Hematopoietic Progenitors and Interleukin-3–Dependent Cell Lines Synthesize Histamine in Response to Calcium Ionophore

By Michel Dy, Anne Arnould, François M. Lemoine, François Machavoine, Hermann Ziltener, and Elke Schneider

The calcium ionophore A23187 promotes histamine synthesis in murine bone marrow cells by increasing the expression of mRNA encoding histidine decarboxylase (HDC), the histamine-forming enzyme. The cells responsible for this biological activity copurify with hematopoietic progenitors in terms of density, light scatter characteristics, and rhodamine retention, similar to interleukin (IL) 3–induced histamine-producing cells. Yet, the effect of calcium ionophore is not mediated by IL-3. The most purified rhodamine-bright bone marrow subset contains 80% cells that respond to calcium ionophore by increased HDC mRNA expression. This high frequency makes the involvement of one particular progenitor subset in histamine synthesis unlikely. The finding that all IL-3–dependent cell lines tested so far exhibit increased histamine production and HDC mRNA expression in response to calcium influx lends further support to this notion. Cell lines requiring other growth factors or proliferating spontaneously lack this ability. Finally, it should be noted that IL-3–dependent cell lines do not produce histamine in response to their growth factor. It might, therefore, be suggested that the pathway transducing the signal for increased histamine synthesis after IL-3 receptor binding in normal hematopoietic progenitors is modified in these cell lines.

IN ADDITION TO its central role during immediate hypersensitivity, histamine has been implicated by several investigators as a mediator of cell growth and/or differentiation. This notion originated from studies by Kahlson and Rosengren, showing that regeneration of injured tissues is concomitant with increased histamine synthesis. It derived further support from the observation that exogenous histamine triggers pluripotent stem cells into cycle and that myeloblasts differentiate in vitro into myelocytes and metamyelocytes in response to this amine. More recently, the demonstration of a new intracellular histamine receptor and the inhibition of cell proliferation by its specific antagonist have led to the assumption that histamine might act as an intracellular messenger of cell proliferation.

Our own evidence for the generation of large amounts of histamine in the hematopoietic environment in response to interleukin (IL)-3 or granulocyte-macrophage colony-stimulating factor (GM-CSF), both in vitro and in vivo, is consistent with the physiological relevance of these observations. We have also established that endogenous histamine production is requisite for the colony-forming unit-spleen (CFU-S) cell cycling promoted by IL-3 or by GM-CSF in combination with IL-1. It is important to note that the histamine secreted in response to the growth factors results entirely from de novo synthesis of histidine decarboxylase (HDC) and not from degranulation of stored histamine. Knowing that mast cells or basophils, as well as other end cells of the bone marrow do not produce histamine in response to IL-3 or GM-CSF, could thus be hypothesized that histamine synthesis was associated with IL-3–induced cell proliferation and/or differentiation in various hematopoietic progenitors.

In accordance with this idea, we show in the present study that the calcium ionophore A23187 can promote increased HDC mRNA expression in almost all low-density bone marrow cells with high Rh retention, despite the heterogeneity of this population, which comprises a variety of progenitors with partially lineage-restricted potential. In the same line of evidence, we demonstrate that several IL-3–dependent cell lines express this biological activity in response to calcium ionophore.

MATERIALS AND METHODS

Animals, Cytokines, and Reagents

Male or female 6 to 8-week old C57BL/6 mice were obtained from the Centre d’Élevage R. Janvier (Le Genest-St Isle, France). Murine recombinant (mr) IL-3 (specific activity: 1 to 2 x 10⁷ U/mg, as assessed on the NFS-60 cell line) and mrGM-CSF (1 to 2 x 10⁷ U/mg, assessed on the DA-3 cell line) were purchased from R & D System (Oxon, Great Britain). The IgG fractions from sheep antisera against IL-3 and GM-CSF were used at a dilution of 1/50. In these conditions, they neutralized the effect of optimal doses of IL-3 and GM-CSF, respectively. The calcium ionophore A23187 was purchased from Boehringer (Mannheim, Germany) and used at doses ranging from 0.5 to 5 µmol/L α-fluoromethylhistidine (α-FMH), a specific inhibitor of HDC, was kindly provided by Dr J. Kollonitch (Merck, Sharp & Dohme, Rahway, NJ).

Cell Lines

The IL-3–dependent cell lines BAF/B03, FDCP1 and FDCP2 were maintained in RPMI 1640 (GIBCO, Europe, Paisley, Scotland) supplemented with 1% L-glutamine 100X, 100 IU/mL penicillin, 100 µg/mL streptomycin (GIBCO), 10% WEHI-3–conditioned medium and 10% heat-inactivated horse serum (HS; HyClone, Logan, UT). For maintenance of Ea3 cells, horse serum was replaced by 10% heat-inactivated fetal calf serum (FCS; Biological Industries, Kibbutz Beth Haemek, Israel). The IL-6–dependent cell line 7TD1, as well as the IL-2–dependent cell line CTLL-2, were cultured in complete RPMI 1640 medium supplemented with 10% FCS, 5 x 10⁻⁵ mol/L β-mercaptoethanol and optimal doses of mlRIL-6 and 10% Con A-conditioned medium from rat spleen cells, respectively. The WEHI-3 cell line was maintained in the same medium without cytokines. Before use, all cell lines were washed three times in complete culture medium, adjusted to a final concentration of 0.5 x 10⁶ cells/mL, and incubated in the presence of cytokines or A23187 for 4 to 24 hours.

From the CNRS URA 1461, Université René Descartes—Paris V, Hôpital Necker, Paris; CNRS URA 1463, CERVI Hôpital Pitié Salpêtrière, Paris, France, and Biomedical Research Center, Vancouver, Canada.

Submitted July 18, 1995; accepted November 27, 1995.

Supported in part by grants from the ‘‘Association pour la Recherche contre le Cancer’’ (Grant No. ARC 70935) and ‘‘La Ligue Nationale contre le Cancer’’.

Address reprint requests to Michel Dy, PhD, CNRS URA 1461—Hôpital Necker, 161, rue de Sèvres, 75743 Paris Cedex 15, France.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked ‘‘advertisement’’ in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1996 by The American Society of Hematology.
Cell Preparations

Spleen cell suspensions were obtained by disruption with forceps. Mesenteric, inguinal, and axillary lymph nodes were removed and disrupted in a Potter-Elvehjem homogenizer. Bone marrow cells were collected by flushing tibias and femurs with ice-cold Hanks’ Balanced Salt Solution (GIBCO). After centrifugation, cells were suspended in Modified Essential Medium with Earle’s salts (MEM), supplemented with 1% L-glutamine 100X, 1% sodium pyruvate 100X, 1% nonessential amino acids 100X, 100 IU/mL penicillin, 100 µg/mL streptomycin (all from GIBCO), and 10% HS. In some experiments, cells were cultured in S-MEM (Spinner culture) without out calcium (GIBCO). Incubations were performed at a concentration of 10^6 cells/mL at 37°C in 5% CO2. Progenitor-enriched bone marrow cells were obtained by fractionation on a discontinuous Ficoll gradient as previously described. Briefly, the gradient was prepared from Ficoll 400 (Pharmacia Fine Chemicals, Uppsala, Sweden) at concentrations of 10, 14.6, 16.1, 17.7, 19.2, and 23% in 0.1 mol/L sodium phosphate (pH 7.4). A total of 1 to 2 mL of bone marrow cell suspension from five mice were layered on top of the gradient (1.2 mL/layer) and centrifuged for 30 minutes at 23,500g at 4°C. Layer 0 was defined as the interphase between culture medium and the less dense layer, and subsequent interphases were numbered sequentially. Cells located in layer 2 to 4 were then collected, washed, and adjusted to a final concentration of 0.5 x 10^6 cells/mL in the culture medium described earlier.

Sorting of Bone Marrow Cells

A total of 2 x 10^6 low density cells per mL were incubated for 45 minutes at 37°C in MEM containing 0.1 µg/mL rhodamine-123 (Eastman Kodak, Rochester, NY), as previously reported. The excess internalized Rh was removed by incubating the cells further for 30 minutes in MEM at 37°C. Following two washings in MEM, the cells were resuspended at a concentration of 1 to 2 x 10^6/mL in phosphate-buffered saline (PBS) containing 2% FCS. They were then analyzed and sorted with a 2-laser flow cytometer Epics Elite (Coultronics, Margency, France), according to their light scatter properties and fluorescence intensity. The flow rate was 2,000 cells per second. The preselected sorting area contained the majority of immature cells (blast cell window: about 60% of low-density cells). The Rh-bright and Rh-dull fractions comprised respectively 10% of the most and 10% of the less fluorescent cells of the blast cell window. The purity of the sorted cells was controlled by reanalysis of the samples and attained more than 95%.

Histamine and HDC Assays

Histamine concentrations in cell supernatants were currently determined by an automated continuous flow fluorometric technique (lower limit of sensitivity: 0.5 ng/mL) as previously described. Its specificity has been confirmed by the use of other assays, such as the classical bioassay and the radiomunnoassay (Immunotech, Marseille, France). We have also verified that Rh-123 did not interfere with the assay by comparing the histamine production of low-carboxylated of the remaining L-[3H]histidine. To assess the specificity of the assay, each reaction was performed with or without 10^-6 mol/L α-FMH, the specific inhibitor of HDC. After centrifugation, the synthesized [3H]-histamine was separated from [3H]-histidine by ion exchange chromatography on Amberlite CG-50 columns (Prolabo, Paris, France).

Hybridization Probes

The murine HDC probe used for Northern blot analysis and in situ hybridization was kindly provided by Dr A. Ichikawa (Kyoto University, Kyoto, Japan). A 523-bp fragment was subcloned into a PGEM-4 plasmid (Promega Biotec, Madison, WI). The murine β-actin probe (a generous gift from Dr F. Daubry, Villejuif, France), subcloned into a Bluescript transcription vector served as positive control.

Northern Blot Analysis

Total RNA was isolated by a guanidine isothiocyanate procedure, followed by cesium chloride centrifugation. A total of 5 µg of total RNA were electrophoresed in a 1% agarose-formaldehyde gel, transferred to a nylon membrane, and baked for 2 hours at 80°C. HDC and β-actin probes were labeled with [32P]dCTP by random priming, according to the manufacturer’s instructions (Megaprime DNA labeling system, Amersham, Great Britain). Filters were prehydrized for 18 hours in hybridization buffer (HBN: 50% deionized formamide, 5X SSC (1X SSC = 150 mmol/L NaCl, 15 mmol/L Na citrate, pH 7), 5X Denhardt’s, 0.1% sodium dodecyl sulfate (SDS), 125 µg/mL DNA SS) at 42°C and then hybridized in the same conditions for further 18 hours with the radiolabeled probe (10^6 cpm/ mL). Filters were washed once with 1X SSC, 0.1% SDS for 30 minutes at room temperature and twice at 68°C in 0X SSC, 0.1% SDS. Autoradiography was then performed for 72 hours at -80°C. The amount of RNA loaded per lane was verified by rehybridizing the same filters with a β-actin probe.

In Situ Hybridization

Hybridization was performed as previously described. In brief, different cell populations incubated 4 hours or 24 hours in culture medium alone or together with IL-3 or A23187 were cytotoxicified, fixed in 4% paraformaldehyde/PBS, and kept in 70% ethanol until use. The slides were then postfixed in 2% paraformaldehyde/1% glutaraldehyde/PBS for 5 minutes, washed in PBS, acetylated by 0.25% acetic anhydride in 0.1 mol/L triethanolamine buffer (pH 8) for 10 minutes, washed in PBS, dehydrated in ethanol, and air dried. Negative controls were prepared by treating some slides with RNase A (200 µg/mL) and RNase T1 (12.5 U/mL) (Boehringer) in 2X SSC. RNA probes with a specific activity of 10^8 cpm/µg were prepared according to Lawrence and Singer as previously reported. Their fragment length was adjusted to a mass average of approximately 100 to 150 bases by limited alkaline hydrolysis. RNA probes were ethanol-precipitated with yeast tRNA and resuspended in 20 mmol/L dithiothreitol (DTT), 250 µg/mL yeast tRNA, 12.5 µg/ mL DNA, and 106 cpm/30 µL of the RNA probe. The hybridization mixture was denatured, applied to slides, and covered with paraffin. Hybridization was performed at 50°C for 18 hours in a humidified chamber. The subsequent washing procedures were all performed in the presence of 10 mmol/L DTT at 52°C, as reported before. For autoradiography, the slides were dried and coated with NTB2 emulsion (Eastman Kodak). After 3 weeks of exposure, the slides were developed in Dektol (Eastman Kodak), fixed in Unifix (Eastman Kodak), and stained with May–Grünewald Giemsa.
HISTAMINE AND HEMATOPOIETIC PROGENITORS

The specificity of hybridization was assessed by the sense HDC mRNA probe, as well as ribonuclease treatment of some cell preparations. In both cases, there was no significant labeling. Background signal was estimated to be less than 20 silver grains per cell. The integrity of the mRNA was verified by a positive control, performed with a β-actin probe.

Reverse Transcriptase-Polymerase Chain Reaction Analysis

Total RNA was extracted by a modified method of Chomczynski and Sacchi, using TRizol Reagent (Life Technologies, Gaithersburg, MD) according to the manufacturer’s instructions. The primers specific for HDC and β2microglobulin synthesized by Bioprobe (Montreuil, France) had the following sequences: HDC: 5’ primer, TAC GCC GAC TCC TTC ACC; 3’ primer, GCT GGG GCT GTA GAA CT; β2microglobulin: 5’ primer, TGA CCG GCT TGT ATG CTA TC and 3’ primer, CAG TGT GAG CCA GGA TAT AG. Reverse transcription (RT) was performed by a standard procedure using 2 pmol/L of total RNA. A total of 2 μL of RT products (pure or prediluted) were then added to 48 μL of the polymerase chain reaction (PCR) mix to give a final concentration of 50 U/mL Taq DNA polymerase (Life Technologies), 0.25 mmol/L 5’ and 3’ primers, 50 μmol/L of each dNTP, 1.5 mmol/L MgCl2, and 1× PCR buffer (20 mmol/L Tris-HCl pH 8.4, 50 mmol/L KCl). cDNAs were amplified for 30 cycles, each one performed at 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute. Samples were then cooled at room temperature, and 10 μL of RT-PCR products were separated by 1.5% agarose gel electrophoresis in TBE 1X (Tris base 0.089 mol/L, boric acid 0.089 mol/L, EDTA 2 mmol/L, pH 8.3) containing 0.2 μg/mL of ethidium bromide. Fragments of DNA were visualized under ultraviolet (UV) light.

RESULTS

Comparison Between the Effect of Calcium Ionophore and IL-3 on Histamine Synthesis by Bone Marrow Cells

As shown in Fig 1, the calcium ionophore A23187 increased histamine production by unfractionated bone marrow cells with a maximal effect at doses ranging from 1 to 10 μmol/L. The histamine levels generated in these conditions within 24 hours were identical to those induced by IL-3. They were attained by different kinetics illustrated in Fig 2, with a more rapid and transient effect of A23187, resulting in about twice as much histamine after a 4-hour stimulation, relative to IL-3 (8.2 ± 1.6 and 4.7 ± 0.6 ng/10^6 cells, respectively; v 2.2 ± 0.2 ng/10^6 cells in culture medium alone; mean ± SEM from three separate experiments). A23187 and IL-3 did not synergize in terms of histamine production, and their combined effect was only partially additive (data not shown).

The increase in extracellular histamine levels promoted by both IL-3 and A23187 was totally abrogated by α-FMH, a suicide substrate of the histamine-forming enzyme HDC (Fig 3A), ruling out the involvement of mast cell or basophil degranulation in this process. This observation was confirmed by enzymatic assays in cell lysates, which established a striking enhancement of HDC activity after stimulation by IL-3 or calcium ionophore (Fig 3B). In agreement with the kinetics of histamine production, increased HDC mRNA expression was easily detected after a 4-hour stimulation of bone marrow cells with A23187 (Fig 4). IL-3 required 12 to 18 hours to exert a similar effect, as reported before. Although induction of HDC mRNA occurred earlier in response to calcium ionophore than in response to IL-3, we verified whether it involved the growth factor. As shown in Fig 5, histamine production in response to A23187 was not significantly diminished when bone marrow cells were preincubated with antibodies directed against IL-3 and GM-CSF (another inducer of histamine synthesis) at concentrations that completely neutralized their respective antigens. Conversely, calcium deprivation diminished spontaneous, as well as IL-3–induced, histamine production by bone marrow cells.
Fig 3. A23187- or IL-3-induced increase in histamine synthesis by total bone marrow cells. (A) Inhibition of IL-3- or A23187-induced histamine production by α-FMH (mean values ± SEM from three experiments). (B) Effect of IL-3 or A23187 on HDC activity measured in cell lysates after a 24-hour incubation (typical experiment).

Characterization of A23187-Induced Histamine-Producing Cells

Apart from bone marrow cells, only splenocytes responded consistently to calcium ionophore in terms of histamine production. In accordance with the lower incidence of progenitors in this hematopoietic population, they were low producers (7.9 ± 3.0 and 1.5 ± 0.4 ng of histamine per 10⁶ splenocytes, respectively, after a 24-hour incubation with or without calcium ionophore, as compared with 116 ± 21.0 and 8.5 ± 1.1 ng of histamine per 10⁶ bone marrow cells in the same conditions). Cells from lymphoid organs, such as lymph nodes and thymus, did not secrete histamine, whatever the concentrations of calcium ionophore or IL-3. As illustrated in Fig 7, histamine production was 5-fold increased when cells from the low-density layers 2 to 4 of the discontinuous Ficoll gradient (5% to 10% of the starting population) were stimulated with A23187 instead of total bone marrow cells. These cells coenriched with hematopoietic progenitors, such as CFU-S day-8, CFU-C, and mast cell precursors, similar to the IL-3 responder cells we have previously characterized.

To evaluate the proportion of cells actually expressing HDC mRNA at a given time point, we performed in situ hybridization on low-density bone marrow cells stimulated for 4 hours with IL-3 or calcium ionophore, relative to medium controls. In these conditions, 4.8% ± 0.8% and 8.8% ± 2.1% cells were labeled in response to IL-3 and A23187, respectively, relative to 1.87% ± 0.1% in medium controls (means ± SEM from three separate experiments). This difference in the proportion of cells, labeled by the HDC probe
A23187-Induced Histamine Synthesis by IL-3–Dependent Cell Lines

Calcium ionophore promoted a striking histamine production by all IL-3–dependent cell lines tested, whatever the stage of differentiation they were arrested in. It exerted a more pronounced effect on relatively immature BAF/BO3, FDCP1, FDCP2, and Ea3 cells, represented in Fig 9. Conversely, the mast cell-committed MC9 line produced lower amounts of histamine and cell lines, such as CTL2 (IL-2–dependent) or 7TD1 (IL-6–dependent) did not express HDC mRNA in response to calcium ionophore, even when analyzed by RT-PCR (Fig 10). Histamine is also not produced by spontaneously proliferating cell lines, namely WEHI-3, stimulated with calcium ionophore (data not shown). In contrast to normal hematopoietic progenitors, none of the IL-3–dependent cell lines tested produced consistent amounts of histamine in the presence of IL-3 alone. The growth factor did not significantly enhance the effect of calcium ionophore (Fig 9). It should be noted that the histamine levels generated by a given cell line were liable to variations in the course of time and according to their origin, but none of the positive cells ever lost this biological activity.

Like normal hematopoietic cells, IL-3–dependent cell lines responded to A23187 by enhanced HDC mRNA expression, as assessed by Northern blot analysis (data not shown) or RT-PCR (Fig 10). As illustrated by in situ hybridization taking the BAF/BO3 cell line as an example (Fig 11), a 4-hour exposure to calcium ionophore induced HDC mRNA expression in nearly all cells, whereas no significant labeling occurred in culture medium alone.

DISCUSSION

IL-3 and A23187 are excellent inducers of histamine production by bone marrow cells. Both factors exert their action by increasing the expression of mRNA encoding histidine decarboxylase, the enzyme responsible for histamine synthesis. The effect of calcium ionophore was not impaired by anti–IL-3 or anti–GM-CSF antibodies, which makes the involvement of these potential histamine inducers unlikely. Conversely, intracellular calcium levels play at least some part in the histamine synthesis promoted by IL-3, because of the significant decrease observed after calcium deprivation.

Although nascent histamine has been demonstrated in a variety of proliferating tissues devoid of mast cells or basophils,1,26 we paid particular attention to the presence of histamine-containing cells among the different bone marrow subsets tested, inasmuch as A23187 is a potent degranulating agent.27 We knew already from our previous studies on IL-3-induced histamine synthesis that mature components of the mast cell/basophil lineage were not present in the bone marrow subset sorted on the basis of low-density and high-rhodamine retention (Rh-bright), which was most enriched for histamine-producing cells.15 Yet, it could still be argued that histamine synthesis represented an early event in mast cell or basophil differentiation, occurring before these cells had acquired the capacity to store the amine. Our present data are not consistent with such an exclusive participation of early mast cell/basophil precursors in A23187-induced histamine synthesis.

Fig 6. Effect of calcium deprivation on histamine production by bone marrow cells in response to IL-3 or A23187 (mean values ± SEM from three experiments). (□) MEM; (■) MEM without calcium.

Fig 7. Histamine production by total and Ficoll-fractionated bone marrow cells recovered from layers 2 to 6 of a discontinuous Ficoll gradient and incubated for 24 hours in culture medium, IL-3 or $10^{-8}$ mol/L A23187 (mean values ± SEM from three experiments).
Fig 8. HDC mRNA expression in Rh-bright bone marrow cells stimulated for 2 hours with $5 \times 10^{-7}$ mol/L A23187 (A) or IL-3 (B), relative to culture medium alone (C). In situ hybridization was performed on cytocentrifuged cells using an antisense HDC riboprobe. Original magnification ×12,000.
HISTAMINE AND HEMATOPOIETIC PROGENITORS

The finding that all IL-3–dependent cell lines tested, so far, synthesize histamine on exposure to calcium ionophore provides an additional argument in favor of a large participation of hematopoietic progenitors at different stages of maturation in this process, rather than that of a particular lineage. In situ hybridization with the HDC mRNA probe after stimulation with calcium ionophore showed a quite uniform labeling among several cell lines (unpublished data, March 1995), thus ruling out the possibility that only a minor lineage-committed subset was involved in histamine synthesis. In the same line of evidence, the histamine levels generated by the IL-3–dependent mast cell line MC9 were significantly lower. It is also noteworthy that cell lines that proliferate spontaneously or require other growth factors than IL-3 do not express HDC mRNA in response to calcium ionophore. Furthermore, IL-3–dependent cell lines fail to respond to their growth factor in terms of histamine synthesis, suggesting that they lack some element(s), present in normal hematopoietic progenitors, allowing to transduce the signal for increased histamine synthesis after IL-3R binding.

A role for endogenous histamine during cell proliferation and differentiation has first been proposed by Kahlson and Rosengren who demonstrated increased histamine synthesis in rapidly proliferating tissues. These investigators established also that the histamine thus generated was not derived from mast cells or basophils. During the past decade, several major findings have lent further support to this hypothesis.28 With regard to hematopoiesis, several studies provided evidence for the entry of CFU-S into cell cycle on stimulation with exogenous histamine or its H2 receptor agonists.24 Furthermore, it has been reported that histamine induces proliferation and differentiation of normal granulocytic precursors in vitro,2 as well as differentiation of the leukemic cell line HL-60.29 All these data were consistent with a potential role for histamine during hematopoiesis, provided that sufficient amounts of this mediator became available in the hematopoietic microenvironment. Our own studies have established that this is actually the case following stimulation of hematopoietic progenitors with IL-3, GM-CSF,8 or increased calcium influx.

The observation that hematopoietic progenitors themselves are a source of histamine is rather intriguing. Indeed, it is currently believed that normal hematopoietic progenitors are relatively passive elements, under the control of their microenvironment. Yet, our data convey the notion that under certain circumstances (stimulation by IL-3, GM-CSF, or increase in intracellular calcium concentrations), the majority of partially lineage-committed progenitors are capable of producing mediators which will, in turn, influence the differentiation scheme. Thus, the newly synthesized histamine may exert its effect upstream and downstream, participating on the one hand in the entry of CFU-S into cell cycle on stimulation by IL-3 (or GM-CSF + IL-1)12,13 and regulating on the other hand late events of granulocyte maturation.7 Furthermore, IL-4 and IL-6, produced in association with histamine in response to IL-330 and calcium ionophore (unpublished data, March 1995), may participate at various maturational stages.

The discovery of intracellular histamine receptors (H1,2) by Brandes et al has opened new perspectives to this issue. Indeed, according to these investigators, histamine acts as an intracellular messenger of platelet aggregation32,33 via
Fig 11. HDC mRNA expression assessed by in situ hybridization on BAF/B03 cells exposed for 4 hours to $5 \times 10^{-6}$ mol/L A23187 (A) or culture medium (B).

these new receptors that have been identified in the cytoplasm (low-affinity receptors) and in the nucleus (high-affinity receptors). They have generalized this notion, proposing that histamine could play a similar role in cell proliferation. Our recent evidence for both histamine synthesis and uptake by hematopoietic progenitors lends further support to such a possibility.

REFERENCES
2. Byron JW: Mechanism for histamine H2-receptor induced cell-cycle changes in the bone marrow stem cell. Agents Actions 7:209, 1977
19. Ploemacher RE, Brons NHC: Cells with marrow and spleen repopulating activity and forming spleen colonies on day 16, 12, and 8 are sequentially ordered on the basis of increasing rhodamine 123 retention. J Cell Physiol 136:531, 1988
20. Lebel B: A high sampling rate automated continuous flow fluorometric technique for the analysis of nanogram levels of histamine in biological samples. Anal Biochem 133:16, 1983
27. Foreman JC, Mongar JL, Gomperts BD: Calcium ionophores and movement of calcium ions following the physiological stimulus to a secretory process. Nature 245:249, 1973
34. Corbel S, Schneider E, Lemoine F, Dy M: Murine hematopoietic progenitors are capable of both histamine synthesis and uptake. Blood 86:531, 1995
Hematopoietic progenitors and interleukin-3-dependent cell lines synthesize histamine in response to calcium ionophore

M Dy, A Arnould, FM Lemoine, F Machavoine, H Ziltener and E Schneider