

Cord blood-derived human cultured mast cells produce transforming growth factor β_1

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Summary

Background Mast cells frequently accumulate at the site of fibrosis and their contribution has been suspected in the pathogenesis of fibrotic conditions. However, it still remains unknown whether human mast cells synthesize transforming growth factor β_1 (TGF- β_1).

Objective We have investigated whether cord blood-derived human cultured mast cells express messenger RNA (mRNA) for TGF- β and produce bioactive TGF- β_1 .

Methods Mast cells were obtained by culturing mononuclear cells from cord blood in the presence of stem cell factor and interleukin-6. Expression of mRNA for TGF- β_1 was examined by the method of reverse transcriptase-polymerase chain reaction (RT-PCR). Immunocytochemical staining for TGF- β and growth-inhibitory assay using Mv1Lu cells were also performed.

Results The cultured human mast cells constitutively expressed mRNA for TGF- β_1 . With calcium ionophore A23187, the intensity of the PCR-amplified band for TGF- β_1 was not increased. Immunocytochemical staining showed that the cultured mast cells were positive for both latency-associated peptide and activated forms of TGF- β . Bioassay with Mv1Lu cells and R 4-2 mutant cells showed that mast-cell conditioned medium had a bioactivity of TGF- β_1 .

Conclusion Cord blood-derived human cultured mast cells constitutively express mRNA for TGF- β_1 and produce functional TGF- β_1 . Because TGF- β_1 has been shown to be highly fibrogenic, these results may highlight a novel role for human mast cells in tissue fibrosis.

Keywords: mast cell, cord blood, stem cell factor, interleukin-6, transforming growth factor β_1 , reverse transcriptase-polymerase chain reaction, immunocytochemical staining, growth-inhibition assay

Clinical and Experimental Allergy, Vol 29, pp. 105–113. Submitted 6 November 1997; revised 16 December 1997; accepted 5 June 1998.

Introduction

Transforming growth factor β (TGF- β) is a 25-kDa pleiotrophic growth factor that has been shown to inhibit the proliferation of many types of cells, especially those of the epithelial lineage. In addition, TGF- β plays an important role in the pathogenesis of fibrosis [1,2] because it stimulates production of extracellular matrix (ECM) and inhibits

the formation of extracellular proteases. In fact, abnormally large amounts of TGF- β are found to be present in clinical specimens obtained from fibrotic diseases, such as early skin lesions from keloid [3], scleroderma [4,5] and lung fibrosis [6].

Mast cells, which have been recognized as one of the effector cells of immediate-type allergic reactions, may also contribute to the pathogenesis of a variety of non-allergic inflammatory conditions. They frequently accumulate at the site of fibrosis, such as the involved skin of keloid, scleroderma [7] and fibrotic lung tissue [8]. Although contribution of mast cells has been suspected in

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the fibrotic conditions, very few potent mediators capable of stimulating fibroblast growth or ECM deposition have been identified. One of the potential mast cell-derived mediators which possess a fibrogenic activity is tryptase, and it has recently been found to stimulate fibroblast proliferation and ECM production [9–11]. Mast cells have recently been recognized as a potential source of cytokines [12–18], however, little information is available about human mast cells as a source of TGF- β .

We have succeeded recently in obtaining a selective and persistent growth of human mast cells by culturing mononuclear cells from cord blood in the presence of stem cell factor (SCF) and interleukin-6 (IL-6) [19–21], and they may help in studying the functional properties of human mast cells. In the present study, we demonstrated for the first time that cord blood-derived human-cultured mast cells constitutively express mRNA for TGF- β_1 and produce bioactive TGF- β_1 .

Materials and methods

Cell culture

Hep G2 cells (human hepatocellular carcinoma; RIKEN cell bank, Tsukuba, Japan) were maintained in Dulbecco's modified Eagle medium (DMEM; Sankou Pure Chemicals, Tokyo, Japan) supplemented with 10% fetal calf serum (FCS; Cytosystems, Castle Hill, Australia). PC3 cells (human prostate adenocarcinoma; American Type Culture Collection, Rockville, MD, USA) were maintained in Ham's F-12K medium (Dainippon Pharmaceutical, Osaka, Japan) with 10% FCS. Mv1Lu cells (CCL-64; mink lung epithelial cells) and R 4-2 mutant cells, which lack functionally active TGF- β_1 receptors [22], were generously provided by Drs K. Miyazono and M. Kato (The Cancer Institute, Japanese Foundation for Cancer Research, Tokyo, Japan). They were maintained in the same medium for Hep G2 cells. All cells were cultured at 37°C in 5% CO₂.

Culture of cord blood-derived human mast cells

Cord blood-derived human cultured mast cells were obtained as described [19–21] with some modifications. Briefly, mononuclear cells were obtained from heparin-treated umbilical cord blood after centrifugation with Ficoll-paque (Pharmacia Biotech, Uppsala, Sweden), suspended in Media I (modified DMEM component plus 10 μ g/mL insulin, 10 μ g/mL transferrin, 5×10^{-5} mol/L 2-mercaptoethanol, 25 mmol/L HEPES and 2.6 ng/mL NaSeO₃; IBL Men-eki Seibutsu Kenkyujo, Gunma, Japan) containing 10% heat-inactivated FCS, 100 ng/mL human recombinant SCF and 50 ng/mL human recombinant IL-6 (kindly provided by Kirin Brewery Co., Maebashi, Japan), and cultured

in a 37°C 5% CO₂ humidified incubator. Non-adherent cells were passaged in the same medium weekly. The purity of mast cells was determined by staining with May–Grünwald–Giemsa reagents (Muto Pure Chemicals, Tokyo, Japan) and by immunocytochemical staining for mast cell specific proteases, as described below.

Immunocytochemical staining

Cord blood-derived human cultured mast cells were immunocytochemically stained for tryptase and chymase as described [19–21] based on the method reported by Irani *et al.* [23]. Briefly, the cultured mast cells were spun down onto glass slides with a cytocentrifuge (Cytospin 3; Shandon, Pittsburgh, PA, USA) at 20 *g* for 5 min, dried, and fixed with Carnoy's fixative (60% ethanol, 30% chloroform and 10% glacial acetic acid) for 15 min at room temperature. After blocking the endogenous peroxidase with 0.6% H₂O₂ for 30 min, the slides were incubated with biotin-conjugated mouse monoclonal antibody (MoAb) specific to chymase (Chemicon International, Temecula, CA, USA) at 4°C overnight. The slides were then incubated with horseradish peroxidase-conjugated streptavidin (Sigma Chemical, St Louis, MO, USA) for 1 h at room temperature. The colour developed by H₂O₂ and 9-amino-3-ethylcarbazole (Sigma) and positive cells were stained with a brown colour. The slides were further incubated with anti-tryptase MoAb conjugated with alkaline phosphatase (Chemicon) at 4°C overnight. The colour developed by levamisole, Naphthol AS-MX and Fast Blue (Sigma) and positive cells were stained with a blue colour.

To detect TGF- β on the cultured mast cells, the slides were incubated with either goat anti-human TGF- β_1 latency-associated peptide (LAP) antibody (R&D systems, Minneapolis, MN, USA) or rabbit anti-human TGF- β antibody (R&D systems) for 1 h at room temperature. The slides were then incubated with each second antibody conjugated with fluorescein isothiocyanate (FITC) for 1 h at room temperature. The slides were observed using fluorescence microscopy (Olympus, Tokyo, Japan). Immunocytochemical staining for LAP and TGF- β was performed in parallel using Hep G2 cells as positive controls, because they contain abundant TGF- β . To confirm that TGF- β -positive cells were mast cells, the slides were concurrently incubated with mouse MoAb specific to tryptase (Chemicon) at 4°C overnight, and then with tetramethyl rhodamine isothiocyanate (TRITC)-labelled goat anti-mouse IgG antibody (Cappel, Durham, NC, USA). Normal goat IgG or normal rabbit IgG instead of each specific primary Ab was used as a negative control.

Activation of mast cells

A part of the cultured mast cells was used for reverse

transcriptase-polymerase chain reaction (RT-PCR) analysis and the remainder for histamine assay.

Preliminary studies showed that calcium ionophore A23187 effectively stimulated the cultured mast cells and histamine were released in a dose-dependent manner from 0.003 $\mu\text{g/mL}$ to 0.3 $\mu\text{g/mL}$ calcium ionophore A23187 [21]. In this study, 0.3 $\mu\text{g/mL}$ calcium ionophore A23187 (Sigma) in DMSO (the final concentration of DMSO in the conditioned medium was 0.1%) was used as a stimulator. Briefly, after washing with DMEM without FCS three times, the cells were resuspended in the medium with 1% bovine serum albumin (BSA). The cells (1×10^6) were preincubated at 37°C for 5 min, and then incubated at 37°C with calcium ionophore A23187 for up to 24 h as indicated. Experiments incubated for 24 h at 37°C without the stimulation were carried out in parallel as a control.

Histamine measurement

The reaction was stopped by adding 4°C DMEM to the cell suspension, and the cells were centrifuged at 200 *g* for 10 min at 4°C. Perchloric acid was added to both the supernatant and the cell pellet, and the proteins were removed by centrifugation at 2000 *g* for 10 min. Total histamine was assayed in samples after two cycles of freezing and thawing.

Histamine was measured by high-performance liquid chromatography (HPLC) as described [21]. Briefly, test samples were applied to a cation exchange column (Hitachi no. 3011-C, 4 $\times$ 250 mm; Hitachi, Tokyo, Japan) at 50°C, and eluted by a mixture of 0.25% KH_2PO_4 and methanol/water (70:30) at a flow rate of 1 mL/min. The elute was then reacted with *o*-phthal dialdehyde (Sigma) solution (4 mmol/L in H_2O), and detected by a fluorometer (Hitachi F-1050) at excitation 375 nm and emission 460 nm. The data were analysed by a data integrator (Hitachi D-2500). Data on the amount of histamine released in the supernatant were expressed as a net percentage of the total histamine content in the specimen.

mRNA preparation

Total RNA was extracted by the modified method of acid guanidinium thiocyanate (GT)-phenol-chloroform extraction as described [24]. Briefly, the cells (1×10^6) were spun down and suspended with GT solution. Sodium acetate at 2 mol/L pH 4, water-saturated phenol and chloroform isoamylalcohol mixture (49:1) were sequentially added. After centrifugation, the aqueous phase was transferred to a fresh tube and eluted with isopropanol. The RNA pellet was then precipitated in 75% ethanol and the purified RNA was resuspended in distilled water.

Primers

Oligonucleotide primers were synthesized by Kurabou Co. (Kurashiki, Japan) and their sequences are summarized as follows; TGF- β_1 : 5' primer, AGCCC TGGAC ACCAA CTATT GCT; 3' primer, CGGGC CCTGC CCCTA CATT GG; TGF- β_2 : 5' primer, GATTT CCATC TACAA GACCA CGAGG GACTT GC; 3' primer, CAGCA TCAGT TACAT CGAAG GAGAG CCATT CG; TGF- β_3 : 5' primer, CTGGC GGAGC ACAAC GAACT; 3' primer, CGACG CGGTG TGGGG GAATC AT [25]; human granulocyte colony-stimulating factor receptor (G-CSF R): 5' primer, ACCTG GGCAC AGCTG GAGTG G, 3' primer, CAGGC TGCTG TGAGC TGGGT C-3'; and human monocyte colony-stimulating factor receptor (c-fms): 5' primer, CGAGG ACCAG GACGC ACTAC T, 3' primer, GTTGT TGTGT TGGAG GAAGA C [21]. Primers used for tumour necrosis factor α (TNF- α) and β -actin were commercially available from Stratagene (La Jolla, CA, USA).

RT-PCR

One microgram of each RNA was reverse-transcribed to cDNA with Oligo(dT) primer and SuperScript Preamplification System (GIBCO-BRL, Grand Island, NY, USA), according to the manufacturer's instructions. Reaction mixtures, human bone marrow cDNA (Clontech Laboratories, Palo Alto, CA, USA) or non-RT samples were added to a PCR buffer containing 1.5 mmol/L MgCl_2 , 1 U Taq polymerase (GIBCO-BRL), 0.2 mmol/L of each dNTP (Pharmacia Biotech) and the relevant primer (5 $\mu\text{g/mL}$ of each primer) to a total volume of 100 μL . Reaction mixtures were amplified using 28 cycles each on TGF- β_1 and β -actin, and

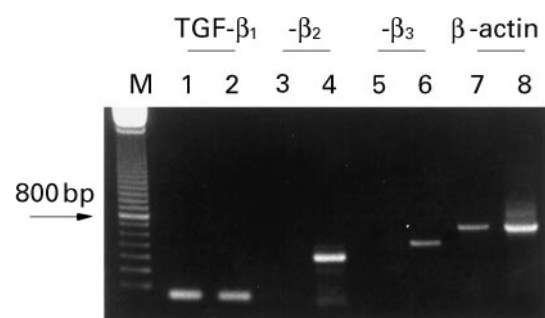


Fig. 1. The messages for TGF- β_1 , - β_2 , and - β_3 from cord blood-derived human cultured mast cells (RT-PCR analysis). Lane M is 100 bp ladder with the 800 bp as a bright band; lanes 1, 3, 5 and 7, human cultured mast cells; lanes 2, 4, 6 and 8, PC3 cells as positive controls. The message for TGF- β_1 with the expected size 157 bp was detected in the cultured mast cells, whereas those for TGF- β_2 (503 bp) and - β_3 (535 bp) were not detected in the mast cells.

35 cycles on others under the following conditions; denaturation at 95°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 1 min. Final extension was at 72°C for 7 min. To visualize the PCR products, they were analysed by an electrophoresis in 1% agarose gel containing 0.5% ethidium bromide (Sigma) and compared with DNA reference markers (100 bp ladder; Pharmacia Biotech). The fluorescent DNA bands were visualized by UV excitation.

Assay for growth-inhibitory activity

Bioactivity of TGF- β in the conditioned medium from calcium ionophore A23187-stimulated cultured mast cells was monitored as an inhibition of DNA synthesis in Mv1Lu cells using a [^3H]thymidine incorporation assay. Namely,

Mv1Lu cells were seeded in triplicate into 24-well tissue culture plates (Corning Glass, Corning, NY, USA) at a cell density of 1×10^5 cells/mL in DMEM containing 1% FCS. Twenty-four hours after seeding, the medium was changed and the test samples were added. After a further 24-h incubation, 0.3 μCi of [^3H]thymidine (6.7 Ci/mmol, New England Nuclear, Boston, MA, USA) was added and the incubation proceeded for another 2 h. The incorporated ^3H radioactivity was counted as described [26,27]. Growth-inhibitory activities of TGF- β_1 purified from human platelets (R&D systems) were also measured under the same procedures as the controls.

To investigate a blocking of the effect of TGF- β , TGF- β neutralizing antibody (R&D systems) at a concentration of 1 $\mu\text{g/mL}$ or 10 $\mu\text{g/mL}$ was added to the cultures. Normal rabbit IgG was used as a negative control.

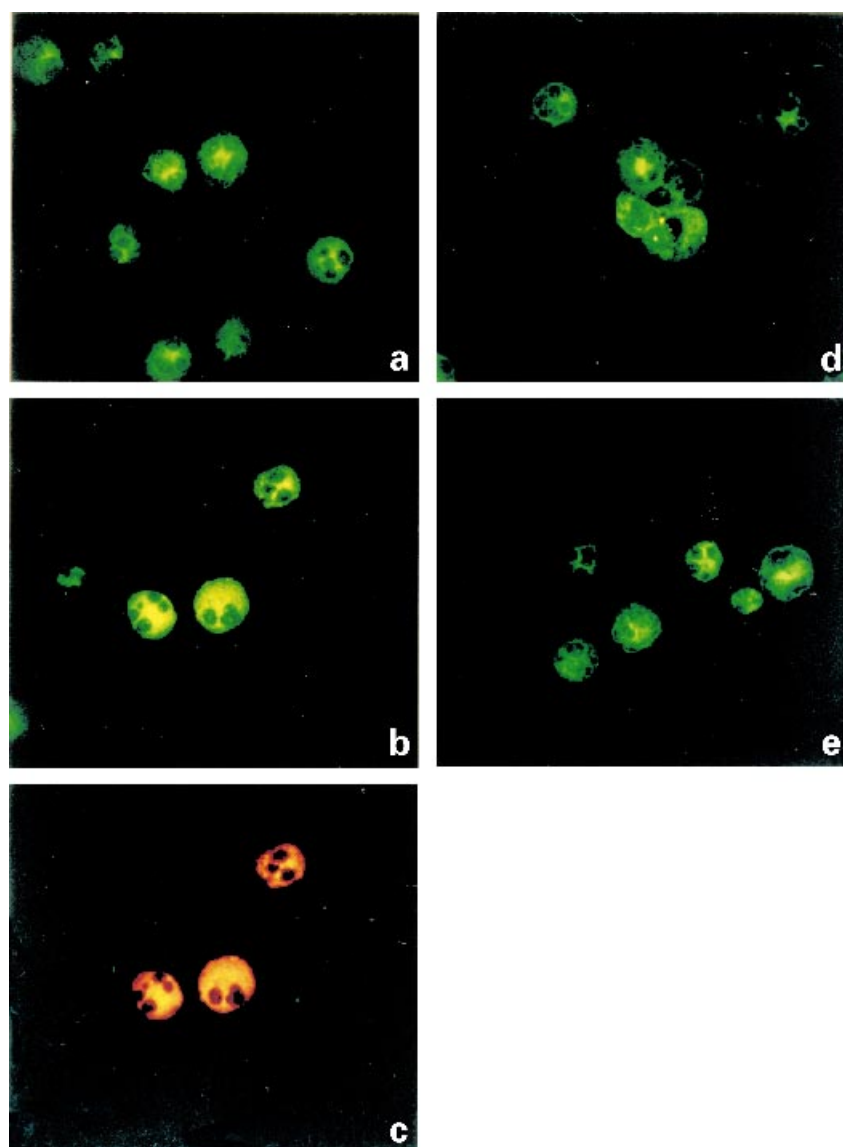


Fig. 2. Immunocytochemical staining for LAP (a) and activated form of TGF- β (b) in human cultured mast cells. Detection of TGF- β was investigated by indirect immunofluorescence microscopy using anti-LAP or anti-TGF- β antibody, and FITC-labelled second antibody. For confirmation that TGF- β positive cells were the mast cells, the slides for TGF- β were concurrently incubated with MoAb specific to tryptase and TRITC-labelled second antibody (c). Immunocytochemical stainings were performed in parallel with Hep G2 cell as a positive control (d, e). The cultured mast cells, which were stained with MoAb specific to tryptase, were identified to be positive for both LAP and activated form of TGF- β .

Statistics

Statistical analyses for the histamine measurement were performed with the analysis of variance (ANOVA) followed by Dunnett's multiple comparison test.

Results

General description

In the presence of 100 ng/mL SCF and 50 ng/mL IL-6, mast cells appeared as early as within 2 weeks of culture. After 8 weeks, immunocytochemical staining showed that all cells were positive for tryptase and/or chymase, indicating that the purity of mast cells reached virtually 100%. The population of chymase-positive cells varied depending on cord blood specimens, and chymase-positive cells were $22.3 \pm 9.2\%$ (mean $\pm$ SEM, $n = 6$) among tryptase-positive cells in the period of 12 weeks.

Expression of mRNA for TGF- β_1 from cord blood-derived human cultured mast cells

It has been demonstrated that there are five distinct forms of TGF- β [28–32]. Among them, TGF- β_1 , - β_2 , and - β_3 have been detected in mammalian cells [33]. TGF- β_1 -specific primers yielded the expected product of 157 bp in the human cultured mast cells (Fig. 1). In the absence of RT, PCR yielded no product, indicating the absence of genomic DNA contamination (data not shown). On the other hand, TGF- β_2 , and - β_3 mRNA were not detected in the cord blood-derived human cultured mast cells. The expected 503-bp and 535-bp products, respectively, were observed in PC3 cells, indicating that the lack of RT-PCR products was not due to defective primers. In addition, RT-PCR products for G-CSF R and *c-fms* were not detected in the human cultured mast cells, indicating that the message for TGF- β_1 was not possibly contaminated cells but by the mast cells (data not shown).

Immunocytochemical staining for TGF- β and LAP

Indirect immunofluorescence microscopy was used to detect whether the cord blood-derived human cultured mast cells were the source of TGF- β . Because TGF- β is known to be synthesized as a latent complex from the producer cells, antibodies to LAP as well as TGF- β were used in the present study. The cultured human mast cells, which were positively stained for tryptase, were identified to be positive for both LAP and activated form of TGF- β by immunocytochemical staining (Fig. 2). With normal goat IgG or normal rabbit IgG instead of each specific primary antibody, the fluorescence on both the cultured mast cells and Hep G2 cells was not detected.

Kinetics of histamine release and expression of mRNA for TGF- β_1

A serial RT-PCR analysis of TGF- β mRNA was performed in parallel with the measurements of histamine release into the supernatant. With 0.3 μ g/mL calcium ionophore A23187, a significant amount of histamine, $76.1 \pm 6.7\%$ (mean $\pm$ SEM, $n = 5$), was released into the supernatant 3 h after the stimulation and showed a plateau up to 24 h. However, the intensity of PCR-amplified band for TGF- β_1 was not increased with the stimulation for up to 24 h. On the other hand, the intensity of the band for TNF- α was increased with a peak at 6 h (Fig. 3), suggesting that A23187 effectively stimulated the mast cells and induced the expression for TNF- α mRNA. TGF- β_2 , and - β_3 mRNA were not detected in the calcium ionophore A23187-stimulated human cultured mast cells.

Effect of the conditioned medium from calcium ionophore A23187-stimulated mast cells on Mv1Lu cell growth

To investigate whether TGF- β_1 which may be produced from the cultured mast cells can contribute biologically, a growth-inhibitory assay for Mv1Lu cells was performed with the supernatant from calcium ionophore A23187-stimulated mast cells. TGF- β has been shown to be secreted

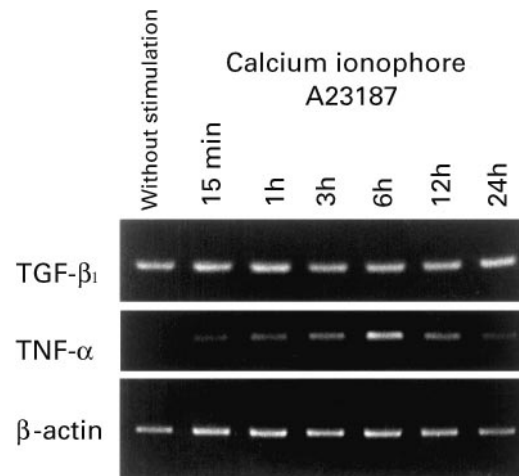
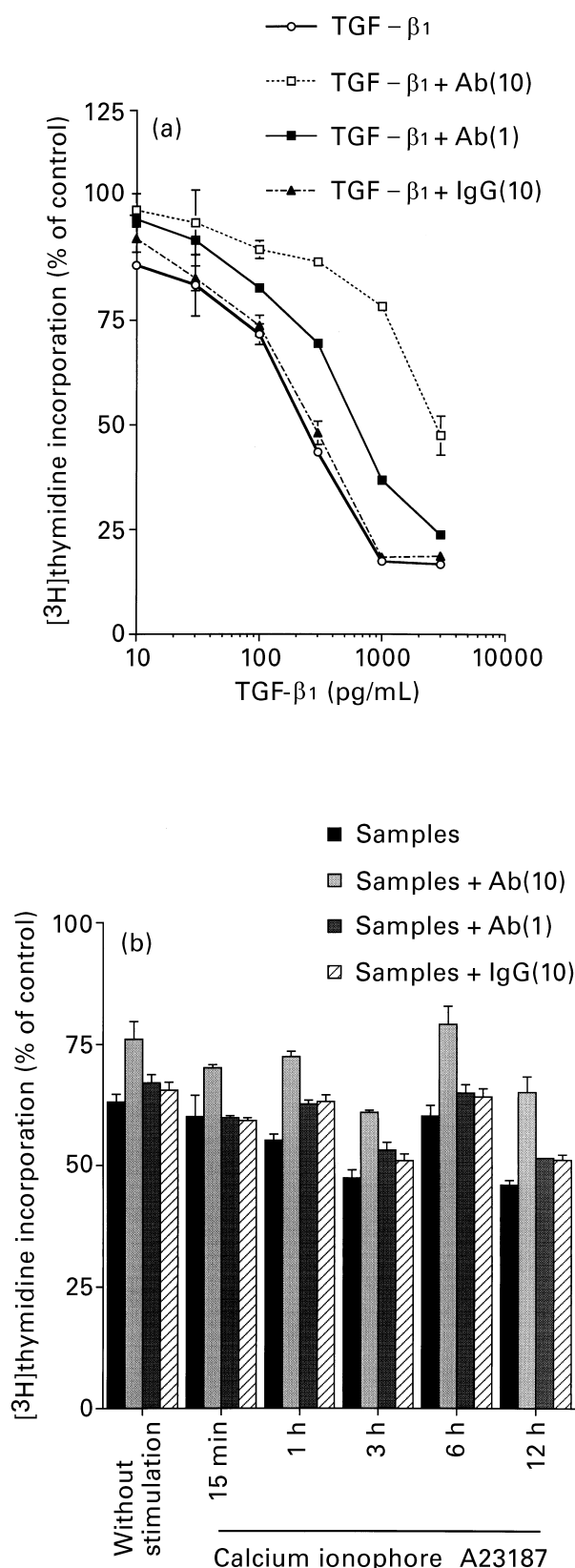


Fig. 3. Kinetics of TGF- β_1 and TNF- α mRNA expression in human cultured mast cells stimulated with calcium ionophore A23187 (RT-PCR analysis). The cells were harvested with 0.3 μ g/mL A23187 in the presence of SCF but without FCS, and a serial RT-PCR analysis of TGF- β_1 and TNF- α mRNA at the indicated time was performed. Lane 1, human cultured mast cells without the stimulation; lanes 2 to 7, the mast cells stimulated with calcium ionophore A23187 for indicated times. The intensity of the PCR-amplified band for TGF- β_1 was not increased, whereas that for TNF- α was increased with a peak at 6 h with the stimulation.



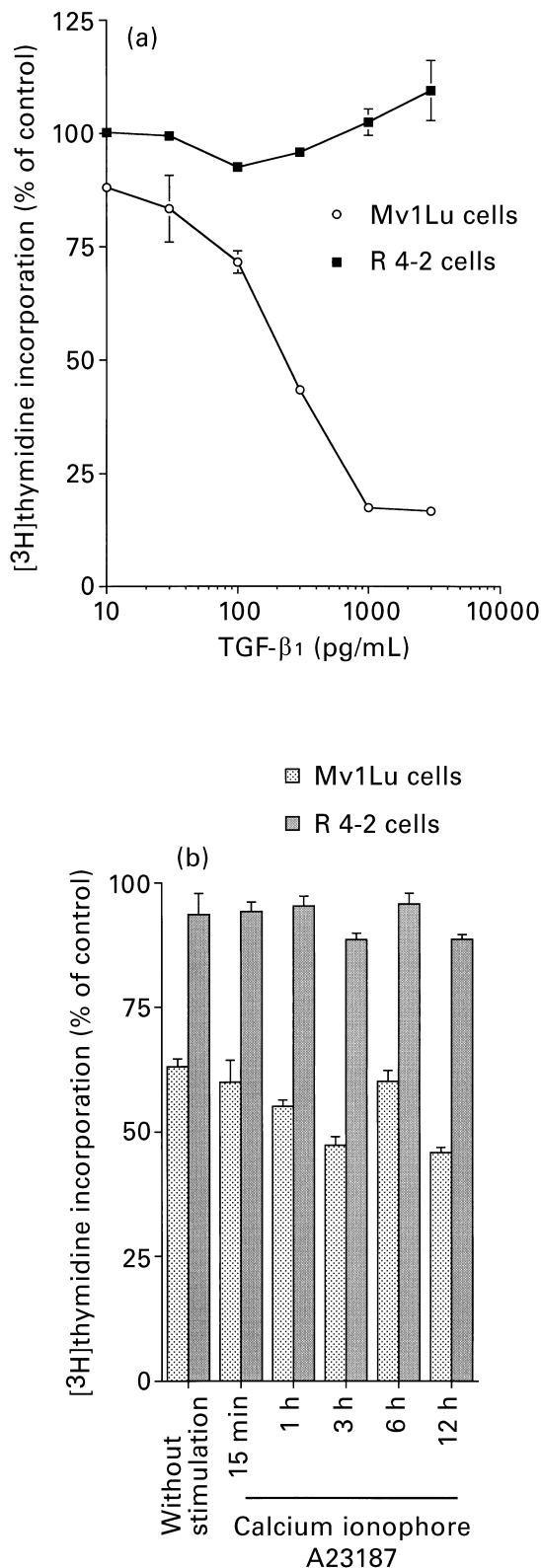
from the producer cells as a biologically latent form, and it can be activated chemically by treatment with acid, alkali or heat [26]. In this study, heat treatment of the samples at 85°C for 15 min was found to inhibit the growth of Mv1Lu cells, whereas non-treated samples showed minimal growth inhibition (data not shown).

The control study showed that TGF- β_1 purified from human platelets inhibited the DNA synthesis on Mv1Lu cells in a concentration-dependent manner (Fig. 4a). Neutralizing antibody against TGF- β has an inhibitory effect on both TGF- β_1 and - β_2 . The antibody, but not isotype matched IgG, abolished the inhibitory effect on TGF- β_1 in a dose-dependent manner (Fig. 4a). As shown in Fig. 4b, the heat-treated samples of the supernatant from the cultured mast cells showed the growth inhibitory activities on Mv1Lu cells. The inhibitory effects from the cultured mast cells were partially blocked by the TGF- β neutralizing antibody in a concentration-dependent manner.

Effect of TGF- β_1 from calcium ionophore A23187-activated mast cells on Mv1Lu cell growth

DNA synthesis in Mv1Lu cells was inhibited by an addition of mast cell-conditioned medium, whereas its growth inhibitory effect was not completely blocked by the TGF- β neutralizing antibody. The bioassay employed in the present study with Mv1Lu cells as responders is sensitive to any isoform of TGF- β . However, the absence of expression for TGF- β_2 and - β_3 mRNA in the cord blood-derived human cultured mast cells by a RT-PCR analysis suggests that the

Fig. 4. Analysis of the biological activity of released TGF- β by Mv1Lu cell growth-inhibition assay. (a) As a control study, growth-inhibitory activity of TGF- β_1 purified from human platelets on Mv1Lu cells was investigated. To investigate the blocking of the effect of TGF- β , TGF- β neutralizing antibody at 1 μ g/mL or 10 μ g/mL or normal rabbit IgG as a negative control was added to the cultures. Each point represents the mean $\pm$ SEM of three separate experiments. TGF- β_1 purified from human platelets inhibited the DNA synthesis on Mv1Lu cells in a concentration-dependent manner. The neutralizing antibody against TGF- β , but not isotype-matched IgG, abolished the inhibitory effect of TGF- β_1 . (b) The cord blood-derived mast cells were incubated with 0.3 μ g/mL A23187 in the presence of SCF but not FCS for the indicated time. Heat-treated samples of the supernatant from the activated human mast cells (1×10^6) were diluted to 500 μ L with DMEM containing 1% BSA, and analysed by Mv1Lu cell growth-inhibition assay in triplicate. TGF- β neutralizing assay with the TGF- β neutralizing antibody was also performed. Each column represents the mean $\pm$ SEM of three separate experiments. The growth inhibitory activity observed in the heat-treated samples was partially blocked by the TGF- β neutralizing antibody in concentration-dependent manners.



human cultured mast cells may only produce TGF- β_1 , which inhibits DNA synthesis of Mv1Lu cells. To confirm this growth inhibitory effect was due to TGF- β_1 released from the mast cells, the effects of TGF- β_1 on R 4-2 cells, which are mutant Mv1Lu cells and lack functionally TGF- β_1 receptors [22], were further investigated. The control study showed that TGF- β_1 purified from human platelets inhibited DNA synthesis of Mv1Lu cells, but not R 4-2 mutant cells (Fig. 5a). As shown in Fig. 5b, the growth inhibitory activity of the heat-treated samples from the cultured mast cells were not observed with R 4-2 cells, indicating that an inhibitory activity shown against Mv1Lu cells was due to TGF- β_1 derived from the human cultured mast cells.

Discussion

TGF- β is a cytokine which plays a central role in fibrosis [1,2]. TGF- β is synthesized by fibroblasts and macrophages, and can stimulate its own production in an autocrine/paracrine fashion [34,35]. Zugmaier *et al.* [36] reported that an injection of a large amount of TGF- β into nude mice caused a generation of interstitial fibrosis. In an experimental model of lung fibrosis, the areas containing activated fibroblasts showed high levels of TGF- β [6].

The number of mast cells has been shown to be increased in tissue fibrosis, such as the involved skin from keloid, scleroderma [7] and fibrotic lung tissue [8]. Interestingly, a large amount of TGF- β has been demonstrated to be present in these tissues [3–6]. However, little information is available about mast cells as a source of this cytokine. Murine mast cells [37,38] and dog mastocytoma cell line [39] have been reported to produce TGF- β_1 . Although human mast cell line HMC-1 has been shown to express mRNA for TGF- β_1 [40], it is still unknown whether human mast cells may produce a bioactive protein of TGF- β_1 .

Several different isoforms of TGF- β , such as TGF- β_1 , - β_2 and - β_3 [33], have been reported to be present in mammalian

Fig. 5. Effect of TGF- β_1 on growth-inhibition assay with Mv1Lu cells. (a) As a control study, growth-inhibitory activities of TGF- β_1 purified from human platelets on Mv1Lu cells and R 4-2 mutant cells were investigated. Each point represents the mean $\pm$ SEM of three separate experiments. The purified TGF- β_1 inhibited DNA synthesis on Mv1Lu cells, but not on R 4-2 mutant cells. (b) Heat-treated samples of the supernatant from 0.3 μ g/mL A23187-stimulated mast cells were analysed in triplicate by growth-inhibition assay using Mv1Lu cells and R 4-2 mutant cells as responders. Each column represents the mean $\pm$ SEM of three separate experiments. The growth inhibitory activity was observed in Mv1Lu cells but not in R 4-2 mutant cells, which lack functionally active TGF- β_1 receptor.

cells. TGF- β_1 is the best-studied protein among the three differentially expressed and regulated human TGF- β isoforms. TGF- β_1 , which is a homodimer of the 112-amino acid carboxyl-terminal peptide, is cleaved from the 391-amino acid precursor [28], and LAP is derived from a dimer of the remaining amino-terminal pro-segment. In the present study, immunocytochemical stainings showed that the cultured human mast cells were positive for both LAP and activated forms of TGF- β .

In this study, expression of mRNA for TGF- β in cord blood-derived human cultured mast cells was examined by a RT-PCR analysis. The message for TGF- β_1 was detected in the cultured mast cells, whereas those for TGF- β_2 and - β_3 were not detected. The cultured human mast cells constitutively expressed mRNA for TGF- β_1 . The intensity of the PCR-amplified band was not increased with calcium ionophore A23187, although a significant histamine release into the supernatant was observed by the stimulation. However, bioassays with Mv1Lu cells and R 4-2 mutant cells as responders indicated that the supernatants from activated human mast cells showed bioactivities of TGF- β_1 . Because mRNA for TGF- β_1 was expressed in cord blood-derived human cultured mast cells constitutively and independently on the stimulation, TGF- β_1 may be released spontaneously from the mast cells into the supernatants during the culture system.

Recent understanding of mast cells as a potential source of cytokines [12–18] has led us to ask whether the assessment of mediators is always an effective measure of the major biological importance of the cells. In the present study, the intensity of the PCR-amplified band for TGF- β_1 was not increased by calcium ionophore A23187, but a significant histamine was released from the mast cells and the intensity of the PCR-amplified band for TNF- α was increased by the stimulation. These results suggest that TGF- β_1 production might be regulated independently of TNF- α production or histamine release.

In conclusion, this study demonstrates for the first time that human mast cells constitutively express mRNA for TGF- β_1 and produce bioactive TGF- β_1 . Because TGF- β_1 has been reported to be highly fibrogenic, human mast cells may contribute to fibrotic reactions by producing TGF- β_1 .

Acknowledgements

We would like to thank Drs Kenji Muraoka and Tatsutoshi Nakahata (Department of Clinical Oncology, Institute of Medical Science, University of Tokyo, Tokyo), Dr Hirohisa Saito (Division of Allergy, National Children's Medical Research Center, Tokyo) and Dr Makoto Yanagida (Pharmaceutical Development Laboratory and Pharmaceutical Research Laboratory, Kirin Brewery Co. Ltd. Maebashi) for their advice and technical support. We would like to

thank Drs Kohei Miyazono and Mitsuyasu Kato (The Cancer Institute, Foundation for Cancer Research, Tokyo) for providing Mv1Lu cells and R 4-2 mutant cells. This work was supported by a research grant from the Ministry of Education, Science and Culture of Japan 08457235.

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