

Serotonin and histamine storage in mast cell secretory granules is dependent on serglycin proteoglycan

Maria Ringvall, PhD,^{a,*} Elin Rönnerberg, MSc,^{a,*} Sara Wernersson, PhD,^a Annette Duelli, MSc,^a Frida Henningsson, PhD,^a Magnus Åbrink, PhD,^b Gianni García-Faroldi, MSc,^c Ignacio Fajardo, PhD,^c and Gunnar Pejler, PhD^a *Uppsala, Sweden, and Malaga, Spain*

Background: Serotonin and histamine are components of human and rodent mast cell secretory granules.

Objective: Serotonin and histamine are stored in the same compartment as serglycin proteoglycan. Here we addressed the possibility that serglycin may be involved in their storage and/or release.

Methods: The storage and release of histamine and serotonin was studied in bone marrow–derived mast cells (BMMCs) and in peritoneal mast cells from wild-type or serglycin^{−/−} mice.

Results: Both serotonin and histamine storage in BMMCs was positively correlated with the degree of mast cell differentiation, and the amount of stored amine was reduced in serglycin^{−/−} BMMCs compared with wild-type controls. The amounts of histamine/serotonin stored were reflected by the expression levels of histidine decarboxylase and tryptophan hydroxylase 1, respectively. Calcium ionophore activation resulted in serotonin/histamine release both from wild-type and serglycin^{−/−} BMMCs. Interestingly, serotonin release was induced in cells lacking intracellular stores of serotonin, suggesting *de novo* synthesis. The knockout of serglycin affected the levels of stored and released mast cell serotonin and histamine to an even larger extent in *in vivo*–derived mast cells than in BMMCs.

Conclusion: These results establish a previously assumed, but not proven, role of serglycin in storage of histamine and, further, establish for the first time that serotonin storage in mast cells is dependent on serglycin proteoglycan. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Mast cells, proteoglycans, serglycin, histamine, serotonin, histidine decarboxylase, tryptophan hydroxylase

From ^athe Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences; ^bthe Department of Medical Biochemistry and Microbiology, Uppsala University; and ^cthe Department of Molecular Biology and Biochemistry, Faculty of Sciences, University of Malaga.

*These authors contributed equally to this work.

Supported by grants from the Swedish Research Council, King Gustaf V's 80th Anniversary Fund, the Mizutani Foundation for Glycoscience, the Göran Gustafsson Foundation, and grant SAF-2005-1812 (Ministerio de Educación y Ciencia, Spain).

Disclosure of potential conflict of interest: G. Pejler has received research support from the Swedish Research Council, King Gustaf V's 80th Anniversary Fund, and the Mizutani Foundation of Glycoscience. M. Åbrink has received research support from the Goran Gustafsson Foundation, VR Medicine, and FORMAS. I. Fajardo has received research support from the Ministerio de Educación y Ciencia, Spain. The rest of the authors have declared that they have no conflict of interest.

Received for publication September 10, 2007; revised November 22, 2007; accepted for publication November 28, 2007.

Reprint requests: Gunnar Pejler, PhD, Swedish University of Agricultural Sciences, Department of Anatomy, Physiology and Biochemistry, BMC, Box 575, 75123 Uppsala, Sweden. E-mail: Gunnar.Pejler@bmc.uu.se.

0091-6749/\$34.00

© 2008 American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2007.11.031

Abbreviations used

BMMC: Bone marrow–derived mast cell
EIA: Enzyme immunoassay
HDC: Histidine decarboxylase
MC: Mast cell
mMCP: Mouse mast cell protease
qPCR: Quantitative PCR
TPH: Tryptophan hydroxylase
WT: Wild-type

Mast cells (MCs) are versatile cells of the immune system, contributing to both to the innate and the adaptive defense toward external insults.¹ In addition, MCs are well known for their deleterious effects in connection with allergic inflammation,² and more recently, MCs have been strongly implicated in autoimmune disease,^{3–5} atherosclerosis,⁶ and cancer.⁷ The effect of MCs in any pathological state is often a consequence of MC degranulation, although degranulation-independent actions have also been described.¹ MC secretory granules contain a wide array of pre-formed mediators. Among the first of these to be identified was histamine, and it is now established that MC granules also contain various cytokines, proteases, and lysosomal enzymes such as β -hexosaminidase.¹ Serotonin was also early identified in MCs, but it was thought for a long time that serotonin storage was limited to rodent MCs. However, recent progress has established that serotonin is also a prominent component of human MCs.⁸ In addition to these compounds, MC granules are known to contain large amounts of proteoglycans, protein cores to which unbranched, sulfated, and thereby negatively charged polysaccharides of glycosaminoglycan type are attached.⁹ In MCs, serglycin constitutes the major type of proteoglycan¹⁰ and, depending on species and MC subtype, glycosaminoglycans of either heparin or chondroitin sulfate type may be attached to the serglycin protein core.¹¹

Considering that the MC secretory granule proteoglycans are heavily negatively charged and that histamine is positively charged, it has been assumed for a long time that they interact within the granule. In support of such a notion, MC activation results in the release of heparin proteoglycan and histamine with similar kinetics,¹² and furthermore, histamine and heparin have been shown to interact in purified systems.^{13,14} However, no formal proof for a dependence of histamine on serglycin proteoglycan for storage and release *in vivo* has been presented.

Serotonin has, apart from its role as neurotransmitter of the central nervous system, a range of effects on the immune system.¹⁵ However, despite the strong implication of serotonin in immune regulation and the likely possibility that serotonin

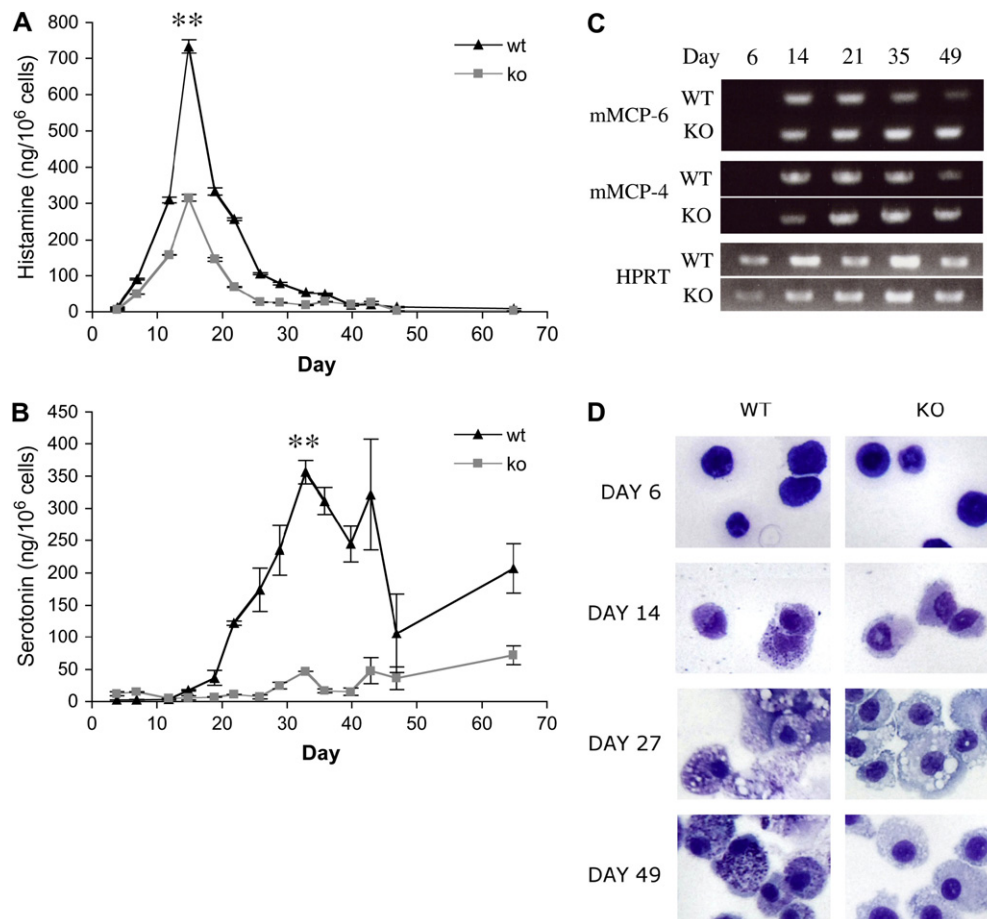


FIG 1. Reduced levels of histamine and serotonin in BMMCs from serglycin null animals. Bone marrow precursor cells were cultured *in vitro* into BMMCs. At the time points indicated, cells (2×10^6) were taken and analyzed for cellular content of histamine (**A**) and serotonin (**B**). The results displayed are from 1 cell culture experiment ($n = 2$; mean of duplicates $\pm$ SD; Student *t* test; $**P < .01$) and are representative of 3 individual experiments. **C**, RT-PCR analysis of BMMCs for expression of the mMCP-4 and mMCP-6 genes. HPRT, Housekeeping control. **D**, May-Grünwald/Giemsa staining of WT and serglycin^{-/-} BMMCs at different time points of cell culture. ko, Knockout.

specifically released from MCs may contribute to such events, the mechanisms that govern the storage and release of MC serotonin are poorly investigated. Here we show that histamine and serotonin are both dependent on serglycin proteoglycan for storage in MCs and for release.

METHODS

Mice

The serglycin^{-/-} strain was described previously.¹⁰ Age-matched wild-type (WT) and serglycin^{-/-} mice (9 weeks) on either C57BL/6 or DBA/1 genetic background (N10) were used. All experiments were approved by the local ethical committee.

MC culture, preparation of samples for histamine and serotonin enzyme immunoassay

Bone marrow-derived MCs (BMMCs) were generated and cultured as described previously.¹⁶ BMMCs (2×10^6) were taken at various time points, washed, pelleted, and stored at -20°C (or -70°C ; for mRNA analysis). Pellets were solubilized in Tyrode buffer/2% Triton X-100 for 30 minutes at room temperature. Lysates were cleared from cell debris by centrifugation, and supernatants were used for cellular histamine and serotonin measurements. To

quantify histamine and serotonin release from BMMCs and peritoneal cells, cells were washed and resuspended in Tyrode buffer and incubated at a density of 5×10^6 cells/mL, $\pm 2 \mu\text{mol/L}$ calcium ionophore A23187 (Sigma-Aldrich, Stockholm, Sweden) for 1 hour at 37°C . Media were cleared from cells by centrifugation, and histamine and serotonin in the supernatant fraction were measured. The cell fraction was solubilized and cleared as described and supernatants used to measure residual histamine and serotonin content. Histamine was quantified with enzyme immunoassay (EIA) EA31 (Oxford Biomedical Research, Oxford, Mich). Serotonin was quantified with EIA BA10-0900 or ultrasensitive EIA BA10-5900 (both from Labor Diagnostika Nord, Nordhorn, Germany).

Peritoneal lavage, preparation of peritoneal fluid and serum

Ice-cold Tyrode buffer (5 to 10 mL) was injected into the peritoneal cavity of mice euthanized by CO_2 . After slight shaking, fluid was drained from the peritoneum with a syringe and an 18G hypodermic needle. Recovered fluid was cleared from cells by centrifugation. Blood samples were incubated at room temperature to clot for 30 minutes before centrifugation.

cDNA synthesis and quantitative PCR

Total RNA from 2×10^6 cultured cells was isolated by using NucleoSpin RNA II (Macherey-Nagel, Düren, Germany), and first-strand cDNA was

synthesized by using random hexamers and SuperScript II kit (Invitrogen, Carlsbad, Calif) according to the manufacturer's instructions. Quantitative PCR (qPCR) was performed with iQ SYBR Green Supermix (BioRad, Hercules, Calif), 200 nmol/L primers, and 200 ng cDNA, using the BioRad miniopticon. PCR cycling conditions included a 95°C heating step of 10 minutes at the beginning of every run. The samples were then cycled 40 times at 95°C for 30 seconds, 57°C for 20 seconds, and 72°C for 20 seconds. Plate reading was performed after each cycle. A melting curve was generated at the end of every run to ensure product uniformity. Primer efficiency was determined by performing qPCR with the following dilutions of cDNA: 1:1, 1:10, and 1:100. The relative amount of cDNA in the experiments was determined in duplicate. Hypoxanthine guanine phosphoribosyl transferase (HPRT) was used as a housekeeping gene to compensate for differences in cDNA amount between samples.

Primers used in qPCR:

HPRT forward 5'-3': GATTAGCGATGAACCTTA
HPRT reverse 5'-3': GACATCTCGAGCTCTTTTCAGTC
Serglycin forward 5'-3': GCAAGGTTATCCTGCTCGGAG
Serglycin reverse 5'-3': GGTCAAACTGTGGTCCCTTCTC
Histidine decarboxylase (HDC) forward 5'-3': GGATTCTGGGTCAAGGACAAGT
HDC reverse 5'-3': AATGCATGAAGTCCGTGGCT
Tryptophan hydroxylase (TPH)-1 forward 5'-3': CTCCGAAAGAGGGAGAGTGACTC
TPH-1 reverse 5'-3': AACAGGCTCACATGATTCTCCTG
TPH-2 forward(1) 5'-3': ATATGGTCAGCCCATTCCTCA
TPH-2 reverse(1) 5'-3': GTACTCCCGCAAGCATGAG
TPH-2 forward(2) 5'-3': TCTAATCTCGAGGATGTGCCGT
TPH-2 reverse(2) 5'-3': GGATGGTTCGGCATCAAGCT
TPH-2 forward(3) 5'-3': ACACCCCGGAACCAGATACA
TPH-2 reverse(3) 5'-3': CCCAGAGACGCTAAGCCTATCTC

The threshold cycle (C_T) values were plotted against log concentration, and primer efficiency was calculated with the slope of the straight line according to the formula efficiency = $10^{(-1/\text{slope})} - 1$.¹⁷ The method used to calculate the relative amount of cDNA was the comparative C_T method. The C_T values obtained were calculated according to the User Bulletin #2: ABI PRISM 7700 Sequence Detection System (P/N 4303859, Applied Biosystems, Foster City, Calif).

Flow cytometry

Peritoneal cells were washed in PBS containing 0.5% FCS, and samples of 3×10^5 cells were stained for 30 minutes on ice with fluorescein isothiocyanate-conjugated rat IgG_{2a} antimouse CD117, phycoerythrin-conjugated rat IgG_{2a} antimouse CD34, or their respective isotype-matched control antibodies (ImmunoTools, Friesoythe, Germany). After staining, cells were washed 3 times and resuspended in 0.5 mL PBS/0.5% FCS. Data from 10,000 cells per sample were collected and analyzed by using a FACScan flow cytometer and the CELLQuest software (Becton Dickinson, San Jose, Calif).

HPLC

Quantification of intracellular histamine, serotonin, and polyamines in BMMC samples by HPLC was performed as previously described.¹⁸

RESULTS

To study the role of serglycin in storage of serotonin and histamine in MCs, MCs were first obtained by *in vitro* differentiation of serglycin^{+/+} and serglycin^{-/-} bone marrow precursors into MCs—that is, BMMCs. Cells were taken at various time points during the differentiation process and were analyzed for intracellular content of serotonin and histamine by EIA. Histamine (Fig 1, A) and serotonin (Fig 1, B) were essentially undetectable in both WT and knockout cells at early time points but showed a dramatic increase at day ~7 (histamine) and day ~19 (serotonin) of culture. RT-PCR analysis showed that MC protease (mMCP)-4

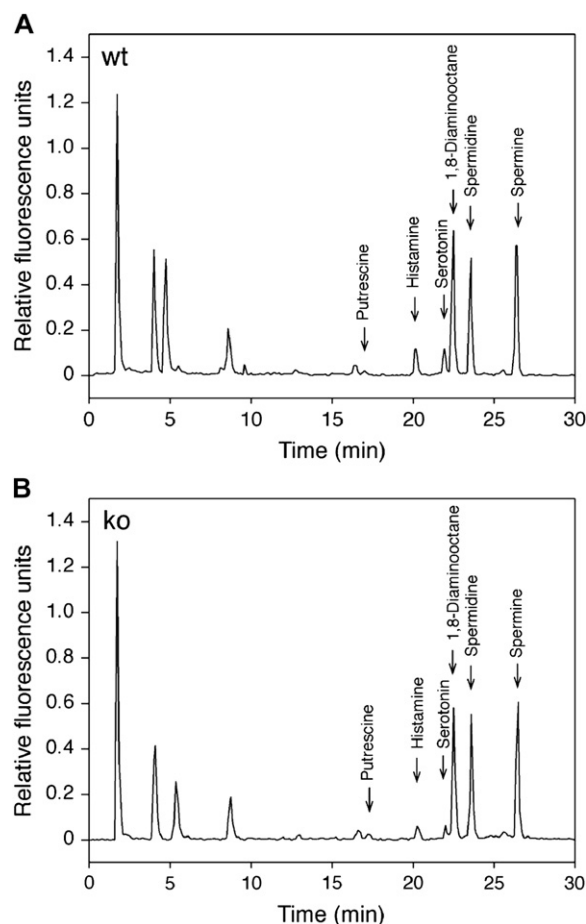


FIG 2. Histamine, serotonin, and polyamines levels in BMMCs. Serglycin^{+/+} (A; WT) or serglycin^{-/-} (B; knockout [KO]) bone marrow precursor cells were cultured *in vitro* into BMMCs. After 18 days, cells (4×10^6) were taken and analyzed for cellular contents of histamine, serotonin, putrescine, spermidine, and spermine by HPLC. 1,8-Diaminooctane was used as an internal standard added to the cell extract before derivatization with dansyl chloride.

and mMCP-6 gene expression, with the expression of these genes being markers of MC differentiation,^{19,20} was undetectable at early stages of culture (day 6), but markedly increased from day ~14 (Fig 1, C). Moreover, in agreement with previous reports,^{10,16} WT and serglycin^{-/-} BMMCs appeared to express equal levels of the mMCP-4 and mMCP-6 genes (Fig 1, C), indicating that the lack of serglycin did not result in defective MC differentiation. Interestingly, the levels of both serotonin and histamine were considerably lower in cells derived from serglycin^{-/-} than from WT mice, indicating that the storage of both amines is dependent on serglycin. A dependence of histamine/serotonin on serglycin for storage was seen in BMMCs derived from mice of both C57BL/6 and DBA/1 backgrounds (not shown). An independent method, HPLC, confirmed that the levels of histamine and serotonin are reduced in BMMCs from serglycin^{-/-} mice compared with WT controls (Fig 2). The HPLC analysis in addition showed that the levels of the ornithine-derived polyamines, putrescine, spermidine, and spermine were not affected by the lack of serglycin (Fig 2).

To see whether the onset of serotonin/histamine storage showed a correlation with development of MC-like morphology,

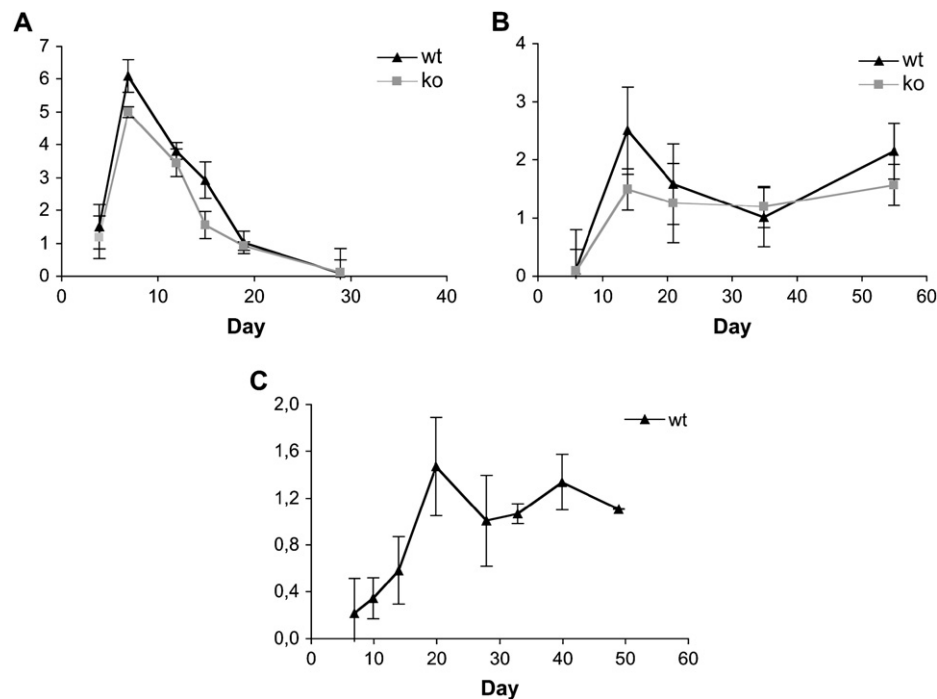


FIG 3. Expression of HDC, TPH-1, and serglycin in BMMCs. WT or knockout (*ko*) bone marrow precursor cells were cultured *in vitro* into BMMCs and were analyzed for levels of transcript for HDC (**A**; the levels of expression relative to that of WT cells at day 19), TPH-1 (**B**; the levels of expression relative to that of WT cells at day 35), and serglycin (**C**; the levels of expression relative to that of cells at day 28) by qPCR. The results displayed are from 1 cell culture experiment ($n = 2$; mean of duplicates $\pm$ SD) and are representative of 2 individual experiments.

cells were stained with May-Grünwald/Giemsa. Indeed, serglycin^{+/+} cells, starting at day ~14, showed clear signs of MC-like morphology, with an abundance of granule-like vesicles (Fig 1, D). At later stages of cultures, a gradual increase in staining was seen. In agreement with previous studies,^{10,21} serglycin^{-/-} cells stained poorly with May-Grünwald/Giemsa at all time points (Fig 1, D).

Next, the correlation between the storage levels of histamine/serotonin and the mRNA expression of the corresponding biosynthetic enzymes—that is, HDC and TPH—was studied by qPCR. Out of the 2 isoforms of TPH, TPH-1 and TPH-2, TPH-1 appeared to be the predominant isoform expressed in BMMCs, whereas TPH-2 gene expression was undetectable using 3 different primer sets (see Methods; not shown). As shown in Fig 3, HDC and TPH-1 gene expression was low at early time points, but both transcripts were upregulated during the culture period. Maximal HDC gene transcription occurred at day ~7, whereas TPH-1 gene expression was somewhat delayed, with maximal expression reached at day ~15. The levels of HDC and TPH-1 transcript were similar in WT and serglycin^{-/-} BMMCs (Fig 3, A and B). Serglycin gene expression was detected at early time points during the cell culture period. However, an up-regulated expression was seen at day ~20, and continuous high expression was seen throughout the cell culture period (Fig 3, C).

A comparison of the levels of stored histamine and levels of HDC gene transcript revealed that maximal HDC transcription occurred somewhat earlier (day ~7) than maximal histamine storage (day ~15; Figs 1, A, and 3, A). Another observation was that the histamine levels dropped markedly during prolonged culture periods, accompanied by reduced transcription of the HDC

gene (Fig 3, A). The reduction in histamine and HDC levels was not an effect of a general reduction in secretory granule content, as evidenced by morphologic criteria (Fig 1, D), expression of MC proteases (Fig 1, C), levels of serglycin gene expression (Fig 3, C), and the ability to release β -hexosaminidase (not shown). Addition of stem cell factor (50 ng/mL) to the culture medium only marginally prevented the time-dependent reduction of histamine storage in BMMCs (not shown). Similar to histamine/HDC, the onset of serotonin storage was somewhat delayed compared with TPH-1 mRNA expression (Figs 1, B, and 3, B). In contrast with histamine/HDC, however, serotonin storage and TPH-1 gene expression were maintained at a higher level throughout the cell culture period (Fig 3, B).

The release of histamine and serotonin in response to BMMC degranulation was studied. Addition of calcium ionophore A23187 caused the release of histamine and serotonin from both WT and serglycin^{-/-} BMMCs, and for histamine, the amounts released (Fig 4, A) from BMMCs taken at various time points of cell culture showed a correlation with the levels stored (Fig 1, A). In contrast, the amounts of serotonin released from BMMCs were similar at all time points of cell culture, independently of the levels of stored serotonin (Fig 4, B). In fact, addition of A23187 to day 14 cells—that is, at a time point when serotonin storage was almost undetectable (Fig 1, B)—caused a robust secretion of serotonin from both WT and serglycin null BMMCs (Fig 4, B), suggesting that the released serotonin is a result of *de novo* synthesis rather than release from preformed stores, the latter notion in line with previous reports.^{22,23}

Together, these results point to a role for serglycin proteoglycan in regulating the storage of both serotonin and histamine in

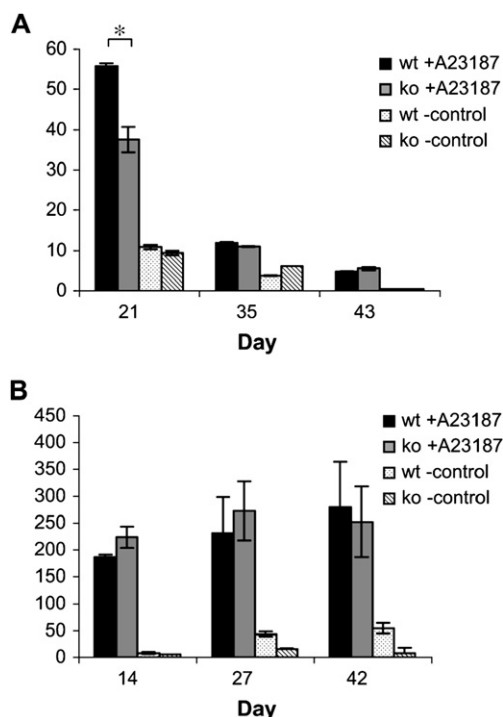


FIG 4. Release of histamine and serotonin from BMMCs. BMMCs, taken at the time points of culture indicated, were stimulated with calcium ionophore A23187 ($n = 2$; mean of duplicates $\pm$ SD; Student t test; $*P < .05$). Conditioned media were collected and analyzed for histamine (A) and serotonin (B) by EIA. As controls, media from nonstimulated cells were analyzed. The results are representative of 3 individual experiments. ko, Knockout.

BMMCs. To address whether this is also seen *in vivo*, peritoneal cells were obtained from WT and serglycin^{-/-} mice and were analyzed for histamine and serotonin content. As shown in Fig 5, the levels of both histamine (Fig 5, A) and serotonin (Fig 5, B) were profoundly decreased in serglycin^{-/-} compared with WT peritoneal cells. Moreover, the amounts of released histamine and serotonin in response to calcium ionophore were dramatically reduced in cells from serglycin^{-/-} animals (Fig 5, A and B). Importantly, the release of both histamine and serotonin in response to calcium ionophore was accompanied by a corresponding reduction in the intracellular content of the respective amine (Fig 5, A and B), indicating release from intracellular stores. However, the amounts of serotonin released exceeded the amounts stored (Fig 5, B), indicating that *de novo* synthesized serotonin contributed to the total released amine.

These results suggest that the effect of serglycin proteoglycan on histamine/serotonin storage is even larger in *in vivo*-derived MCs than in BMMCs. On the other hand, the peritoneal cell population is a mixture of lymphocytes, macrophages, and MCs (~1% to 3% MCs), and it could not be excluded that the reductions in histamine and serotonin are a result of a reduction in the number of MCs in the peritoneal cavity. Therefore, MC numbers were quantified by flow cytometry, using CD117 (c-kit) as a surface marker. This analysis revealed an approximately equal number of MCs in the peritoneum of WT and serglycin^{-/-} mice (Fig 5, C). The presence of another MC marker, CD34, on CD117⁺ cells was confirmed by double-staining (data not shown). Interestingly, the CD117⁺ cells from serglycin^{-/-} mice

demonstrated a dramatic decrease in granularity, as shown by low values of side scatter detection compared with WT counterparts (Fig 5, D). Clearly, this finding is well in line with the secretory granule defects reported previously in serglycin null MCs.^{10,21} Together, these results indicate that the reduction of histamine and serotonin in peritoneal cells from serglycin null animals is a result of decreased storage in the individual MCs, and not an effect of decreased MC numbers.

To assess whether serglycin affects also the extracellular levels of histamine and serotonin, their levels were quantified in both the peritoneal fluid and serum samples from WT and serglycin null animals (Fig 6). Indeed, serglycin^{-/-} mice exhibited a tendency of reduced levels of histamine and serotonin in both peritoneal fluid and serum, but a statistically significant reduction was seen only for histamine levels in serum.

DISCUSSION

Serglycin is expressed by a multitude of hematopoietic cells, including MCs, macrophages, neutrophils, cytotoxic T lymphocytes, and platelets. In most of these cell types, serglycin proteoglycan is located in secretory vesicles, and recent data obtained through the knockout of the serglycin gene have demonstrated an important role of serglycin in promoting storage of a variety of granule compounds, such as MC proteases,^{10,21} granzyme B in cytotoxic T lymphocytes,²⁴ and neutrophil elastase.²⁵ Here we expand the repertoire of secretory granule compounds being dependent on serglycin by showing that histamine and serotonin storage in MCs is dependent on serglycin proteoglycan. Importantly, we demonstrate the dependency on serglycin both in BMMCs and in MCs derived from *in vivo* sources.

We cannot be entirely certain of the exact mechanism by which these amines depend on serglycin. One possibility would be that the lack of serglycin affects the rate of histamine and serotonin synthesis by causing a reduced expression of their corresponding biosynthetic enzymes, HDC and TPH. However, because the expression of the HDC and TPH-1 genes was comparable in WT and serglycin^{-/-} BMMCs, this does not appear to be the case. A more likely possibility is therefore that histamine and serotonin storage in MCs is mediated through direct interaction with serglycin proteoglycan in the granules. In support of such a notion, histamine has been shown to interact electrostatically with heparin, 1 of the 2 glycosaminoglycan types that may be attached to the serglycin protein core,¹¹ the other being chondroitin sulfate. Also, previous studies have shown that MCs lacking expression of N-deacetylase/N-sulfotransferase-2, an enzyme involved in the sulfation of heparin, have a reduced histamine content.^{26,27} Considering the structural similarity between histamine and serotonin, it appears likely that their dependence on serglycin is explained by similar mechanisms. We may therefore suggest that serotonin engages in electrostatic binding with the glycosaminoglycans attached to the serglycin protein core, presumably through the interaction of its positively charged ammonium ion with negative charges on the glycosaminoglycan. A third possibility would be that the storage of MC histamine and serotonin depends on interaction with compounds other than serglycin and that such compounds, in turn, are serglycin-dependent.

If indeed histamine and serotonin are synthesized at the same rate in WT and knockout MCs, an important question concerns the fate of the amines when serglycin is absent. A likely scenario

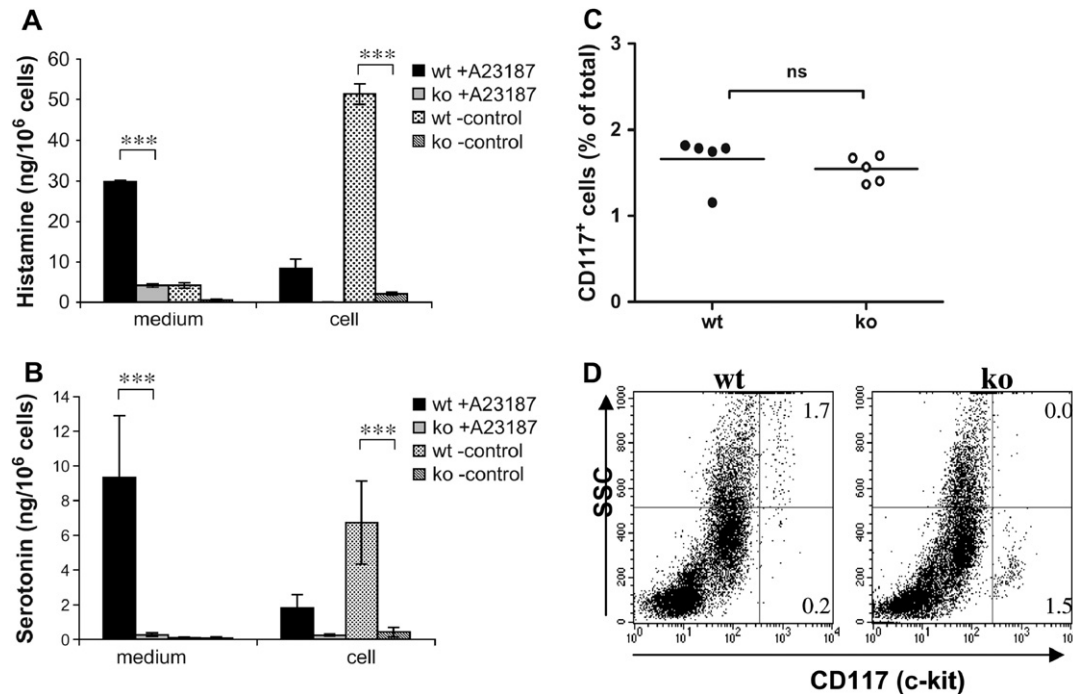


FIG 5. Reduced storage and release of histamine and serotonin in peritoneal cells from serglycin null animals. Peritoneal cells from WT or knockout (KO) mice were stimulated *in vitro* with A23187 for 60 minutes. Conditioned medium from stimulated and control cells were analyzed for content of histamine (**A**) and serotonin (**B**). Cellular levels (*cell*) of histamine (**A**) and serotonin (**B**) were analyzed in A23187-activated or nontreated cells ($n = 4$; mean values $\pm$ SD; Student t test; *** $P < .001$). **C**, The percentage of CD117⁺ cells in peritoneum was determined by flow cytometry ($n = 5$). **D**, Cell complexity (granularity) of CD117⁺ cells is demonstrated by side scatter (SSC) detection (representative *dot blots*; $n = 5$). ns, Not significant.

would be that they are secreted to the extracellular space, thus causing an excessive accumulation of serotonin and histamine. However, we did not see any increased spontaneous release of either serotonin or histamine in cell cultures from serglycin^{-/-} animals, nor did we see any accumulation of amine in either the peritoneal cavity or serum. In fact, their extracellular levels appeared to be reduced in animals lacking serglycin. We therefore favor the possibility that the lack of serglycin causes an increase in the intracellular turnover of histamine and serotonin.

During the BMMC culture experiments, we noted that histamine storage was low in bone marrow precursor cells, but was dramatically elevated during differentiation into MC phenotype. This is in agreement with previous studies demonstrating high levels of histamine in ~2-week BMMC cultures but only low levels at earlier stages of culture.²⁸ However, we noted that the levels of histamine were decreased on prolonged cell culture, probably as a consequence of reduced HDC expression. This is in apparent contrast with the *in vivo*-differentiated MCs, which contain high levels of histamine, and underscores that the phenotype of *in vitro*-differentiated BMMCs does not exactly match the phenotype *in vivo*. Interestingly, although histamine storage was reduced in more mature BMMC cultures, the levels of serotonin were maintained at a high level and the TPH-1 gene was expressed continuously throughout the culture period. We also noted that the onset of serotonin storage occurred considerably later during the BMMC differentiation process than storage of histamine, thus suggesting that serotonin storage is a characteristic of more mature MCs whereas histamine storage is initiated earlier during MC differentiation. Also of interest is a recent report showing that

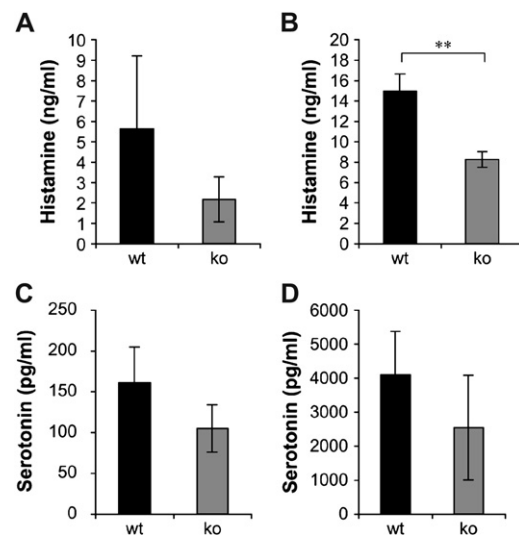


FIG 6. Extracellular levels of histamine and serotonin in the peritoneum and serum of WT and serglycin^{-/-} animals. Peritoneal lavage fluid and serum were collected from WT and serglycin^{-/-} (knockout [KO]) animals ($n = 5$ for peritoneal fluid; $n = 3$ for serum) and were analyzed for histamine and serotonin content by EIA. **A**, Histamine levels in the peritoneum. **B**, Histamine levels in serum. **C**, Serotonin levels in the peritoneum. **D**, Serotonin levels in serum. ** $P < .01$ (Student t test).

serotonin storage is increased in MCs lacking HDC expression,²⁹ introducing the possibility that histamine and serotonin may compete for “space” in the granules, perhaps by competing for binding to the glycosaminoglycans attached to the serglycin protein core.

Previous studies have suggested the existence of TPH in MCs,^{8,30,31} but before this study, it was not known whether MCs preferentially express the TPH-1 or TPH-2 isoform. Here we show that BMHCs express TPH-1 rather than TPH-2, and we also show that TPH-1 is upregulated during the differentiation of bone marrow precursor cells into MC phenotype, thus suggesting that TPH-1 gene expression is tightly coupled to the MC differentiation process. Previous studies have indicated that the TPH-2 gene is preferentially expressed in the central nervous system, whereas TPH-1 usually has a peripheral distribution.³²⁻³⁴ Our results thus conform to the notion of tissue segregation between TPH-1 and TPH-2.

Clinical implications: Given the huge effect of histamine and serotonin in allergic and other clinical settings, their dependence on serglycin proteoglycan may be exploited for therapeutic purposes.

REFERENCES

- Galli SJ, Kalesnikoff J, Grimaldeston MA, Piliponsky AM, Williams CM, Tsai M. Mast cells as "tunable" effector and immunoregulatory cells: recent advances. *Annu Rev Immunol* 2005;23:749-86.
- Boyce JA. The role of mast cells in asthma. *Prostaglandins Leukot Essent Fatty Acids* 2003;69:195-205.
- Lee DM, Friend DS, Gurish MF, Benoist C, Mathis D, Brenner MB. Mast cells: a cellular link between autoantibodies and inflammatory arthritis. *Science* 2002;297:1689-92.
- Chen R, Ning G, Zhao ML, Fleming MG, Diaz LA, Werb Z, et al. Mast cells play a key role in neutrophil recruitment in experimental bullous pemphigoid. *J Clin Invest* 2001;108:1151-8.
- Secor VH, Secor WE, Gutekunst CA, Brown MA. Mast cells are essential for early onset and severe disease in a murine model of multiple sclerosis. *J Exp Med* 2000;191:813-22.
- Sun J, Sukhova GK, Wolters PJ, Yang M, Kitamoto S, Libby P, et al. Mast cells promote atherosclerosis by releasing proinflammatory cytokines. *Nat Med* 2007;13:719-24.
- Coussens LM, Raymond WW, Bergers G, Laig-Webster M, Behrendtsen O, Werb Z, et al. Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. *Genes Dev* 1999;13:1382-97.
- Kushnir-Sukhov NM, Brown JM, Wu Y, Kirshenbaum A, Metcalfe DD. Human mast cells are capable of serotonin synthesis and release. *J Allergy Clin Immunol* 2007;119:498-9.
- Stevens RL, Austen KF. Recent advances in the cellular and molecular biology of mast cells. *Immunol Today* 1989;10:381-6.
- Åbrink M, Grubic M, Pejler G. Serglycin is essential for maturation of mast cell secretory granule. *J Biol Chem* 2004;279:40897-905.
- Kolset SO, Prydz K, Pejler G. Intracellular proteoglycans. *Biochem J* 2004;379:217-27.
- Yurt RW, Leid RW Jr, Spragg J, Austen KF. Immunologic release of heparin from purified rat peritoneal mast cells. *J Immunol* 1977;118:1201-7.
- Chuang WL, Christ MD, Peng J, Rabenstein DL. An NMR and molecular modeling study of the site-specific binding of histamine by heparin, chemically modified heparin, and heparin-derived oligosaccharides. *Biochemistry* 2000;39:3542-55.
- Rabenstein DL, Bratt P, Peng J. Quantitative characterization of the binding of histamine by heparin. *Biochemistry* 1998;37:14121-7.
- Mossner R, Lesch KP. Role of serotonin in the immune system and in neuroimmune interactions. *Brain Behav Immun* 1998;12:249-71.
- Henningsson F, Hergeth S, Cortelius R, Åbrink M, Pejler G. A role for serglycin proteoglycan in granular retention and processing of mast cell secretory granule components. *FEBS J* 2006;273:4901-12.
- Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 2001;29:e45.
- Fajardo I, Urdiales JL, Paz JC, Chavarria T, Sanchez-Jimenez F, Medina MA. Histamine prevents polyamine accumulation in mouse C57.1 mast cell cultures. *Eur J Biochem* 2001;268:768-73.
- Gurish MF, Ghildyal N, McNeil HP, Austen KF, Gillis S, Stevens RL. Differential expression of secretory granule proteases in mouse mast cells exposed to interleukin 3 and c-kit ligand. *J Exp Med* 1992;175:1003-12.
- Pejler G, Åbrink M, Ringvall M, Wernersson S. Mast cell proteases. *Adv Immunol* 2007;95:167-255.
- Braga T, Grubic M, Lukinius A, Hellman L, Åbrink M, Pejler G. Serglycin proteoglycan is required for secretory granule integrity in mucosal mast cells. *Biochem J* 2007;403:49-57.
- Theoharides TC, Kops SK, Bondy PK, Askenase PW. Differential release of serotonin without comparable histamine under diverse conditions in the rat mast cell. *Biochem Pharmacol* 1985;34:1389-98.
- Buckley MG, Coleman JW. Cycloheximide treatment of mouse mast cells inhibits serotonin release: evidence of a requirement for newly synthesized protein in the exocytotic response. *Biochem Pharmacol* 1992;44:659-64.
- Grubic M, Braga T, Lukinius A, Eloranta ML, Knight SD, Pejler G, et al. Serglycin-deficient cytotoxic T lymphocytes display defective secretory granule maturation and granzyme B storage. *J Biol Chem* 2005;280:33411-8.
- Niemann CU, Åbrink M, Pejler G, Fischer RL, Christensen EI, Knight SD, et al. Neutrophil elastase depends on serglycin proteoglycan for localization in granules. *Blood* 2007;109:4478-86.
- Forsberg E, Pejler G, Ringvall M, Lunderius C, Tomasini-Johansson B, Kuschel-Gullberg M, et al. Abnormal mast cells in mice deficient in a heparin-synthesizing enzyme. *Nature* 1999;400:773-6.
- Humphries DE, Wong GW, Friend DS, Gurish MF, Qiu WT, Huang C, et al. Heparin is essential for the storage of specific granule proteases in mast cells. *Nature* 1999;400:769-72.
- Levi-Schaffer F, Dayton ET, Austen KF, Hein A, Caulfield JP, Gravalles PM, et al. Mouse bone marrow-derived mast cells cocultured with fibroblasts: morphology and stimulation-induced release of histamine, leukotriene B₄, leukotriene C₄, and prostaglandin D₂. *J Immunol* 1987;139:3431-41.
- Csaba G, Kovacs P, Buzas E, Mazan M, Pallinger E. Serotonin content is elevated in the immune cells of histidine decarboxylase gene knock-out (HDCKO) mice: focus on mast cells. *Inflamm Res* 2007;56:89-92.
- Lovenberg W, Levine RJ, Sjoerdsma A. A tryptophan hydroxylase in cell-free extracts of malignant mouse mast cells. *Biochem Pharmacol* 1965;14:887-9.
- Mathiau P, Bakalara N, Aubineau P. Tryptophan hydroxylase can be present in mast cells and nerve fibers of the rat dura mater but only mast cells contain serotonin. *Neurosci Lett* 1994;182:133-7.
- Cote F, Thevenot E, Fligny C, Fromes Y, Darmon M, Ripoche MA, et al. Disruption of the nonneuronal tph1 gene demonstrates the importance of peripheral serotonin in cardiac function. *Proc Natl Acad Sci U S A* 2003;100:13525-30.
- Patel PD, Pontrello C, Burke S. Robust and tissue-specific expression of TPH2 versus TPH1 in rat raphe and pineal gland. *Biol Psychiatry* 2004;55:428-33.
- Walther DJ, Peter JU, Bashammakh S, Hortnagl H, Voits M, Fink H, et al. Synthesis of serotonin by a second tryptophan hydroxylase isoform. *Science* 2003;299:76.