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In a Concerted Action Kit Ligand and Interleukin 3 Control the Synthesis of Serotonin in Murine Bone Marrow-derived Mast Cells

UP-REGULATION OF GTP CYCLOHYDROLASE I AND TRYPTOPHAN 5-MONOOXYGENASE ACTIVITY BY THE Kit LIGAND*

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Irmgard Ziegler‡§, Lothar Hültner¶, Dietmar Egger¶, Bettina Kempkes‡, Reinhard Mailhammer¶, Steven Gillis‖, and Wolfgang Rödl‡

From the ‡GSF-Institut für Klinische Molekularbiologie und Tumorgenetik and the ¶GSF-Institut für Experimentelle Hämatologie, D-8000 München 70, Germany and ‖Immunex Corp., Seattle, Washington 98101

Mouse bone marrow-derived mast cells (BMMC) store and release serotonin whose synthesis is initiated by tryptophan 5-monooxygenase. (6*R*)-H_4biopterin serves as the natural cofactor for this reaction. GTP cyclohydrolase I catalyzes the first and rate-limiting step of its synthesis. In this study we demonstrate that among a panel of growth-promoting cytokines including kit ligand (KL), interleukin 3 (IL-3), IL-4, IL-9, and nerve growth factor, KL selectively enhances the synthesis of H_4biopterin through up-regulation of GTP cyclohydrolase I activity to 6.2-fold levels. The activities of the subsequent enzymes 6-pyruvoyl-H_4pterin synthase and sepiapterin reductase remain unaffected. The activity of tryptophan 5-monooxygenase was selectively enhanced 4.5-fold by the combination of IL-3 with KL. All other factors could not substitute for KL. The constitutive high activity of aromatic L-amino acid decarboxylase is not different in cells cultured in IL-3 and/or KL. In consequence, the concerted action of IL-3 and KL on the GTP cyclohydrolase I and the tryptophan 5-monooxygenase reaction enhances the production of serotonin to about 20-fold levels. Additionally, KL specifically causes the release of about half of total serotonin produced. Hence, our data demonstrate a novel role of these cytokines for the function of mouse BMMC and provide a coherent view of the regulation of serotonin synthesis in this cell type.

It is well established that tryptophan 5-monooxygenase (EC 1.14.16.4) is the first and rate-limiting enzyme of 5-hydroxytryptamine (serotonin) biosynthesis, catalyzing the formation of 5-hydroxytryptophan from L-tryptophan. In the subsequent step, 5-hydroxytryptophan is metabolized to serotonin by the L-aromatic amino acid decarboxylase (EC 4.1.1.28). (6*R*)-H_4biopterin serves as the natural and immediate electron donor for the hydroxylation of L-tryptophan, and molecular oxygen is used as an oxidizing agent (for review, see Ref. 1). Previous studies have shown that during the course of this hydroxylation reaction in murine BMMC[1] the 7-isomer is formed (2), sharing the same mechanism with the L-phenylalanine 4-monooxygenase reaction (3). The cofactor (6*R*)-H_4biopterin is synthesized *de novo* from GTP in all tissues that are competent for hydroxylation of aromatic L-amino acids such as tryptophan, phenylalanine, or tyrosine and thus, are competent for biosynthesis of serotonin, dopamine, and catecholamines, or for degradation of phenylalanine (4). The first committed step in the biosynthetic pathway is catalyzed by GTP cyclohydrolase I. Its activity determines the formation of dihydroneopterin triphosphate from GTP (Ref. 5; for review, see Ref. 6). 6-Pyruvoyl-H_4pterin synthase subsequently eliminates the triphosphate from this first intermediate and catalyzes the intramolecular reaction, yielding 6-pyruvoyl-H_4pterin. Both keto groups of this metastable intermediate are reduced by NADPH. Sepiapterin reductase (EC 1.1.1.153) can catalyze the reduction of both C1′ and C2′-oxo functions (7) to yield the final product H_4biopterin, which is generally accepted to represent the 6-isomer in the *R* configuration.

Serotonin synthesis is found in the brain, where it functions as a neurotransmitter (for review, see Ref. 8), and in the pineal gland (9), where it is the precursor of melatonin. Serotonin is also found in neurons of the myenteric plexus and in enterochromaffin cells (10). Accordingly, tryptophan 5-monooxygenase activity (for review, see Ref. 1) and GTP cyclohydrolase I activity (4) appear to be constitutively expressed in these tissues.

Rodent mast cells store and release serotonin as an important component of the acute broncho- and vasospastic response following allergen challenge (for review, see Ref. 11). In cultured mouse mast cells derived from bone marrow, spleen, or fetal liver, serotonin concentrations were reported to range from 2.7 to 4.1 nmol/10^6 cells (12). In all those studies of synthesis, storage, and/or release of serotonin by mast cells, the cells were allowed to take up either the immediate precursor, 5-hydroxytryptophan (12–14), or serotonin itself (15). Even though a tryptophan 5-monooxygenase was characterized from the mouse mastocytoma clone P815 and found to represent a different entity from brain enzyme (16,

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§ To whom correspondence should be addressed: GSF-Institut für Klinische Molekularbiologie und Tumorgenetik, Marchioninistr. 25, D-8000 München 70, Germany.

[1] The abbreviations and trivial names used are: BMMC, bone marrow-derived mast cells; H_4biopterin, 5,6,7,8-tetrahydrobiopterin; biopterin, L-erythro-1′,2′-dihydroxypropylpterin; 6-pyruvoyl-H_4pterin, (6*R*)-(1′,2′-dioxopropyl)-5,6,7,8-tetrahydropterin; IFN-γ, interferon-γ; IL, interleukin; NGF, nerve growth factor; m, murine; r, recombinant; KL, kit ligand; hisKL, recombinant murine kit ligand expressed in *E. coli*; HPLC, high performance liquid chromatography.

17), no information exists on its activity or its regulation in primary murine BMMC. The same holds true for the synthesis and regulation of H_4biopterin that is critically involved as hydroxylation cofactor in the initial step of serotonin synthesis. It has been shown previously that IFN-γ controls GTP cyclohydrolase I activity in human cells associated with the immune system (18). This increase correlates with increased levels of steady state GTP cyclohydrolase I mRNA levels (19). In these cells however, (6*R*)-H_4biopterin does not serve as hydroxylation cofactor but rather modulates IL-2-triggered T cell proliferation (for review, see Ref. 20).

It is well established that IL-3 promotes the proliferation of murine BMMC *in vitro* (21). Homogeneous populations of immature mast cells can be generated from mouse bone marrow and kept for several weeks in a state of factor-dependent growth in cultures supplemented with this cytokine (21, 22). The proliferative action of IL-3 on these primary BMMC and BMMC-derived permanent mast cell lines can be synergistically enhanced by additional cytokines including IL-4 (23), IL-9 (24, 25), IL-10 (26), or NGF (27). In addition, primary BMMC express the product of c-*kit* (28), a receptor tyrosine kinase (29). The ligand for this receptor (KL) has recently been identified (30). KL is able to promote *in vitro* proliferation of BMMC and induces their maturation (28, 31). Recent evidence suggested that KL may also influence the functional activity of mast cells, *e.g.* the release of serotonin from mouse BMMC *in vitro* (13), or the release of histamine in various types of mast cells (32–34). Also the induction of an acute inflammatory response following intradermal injection of KL suggests that this cytokine may control the function of mast cells (35).

In the present study we report that among a panel of cytokines which support the growth of murine BMMC IL-3 orchestrates with KL in increasing serotonin formation to 20-fold levels. The results characterize KL as a key regulatory factor of serotonin biosynthesis in these cells. It selectively up-regulates the activity of GTP cyclohydrolase I, thus optimizing the concentrations of the H_4biopterin cofactor. In addition, KL synergizes with IL-3 in achieving maximum activity of tryptophan 5-monooxygenase. Together with the demonstration of KL-mediated serotonin release, the data provide a coherent view of serotonin regulation in murine BMMC and demonstrate a novel role for these cytokines.

EXPERIMENTAL PROCEDURES

Chemicals—The sources of the chemicals that were used for determination of enzymatic activities and for protein and biopterin determination (36–38) were as described previously. The other materials were obtained from the manufacturers indicated in parentheses: 6,7-dimethyl-5,6,7,8-tetrahydropterin (Schircks, Jona, Switzerland); 5-hydroxytryptophan, *S*(−)-α-hydrazino-3,4-dihydroxy-α-methylbenzenepropanoic acid (carbidopa), *N*-methyl-*N*-2-propanylbenzylamine (pargyline hydrochloride), *N*-methyl-*N*-propargyl-3-(2,4-dichlorophenoxy)propylamine (clorgyline hydrochloride) (Research Biochemicals Inc., Natick, MA); and 5-hydroxytryptamine (serotonin) and L-tryptophan (Sigma). The sources of all other chemicals are listed in Ref. 2 or were of highest analytical grade from Sigma or Merck (Darmstadt, Germany).

Cytokines—The following cytokines were commercially available as listed: murine (m) 2.5 S NGF (Paesel, Frankfurt, Germany), recombinant (r) mIL-4 (Genzyme, Boston, MA). mIL-3 was from Bachem Biochemica (Heidelberg, Germany). Alternatively, as a source of rmIL-3 we used a pretested batch of supernatant derived from X63Ag8-653 myeloma cells transfected with a retroviral vector carrying the the mouse IL-3 gene (39). The XAg8-653 myeloma cells were originally generated at the Basel Institute of Immunology (Basel, Switzerland) and kindly provided by Dr. E. Schmitt (Institute for Immunology, Universität Mainz, Germany), who also sent us purified mIL-9. Pilot studies showed that a final concentration of 0.5% X63Ag8-653 supernatant equaled the saturating effect of 10–20 ng ml^{-1} purified murine rIL-3 obtained commercially from Bachem (Heidelberg, Germany). The expression in yeast cells of rmKL and its purification to apparent homogeneity has been described (40). Some experiments have been performed with a rmKL (hisKL) expressed in *Escherichia coli* and purified by affinity chromatography as described (41). The biological activity of the purified hisKL preparations was assessed and compared with KL in a colorimetric proliferation assay (42, 43), as well as in experiments measuring the capacity to induce H_4biopterin synthesis in mouse BMMC. Due to the lower specific activities of hisKL preparations, about 400 ng ml^{-1} hisKL were required to equal a saturating dose of 100 ng ml^{-1} yeast-derived KL in mast cell cultures (data not shown).

Culture of Primary Mouse BMMC—Homogeneous populations of immature mast cells (>97% Alcian blue$^+$/Safranin$^-$) were obtained from femoral bone marrow cultures of BALB/c mice as described previously (43). As a source of rmIL-3 or native mIL3, either 1% of a pretested batch of supernatant from X63Ag8-653 myeloma cells carrying a transfected mouse IL-3 gene (39) or 10% of pokeweed mitogen-stimulated spleen cell-conditioned medium (concentrated 10 times) have been used. Primary BMMC (*in vitro* age: 3–4 weeks) were cultured at 5×10^5 cells ml^{-1} in RPMI 1640 medium supplemented with 20% fetal calf serum, 2 mM L-glutamine, 10^{-5} M α-thioglycerol, 100 units ml^{-1} penicillin/streptomycin in the presence of mast cell growth factors or factor combinations including KL, rmIL-3, rmIL-4, mIL-9, and/or mNGF. Unless otherwise indicated the mast cells were harvested after an incubation time (37 °C; 10% CO_2 in fully humidified air) of 40 h and stored at −70 °C.

HPLC Determination of Biopterin—Cellular H_4biopterin was determined after acidic iodine oxidation of the reduced forms. Deproteinization by trichloroacetic acid, prepurification by cation exchange chromatography, separation by reversed-phase HPLC, and fluorometric detection have been described previously (38). The modifications of the method were the same as detailed in Ref. 36. The solvent system (2% methanol, 1% acetonitrile, adjusted to pH 2.5 with H_3PO_4) separated 6- and 7-biopterin (2). H_2biopterin can be recovered as biopterin also after oxidation in alkaline solution (4). In this case, oxidation was performed in 0.1 M NaOH, followed by addition of 2 N HCl to bring biopterin back to its cationic form (4). Measurements were carried out in duplicates. For the purpose of statistics, *n* denotes the number of independent experiments.

HPLC Determination of 5-Hydroxytryptophan and Serotonin—5-Hydroxytryptophan was separated by reversed-phase HPLC on a Hypersil ODS column as described (44). It was detected by its fluorescence (excitation 302 nm, emission 340 nm) with a Shimadzu RF 535 HPLC spectrofluorimeter. Serotonin was assayed in two different ways. For direct analysis, the protein of the cell extract containing 50% methanol (see below) was precipitated by perchloric acid (1 M final concentration) and serotonin was separated on a reversed-phase HPLC column (Hypersil ODS) with the mobile phase described in (45). It was fluorimetrically detected (excitation 302 nm, emission 340 nm). Otherwise, serotonin was previously derivatized by *o*-phthaldialdehyde (19 mM). 70 μM sodium tetraborate, pH 9.5, and 2 mM 2-mercaptoethanol were added to the sample dissolved in 50% methanol (see below) and the mixture was incubated for 1 min at 20 °C. The resulting phthalimido derivative was separated by reversed-phase HPLC on a C_{18} Spherisorb ODS column with methanol, 0.02 M sodium acetate (1:1), with addition of 5 mM 1-octanesulfonic acid. It was fluorimetrically detected (excitation 360 nm, emission 455 nm). For the purpose of statistics *n* denotes the number of independent experiments.

Preparation of Cell Extracts for Enzyme Determination—Aliquots of $1–5 \times 10^7$ cells were extracted and separated from low molecular weight components as previously described (18, 36). Protein was estimated by the Coomassie dye binding reagent (Bio-Rad Protein Assay Reagent) according to the manufacturer's instruction.

Enzyme Assays—The activity of GTP cyclohydrolase I was determined by ion pair HPLC after acidic iodine oxidation of the reaction products to neopterin phosphates. Sepiapterin reductase activity was determined by reversed-phase HPLC after acidic iodine oxidation of the reaction product to biopterin. The detailed conditions of these enzyme assays are described (36). 6-Pyruvoyl-H_4pterin synthase was measured by the release of tritiated water from [2′-^{3}H]dihydroneopterin triphosphate and calculated for saturating substrate conditions (36, 37).

The activity of tryptophan 5-monooxygenase was determined on

the basis of 5-hydroxytryptophan formation from L-tryptophan. In aliquots containing 30–50 μg of protein, enzyme activity that had been lost during manipulation of the cell extracts was restored by preincubation with Fe^{2+} and dithioerythritol and then assayed as described in Ref. 17. 6,7-Dimethyl-H_4pterin (200 μM) was used as cofactor, and L-tryptophan was added at saturating substrate concentrations (400 μM). Carbidopa (20 μM) was added for inhibition of aromatic L-amino acid decarboxylase. The reaction was terminated by addition of perchloric acid (17), and 5-hydroxytryptophan was determined by HPLC.

The activity of aromatic L-amino acid decarboxylase was determined by measuring serotonin formation from 5-hydroxy-L-tryptophan. Aliquots containing 10–20 μg of protein were assayed as described (46). Pargyline (100 μM) was added as inhibitor of monoamine oxidase. The reaction was terminated by addition of methanol (1:1, v/v) and serotonin was determined by HPLC.

Synthesis of 5-Hydroxytryptophan from Tryptophan in the Cells—Murine BMMC (5×10^6 cells ml^{-1}) were cultured for 22 h in either IL-3, KL, or a combination of both cytokines. The conversion of L-tryptophan into 5-hydroxytryptophan by the cells was then determined by incubation of 10-ml aliquots (5×10^6 cells), which were supplemented with L-tryptophan (100 μM). Decarboxylation of 5-hydroxy-L-tryptophan was inhibited by addition of carbidopa (50 μM). At the times indicated, the cells were centrifuged and extracted with 100 μl of 0.5 M perchloric acid. The extract was subjected to HPLC analysis.

Synthesis of Serotonin from L-Tryptophan by Cells and Its Release—Murine BMMC were cultured as described for the assay of 5-hydroxytryptophan synthesis. After 22 h the cultures were supplemented with L-tryptophan (100 μM) and pargyline (100 μM) or clorgyline (100 μM) as monoamine oxidase inhibitors. The effect of both inhibitors were comparable (not shown). Aliquots of 5×10^6 cells were centrifuged at t_o and after an 8-h exposure to the experimental conditions. They were then sonicated in 250 μl of Tyrode's buffer, with a Branson sonifier B-12 (three pulses, 20 s, setting 3). After centrifugation (15,000 × *g*, for 5 min at 4 °C), 200-μl aliquots were evaporated to dryness and kept with 500 μl of methanol/water (1:1) at −20 °C until HPLC determination. For determination of released serotonin, 200-μl aliquots of the culture supernatant were lyophilized and extracted in 500 μl of methanol/H_2O (1:1). Protein was precipitated with perchloric acid (1 M final concentration). The supernatant was subjected to HPLC analysis. The serotonin level of the medium, which is contributed by fetal calf serum (12), was determined to 0.5 nmol ml^{-1} and subtracted. For calculation of serotonin production, the values for t_o were subtracted.

RESULTS

Production of Reduced Biopterins (H_2biopterin and H_4biopterin)—Biopterin in murine BMMC that were grown with KL, IL-3, or in combination of IL-3 with IL-4, IL-9, or NGF, was present in the fully reduced form, H_4biopterin. Fig. 1 demonstrates that among this panel of cytokines, KL selectively caused an increase in H_4biopterin (6-H_4biopterin plus 7-H_4biopterin) amount to about 4-fold levels. The combination of IL-3 with KL did not further increase the amounts of total H_4biopterin.

As previously shown for primary mouse BMMC cultured with IL-3, 6-biopterin is associated with its 7-isomer that assigns to 31.9 (± 7.7)%. It is formed during the tryptophan 5-monooxygenase reaction (2). Combinations of IL-3 with IL-4, IL-9, or NGF increased neither total H_4biopterin production nor the relative contribution of the 7-isomer (Fig. 1). The KL-mediated increase in cellular H_4biopterin accounted to the 6-isomer only, while the absolute concentration of the 7-isomer remained as low as in IL-3-exposed cells, averaging 2 pmol/10^6 cells. Hence, its relative contribution to total biopterin decreased to <10%. The combination of KL with IL-3, which did not further enhance the KL-induced synthesis of total H_4biopterin, however, increased the portion of the 7-isomer more than 3-fold. The data indicated that KL up-regulates the biosynthetic pathway leading to H_4biopterin, whereas IL-3 affected the generation of the 7-isomer during the tryptophan 5-monooxygenase reaction. In order to address the regulation of H_4biopterin synthesis by cytokines, for better clarity only the total of the 6-and the 7-isomer will be presented below in more detail.

Both KL and IL-3 induced H_4biopterin synthesis in a dose-dependent manner. Maximum levels were achieved in the range of 100–200 ng KL ml^{-1} or with 20 ng IL-3 ml^{-1} (Fig. 2*A*) corresponding to their respective maximum proliferation-stimulating activities at these concentrations (data not shown). Maximum H_4biopterin production however, was markedly different between cells cultured with KL or IL-3 (Fig. 2*A*). The time courses of H_4biopterin synthesis were comparable between mast cells grown in KL or IL-3, culminating after 40 h, however, at markedly different levels (Fig. 2*B*).

Activity of H_4biopterin-synthesizing Enzymes—In order to determine the step of H_4biopterin synthesis that is modulated by KL, the specific activities of GTP cyclohydrolase I, 6-pyruvoyl-H_4pterin synthase and sepiapterin reductase were compared in extracts of BMMC cultured in either IL-3 or KL. Fig. 3 shows that KL increased the activity of GTP cyclohydrolase I to 6.2 (± 1.7 S.D.)-fold levels. Combination of KL with IL-3 did not further enhance the activity (data not shown). 6-Pyruvoyl-H_4pterin synthase and sepiapterin reductase remained unaffected and were the same in IL-3- and KL-grown cells (Fig. 3). These data demonstrated that the up-regulation of (6*R*)-H_4biopterin synthesis by KL is caused by increased activity of the initial and rate-limiting enzyme GTP cyclohydrolase I.

In conclusion, the data show that KL provides increased cofactor concentrations for the tryptophan 5-monooxygenase reaction. With both the non-neuronal and the neuronal enzyme the *in vitro* K_m value for (6*R*)-H_4biopterin is 22–28 μM (47). Assuming a homogeneous distribution of H_4biopterin and a cell volume of $\sim 8 \times 10^{-10}$ ml, the cofactor concentration amounts to ~8 μM in IL-3-grown cells, which increases to ~30 μM after cultivation in KL. Thus, alterations in (6*R*)-H_4biopterin levels occur around the K_m values that result in maximum changes of tryptophan 5-monooxygenase activity.

Activity of L-Tryptophan 5-Monooxygenase—Tryptophan 5-monooxygenase from neuronal (8, 48) and non-neuronal source (17) is a very unstable enzyme and loses its catalytic activity during the handling procedures after extraction of the cells. Full activity of the enzyme was restored by preincubation with Fe^{2+} and dithioerythritol, providing reproducible values and quantitative information on the enzyme under investigation (17). In murine BMMC that were grown for 40 h with either IL-3 or KL, the restored activities amounted to 47.6 (±7.4) and 50.8 (± 13.8) pmol mg^{-1} min^{-1}, respectively. The combined treatment of the cells with IL-3 and KL synergistically increased the specific activity of their tryptophan 5-monooxygenase to about 5-fold levels (Fig. 4). In combination with IL-3, none of the additional cytokines tested could substitute for KL (Fig. 4).

Activity of Aromatic L-Amino Acid Decarboxylase—The activity of aromatic L-amino acid decarboxylase reacting with 5-hydroxy-L-tryptophan as a substrate was about 12–15-fold as compared to the maximum activity of tryptophan 5-monooxygenase. It was not different between cells previously exposed for 40 h to IL-3, KL, or the combination of both cytokines (Table I). These data agree with the generally accepted view that the decarboxylase is not the rate-limiting step in serotonin biosynthesis and demonstrate that its activity is not changed through different cytokine treatments of murine BMMC.

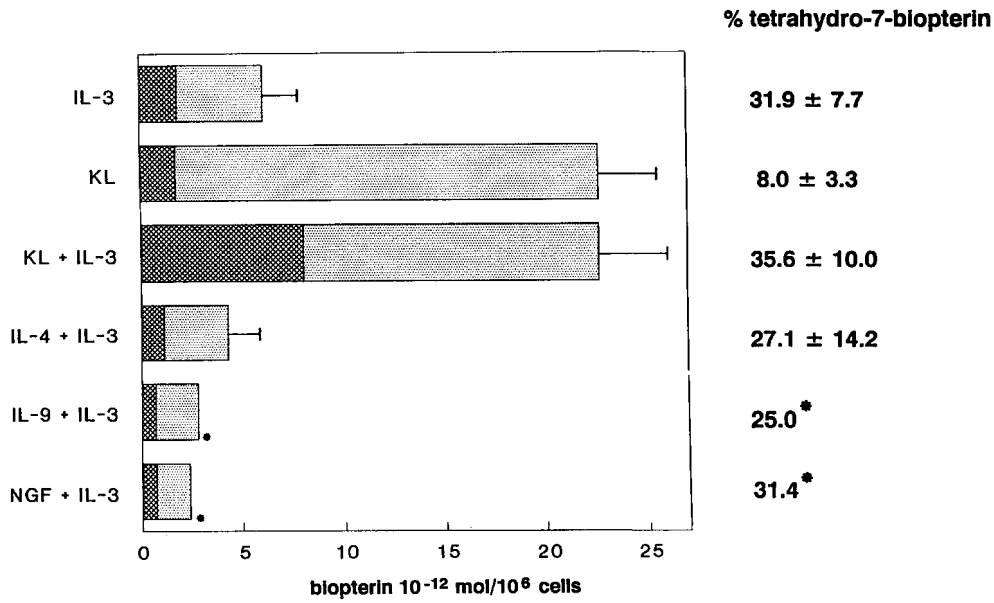

FIG. 1. **Production of biopterin in murine BMMC cultured in the presence of different growth factors.** Cells were seeded at 5 × 10^5 ml^{-1} and harvested after 40 h from the cultures grown to 0.7–1 × 10^7 cells ml^{-1}. Biopterin was determined by HPLC after acidic (H_4biopterin) and alkaline (H_2biopterin) oxidation as described under "Experimental Procedures." The differential oxidation showed that all biopterin was present in its fully reduced form, H_4biopterin. Values are means of total 6-biopterin plus 7-biopterin. **T**, ± S.D.; *, means of two determinations. For 7-biopterin, only the mean value is depicted. Percentage of 7-biopterin from total biopterin is separately listed. For IL-3 $n = 6$; for KL $n = 7$; for IL-3 + KL $n = 3$; for IL-3 + IL-4 $n = 3$. ▩, 7-biopterin; □, 6-biopterin. The following growth factor concentrations were used: KL (100 ng ml^{-1}), IL-3 (20 ng ml^{-1}), IL-4 (400 units ml^{-1}), IL-9 (40 units ml^{-1}), NGF (500 ng ml^{-1}).

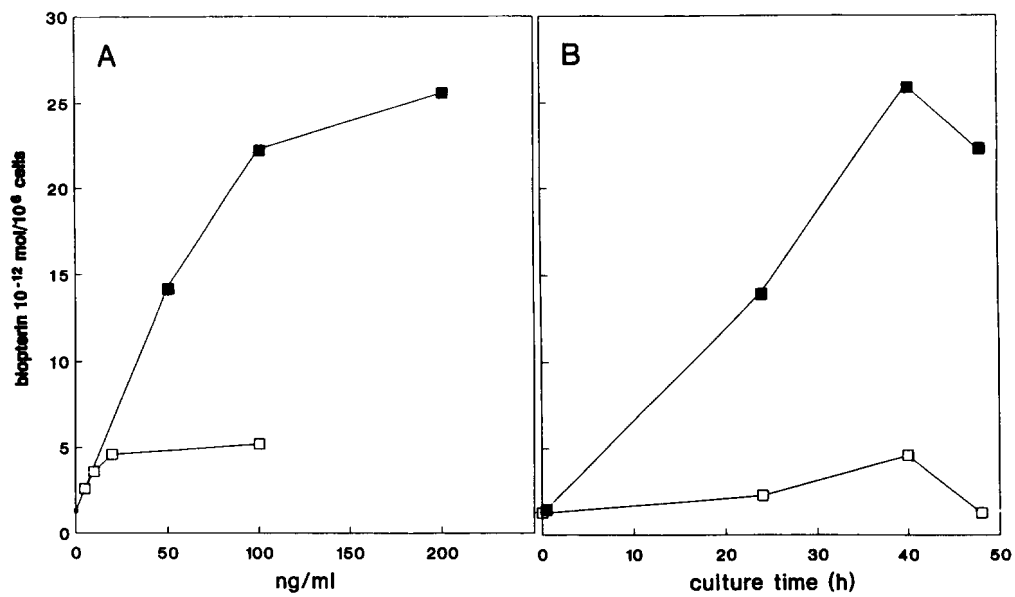

FIG. 2. **Production of biopterin in murine BMMC cultured with IL-3 or KL.** *A*, effect of graded concentrations of IL-3 and KL; *B*, time course; IL-3 (□), KL (■). Cells were seeded, supplemented with the growth factors, and harvested as described in the legend to Fig. 1. For biopterin determination, also see legend of Fig. 1. Values represent the total of 6-biopterin plus 7-biopterin.

Cellular Production of 5-Hydroxy-L-tryptophan—During exposure to the experimental conditions, the production of 5-hydroxytryptophan from L-tryptophan increased in a linear manner. As calculated from the regression analysis, murine BMMC grown with IL-3 or with KL produced 1.07 and 1.42 pmol of 5-hydroxytryptophan/h/10^6 cells, respectively (Fig. 5). In cells that were cultured with the combination of both cytokines, the production of 5-hydroxytryptophan was increased to 3.21 pmol/h/10^6 cells.

Cellular Production and Release of Serotonin—The joint up-regulation of GTP cyclohydrolase and tryptophan 5-monooxygenase activities by KL suggested that this cytokine is highly significant for the regulation of serotonin biosynthesis in murine BMMC. Fig. 6 shows that indeed maximum serotonin production was performed by the cells that had been induced by the concerted action of both IL-3 and KL. In cells grown with KL alone, serotonin production was reduced to 38% and in those cultured with IL-3 alone to 5%, respectively. The data, moreover, demonstrate that in the supernatant of BMMC cultured with IL-3 alone, only traces of serotonin above background levels could be detected. In the presence of KL applied either alone or in combination with IL-3, 45 and 54%, respectively, of total serotonin were released. Thus, the synergistic effect of IL-3 and KL up-regulates the activity of

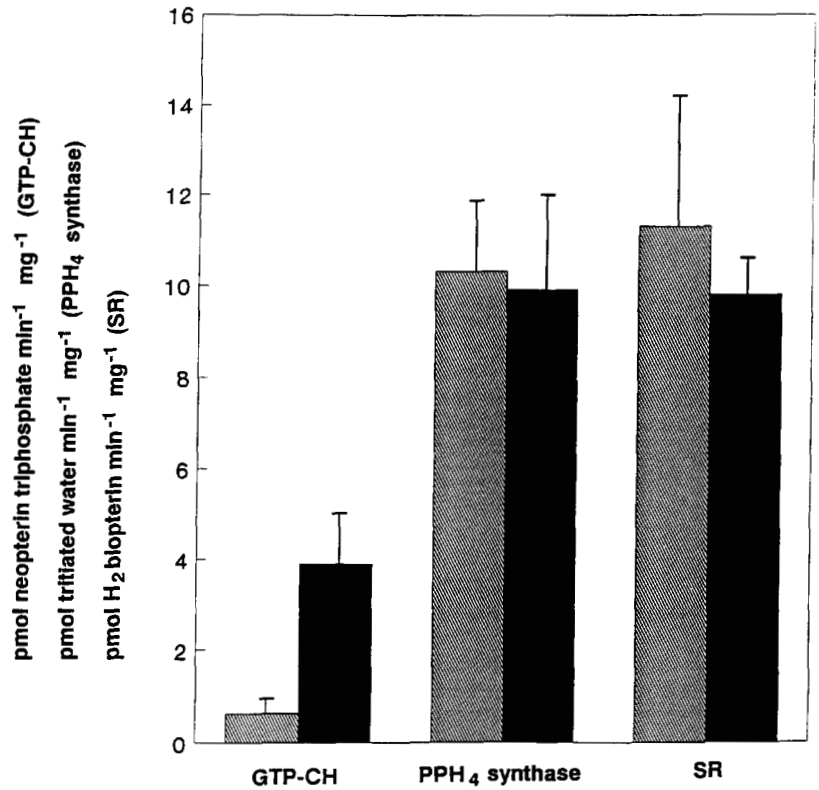

FIG. 3. **Activities of the (6*R*)-H_4biopterin-synthesizing enzymes GTP cyclohydrolase I (*GTP-CH*), 6-pyruvoyl-H_4pterin synthase (*PPH_4 synthase*), and sepiapterin reductase (*SR*) in murine BMMC cultured in the presence of IL-3 or KL.** Cells were seeded, supplemented with the growth factors, and harvested as described in the legend to Fig. 1. ▨, IL-3; ■, KL. Values are means ± S.D. (T; n = 3).

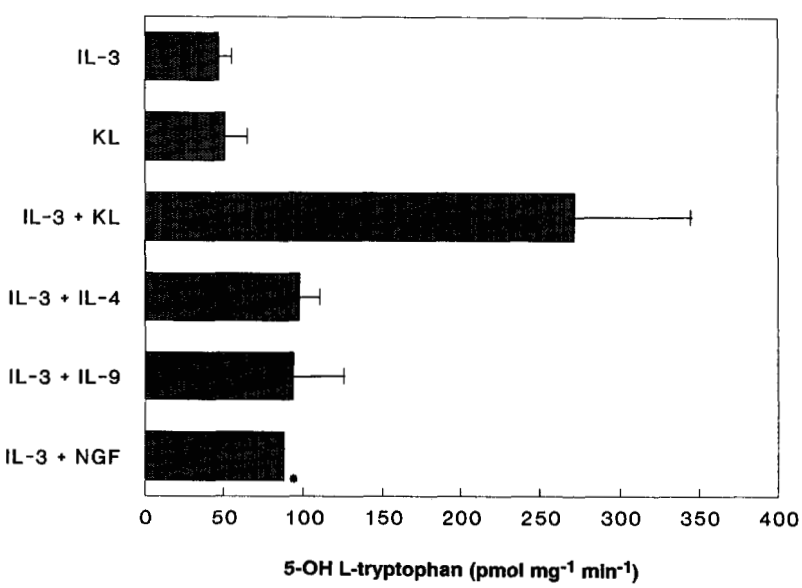

FIG. 4. **Activity of tryptophan 5-monooxygenase in murine BMMC cultured in the presence of different growth factors.** Cells were seeded, supplemented with the growth factors, and harvested as described in the legend to Fig. 1. Values are means ± S.D. (T; n = 6). *, means of two determinations.

TABLE I

Activity of aromatic L-amino acid decarboxylase in murine BMMC cultured in the presence of IL-3, KL, or both growth factors

The cells were seeded, supplemented with the growth factors, and harvested as described in the legend of Fig. 1.

Growth factor	Exp. 1	Exp. 2	Exp. 3	Exp. 4
	nmol serotonin mg^{-1} min^{-1}			
IL-3	2.3	2.0	2.2	3.2
KL	2.7	2.2	1.6	2.9
IL-3 + KL	2.5	3.0	1.8	2.9

tryptophan 5-monooxygenase, resulting in a 20-fold increase in serotonin formation as compared to IL-3 alone. Finally, KL *per se* effects release of the mediator, a process that is only minimal in IL-3-grown cells.

DISCUSSION

In this study, we have identified novel functional roles for KL and IL-3. Among a panel of growth factors that induce proliferation in murine BMMC, interaction of KL with its tyrosine kinase receptor selectively enhances the synthesis of (6*R*)-H_4biopterin through up-regulation of GTP cyclohydrolase I activity, leaving the subsequent enzymes of H_4biopterin

synthesis unaffected. This provides optimum cofactor concentrations for hydroxylation of tryptophan. Moreover, in synergistic action with IL-3, KL up-regulates the activity of tryptophan 5-monooxygenase, without affecting L-amino acid oxidase activity. Fig. 7 summarizes the novel role of KL and its concerted action with IL-3. The figure provides a coherent view of serotonin biosynthesis and its control.

The enzymatic data fully explain the pattern of 6-biopterin and 7-biopterin in murine BMMC insofar as KL *per se* only up-regulates the levels of (6*R*)-H_4biopterin, the end product of the biosynthetic pathway. Combination with IL-3 has no effect on total biopterin amount but increases the portion of 7-biopterin from 8.0 to 35.6% (Fig. 1). The 7-isomer is only formed during ongoing hydroxylation of the aromatic amino acid, as was shown in case of the phenylalanine 4-monooxygenase reaction in liver (3) and the hydroxylation of tryptophan in murine BMMC (2). In consequence, up-regulation of tryptophan 5-monooxygenase activity, which depends on both KL and IL-3 (Fig. 4), markedly increased the portion of the 7-isomer as compared to cells that were grown with KL alone. This isomer is a potential inhibitor of aromatic amino acid hydroxylase (49) and may thus serve for final down-regulation of ligand-induced serotonin production in murine BMMC.

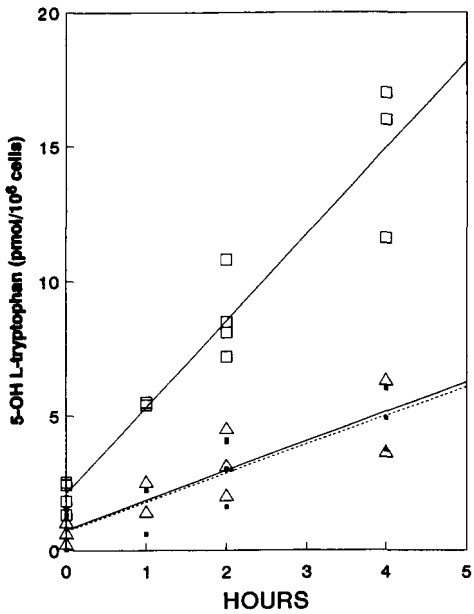

FIG. 5. **Synthesis of 5-hydroxytryptophan in murine BMMC cultured in the presence of IL-3, KL, or both growth factors.** Cells were seeded and supplemented with the growth factors as described in the legend to Fig. 1. After 22 h they were subjected to the conditions described under "Experimental Procedures." ⊡, IL-3; △, KL; □, IL-3 + KL. Regression coefficients/correlation coefficients were as listed (IL-3: 1.07/0.87; KL: 1.41/0.86; IL-3 + KL: 3.21/0.96).

It has been reported previously (13, 32–34) that KL induces and/or enhances the release of serotonin and histamine by murine and human mast cells. The efficiency of this growth factor-mediated increase, however, was generally limited to a 20–30% increase, as compared to control cells. The experimental conditions obviously provided only limited supply with substrates for serotonin synthesis. In our studies, saturating tryptophan supply avoided this limitation and we found that KL increased the release of serotonin from virtually nonexistent levels (in IL-3-grown cells) to about 50% of total serotonin production. It may be suggested that previous replenishment of storage sites, *e.g.* granules, is required prior to a maximum release.

The up-regulation of GTP cyclohydrolase and tryptophan 5-monooxygenase activities and, thus, of serotonin synthesis by KL alone or in combination with IL-3, is necessarily restricted to cells that express the specific receptor(s). In the mouse mutant genotypes W/W^v and Sl/Sl^d, expression of functional kit receptors (50) and KL (51), respectively, is severely impaired. Nevertheless, H_4biopterin and serotonin synthesis in brain as well as H_4biopterin production and phenylalanine degradation in liver were unaffected (data not

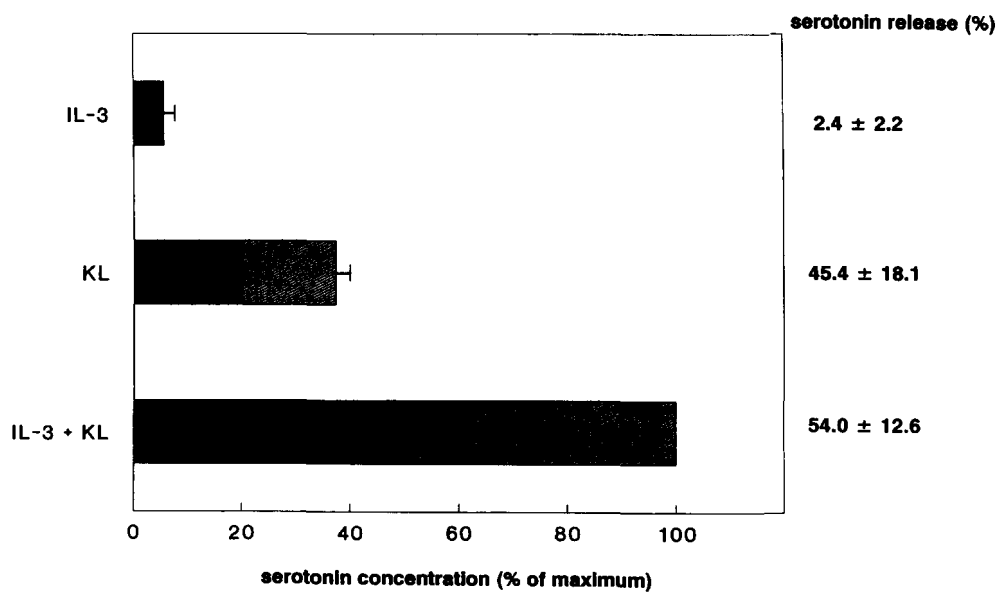

FIG. 6. **Synthesis and release of serotonin in murine BMMC cultured in the presence of IL-3, KL, or both growth factors.** Cells were seeded and supplemented with the growth factors as described in the legend to Fig. 1. After 22 h they were subjected to the conditions as described under "Experimental Procedures." ■, cellular serotonin; ▨, released serotonin. Values are means ± S.D (**T**). In individual experiments total serotonin concentrations (intracellular + supernatant serotonin) were normalized to the maximum serotonin concentrations determined in cultures containing both IL-3 and KL. Maximum total production obtained with the combination of IL-3 and KL was 3.0, 2.4, and 7.2 pmol/h/10^6 cells in three different experiments.

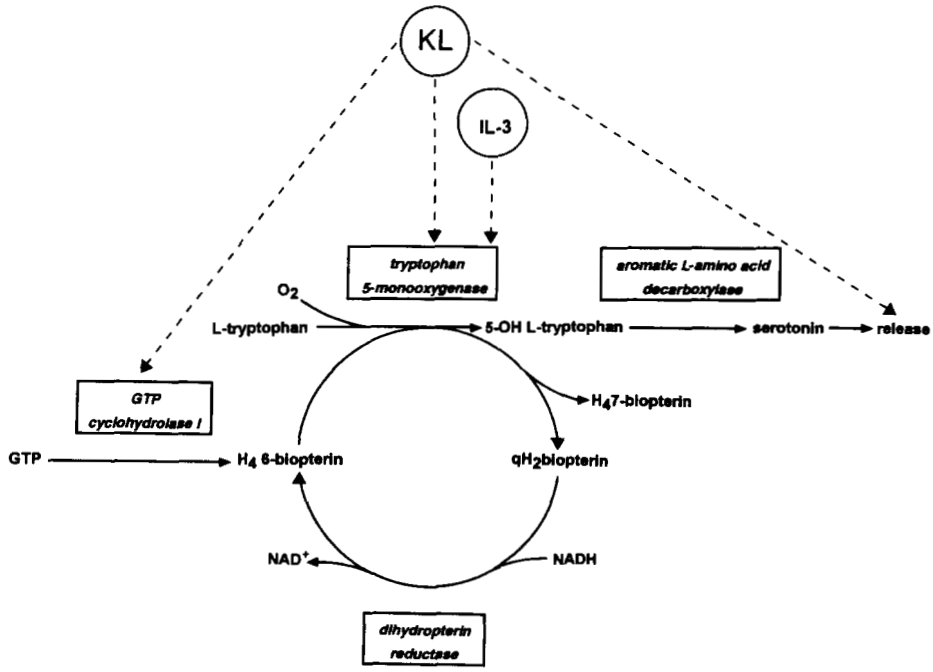

FIG. 7. **Control of serotonin synthesis and release in murine BMMC.** *Dashed arrows*, target of growth factor-mediated enhancement; *solid arrows*, reaction sequence. Formation of quinonoid H_2biopterin during the hydroxylation reaction and its recycling to $H_4$6-biopterin is described in Ref. 1, and the formation of $H_4$7-biopterin during the hydroxylation of tryptophan in Ref. 2. The *de novo* synthesis of $H_4$6-biopterin from GTP is reviewed in Ref. 6.

shown). Theoretically, a residual function of the ligand/receptor system in these mutants may be sufficient for persistence of the relevant enzyme activities. It has been shown previously that IFN-γ causes an up-regulation of GTP cyclohydrolase activity in human cells associated with the immune system that correlates with increased mRNA expression (18, 19). In the light of these data, it appears more likely that the relevant enzymes are subjected to a cell lineage-specific regulation. This is supported by the fact that two species of tryptophan 5-monooxygenase mRNA are expressed differently in various tissues and cell lines (52). The tissue-specific expression of multiple forms of a protein kinase-dependent activator protein (53) further contributes to the control of the monooxygenases. Moreover, existence of multiple GTP cyclohydrolase mRNA species and their tissue-specific expression (54, 55) points to a cell lineage-specific control of cofactor synthesis. The question of whether KL induces the expression of specific enzyme isoforms in BMMC or whether this cytokine post-translationally activates tryptophan 5-monooxygenase and/or GTP cyclohydrolase awaits further investigation.

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