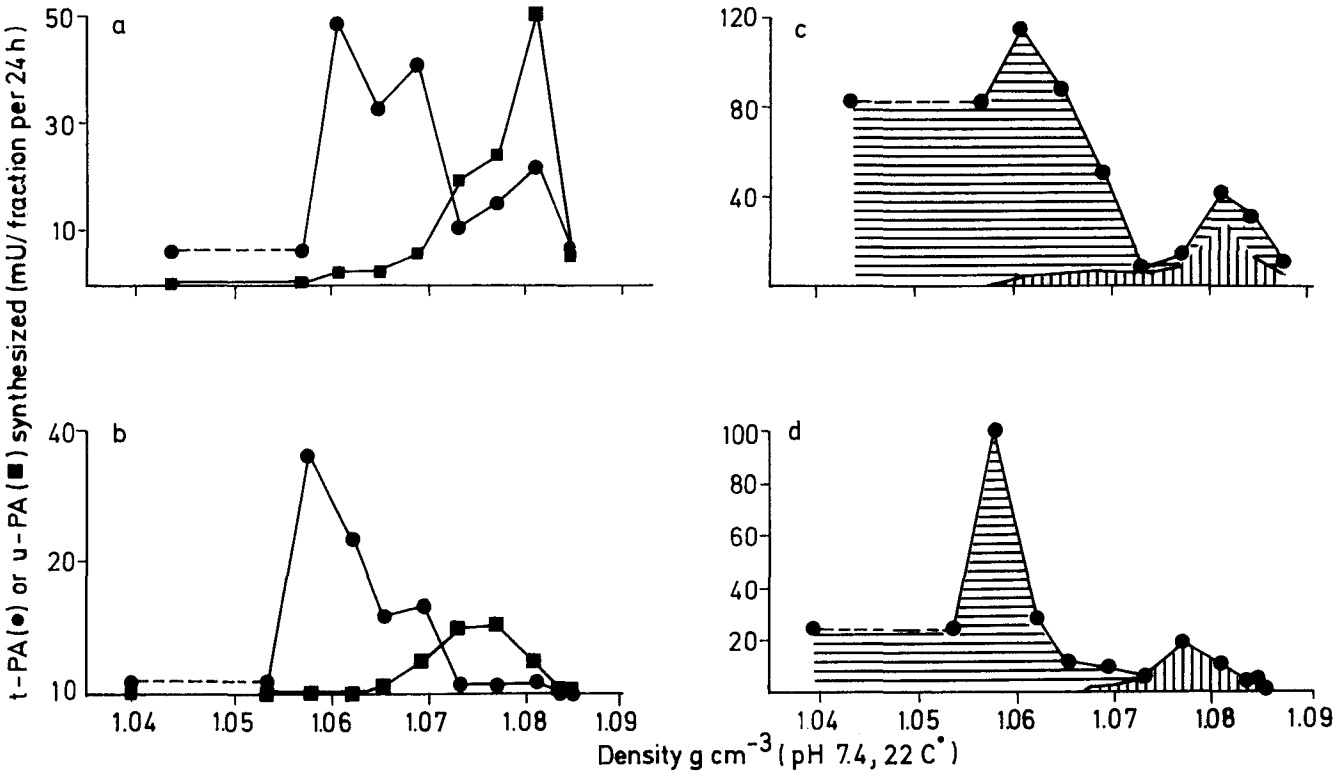

Fig. 1. **a** and **b** Equilibrium density distribution profiles of two normal marrow specimens, showing t-PA (●) and u-PA (■) expressed as units of enzyme per fraction per density increment. Cells producing t-PA have a relatively lower density than those producing u-PA. In both cases, the t-PA peaks were biphasic. **c** and **d** Density distribution profiles of the same marrow specimens, showing the relationship between total units of plasminogen activator/10^7 cells produced in 24 h and buoyant density. *Vertical hatching* indicates u-PA and *horizontal hatching* indicates t-PA (*dashed lines* represent adjacent fractions which have been pooled)

approximately 1.060 g cm^{-3}) was observed, thus indicating that although these cells can be divided into two populations on the basis of their density, they constituted a single population on the basis of the rate of enzyme production per cell.

The bimodal distribution in the t-PA-producing cell populations was observed in 7 out of 11 cases. The mean densities for the two t-PA-containing peaks were 1.063 and 1.072 g cm^{-3}, respectively.

The u-PA, in contrast, was produced by cells which fractionated in either a single peak or one which had a low broad profile. The mean density of the u-PA-producing cells was 1.076 g cm^{-3}. In vitro agar bone marrow cultures of the cells in the different gradient fractions revealed that the first and second t-PA-producing cell populations most closely resembled CFU-GM and cluster-forming cells, respectively. The modal density for myeloblasts was found to be between the two t-PA-producing cell peaks. Promyelocytes and myelocytes had modal densities of 1.069 and 1.072 g cm^{-3}, respectively. These populations appeared to correspond to the second peak of t-PA, which had a density of 1.072 g cm^{-3}. The u-PA-producing cells with a density of 1.076 g cm^{-3} corresponded to the neutrophil-granulocyte population.

In order to assess more directly the cellular species secreting each enzyme type, the low-density bone marrow cells (less than 1.063 g cm^{-3}) were cultured in semi-solid agar for varying periods of time. Developing clones were examined for the species of enzyme produced. Developing clones on day 3 produced t-PA exclusively. Clones examined on days 6–9 secreted both enzyme species; by day 11, large neutrophil and/or macrophage colonies were present and all the secreted enzyme was u-PA. Macrophages were also found to produce u-PA.

D. Discussion

These results showed that the secretion of t-PA and u-PA by haemopoietic cells was a differentiation-linked property, with t-PA being produced by primitive progenitors and u-PA being secreted by more differentiated cells (Wilson and Francis 1987).

It has been shown that a variety of human cells release plasminogen activators of either the t-PA or the u-PA type (Tucker et al. 1978; Vetterlein et al. 1979; Wilson et al. 1980). Apart from the involvement of t-PA in the fibrinolytic system (Collen et al. 1983), the physiological role of these two enzymes is obscure. The enzymes are produced by a wide variety of cells, including macrophages (Vassali et al. 1977) and neutrophil polymorphonuclear leucocytes (Granelli-Piperno et al. 1977; Wilson et al. 1983), where they may be involved in a number of processes, including proteolysis of inflammatory exudates, generation of chemotactic peptides and processes that require regulated local proteolysis (Reich 1978).

Tissue plasminogen activator was secreted by two populations of bone marrow cells, one with a mean density of 1.063 g cm^{-3} and the other with one of 1.072 g cm^{-3}. The first population corresponds to the modal density for CFU-GM. We cannot exclude the possibility that other progenitor types also produce t-PA. Analysis of the density distribution of the fractionated cells suggested that myelocytes and possibly promyelocytes were responsible for the second t-PA peak. Urokinase was secreted by cells with a density of between 1.067 and 1.082 g cm^{-3}. Promyelocytes, myelocytes and neutrophil granulocytes are present in these fractions. It thus appears either that the promyelocytes and myelocytes produce both types of enzyme or that the switch from t-PA to u-PA production occurs over a range of maturation stages.

The functional properties of AML clonogenic cells have been shown to relate to response to chemotherapy (Moore et al. 1974; Francis et al. 1981 b; McCulloch et al. 1982). The results indicating that early normal progenitor cells secrete t-PA (Wilson and Francis 1987), together with the previous observation (Wilson et al. 1983) that patients whose AML cells secreted t-PA alone failed to respond to chemotherapy, suggest that these patients have an accumulation of a primitive t-PA progenitor secreting cell population.

While it appears that neutrophils and macrophages require u-PA for their role in inflammation, the reason why primitive haemopoietic cells produce t-PA is less readily apparent. The production of proteases by progenitor cells may be necessary to provide a local proteolytic mechanism for generating biologically active peptides. In addition, stem cells are endowed with the capacity for migration and implantation in specific haemopoietic sites, and this process could also require proteolytic enzyme secretion.

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