ARTICLE
Journal of Cellular Biochemistry 108:261–271 (2009)

Journal of Cellular Biochemistry

Polyamines Affect Histamine Synthesis During Early Stages of IL-3-Induced Bone Marrow Cell Differentiation

Gianni García-Faroldi,[1] Florencia Correa-Fiz,[1] Hicham Abrighach,[1] María Berdasco,[2] Mario F. Fraga,[2] Manel Esteller,[2] José L. Urdiales,[1] Francisca Sánchez-Jiménez,[1] and Ignacio Fajardo[1*]

[1] *Faculty of Sciences, Department of Molecular Biology and Biochemistry, University of Málaga, CIBER de Enfermedades Raras (CIBER-ER), Campus de Teatinos s/n, 29071 Málaga, Spain*

[2] *Cancer Epigenetics Laboratory, Spanish National Cancer Centre, Melchor Fernández Almagro 3, 28029 Madrid, Spain*

ABSTRACT

Mast cells synthesize and store histamine, a key immunomodulatory mediator. Polyamines are essential for every living cell. Previously, we detected an antagonistic relationship between the metabolisms of these amines in established mast cell and basophilic cell lines. Here, we used the IL-3-driven mouse bone marrow-derived mast cell (BMMC) culture system to further investigate this antagonism in a mast cell model of deeper physiological significance. Polyamines and histamine levels followed opposite profiles along the bone marrow cell cultures leading to BMMCs. α-Difluoromethylornithine (DFMO)-induced polyamine depletion resulted in an upregulation of histidine decarboxylase (HDC, the histamine-synthesizing enzyme) expression and activity, accompanied by increased histamine levels, specifically during early stages of these cell cultures, where an active histamine synthesis process occurs. In contrast, DFMO did not induce any effect in either HDC activity or histamine levels of differentiated BMMCs or C57.1 mast cells, that exhibit a nearly inactive histamine synthesis rate. Sequence-specific DNA methylation analysis revealed that the DFMO-induced HDC mRNA upregulation observed in early bone marrow cell cultures is not attributable to a demethylation of the gene promoter caused by the pharmacological polyamine depletion. Taken together, the results support an inverse relationship between histamine and polyamine metabolisms during the bone marrow cell cultures leading to BMMCs and, moreover, suggest that the regulation of the histamine synthesis occurring during the early stages of these cultures depends on the concentrations of polyamines. J. Cell. Biochem. 108: 261–271, 2009.

KEY WORDS: BIOGENIC AMINES; INFLAMMATORY MEDIATORS; MAST CELLS; METABOLIC INTERPLAY

Mast cells, considered a part of the immune system, are known for their implication in a large number of physiopathological processes including innate and acquired immunity, allergy, inflammatory arthritis and asthma [Galli et al., 2005]. After leaving the bone marrow as immature precursors, mast cells complete their differentiation within peripheral tissues under the influence of the microenvironment [Metcalfe et al., 1997]. Differentiation and maturation include the synthesis and storage of an array of inflammatory mediators including histamine, several mast cell proteases (mainly tryptases and chymases) and diverse cytokines. The types and amounts of the mediators synthesized and accumulated are largely dependent on the factors to which the cells are exposed, thus yielding a broad phenotype heterogeneity, which differs among tissues and species [Schwartz and Huff, 1998]. Out of the mast cell mediators, histamine is one of the most abundant and important due to its implication in many mast cell-associated diseases. Histamine synthesis involves the catalytic decarboxylation of histidine by histidine decarboxylase (HDC, EC 4.1.1.22), an enzyme that presents particularly complex and poorly understood expression and activity control mechanisms [Moya-Garcia et al., 2005]. As evidenced by several works performed with HDC knock out mice, the presence of this enzyme is of critical relevance for the correct development of mast cells [Ohtsu et al., 2001; Wiener et al., 2002]. However, very little is known concerning the regulation of the expression and activity of HDC during the differentiation of mast cells.

Grant sponsor: Ministerio de Ciencia y Tecnología (Spain); Grant number: SAF-2008-02522; Grant sponsor: Centro de Investigación Biomédica en Red (CIBER) de Enfermedades Raras (CIBER-ER); Grant sponsor: Junta de Andalucia (Spain); Grant number: BIO 267.

*Correspondence to: Dr. Ignacio Fajardo, Faculty of Sciences, Department of Molecular Biology and Biochemistry, University of Málaga, Campus de Teatinos s/n, 29071 Málaga, Spain. E-mail: ifajardo@uma.es

Received 5 November 2008; Accepted 20 May 2009 • DOI 10.1002/jcb.22246 • © 2009 Wiley-Liss, Inc.
Published online 26 June 2009 in Wiley InterScience (www.interscience.wiley.com).

The ornithine-derived polyamines (putrescine, spermidine, and spermine) are small polycations present in all living organisms, that are absolutely necessary for cell survival. Polyamine metabolism consist of the concerted action of diverse enzymes and transporters that carry out the synthesis, catabolism, uptake and/or elimination of a broad number of metabolites, all to assure accurate polyamine intracellular concentrations. Among the enzymes involved in polyamine biosynthesis, it is widely recognized that ornithine decarboxylase (ODC, EC 4.1.1.17) is the key one, being its expression and activity subject to extraordinary regulation [Cohen, 1998; Medina et al., 2003]. Polyamines have been implicated in a large number of cellular processes, including functioning of ion channels, nucleic acid packaging, DNA replication, apoptosis, transcription and translation [Childs et al., 2003]. It is thought that the contribution of polyamines to these processes arises mainly from their ability to stabilize negatively charged biomolecules such as nucleic acids, proteins and cell membranes [Cohen, 1998; Ruiz-Chica et al., 2001; Medina et al., 2003]. Despite the essential role of polyamines, their specific functions remain quite obscure, and only a few functions have been identified. Perhaps the clearest example of a polyamine-specific function is the involvement of spermidine in the formation of hypusine in eukaryotic translation initiation factor-5A, a unique post-translational modification required for the activity of the molecule [Park et al., 1993].

In previous studies, working with the established cell lines C57.1, P-815, RBL-1, and RBL-2H3, which exhibit some phenotypic features resembling those of mast cells and basophils, we detected an interplay between histamine and polyamine metabolisms. We observed that the exposure of these cells to a treatment consisting of a combination of the phorbol ester PMA and the glucocorticoid dexamethasone, which induces the expression and activity of HDC, resulted in a simultaneous decrease in the expression and activity of ODC, leading to a reduction of total polyamine concentrations [Fajardo et al., 2001a]. On the other hand, the increase of intracellular histamine concentration in C57.1 cells by a treatment with exogenous histamine resulted in a decrease of intracellular polyamines, through a mechanism involving a reduction of polyamine uptake and an induction of the spermidine–spermine acetyl transferase activity [Fajardo et al., 2001b]. Taken together, these results suggested an antagonistic relationship between polyamine and histamine metabolisms in these mast cell lines. Established mast cell lines show however only a limited number of mast cell specific features, that is, they express only a restricted amount of mast cell mediators, and the results obtained by studying these cell lines are often difficult to interpret in terms of their biological significance. A more appropriate approach to the study of mast cell biology, that is, the obtention of cultures of mast cells closer to the mast cells that reside in the body, is provided by the preparation of mast cells by the differentiation of hematopoietic precursors. Indeed, different models exist that allow the obtention of cultures of mast cells with different specific sub-phenotypes. For example, it is possible to prepare human cord blood-derived mast cells (CBMCs), which exhibits a MC_{TC} (tryptase/chymase) phenotype, by culturing umbilical cord blood mononuclear cells in the presence of stem cell factor (SCF) [Nilsson et al., 1996], or mouse bone marrow-derived mast cells (BMMCs) with either a connective tissue- or a mucosal-type phenotype by culturing bone marrow cells in the presence of either IL-3 alone or a mixture of IL-3, IL-9, SCF, and TGF-β, respectively [Braga et al., 2007]. Working with IL-3-driven BMMCs, in collaboration with Dr. Pejler group (Uppsala, Sweden), we recently observed a marked increase of HDC mRNA expression and histamine levels on early stages of the cultures, that dropped down after prolonged (more than 2 weeks) culture times [Ringvall et al., 2008]. This result, together with our results observed in established mast cell lines, led us to explore if a relationship between histamine and polyamine metabolisms exist in this mast cell culture system, that resembles a further biological significance. The results presented here support the existence of an antagonistic interplay between histamine and polyamine metabolisms in this cellular model and, moreover, suggest that polyamine levels can affect the regulation of the histamine synthesis that takes part during the early stages of the bone marrow cell cultures that lead to BMMCs.

MATERIALS AND METHODS

MATERIALS

Cell culture media, fetal bovine serum, antibiotics and L-glutamine were purchased from BioWhittaker (Cambrex, UK). Plastics for cell culture were supplied by NUNC (Roskilde, Denmark). Rabbit polyclonal antibodies against HDC (antiserum K9503) were a kind gift from Dr. Lo Persson (University of Lund, Sweden). Rabbit polyclonal antibodies against mouse mast cell protease (mMCP)-6 and mouse carboxypeptidase A (CPA) were a kind gift from Dr. Gunnar Pejler (SLU, Uppsala, Sweden). Mouse monoclonal antibodies against β-actin (clone AC-15) were from Sigma–Aldrich (St. Louis, MO). L-[^{14}C]histidine (50 μCi/ml, >300 mCi/mmol) was supplied by GE Healthcare (Barcelona, Spain). α-Difluoromethylornithine (DFMO) was a kind gift of Dr. Patrick Woster (Wayne State University) who obtained it from Ilex Oncology (San Antonio, TX). Acetonitrile and methanol for HPLC were from Romil (Teknokroma, Spain). All buffer salts and other chemicals were purchased either from Merck (VWR International) or Sigma–Aldrich.

CELL CULTURE

BMMCs were obtained essentially as previously described [Henningsson et al., 2002]. Briefly, C57BL/6 mice (female, 9–11 weeks old) were sacrificed by cervical dislocation (a method approved by the local ethical committee), and femur and tibia bone marrow cells were extracted and cultured at 37°C in a humidified atmosphere containing 5% CO_2 in 50% DMEM supplemented with 10% heat-inactivated fetal bovine serum, 50 μg/mL gentamycin sulfate and 2 mM L-glutamine and 50% WEHI-3B conditioned medium (as a source of IL-3). The cells were kept at a density of 0.5×10^6 cells/ml, and the culture medium was changed every third day. Mast cell maturation was judged by either toluidine blue or May-Grünwald/Giemsa staining, FcεRI and CD117 (c-kit) surface expression, histamine content, mMCP-6 expression and the ability of the cells to degranulate upon stimulation with IgE/anti-IgE. WEHI-3B-conditioned medium was produced by seeding WEHI-3B cells (0.5×10^6 cells/ml) into the medium described above and incubating them for 3 days.

C57.1 cells were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 50 μg/ml gentamycin sulfate and 2 mM L-glutamine.

FLOW CYTOMETRY ANALYSIS

Cells were washed in phosphate buffered saline (PBS; 10 mM phosphate, 137 mM NaCl, 2.68 mM KCl (pH 7.4)) and were fixed at room temperature with 2% paraformaldehyde (in PBS) for 20 min before staining. Next, cells were treated for 5 min with blocking buffer (2% bovine serum albumin, 10% fetal calf serum in PBS) on ice. Afterwards, 1×10^6 cells were stained for 20 min on ice with fluorescein isothiocyanate-conjugated rat IgG2b anti-mouse CD117 (c-kit), phycoerythrin-conjugated armenian hamster IgG anti-mouse FcεRI, or their respective isotype-matched control antibodies (all from eBioscience) using the same blocking buffer. After staining, cells were washed twice with PBS and were analyzed by using a FACSort flow cytometer and the CELLQuest software (BD Biosciences), collecting 10,000 cells per sample.

INTRACELLULAR AMINE DETERMINATION BY HPLC

The intracellular content of putrescine, histamine, spermidine and spermine was simultaneously determined by fluorimetry after separation of their dansyl derivatives by reversed-phase HPLC as previously described [Matés et al., 1992], with some modifications. Briefly, cell pellets (4×10^6) were suspended in 400 μL of PBS containing 6 microM 1,8-diaminooctane (used as internal standard), amines were extracted at room temperature (RT) with 1,100 μl of 0.34 N $HClO_4$ by vigorous vortexing and extracts were centrifuged at 12,000*g* for 15 min. Subsequently, pellets were redissolved in 200 μl of 0.3 N NaOH and stored at −20°C until quantification of protein contents with the Biorad Protein Assay kit (BioRad, Hercules). Amines in the supernatants were derivatized to their dansyl derivatives by adding 600 μl of dansyl chloride (10 mg/ml; dissolved in acetone) plus 600 μl of a saturated solution of Na_2CO_3 and incubating for 1 h at 60°C. Then, free dansyl chloride was removed by adding 10 μl of L-proline (100 mg/ml) and incubating 30 min at RT. Afterwards, dansyl-derivatives were extracted with 1.5 ml toluene and, after removal of toluene in a Speedvac system (Savant Instrument, NY), were resuspended in 160 μl of a mixture of acetonitrile:water (70:30). Samples were kept at −20°C until their injection (20 μl) into a System Gold Solvent Module (model 125, Beckman Instruments, USA) equipped with a 250 × 4 mm LiChrosorb C18 reversed-phase column (Merck, Germany). Column equilibration and running conditions used were those described previously [Matés et al., 1992]. Eluates from the column were detected by an attached fluorescence detector (Waters Millipore model 420, USA) set at λ-excitation = 338 nm and λ-emission = 425 nm. The entire system was controlled and data handling was carried out by a System Gold software package. Quantification of the amines was performed using a relative calibration procedure, as described previously [Smith and Davies, 1985]. Results were normalized to total cellular protein contents and are expressed as pmol of each amine/μg protein.

EXTRACELLULAR HISTAMINE DETERMINATION

Histamine quantification in cell culture supernatants was performed by ELISA with the use of a kit from DRG (Germany), following the instructions of the manufacturer. Results were normalized to total cellular protein contents and are expressed as pmol of histamine/μg protein.

RNA ANALYSIS

Northern blot analysis was performed as described elsewhere [Sambrook et al., 1989], with the following modifications. Cells to be analyzed for RNA were pelleted and stored at −80°C until required. GenElute Mammalian Total RNA Miniprep kits (Sigma–Aldrich) were used for the extraction of total RNA. Total RNA corresponding to 6×10^6 cells per sample was extracted and subsequently precipitated by adding 2 M LiCl and incubating at 4°C overnight. Precipitated RNA was then dissolved in 20 μl of 50% formamide, 6% formaldehyde, 1× MOPS buffer (20 mM MOPS, 1 mM EDTA, 5 mM sodium acetate, pH 7) and heated at 60°C for 10 min. Next, 25 μl of 50% glycerol, 1 mM EDTA, 0.024% bromophenol blue were added to each sample, and RNA was fractionated on 1% agarose denaturing formaldehyde gels. Once electrophoresis was completed, RNA was blotted to nylon membranes (Schleicher & Schüell, Germany) using established capillary blotting methods. DNA probes for Northern blot analysis were labeled with [α-^{32}P]dCTP (3,000 mCi/mmol; GE Healthcare) using a random primer labeling kit as advised (HighPrime; Roche, Germany). A full-length (2.3 kb) HDC cDNA fragment obtained after digestion of plasmid prHDC 1-656 [Olmo et al., 2000] was used as the template for an HDC-specific probe. A 524 bp fragment of β-actin obtained by RT-PCR, as described previously [Chaves et al., 2007], was used as a β-actin-specific probe. Hybridizations were performed overnight at 42°C using standard methods [Sambrook et al., 1989], and membranes were washed to high stringency in 0.4× SSC (1× SSC is 0.15 M NaCl, 0.015 sodium citrate pH 7), 0.1% SDS at 50°C. After exposure to X-Omat LS autoradiographic film (Kodak), blots were stripped by washing them twice with 0.1× SSC, 0.1% SDS at 65°C for 15 min before rehybridization with other probes. Quantitation of the observed bands was performed with the Quantity One software (BioRad).

WESTERN BLOT ANALYSIS

Cell extracts were prepared by adding 1× SDS-sample buffer (65 mM Tris–HCl containing 2% SDS, 10% glycerol, 5% 2-mercaptoethanol and 0.05% bromophenol blue) directly to the cells pellets (100 μl per 1×10^6 cells) and boiling for 5 min. Samples (20 μl) were subjected to SDS–PAGE on 10% polyacrylamide gels and were subsequently blotted onto polyvinylidene difluoride membranes (GE Healthcare), followed by blocking overnight in PBS/0.1% Tween-20 containing 10% non-fat milk at 4°C. All of the following steps were performed at RT. Next, membranes were incubated for 1–2 h with one of the following rabbit primary antibodies diluted in PBS/0.1% Tween 20 containing 2% BSA: antiserum towards HDC (K9503; 1:1,500), antiserum towards mMCP-6 (1:2,000) and antiserum towards CPA (1:2,000). After washing extensively with PBS/0.1% Tween 20, the membranes were incubated for 1 h with HRP-conjugated anti-rabbit IgG (GE Healthcare) diluted 1:5,000 in PBS/0.1% Tween 20 containing 10% non-fat milk. After extensive washing with PBS/0.1% Tween 20, membranes were developed with the Supersignal West Pico

Chemiluminescent Substrate system (Pierce Biotechnology, USA) according to the instructions of the manufacturer. Normalization for sample loads was performed by re-probing membranes with an anti β-actin mouse monoclonal antibody (Sigma–Aldrich).

HISTIDINE DECARBOXYLASE ACTIVITY

HDC enzymatic activity was measured by following the release of $^{14}CO_2$ from L-[^{14}C]-labeled histidine as we have previously described [Fajardo et al., 2001a]. Results are expressed as pmol of CO_2 released/h/μg of total cellular protein content.

ANALYSIS OF SEQUENCE-SPECIFIC DNA METHYLATION STATUS

The methylation status of specific genomic DNA sequences was established by bisulfite genomic sequencing. Genomic DNA (1–2 μg) from cell cultures was denatured with NaOH at a final concentration of 0.3 M for 15 min at 37°C. Denatured DNA was incubated in a freshly prepared mix of 3.7 mM sodium bisulfite/1.1 mM hydroquinone, pH 5.0, at 50°C for 16 h. The modified DNA was purified using the DNA Cleanup kit (Promega) and was precipitated to remove unreacted bisulfite with cold ethanol in the presence of 0.15 mg/ml glucogen and 3 M ammonium acetate (pH 7.0). Finally, the pellet was resuspended in 25 μl of water and stored at 4°C.

The sequence of interest in the bisulfite-reacted DNA was amplified by PCR using the specific primers listed in Table I. Each amplification reaction was normally carried out on 1 μl of DNA for 40 cycles using standard conditions, with denaturation at 94°C, annealing at 56–58°C, and extension at 72°C. The amplified products were gel purified (Qiagen) and cloned in the pGEM-t-easy vector (Promega) by following Molecular Biology standard protocols. Automatic sequencing of 10 colonies for each sequence was performed to obtain data on the methylation status of every single CpG dinucleotide.

STATISTICAL ANALYSIS

Statistical significance for amine determinations and HDC activity analysis was determined by the Student's paired samples *t*-test (two-tailed) using the GraphPad software. Values of $P < 0.05$ were considered to be significant.

RESULTS

First, to define the evolution of the differentiation of bone marrow precursor cells into BMMCs under our experimental conditions, we assessed the expression levels of the specific mast cell markers carboxypeptidase A (CPA) and mouse mast cell protease-6 (mMCP-6), and also the expression of the surface receptors FcεRI and c-kit, at several time points along the cell culture. As shown in Figure 1A, major phenotypic changes were already observed within the first week of culture, as CPA and mMCP-6 were detectable on days 4 and 7, respectively. After their onset, CPA and mMCP-6 levels increased gradually, peaked at around days 14 and 18, respectively, and remained unchanged afterwards, demonstrating the ability of BMMCs to synthesize and maintain constant levels of these two mediators permanently during their differentiation from bone marrow precursor cells. In line with these findings, the expression of FcεRI and c-kit analysis showed an increasingly number of mast cells (c-kit (+)/FcεRI (+)) starting on day 7 (Table II) that was the major cell population after 3 weeks of culture. Interestingly, a minor population of cells consistent with a basophil phenotype (c-kit (−)/FcεRI (+)) remained unchanged along the whole culture time.

TABLE I. Sequences (5′ to 3′) of Primers Used for DNA Methylation Status Analysis.

Name	Forward/reverse	Sequence	Fragment length (bp)
HDC1f	Forward	TTgTTTTTTAgTTTTgTTTgTTg	516
HDC1r	Reverse	TAATTTCAACCTCTCTTATCCC	
HDC2f	Forward	gTTTgAAggAAgggATTTTATg	335
HDC2r	Reverse	CAAAAATTCAAAAAAAACACCA	

Our previous studies showed an early expression of HDC at the level of mRNA followed by an increase of intracellular levels of histamine within the first 2 weeks of the bone marrow cell cultures that lead to BMMCs [Ringvall et al., 2008]. However, mRNA expression is not always accompanied by enzymatic activity and HDC enzymatic activity was not determined in this study. We therefore determined both the intracellular levels of histamine and HDC enzymatic activity during time-course of this cell culture. Similarly to CPA and mMCP-6, and in line with our previous study, intracellular histamine levels exhibited a quick increase that peaked at day 14, and showed a gradual decrease to reach a minimum at around day 30 (Fig. 1B). The time-course of HDC enzymatic activity was anticipated to intracellular histamine levels, being detectable during the first 2 weeks of culture (days 4, 7, 11, and 14) and almost non-detectable afterwards. To address the possibility that the gradual intracellular histamine decrease is the consequence of its release into the medium, we determined extracellular histamine levels. As shown in Figure 1C, extracellular histamine levels followed a profile very similar to that of the HDC enzymatic activity, peaking at day 7 and suffering a gradual decrease to remain almost unchanged after day 18. Taken together, these results demonstrate that histamine synthesis occurs early during the bone marrow cell cultures conducted to obtain BMMCs and provide evidence that these cells, at least under our experimental conditions, can store only a limited amount of the histamine produced. Furthermore, the results indicate that these cells seem unable to maintain constant histamine levels along their culture, as this amine is gradually lost once the HDC activity decays.

Next, to investigate the existence of a relationship between histamine and polyamine metabolisms in these cells, we explored the evolution of polyamine levels along the cell culture. As shown in Figure 1, polyamine levels were opposite to that of histamine (compare Fig. 1D with B,C); polyamines experimented an initial marked decrease (days 4–11) followed by a gradual increase (days 11–24), all in parallel with the initial increase and subsequent decrease of histamine. Altogether, these results suggest an inverse relationship between histamine and polyamine metabolisms during the cell cultures conducted to differentiate bone marrow precursor cells into BMMCs. To test this hypothesis further, we studied whether

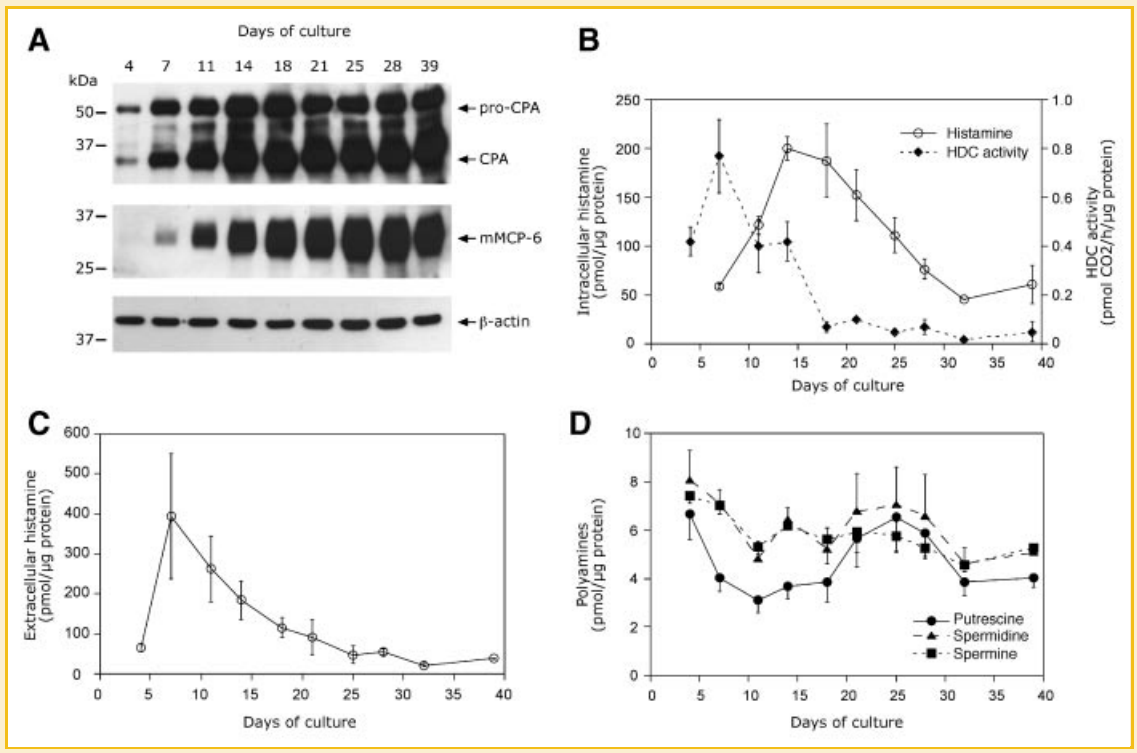

Fig. 1. Evolution of mast cell markers and polyamines during bone marrow cell differentiation into BMMCs. Bone marrow precursor cells were cultured in vitro into BMMCs as described in Materials and Methods Section. At the time points indicated, cells (4×10^6) were taken and analyzed for: (A) expression of CPA and mMCP-6 by Western blot (β-actin was used as a control of sample loads); (B) intracellular content of histamine (n = 3) and HDC enzymatic activity (n = 4); (C) extracellular levels of histamine (n = 4); (D) intracellular levels of polyamines (putrescine, spermidine and spermine; n = 3). The results displayed are means ± SEM. Westerns blots are representative of three independent experiments.

an enhancement of the initial decrease of polyamine levels at the beginning of the bone marrow cell cultures that lead to BMMCs has any effect on histamine levels. Thus, BMMCs were obtained in the presence of α-difluoromethylornithine (DFMO; an irreversible inhibitor of ODC), and both polyamine and histamine levels were measured at several time points along the cell culture. DFMO efficiently inhibited polyamine synthesis from ornithine, as indicated by the fact that putrescine was not detected in treated cells (not shown). Spermidine levels were largely reduced after the treatment (Fig. 2A), and spermine levels tended to increase probably due to an enhancement of the uptake from the extracellular medium [Seiler and Dezeure, 1990], although this increase was significant only on day 11 (Fig. 2B). In summary, by using DFMO, total polyamines experimented a large reduction at the expense of a great decrease in putrescine and spermidine, an effect consistent with previous works in which DFMO have been used to deplete polyamines [Seiler and Dezeure, 1990]. This decrease of total polyamine levels did not induce a major effect in cell proliferation, with cell numbers at each time point of the cell culture being only slightly reduced for the treated cells as compared with the untreated ones (not shown). The latter result is not unexpected, since the proliferation rate of these cells is very low, with a population doubling time of more than 4 days. In addition, CPA and mMCP-6 protein levels were not affected (not shown). However, the polyamine decrease resulted in a mild, but significant increase of the intracellular histamine level specifically on day 11 (Fig. 2C), and resulted in a substantial increase (2.3-fold) of the extracellular histamine levels on days 7 and 11 (Fig. 2D), all around the HDC

TABLE II. Time-Course Expression of FcεRI and CD117 During Bone Marrow Cell Differentiation Into BMMCs

	Days of culture					
Phenotype	4	7	11	14	18	21
CD117 (−)/FcεRI (−)	94.3 ± 1.3	83.3 ± 3.9	49.7 ± 11.4	25.2 ± 8.7	16.5 ± 7.0	9.5 ± 4.0
CD117 (+)/FcεRI (−)	2.2 ± 0.8	4.5 ± 1.6	11.3 ± 2.5	3.3 ± 0.5	5.5 ± 2.6	2.4 ± 0.7
CD117 (−)/FcεRI (+)	2.0 ± 0.6	3.6 ± 1.9	2.2 ± 0.1	2.9 ± 1.6	2.9 ± 0.8	3.6 ± 1.8
CD117 (+)/FcεRI (+)	1.5 ± 0.4	7.8 ± 2.9	36.7 ± 13.8	58.8 ± 13.4	75.1 ± 8.0	84.4 ± 5.1

Bone marrow precursor cells were cultured in vitro into BMMCs as described in Materials and Methods Section. At the indicated days, cells where taken and analyzed for the surface expression of FcεRI and CD117 (c-kit) by flow cytometry as described in Materials and Methods Section. Results displayed represent the % of the cells showing each phenotype and are means ± SEM of four independent experiments.

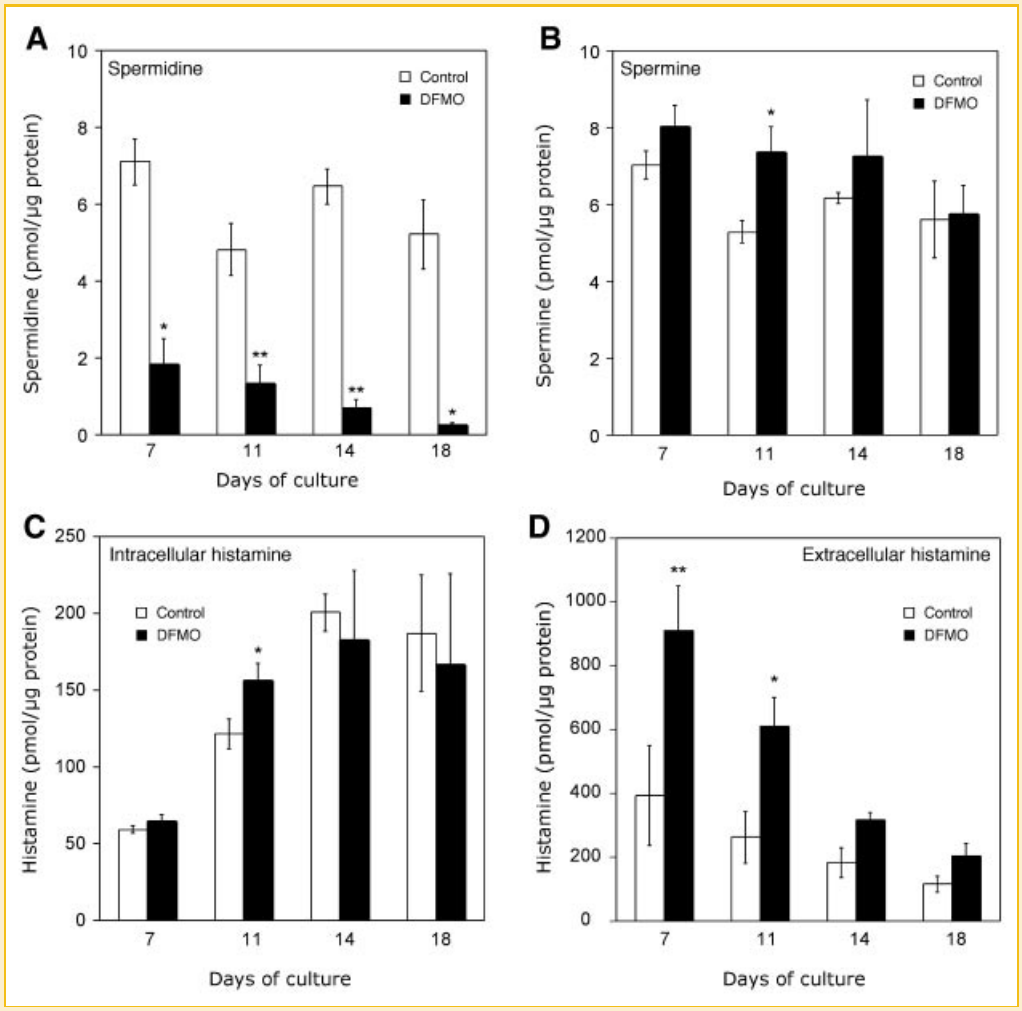

Fig. 2. Effect of DFMO on polyamines and histamine levels during bone marrow cell differentiation into BMMCs. Bone marrow precursor cells were cultured in vitro into BMMCs as described in Materials and Methods Section. On day 4, 5 mM DMFO was incorporated into the culture medium and maintained for the rest of the culture. At the time points indicated, cells (4×10^6) were taken and analyzed for spermidine (A), spermine (B), intracellular histamine (C), and extracellular histamine (D) as described in Materials and Methods Section. The results displayed are means ± SEM of four independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$ compared with untreated control cells by Student's paired sample *t*-test (two-tailed).

enzymatic activity peak (Fig. 1B) and in an early stage of the bone marrow cell culture where the majority of the present cells are still far from being differentiated BMMCs (Table II). To determine if the DFMO treatment has any effect on histamine levels of BMMCs that have completed their in vitro differentiation, BMMCs of 21 days were treated with DFMO for four days and polyamines and histamine levels were analyzed. In these cells, the reduction of total polyamines did not provoke any significant alteration of either histamine levels (Table III) or HDC enzymatic activity (not shown). Furthermore, similar results were obtained in C57.1 cells regarding both histamine levels (Table IV) and HDC enzymatic activity (not shown). These cells consist of an established growth-factor-independent mast cell line derived from C57BL/6J mouse bone marrow cells [Young et al., 1987], that resemble many features of BMMCs, and with the ability to synthesize histamine upon different stimuli as we have previously proved in our laboratory [Fajardo

TABLE III. Polyamine and Histamine Levels in 21-Day-Old BMMCs After Treatment With DFMO

Amine	Control	DFMO
Putrescine	9.63 ± 1.57	ND
Spermidine	8.77 ± 1.38	1.87 ± 0.47*
Spermine	6.39 ± 0.64	7.67 ± 0.88
Intracellular histamine	104.02 ± 15.79	83.45 ± 10.07
Extracellular histamine	66.99 ± 24.74	53.46 ± 16.48

BMMCs were prepared as described in Materials and Methods Section. On day 21, cells were incubated in the absence or presence of 5 mM DFMO during 4 days. Intracellular polyamines and both intracellular and extracellular histamine levels were measured as described in Materials and Methods Section. Results are expressed as pmol/µg protein and are mean ± SEM of four independent experiments.
ND, non-detected.
$^{*}P < 0.05$ compared with untreated control cells by Student's paired sample *t*-test (two-tailed).

TABLE IV. Polyamine and Histamine Levels in C57.1 Mast Cells After Treatment With DFMO

Amine	Control	DFMO
Putrescine	ND	ND
Spermidine	8.29 ± 0.40	0.52 ± 0.10*
Spermine	6.02 ± 0.57	5.32 ± 0.52*
Intracellular histamine	6.89 ± 1.95	3.11 ± 1.57
Extracellular histamine	28.83 ± 11.3	53.00 ± 17.70

C57.1 cells were incubated in the absence or presence of 5 mM DFMO during 4 days. Intracellular polyamines and both intracellular and extracellular histamine levels were measured as described in Materials and Methods Section. Results are expressed as pmol/μg protein and are mean ± SEM of three independent experiments.
ND, non-detected.
*$P < 0.05$ compared with untreated control cells by Student's paired sample t-test (two-tailed).

et al., 2001a]. Taken together, the results suggest the possibility that polyamine levels are somehow able to modulate the synthesis of histamine specifically during the initial stages of the bone marrow cell cultures leading to BMMCs, in which an active process of histamine synthesis takes part.

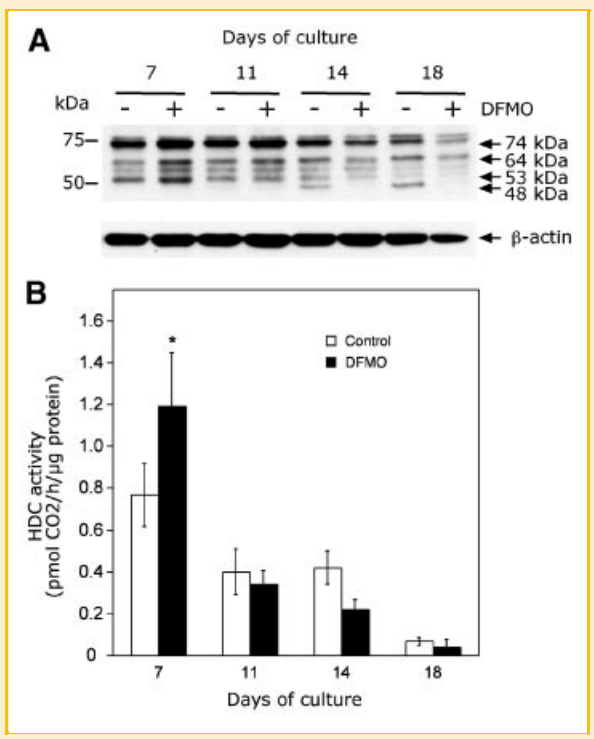

Fig. 3. Effect of DFMO on HDC protein expression and enzymatic activity during bone marrow cell differentiation into BMMCs. Bone marrow precursor cells were cultured in vitro into BMMCs as described in Materials and Methods Section. On day 4, 5 mM DMFO was incorporated into the culture medium and maintained for the rest of the culture. At the time points indicated, cells (4×10^6) were taken and analyzed for HDC protein expression by Western blot (A) and HDC enzymatic activity (B). Western blot is a typical representative experiment. Enzymatic activity values are means ± SEM of four independent experiments. *$P < 0.05$ compared with untreated control cells by Student's paired sample t-test (two-tailed).

To investigate whether the observed histamine increase upon treatment with DFMO is the result of an alteration of the expression and/or activity of HDC, BMMCs were obtained in the presence of DFMO, and HDC protein levels and enzymatic activities were determined at several time points of the cell culture. As shown in Figure 3A, several immunoreactive bands with molecular weights (74–48 kDa) apparently consistent with previous reports [Dartsch et al., 1998, 1999; Fajardo et al., 2001a] were detected with a specific anti-HDC antibody. Out of the several forms of HDC detected in the immunoblot, it is accepted that the enzymatic activity is mainly due to the 53 kDa form. In control cells, this band was detected on days 7, 11, and 14 but not on day 18, in agreement with the enzymatic activities shown in Figure 1B. On the other hand, DFMO treatment resulted in a marked increase in the intensity of several HDC bands on day 7, had no effect on day 11 and provoked a decrease on days 14 and 18 (Fig. 3A). Importantly, the enzymatic activity also increased significantly on day 7, but was not significantly altered on any of the other days investigated (Fig. 3B). These results indicate that the transient increase in histamine levels observed in early bone marrow cell cultures driven to BMMCs after treatment with DFMO (Fig. 2C,D) could be the result of an accumulation of enzymatically active HDC protein, specifically during these initial stages of the cultures.

We next assessed whether the accumulation of active HDC observed in 7-day-old DFMO treated cells (Fig. 3B) could be the result of an increased level of HDC-mRNA. As shown in Figure 4A, HDC-mRNA levels increased almost threefold after treatment of these cells with DFMO. Then, we sought to find the molecular mechanism behind this HDC-mRNA upregulation. Information about HDC mRNA synthesis regulation in mast cells and other hematopoietic related cells is however very scarce. In mastocytomas, it was demonstrated that the expression of the HDC gene is subject to transcriptional regulation by CpG methylation in the promoter region [Kuramasu et al., 1998; Suzuki-Ishigaki et al., 2000], and a previous report described the ability of DFMO to provoke DNA demethylation [Frostesjo et al., 1997]. Thus, in an attempt to address the mechanism underlying the observed HDC overexpression caused by DFMO, we determined if the methylation state of the CpG dinucleotides located at the HDC promoter is altered by DFMO. As shown in Figure 4B, in control cells, several CpG dinucleotides around the transcription start site (positions −107, −57, +23, +118, +127, and +139) were found to be mainly demethylated, a result consistent with the ability of these cells to express HDC. However, DFMO did not induce any clear change in the methylation state of the CpG dinucleotides studied, suggesting that there must be other mechanisms underlying the upregulation of HDC-mRNA provoked by DFMO.

DISCUSSION

Among the broad array of inflammatory mediators synthesized and stored by mast cells, histamine is significantly important due to its recognized capability to modulate the immune response and also because of its deleterious effects during allergic and other inflammatory reactions [Schneider et al., 2002]. In addition, histamine synthesis has an essential role for the correct differentia-

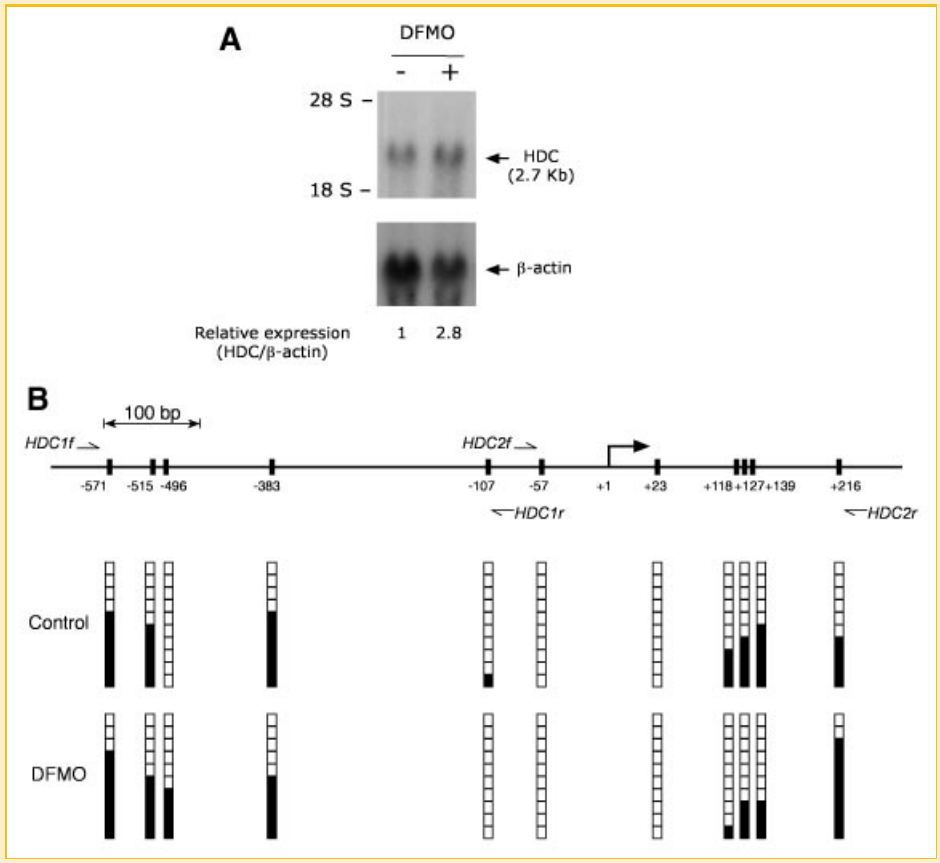

Fig. 4. Effect of DFMO on HDC mRNA expression and HDC gene promoter methylation status of 7-day-old IL-3-driven bone marrow cells. Bone marrow precursor cells were cultured in vitro into BMMCs as described in Materials and Methods Section. On day 4, 5 mM DMFO was added. On day 7, cells (4×10^6) were taken and analyzed for HDC mRNA expression by Northern blot (A). Expression of HDC mRNA was normalized to β-actin expression using the QuantityOne software (BioRad) and results are indicated underneath. B: The methylation status of each CpG dinuclotide around the HDC gene transcription start site was determined in untreated or DFMO-treated cells using sodium bisulfite genomic sequencing as described in Materials and Methods Section. A schematic map around the transcription start site is represented at the top. The transcription start site (position +1) is marked by the bent arrow. CpG sites are represented as short vertical lines and their relative positions to the transcription start site are indicated by the numbers underneath. Location of primers used to amplify the sequenced regions is also indicated. The methylation status of each CpG site for each of the 10 clones analyzed is displayed as an open (demethylated) or closed (methylated) square.

tion and maturation of mast cells, as demonstrated by several works performed with HDC knock out mice [Ohtsu et al., 2001; Wiener et al., 2002]. Expression of the HDC gene has been studied in different cell types and tissues such as gastric carcinoma cells, mastocytoma cells, oxyntic mucosa and fetal liver, and it has been shown that it responds to a number of signals, including gastrin, phorbol esters, glucocorticoids or demethylation of its promoter sequences [Kawai et al., 1992; Ohgoh et al., 1993; Hocker et al., 1997a,b; Suzuki-Ishigaki et al., 2000]. However, little is known about the regulation of histamine synthesis in mast cells and other hematopoietic derived cells like basophils and macrophages. Previously, working with established basophilic and mast cell lines, we described that treatments able to provoke an increase in histamine synthesis and levels led to a decrease in polyamine levels, suggesting that there exists some kind of antagonistic interplay between histamine and polyamine metabolisms in these cell types [Fajardo et al., 2001a,b]. This work was undertaken to address if such an antagonistic relationship does exist, and to explore its role in connection with histamine synthesis, in BMMCs, a widely used in vitro model to obtain mast cells in culture from their cell precursors in the bone marrow, that represents a more biologically relevant cellular model of mast cells as compared with mast cell lines.

That polyamine and histamine levels followed an opposite evolution along the cell cultures conducted to obtain BMMCs from bone marrow precursor cells, suggests that the metabolisms of these amines are antagonistic. Importantly, the expression profile of the specific mast cell markers CPA and mMCP-6 did not show any clear correlation with polyamine levels, indicating that the suggested antagonistic relationship between histamine and polyamines is specific. On the other hand, the fact that an alteration of polyamine levels by direct inhibition of their synthesis with DFMO resulted in an accumulation of enzymatically active HDC and histamine during the first stages of the culture, suggests that polyamine concentrations could be involved in the regulatory mechanisms of the histamine synthesis that happens at these stages. Such a proposed role of polyamines seems again to be specific for histamine, since no effect on CPA and mMCP-6 protein levels was observed after treatment with DFMO. In addition, our data suggest that this

proposed role of polyamines in regulating histamine synthesis takes part only if such a synthesis is active, that is, if the cells encounter the appropriate context of cellular program and signals that lead to the active histamine synthesis, because DFMO alone was not able to induce histamine synthesis in cells where such a synthesis was nearly inactive (differentiated BMMCs and C57.1 cells).

The mechanism by which polyamine concentrations could contribute to the modulation of histamine synthesis is unclear. Our results show that mRNA levels were increased on day 7 upon treatment with DFMO, an effect that could explain the increased levels of HDC protein and enzymatic activity observed. It is known that the methylation state of DNA is one of the principal mechanisms implicated in the control of gene expression during many biological processes, regulating the interaction of many transcription factors with the promoter regions of genes [Goldberg et al., 2007]. In this context, it is remarkable that the expression of the HDC gene has been reported to be subject to transcriptional regulation by CpG methylation in the promoter region [Kuramasu et al., 1998; Suzuki-Ishigaki et al., 2000]. Indeed, the level of HDC expression in several cell lines was correlated to the number of demethylated CpG sites around the transcription start site of the HDC gene [Suzuki-Ishigaki et al., 2000]. Furthermore, in the latter work it was found that one CpG site (position –107 from the transcription start site) located next to a GC box underwent demethylation in mouse mastocytoma P815 cells after incubation in mouse peritoneal cavity, a treatment that induces these immature non-expressing-HDC mast cells to express high levels of HDC mRNA. On the other hand, in a previous study [Frostesjo et al., 1997], it was found that the differentiation of F9 teratocarcinoma cells into a parietal endoderm-like phenotype, upon polyamine depletion by DFMO treatment, involved DNA demethylation as a consequence of DNA methyltransferase inhibition by an increased level of decarboxylated *S*-adenosyl-methionine. All of these data led us to investigate if the upregulation of HDC mRNA observed in 7-day-old DFMO-treated IL-3-driven-bone marrow cells is the result of an alteration in the methylation state of the CpG dinucleotides located at the HDC promoter. However, no significant changes were found in the methylation state of the HDC gene promoter, indicating that other mechanisms responsible for the increased levels of HDC mRNA and/or HDC protein and activity must be operating. Apart from the observation that nuclear factor E2 seems to be indirectly involved [Ohtsu et al., 1996], the regulation of the HDC gene in hematopoietic cells is largely unknown. Thus, the elucidation of how polyamine concentrations could be contributing to the regulation of the HDC gene expression will require considerable effort.

The possibility that polyamine concentrations can exert a critical role in the control of the immune response has long been considered. Due to their implication in cellular processes essential for cell viability, intracellular polyamines are indispensable for the correct functioning of immune cells. In support of this notion, it has been described that polyamines are required for the appropriate differentiation of cytolytic T lymphocytes [Bowlin et al., 1987], the production of immunoglobulins by B lymphocytes [Pasquali et al., 1988] and the stimulated release of superoxide by monocytes and macrophages [Kaczmarek et al., 1992; Messina et al., 1992]. On the other hand, there is evidence indicating that polyamines may play an inhibitory role in certain immune functions. For example, polyamines have been shown to suppress neutrophil locomotion [Ferrante, 1985], NK cell activity [Chamaillard et al., 1993] and NO production in murine macrophages [Szabo et al., 1994]. Although it has been suggested that these inhibitory roles of polyamines are due to the action of serum polyamine oxidases rather than being a direct effect, numerous reports have demonstrated other polyamine inhibitory actions polyamine oxidases-independent. For example, both spermine and—to a lesser extent—spermidine inhibit the activation and production of cytokines like TNF by the macrophage-like cell line RAW 264.7 [Zhang et al., 1997], inhibit the secretion of TNFα and MCP-1 in LPS-stimulated NR8383 macrophages [Perez-Cano et al., 2003], and cause loss of the innate immune response to *Helicobacter pylori* by inhibition of inducible nitric-oxide synthase translation [Bussiere et al., 2005]. Furthermore, it has been shown that spermine prevents macrophage differentiation from the human myeloid leukemia HL-60 cells [Gavin et al., 2004], and inhibition of polyamine biosynthesis by DFMO enhances nitric oxide production in LPS-activated J774 macrophages [Baydoun and Morgan, 1998] and upregulates COX-2 mRNA levels in Caco-2 human colon adenocarcinoma cells [Parker and Gerner, 2002]. All these inhibitory roles of polyamines, together with the fact that they are accumulated at the inflammation sites as a consequence of tissue regeneration and cell damage, support the hypothesis proposed by Zhang et al. [2000] that extracellular polyamine levels could constitute a signal to prevent the tissue damage provoked by an excessive inflammatory response [Zhang et al., 2000]. Mast cells—and other histamine producing cells—are known to be recruited to inflammation sites as part of the immune response and the coexistence of this cell types and local augmented polyamine levels is therefore very likely. Our previous results obtained with established basophilic and mast cell lines [Fajardo et al., 2001a,b] together with the findings reported here, suggest that the metabolisms of histamine and polyamines are antagonistic in these cell types. Furthermore, we have observed that DFMO-induced polyamine depletion provokes an enhancement of histamine synthesis at cellular stages in which such a synthesis is active. All of these data allow us to hypothesize that polyamine levels may influence mast cell—and perhaps other cell types—histamine synthesis in a negative manner. Considering the potent pro-inflammatory properties of histamine, we speculate that a negative role of polyamines in histamine synthesis could constitute another mechanism by which local augmented polyamine levels at inflammation sites would prevent the harmful effects of an excessive inflammatory response.

ACKNOWLEDGMENTS

Thanks are due to Dr. Gunnar Pejler (SLU, Uppsala, Sweden) for providing the anti-mMCP-6 and anti-CPA antibodies. We thank Dr. Lo Persson (University of Lund, Sweden) for providing the anti-HDC antibody and Dr. Patrick Woster (Wayne State University, USA) for supplying DFMO. We are grateful to Dr. Miguel Ángel Medina (University of Málaga, Spain) for critically reviewing the manuscript and to Dr. María F Suárez (University of Málaga, Spain) for helpful discussions. The CIBER-ER is an initiative of the Instituto de Salud Carlos III (ISCIII, Spain).

REFERENCES

Baydoun AR, Morgan DM. 1998. Inhibition of ornithine decarboxylase potentiates nitric oxide production in LPS-activated J774 cells. Br J Pharmacol 125:1511–1516.

Bowlin TL, McKown BJ, Sunkara PS. 1987. Increased ornithine decarboxylase activity and polyamine biosynthesis are required for optimal cytolytic T lymphocyte induction. Cell Immunol 105:110–117.

Braga T, Grujic M, Lukinius A, Hellman L, Abrink M, Pejler G. 2007. Serglycin proteoglycan is required for secretory granule integrity in mucosal mast cells. Biochem J 403:49–57.

Bussiere FI, Chaturvedi R, Cheng Y, Gobert AP, Asim M, Blumberg DR, Xu H, Kim PY, Hacker A, Casero RA, Jr., Wilson KT. 2005. Spermine causes loss of innate immune response to Helicobacter pylori by inhibition of inducible nitric-oxide synthase translation. J Biol Chem 280:2409–2412.

Chamaillard L, Quemener V, Havouis R, Moulinoux JP. 1993. Polyamine deprivation stimulates natural killer cell activity in cancerous mice. Anticancer Res 13:1027–1033.

Chaves P, Correa-Fiz F, Melgarejo E, Urdiales JL, Medina MA, Sanchez-Jimenez F. 2007. Development of an expression macroarray for amine metabolism-related genes. Amino Acids 33:315–322.

Childs AC, Mehta DJ, Gerner EW. 2003. Polyamine-dependent gene expression. Cell Mol Life Sci 60:1394–1406.

Cohen SS. 1998. A guide to the polyamines. New York: Oxford University Press.

Dartsch C, Chen D, Persson L. 1998. Multiple forms of rat stomach histidine decarboxylase may reflect posttranslational activation of the enzyme. Regul Pept 77:33–41.

Dartsch C, Chen D, Hakanson R, Persson L. 1999. Histidine decarboxylase in rat stomach ECL cells: Relationship between enzyme activity and different molecular forms. Regul Pept 81:41–48.

Fajardo I, Urdiales JL, Medina MA, Sanchez-Jimenez F. 2001a. Effects of phorbol ester and dexamethasone treatment on histidine decarboxylase and ornithine decarboxylase in basophilic cells. Biochem Pharmacol 61:1101–1106.

Fajardo I, Urdiales JL, Paz JC, Chavarria T, Sanchez-Jimenez F, Medina MA. 2001b. Histamine prevents polyamine accumulation in mouse C57.1 mast cell cultures. Eur J Biochem 268:768–773.

Ferrante A. 1985. Inhibition of human neutrophil locomotion by the polyamine oxidase-polyamine system. Immunology 54:785–790.

Frostesjo L, Holm I, Grahn B, Page AW, Bestor TH, Heby O. 1997. Interference with DNA methyltransferase activity and genome methylation during F9 teratocarcinoma stem cell differentiation induced by polyamine depletion. J Biol Chem 272:4359–4366.

Galli SJ, Kalesnikoff J, Grimbaldeston MA, Piliponsky AM, Williams CM, Tsai M. 2005. Mast cells as ''tunable'' effector and immunoregulatory cells: Recent advances. Annu Rev Immunol 23:749–786.

Gavin IM, Glesne D, Zhao Y, Kubera C, Huberman E. 2004. Spermine acts as a negative regulator of macrophage differentiation in human myeloid leukemia cells. Cancer Res 64:7432–7438.

Goldberg AD, Allis CD, Bernstein E. 2007. Epigenetics: A landscape takes shape. Cell 128:635–638.

Henningsson F, Ledin J, Lunderius C, Wilen M, Hellman L, Pejler G. 2002. Altered storage of proteases in mast cells from mice lacking heparin: A possible role for heparin in carboxypeptidase A processing. Biol Chem 383:793–801.

Hocker M, Henihan RJ, Rosewicz S, Riecken EO, Zhang Z, Koh TJ, Wang TC. 1997a. Gastrin and phorbol 12-myristate 13-acetate regulate the human histidine decarboxylase promoter through Raf-dependent activation of extracellular signal-regulated kinase-related signaling pathways in gastric cancer cells. J Biol Chem 272:27015–27024.

Hocker M, Zhang Z, Merchant JL, Wang TC. 1997b. Gastrin regulates the human histidine decarboxylase promoter through an AP-1-dependent mechanism. Am J Physiol 272:G822–G830.

Kaczmarek L, Kaminska B, Messina L, Spampinato G, Arcidiacono A, Malaguarnera L, Messina A. 1992. Inhibitors of polyamine biosynthesis block tumor necrosis factor-induced activation of macrophages. Cancer Res 52:1891–1894.

Kawai H, Ohgoh M, Emoto S, Ohmori E, Imanishi N, Yatsunami K, Ichikawa A. 1992. Synergistic effects of 12-O-tetradecanoylphorbol-13-acetate and dexamethasone on de novo synthesis of histidine decarboxylase in mouse mastocytoma P-815 cells. Biochim Biophys Acta 1133:172–178.

Kuramasu A, Saito H, Suzuki S, Watanabe T, Ohtsu H. 1998. Mast cell-/basophil-specific transcriptional regulation of human L-histidine decarboxylase gene by CpG methylation in the promoter region. J Biol Chem 273:31607–31614.

Matés JM, Márquez J, García-Caballero M, Núñez de Castro I, Sánchez-Jiménez F. 1992. Simultaneous fluorometric determination of intracellular polyamines separated by reversed-phase high-performance liquid chromatography. Agents Actions 36:17–21.

Medina MA, Urdiales JL, Rodriguez-Caso C, Ramirez FJ, Sanchez-Jimenez F. 2003. Biogenic amines and polyamines: Similar biochemistry for different physiological missions and biomedical applications. Crit Rev Biochem Mol Biol 38:23–59.

Messina L, Spampinato G, Arcidiacono A, Malaguarnera L, Pagano M, Kaminska B, Kaczmarek L, Messina A. 1992. Polyamine involvement in functional activation of human macrophages. J Leukoc Biol 52:585–587.

Metcalfe DD, Baram D, Mekori YA. 1997. Mast cells. Physiol Rev 77:1033–1079.

Moya-Garcia AA, Medina MA, Sanchez-Jimenez F. 2005. Mammalian histidine decarboxylase: From structure to function. Bioessays 27:57–63.

Nilsson G, Blom T, Harvima I, Kusche-Gullberg M, Nilsson K, Hellman L. 1996. Stem cell factor-dependent human cord blood derived mast cells express alpha- and beta-tryptase, heparin and chondroitin sulphate. Immunology 88:308–314.

Ohgoh M, Yamamoto J, Kawata M, Yamamura I, Fukui T, Ichikawa A. 1993. Enhanced expression of the mouse L-histidine decarboxylase gene with a combination of dexamethasone and 12-O-tetradecanoylphorbol-13-acetate. Biochem Biophys Res Commun 196:1113–1119.

Ohtsu H, Kuramasu A, Suzuki S, Igarashi K, Ohuchi Y, Sato M, Tanaka S, Nakagawa S, Shirato K, Yamamoto M, Ichikawa A, Watanabe T. 1996. Histidine decarboxylase expression in mouse mast cell line P815 is induced by mouse peritoneal cavity incubation. J Biol Chem 271:28439–28444.

Ohtsu H, Tanaka S, Terui T, Hori Y, Makabe-Kobayashi Y, Pejler G, Tchougounova E, Hellman L, Gertsenstein M, Hirasawa N, Sakurai E, Buzas E, Kovacs P, Csaba G, Kittel A, Okada M, Hara M, Mar L, Numayama-Tsuruta K, Ishigaki-Suzuki S, Ohuchi K, Ichikawa A, Falus A, Watanabe T, Nagy A. 2001. Mice lacking histidine decarboxylase exhibit abnormal mast cells. FEBS Lett 502:53–56.

Olmo MT, Urdiales JL, Pegg AE, Medina MA, Sánchez-Jiménez F. 2000. In vitro study of proteolytic degradation of rat histidine decarboxylase. Eur J Biochem 267:1527–1531.

Park MH, Wolff EC, Folk JE. 1993. Is hypusine essential for eukaryotic cell proliferation? Trends Biochem Sci 18:475–479.

Parker MT, Gerner EW. 2002. Polyamine-mediated post-transcriptional regulation of COX-2. Biochimie 84:815–819.

Pasquali JL, Mamont PS, Weryha A, Knapp AM, Blervaque A, Siat M. 1988. Immunosuppressive effects of (2R,5R)-6-heptyne-2,5-diamine an inhibitor of polyamine synthesis. I. Effects on mitogen-induced immunoglobulin production in human cultured lymphocytes. Clin Exp Immunol 72:141–144.

Perez-Cano FJ, Franch A, Castellote C, Castell M. 2003. Immunomodulatory action of spermine and spermidine on NR8383 macrophage line in various culture conditions. Cell Immunol 226:86–94.

Ringvall M, Ronnberg E, Wernersson S, Duelli A, Henningsson F, Abrink M, Garcia-Faroldi G, Fajardo I, Pejler G. 2008. Serotonin and histamine storage in mast cell secretory granules is dependent on serglycin proteoglycan. J Allergy Clin Immunol 121:1020–1026.

Ruiz-Chica J, Medina MA, Sanchez-Jimenez F, Ramirez FJ. 2001. Raman study of the interaction between polyamines and a GC oligonucleotide. Biochem Biophys Res Commun 285:437–446.

Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular cloning. A laboratory manual. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Schneider E, Rolli-Derkinderen M, Arock M, Dy M. 2002. Trends in histamine research: New functions during immune responses and hematopoiesis. Trends Immunol 23:255–263.

Schwartz LB, Huff FT. 1998. Biology of mast cells. In: Middleton EJ, Reed CE, Ellis EF, Adkinson NFJ, Yunginger JW, Busse WW, editors. Allergy. Principles and practice. St. Louis, Missouri: Mosby. pp. 261–276.

Seiler N, Dezeure F. 1990. Polyamine transport in mammalian cells. Int J Biochem 22:211–218.

Smith MA, Davies PJ. 1985. Separation and quantitation of polyamines in plant tissue by high performance liquid chromatography of their dansyl derivatives. Plant Physiol 78:89–91.

Suzuki-Ishigaki S, Numayama-Tsuruta K, Kuramasu A, Sakurai E, Makabe Y, Shimura S, Shirato K, Igarashi K, Watanabe T, Ohtsu H. 2000. The mouse L-histidine decarboxylase gene: Structure and transcriptional regulation by CpG methylation in the promoter region. Nucleic Acids Res 28:2627–2633.

Szabo C, Southan GJ, Thiemermann C, Vane JR. 1994. The mechanism of the inhibitory effect of polyamines on the induction of nitric oxide synthase: Role of aldehyde metabolites. Br J Pharmacol 113:757–766.

Wiener Z, Andrasfalvy M, Pallinger E, Kovacs P, Szalai C, Erdei A, Toth S, Nagy A, Falus A. 2002. Bone marrow-derived mast cell differentiation is strongly reduced in histidine decarboxylase knockout, histamine-free mice. Int Immunol 14:381–387.

Young JD, Liu CC, Butler G, Cohn ZA, Galli SJ. 1987. Identification, purification, and characterization of a mast cell-associated cytolytic factor related to tumor necrosis factor. Proc Natl Acad Sci USA 84:9175–9179.

Zhang M, Caragine T, Wang H, Cohen PS, Botchkina G, Soda K, Bianchi M, Ulrich P, Cerami A, Sherry B, Tracey KJ. 1997. Spermine inhibits proinflammatory cytokine synthesis in human mononuclear cells: A counter-regulatory mechanism that restrains the immune response. J Exp Med 185:1759–1768.

Zhang M, Wang H, Tracey KJ. 2000. Regulation of macrophage activation and inflammation by spermine: A new chapter in an old story. Crit Care Med 28:N60–N66.