

MURINE AND HUMAN HEMATOPOIETIC COLONY FORMATION: A POSSIBLE REGULATORY ROLE FOR INTRACELLULAR HISTAMINE

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Increasing number of data suggests that locally produced histamine is involved in regulation of hematopoiesis. In this study the granulocyte/macrophage (CFU-GM) colony formation by normal murine or human bone marrow cells, leukaemic colony formation (CFU-L) by a murine leukemia cell line (WEHI 3B), and colony formation by bone marrow cells from patients with chronic myeloid leukemia (CML) have been examined. We detected mRNA and protein expression of histidine decarboxylase (HDC), the only enzyme responsible for histamine synthesis both in normal bone marrow progenitor cells and in leukaemic progenitors. The significance of *in situ* generated histamine was shown on colony formation by inhibitory action of α FMH (blocking HDC activity, i.e. *de novo* histamine formation) and by N,N-diethyl-2-{4-(phenylmethyl)phenoxy}-ethanamine-HCl (DPPE) disturbing the interference of histamine with intracellular binding sites. These data provide further confirmation of the role of histamine in development and colony formation of bone marrow derived cells.

Keywords: Histamine – colony – hematopoiesis

INTRODUCTION

Hematopoiesis is a multistep process for the production of mature blood cells, whereby multipotent hematopoietic stem cells differentiate into various pluri-, bi- or unipotent progenitors. Growth and differentiation of these progenitors are regulated by specific hematopoietic growth factors that can be quantitatively assayed by their colony formation *in vitro*. Colony formation results from the combined events of cell proliferation and differentiation. One of the cell-cycle promoters, interleukin-3 (IL-3), acting through signalling pathways different from those of GM-CSF [1], has been shown to stimulate colonies of murine myeloid progenitor cells (CFU-GM: granulocyte, macrophage colony forming units) [2, 3] by enhancing the synthesis of histamine in the low density, progenitor-enriched fraction of murine bone marrow [2, 4]. Exogenous histamine, too, stimulates the division of progenitor cells through H2 histamine receptors [5]. Murine hematopoietic progenitors also take up exoge-

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nous histamine [6]; inhibition of the influx and efflux of histamine by receptor antagonists [7] in these cells has been described. Dual signal transduction of histamine H₂ receptors [15], has been reported in murine CFU-L cells that seem to utilize both cyclic-AMP and phospholipase C pathways in response to H₂ receptor activation (for maintaining the cells in the cell cycle).

The content of histamine in cells thus reflects synthesis, degradation and transport processes. Any change in the synthesis of histamine by inhibiting the activity of the histamine forming enzyme, histidine decarboxylase, or in its action by blocking receptor-ligand interaction would be expected to influence colony formation of bone marrow progenitor cells. By manipulating synthesis and receptor occupancy, the sensitivity to histamine of malignant and normal colony forming cells can be compared. For this purpose, we studied GM colony formation by normal murine or human bone marrow cells, leukaemic colony formation (CFU-L) by a murine leukemia cell line (WEHI 3B), and colony formation by bone marrow cells from patients with chronic myeloid leukemia (CML).

MATERIALS AND METHODS

Clonogenic cells in culture

Assay of CFU-GM

Bone marrow cells were obtained from the femora of normal BDF1 hybrid mice or from normal human donor bone marrow after written consent of the patient. Cells suspended in McCoy 5A medium (Csertex, Hungary) were supplemented with horse serum (10%) for murine cells and with fetal calf serum (10%) (Sigma, Hungary) for human cells. As a source of growth factors, supernatants from cultures of WEHI 3B myelomonocytic leukemia cells, containing IL-3 (Sigma, Hungary), or from human bladder carcinoma cells (5637) were added to murine and human cells, respectively (final concentration, 10%).

4×10^4 murine bone marrow cells/ml or 1×10^5 human mononuclear bone marrow cells/ml were plated in 0.9% final concentration of methylcellulose (Fluka, Wien) and incubated at 37 °C in 5% CO₂. The number of colonies was counted on day 7 (murine) and on day 9 (human). Aggregates of more than 50 cells were regarded as a colony.

Assay of CFU-L

Cells from WEHI cultures were assayed under conditions similar to those for CFU-GM, except for the absence of conditioned medium. Bone marrow cells from CML patients were assayed in the presence of 10% conditioned medium.

Morphological demonstration of histidine decarboxylase (HDC)

The DNA probe for *in situ* hybridization was prepared from the PCR product of HDC-cDNA with primers 5'-AATCTTCAAGCACATGTC-3' (sense) and 5'-CTGGATAGTGGCCGGGATGA-3' (antisense). The biotin-labelled [8] HDC-DNA probe was applied to formaldehyde-fixed colony cells cytocentrifuged onto coated slides. Texas red-avidin conjugate was prepared to assess fluorescence under a confocal microscope (BioRad MC1024).

For the immunohistochemical detection of HDC in bone marrow colonies of progenitor cells, a primary antibody was raised in chickens against the 318-325 (VKD-KYKQLQ) residues of the full HDC protein [9]. FITC-labelled anti-chicken antibody was used for localization of the enzyme protein.

The combination of *in situ* hybridization and immunohistochemistry resulted in an interference colour of yellowish-brown in cells expressing both the gene and the HDC enzyme.

Influencing the amount and action of cell-bound histamine

Histidine decarboxylase (HDC), the enzyme synthesizing histamine, was irreversibly inhibited by alpha-fluoromethyl-histidine (α FMH, kindly gifted by J. Kollonitsch) [10]. The concentrations of α FMH employed were 10^{-3} M – 10^{-7} M. N,N-diethyl-2-{4-(phenylmethyl)phenoxy}ethanamine-HCl (DPPE, produced by Dr. F. Hudecz) [11] an inhibitor of intracellular histamine binding in microsomes and nuclei, was employed to decrease the intracellular effects of histamine by inhibiting its access to acceptor sites. The concentration range of DPPE was 10^{-3} M – 10^{-7} M. The viability of the cells treated with both reagents was controlled by dye (trypan blue) exclusion tests.

Assessment of colony formation and statistical analysis

The number of colonies resulting from α FMH- or DPPE-treatment were compared to control (untreated) colonies as follows: test/control $\times 100$. Standard error (S.E.) and Student's two-tailed test were employed for statistical evaluation of data.

RESULTS

Figure 1 demonstrates the presence and gene expression of HDC in bone marrow colony cells of normal (1A) and of leukemic murine progenitors (1B). Gene expression (in insert), or the protein itself, was not uniformly present in all cells; the frequency of immunopositivity for the HDC protein was about 30% and 55% in murine CFU-GM and CFU-L cells, respectively. In human cells the corresponding values

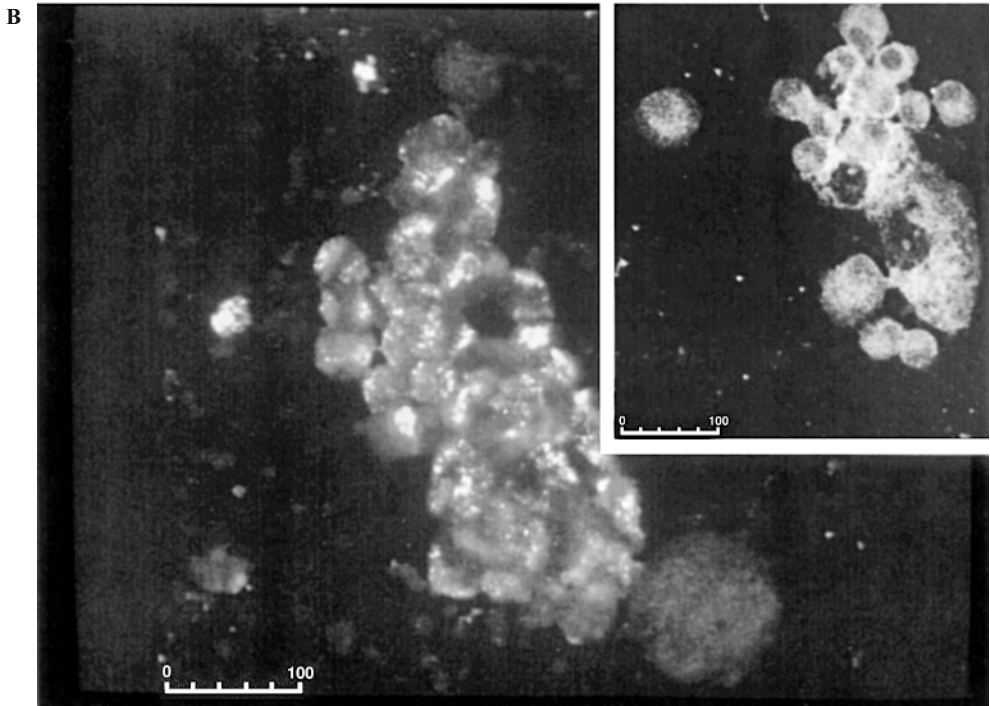
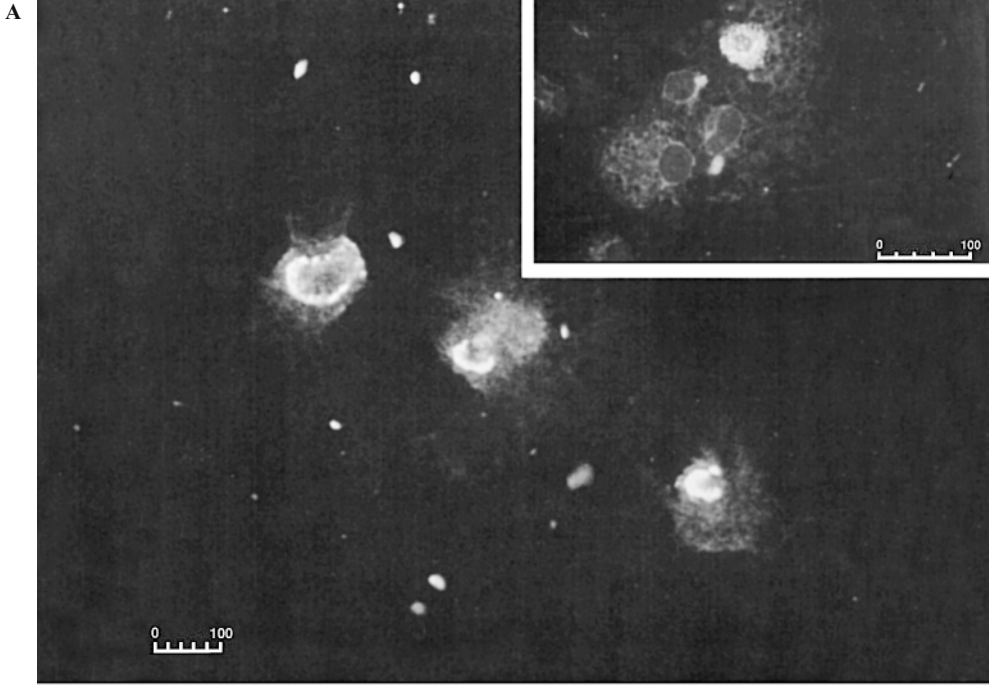


Fig. 1. Demonstration of histidine decarboxylase (HDC) protein by immunofluorescence (main picture, indirect FITC fluorescence) and mRNA by *in situ* hybridization (insert, biotin-Texas red) in murine CFU-GM (granulocyte, macrophage) colony cells (A) and in murine CFU-L (leukemic) (WEHI-3B) cells (B). For details see Materials and Methods.

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were about 25% (CFU-GM cells) and about 70% (CFU-L cells). In a proportion of cells HDC expression (green fluorescence) was not always accompanied by HDC gene expression (red fluorescence).

The effective concentration range of α FMH to inhibit the murine and human CFU-L colony formation was 10^{-3} – 10^{-4} M (see also Table 1). Both kinds of leukemic cells were more sensitive to α FMH as compared to normal human or murine progenitors (Table 1). The effective range of DPPE (minimum 30% inhibition in colony formation) was 10^{-3} M – 10^{-5} M, and 10^{-3} M – 10^{-6} M, in human and murine samples, respectively. (Optimal concentrations for both FMH and DPPE were determined earlier.) At higher concentrations DPPE was cytotoxic to both murine and human cells. Lower ($<10^{-7}$ M) concentrations of DPPE, that did not significantly inhibit colony numbers of human cells were inhibitory to murine cells (Table 1). A higher reactivity of leukaemic cells to DPPE was observed as compared to normal cells. DPPE (10^{-4} M) significantly inhibited human colonies derived from CML patients (human CFU-L).

Table 1
Effects of FMH and DPPE on the colony formation of CFU-GM/CFU-L cells of murine and of human origin

FMH (M)	Murine		Human	
	CFU-GM	CFU-L	CFU-GM#	CFU-L
10^{-3}	57±5**	56±1*	74	61±3*
10^{-4}	77±2*	47±6*	77	54±5*
10^{-5}	80±13	65±8*	99	68±9
10^{-6}	84±10	83±11	89	73±4*
10^{-7}	79±16	106±0	94	75±17
DPPE (M)				
10^{-4}	43±11*	41±1*	70	51±0*
10^{-5}	75±4*	50±5*	76	71±11
10^{-6}	67±5*	72±3*	88	82±36
10^{-7}	90±0*	69±4*	99	101±14

The results are expressed in percentage of control, # mean ± SE, n = 5.

mean of three experiments.

Asterisks represent data with statistical significance ($p < 0.05$)

DPPE and α FMH were inhibitory when the agents were added to plates on day 0. However when added on day 5, no significant inhibition of colony formation by these agents was observed (data not shown).

DISCUSSION

In addition to metabolic degradation, the intracellular concentration of histamine is a function of synthesis and cell transport. Histamine has been shown to promote the growth of some cells via membrane H₂ receptors [5–7], for example, some experimental mammary carcinoma cells [12, 13], and hematopoietic progenitor cells. The H₂ receptor antagonist, cimetidine [6] does not block the uptake of histamine in murine CFU-S cells, but inhibits IL-3 induced DNA synthesis [7]. Cimetidine [14] inhibits (40–50%) murine colonies of CFU-L cells (WEHI 3B) but is less inhibitory (20–30%) to normal CFU-GM colonies. IL-3 promotes cells to enter the cell cycle by increasing histamine synthesis [3] via increased HDC-mRNA expression [4]. In our study the irreversible inhibition of *de novo* HDC by α FMH [13] resulted in decreased murine and human colonies. The inhibition of HDC in human CFU-L cells resulted in a greater decrease in colony number than in normal CFU-GM cells. CFU-L (CML) cells were inhibited by α FMH at 10^{-5} M, a concentration that does not inhibit human CFU-GM cells. Murine cells also showed significantly higher sensitivity to the inhibitory effect of α FMH than human cells. CFU-L had a more pronounced reduction in colony number at an α FMH concentration of 10^{-4} M. The differential sensitivity to α FMH also may be suggesting an existing HDC pool, in addition to *de novo* synthesized enzymes, and may explain the observed larger amount of HDC in leukemic cells, as well as missing transcripts of the gene in many of the cells (Fig. 1A, B).

Similar to their sensitivity to α FMH, CFU-L cells were more sensitive to inhibition by DPPE (10^{-5} M). At concentrations of 10^{-4} M human chronic myeloid leukemic cells were also more sensitive to DPPE than CFU-GM. Higher DPPE concentrations (10^{-3} M) are universally cytotoxic.

Our present understanding on DPPE action involves its binding to antiestrogen (tamoxifen) binding sites, mostly on P450 monooxygenases, DPPE antagonizes histamine binding to these microsomal and nuclear moieties. Perturbation of histamine/P450 complexes by DPPE might be responsible for modulatory action of DPPE on proliferation [18, 19]. Recent data strongly suggest, that chemopotentiating effect of DPPE is directly related to its capacity for preferential attachment to CYP2D6, CYP3A4 and CYP1A1 [19, 20] inhibiting these isoenzymes to metabolize antineoplastic drugs.

Inhibition of colony formation by DPPE might be explained by the disturbance of the effect of intracellular action of histamine on proliferation of both leukaemic and normal progenitors. It is hoped that direct binding and competition studies with labelled histamine and DPPE may elucidate the DPPE-sensitive and -insensitive (intracellular and/or autocrine) influences of endogenous histamine on colony formation of hematopoietic progenitors.

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