JCI The Journal of Clinical Investigation

Increased IL-6-production by cells isolated from the fibrous bone dysplasia tissues in patients with McCune-Albright syndrome.

T Yamamoto, … , T Kishimoto, S Okada

J Clin Invest. 1996;98(1):30-35. https://doi.org/10.1172/JCI118773.

Research Article

McCune-Albright syndrome (MAS) is characterized by café-au-lait spot, multiple endocrine hyperfunction, and polyostotic fibrous dysplasia. A somatic point mutation of Gsalpha protein was reported to decrease GTPase activity, leading to increase in the GSalpha-associated hormone actions via cAMP. IL-6 is known to stimulate osteoclast formation and in the IL-6 promoter, a cAMP responsive element has been identified. In this paper, we investigated the role of IL-6 in the bone lesions of MAS, using the isolated fibrous cells from the polyostotic fibrous dysplasia tissues in bones of the two patients with MAS. Bone biopsy specimen revealed the increased osteoclast in number. In both patients, a GSalpha mutation (Arg201 -> His) was identified in the cultured fibrous cells. Intracellular cAMP content and IL-6 secretion by the patient cells were increased. Rp-8Br-cAMP significantly inhibited IL-6 production in the patient cells, while it had no effect on normal control. The addition of dibutyryl cAMP significantly increased the synthesis of IL-6 in normal control cells. In contrast, no effect of dibutyryl cAMP on IL-6 synthesis was observed in the cells from one of the MAS patients. These data suggest that IL-6 is, at least, one of the downstream effectors of cAMP and that the increased IL-6 synthesis has a pathogenic role in the bone lesions of MAS patients via increasing the number of osteoclasts. These […]

Increased IL-6 Production by Cells Isolated from the Fibrous Bone Dysplasia Tissues in Patients with McCune-Albright Syndrome

Takehisa Yamamoto, Keiichi Ozono,[‡] Soji Kasayama,* Kosei Yoh,[§] Kazuo Hiroshima,[‖] Miki Takagi,* Sayuri Matsumoto, Toshimi Michigami, Kanji Yamaoka, Tadamitsu Kishimoto,* and Shintaro Okada

*Departments of Pediatrics and *Internal Medicine III, Faculty of Medicine, Osaka University, Osaka 565; [‡]Department of Environmental Medicine, Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi 590-02; [§]Department of Orthopedics, Hyogo Medical College, Nishinomiya 663; and [‖]Osaka National Hospital, Osaka 540, Japan*

Abstract

McCune-Albright syndrome (MAS) is characterized by café-au-lait spot, multiple endocrine hyperfunction, and polyostotic fibrous dysplasia. A somatic point mutation of $G_S\alpha$ protein was reported to decrease GTPase activity, leading to increase in the $G_S\alpha$-associated hormone actions via cAMP. IL-6 is known to stimulate osteoclast formation and in the IL-6 promoter, a cAMP responsive element has been identified. In this paper, we investigated the role of IL-6 in the bone lesions of MAS, using the isolated fibrous cells from the polyostotic fibrous dysplasia tissues in bones of the two patients with MAS. Bone biopsy specimen revealed the increased osteoclast in number. In both patients, a $G_S\alpha$ mutation ($Arg^{201}\rightarrow His$) was identified in the cultured fibrous cells. Intracellular cAMP content and IL-6 secretion by the patient cells were increased. Rp-8Br-cAMP significantly inhibited IL-6 production in the patient cells, while it had no effect on normal control. The addition of dibutyryl cAMP significantly increased the synthesis of IL-6 in normal control cells. In contrast, no effect of dibutyryl cAMP on IL-6 synthesis was observed in the cells from one of the MAS patients. These data suggest that IL-6 is, at least, one of the downstream effectors of cAMP and that the increased IL-6 synthesis has a pathogenic role in the bone lesions of MAS patients via increasing the number of osteoclasts. These results may provide a new strategy for the therapy of MAS patients. (*J. Clin. Invest.* 1996. 98:30–35.) Key words: fibrous dysplasia • $G_S\alpha$ protein • cAMP • osteoclast • Paget's disease

Introduction

McCune-Albright syndrome (MAS)[1] is characterized by café-au-lait spot, polyostotic fibrous dysplasia, and multiple endocrine hyperfunction, such as precocious puberty, hyperthyroidism, autonomous adrenal hyperplasia, and growth hormone (GH)-secreting pituitary adenoma (1). Hypophosphatemic rickets is recognized as a rare complication of MAS (2). As the cause of this disease, an activating point mutation of $G_S\alpha$ protein is reported in various tissues, including ovary, thyroid gland, adrenal gland, skin lesions, and GH-secreting pituitary tumor (3–5). Decrease in GTPase activity due to this mutation results in the increase of cyclic adenosine 3′, 5′-monophosphate (cAMP) levels in the endocrine organs and activation of the $G_S\alpha$ protein–associated hormone actions (6).

The mutation of $G_S\alpha$ protein has also been found in the bone lesions in MAS patients, consisting of the spindle-like fibrous cells in the bone marrow space and the increased woven bone (7). This mutation is present irrespective of polyostotic (7) or monostotic bone lesions (8). Recently, it was reported that one of the bisphosphonate derivatives (pamidronate) was effective on the bone lesions of MAS patients (9). Since this drug is also effective in inhibiting osteoclastic bone resorption in Paget's disease (10) or osteoporosis (11), increased osteoclastic bone resorption seems to be linked to the pathophysiology of the bone disease in MAS patients. However, the mechanism of increased bone resorption by $G_S\alpha$ mutation has not been investigated.

IL-6 is a cytokine involved in the differentiation of osteoclasts in vitro (12) and in vivo (13). IL-6 is also an important factor for the trigger of postmenopausal osteoporosis (14). In Paget's disease, IL-6 is considered to be a causal factor for increased osteoclastic bone resorption (15, 16). It is demonstrated that a cAMP responsive element exists in the promoter region of IL-6 gene (17). These lines of evidence enable us to speculate that IL-6 synthesis is enhanced by the increased amount of cAMP content associated with $G_S\alpha$ mutation and that increased IL-6 enhances bone resorption. In this paper, we investigated IL-6 production by the fibrous cells of MAS patients, which possessed a point mutation of $G_S\alpha$ gene, and also measured the osteoclast number in the bone biopsy specimen.

Methods

Materials. Dulbecco's modified minimal essential medium (DMEM) was obtained from Gibco Laboratories (Grand Island, NY), while 24-well culture plates were obtained from Iwaki Glass (Osaka, Japan). Collagenase, DNase type I, and isobutylmethyl xanthine were obtained from Sigma Immunochemicals (St. Louis, MO). A restriction enzyme, NlaIII, was purchased from New England Biolabs Inc. (Beverly, MA). Rp-8-bromo-adenosine 3′,5′-cyclic monophosphothioate (Rp-8Br-cAMP) was purchased from Biolog Life Science Institute

Address correspondence to Dr. T. Yamamoto, Department of Pediatrics, Faculty of Medicine, Osaka University, 2-2 Yamadaoka, Suita City, Osaka 565, Japan. Phone: 81-6-879-3937; FAX: 81-6-879-3939.

Received for publication 9 February 1996 and accepted in revised form 18 April 1996.

1. *Abbreviation used in this paper:* MAS, McCune-Albright syndrome.

J. Clin. Invest.

0021-9738/96/07/■■■■/035 $2.00
Volume 98, Number 1, July 1996, 30–35

(Bremen, Germany). Dibutyryl cAMP (dbcAMP) was purchased from Yamasa Shoyu Co. (Choshi, Japan). Human 1-34 parathyroid hormone (h-PTH) was obtained from Asahi Kasei Co. (Osaka, Japan).

Study subjects. Patient A was a female who visited Osaka University Hospital, complaining of skin pigmentation (café-au-lait spot) and genital bleeding at the age of 1 yr and 2 mo. Physical examination revealed the signs of precocious puberty (breast development and increased bone maturation). Laboratory investigation showed elevated serum estradiol (E2) levels, and ovarian cyst was detected by echosonography. Roentgenogram revealed fibrous dysplasia in her metacarpal bones. We diagnosed her with MAS and treated her with cyproterone-acetate from 2 to 10 yr of age. At 9 yr of age, hypophosphatemia was noted. She was admitted to Osaka University Hospital at 14 yr of age for detailed examination of her bone disease. Laboratory data at the admission were as follows. Serum calcium and phosphate concentrations were 9.2 mg/dl (normal 8.4–10.0) and 2.9 mg/dl (2.9–4.8), respectively. Serum alkaline phosphatase activity was increased (3,026 IU/liter [69–185]). The serum concentration of 25-hydroxyvitamin D (25OHD) was 10.7 ng/ml (9–29) and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) level was low (17.2 pg/ml [13–79] in the presence of hypophosphatemia). Serum PTH and calcitonin concentrations were 317 pg/ml (160–520) and 29 pg/ml (< 100), respectively. Nephrogenous cAMP value was 1.77 nmol/100 ml glomerular filtration rate (0.92–2.2). Serum osteocalcin concentration was elevated (80 ng/ml [16–29]). Urinary excretion of pyridinoline and deoxypyridinoline were also increased (791 pmol/μmol Cr [70–160] and 137 pmol/μmol Cr [10–25], respectively). Serum concentrations of sodium, potassium, glucose, total protein, Cr, and albumin were normal. Her serum level of E2 was 149 pg/ml (20–60). LH and FSH values were 2.1 mU/ml (1.8–5.2) and 2.5 mU/ml (2.9–8.2), respectively. Serum level of TSH was suppressed to 0.05 μU/ml (0.5–3.7), but free triiodothyronine (T3) 5.4 pg/ml (2.4–4.6) and free thyroxin (T4) 0.9 ng/dl (0.8–1.4) levels were normal, suggesting subclinical hyperthyroidism (18). ^{123}I scintigraphy revealed increased uptake in her right lobe of thyroid gland. At 14 yr of age, transiliac bone biopsy was performed, after administration of tetracycline hydrochloride twice at an interval of a week.

Patient B was a male diagnosed as MAS at 6 yr of age, because of skin pigmentation, bone fracture, and deformity. He was also suffering from hyperthyroidism. He was treated by thiamazole from the age of 6 yr, being in euthyroid states. At the age of 20, an operation to correct bone deformity and bone biopsy was done at Osaka National Hospital. At that time, serum calcium and phosphate concentrations were 8.7 and 3.0 mg/dl, respectively. Serum alkaline phosphatase activity was remarkably increased 2,135 IU/liter. At the age of 22, he died of heart failure.

Blood and urinary parameters. Serum and urinary constituents were measured by standard techniques in Osaka University Hospital and Osaka National Hospital. Serum PTH concentration was determined by a radioimmunoassay (Yamasa Shoyu Co.), and the serum osteocalcin concentration was determined by an immunoradiometric method (Mitsubishi Kagaku BCL Co., Ibaraki, Japan). Serum $1,25(OH)_2D$ and 25OHD levels were measured by a radioreceptor assay and competitive protein binding assay, respectively (19). Urinary concentrations of pyridinoline and deoxypyridinoline were determined by HPLC method (Mitsubishi Kagaku BCL Co.).

Bone biopsy. In patient A, bone specimen was fixed by 70% ethanol for 3 wk. After Villanueva bone stain, the 40-μm bone section was made by grinding method (20). In patient B, decalcified section was prepared after the treatment of EDTA, then the specimen was stained with hematoxylin and eosin. The number of osteoclasts on the bone surface surrounding the fibrous bone dysplasia was measured.

Cell culture. Affected bone fragments from the two patients with MAS and three normal controls were digested by collagenase (2 mg/ml) and DNase I (0.1 mg/ml) in PBS for 2 h at 37°C. Released cells were washed twice by DMEM supplemented with 10% FCS and then cultured as described before (21). For normal controls (patients C, D, and E), bone fragments from the patients without any metabolic bone disease were obtained at the operation with the informed consent of the patients or their parents. The age and the disease of normal control patients were as follows: F was a 65-yr-old male, fracture of left femur; E was a 17-yr-old male, osteoid osteoma; and D was a 2-yr-old female, pes equinovarus.

cAMP measurement. Cells were plated at 2×10^4/well in the 24-well multiplates. After reaching confluence, DMEM containing BSA (1 mg/ml) and isobutylmethylxanthine (0.5 mM) were supplied. After the incubation for 15 min, cAMP was extracted by 0.1 N HCl and kept frozen before cAMP measurement by radioimmunoassay. In some experiments, cells treated with 0.5 mM isobutylmethyl xanthine were incubated with 10^{-7} M h-PTH for 15 min. Then, the cAMP contents were measured.

Characterization of the fibrous cells. Alkaline phosphatase activity was measured in the cell sonicates as previously reported (21). Osteocalcin synthesis was measured in the culture media by an immunoradiometric assay, after incubation with 10^{-8} M $1,25(OH)_2D_3$ for 72 h in the 2% FCS-supplemented DMEM.

Cytokine production by the cultured cells. Cells at confluence were supplemented with 0.1% BSA-DMEM and cultured for 48 h. The concentrations of IL-6 (Genzyme Corp., Cambridge, MA), IL-11, IL-1α, and IL-1β (R&D Systems, Minneapolis, MN) were determined by ELISA. In some experiments, the cells were treated with dbcAMP (10^{-3} M) or Rp-8Br-cAMP (10^{-3}–10^{-4} M). The amount of IL-6 and IL-11 production was adjusted by DNA contents measured by a fluorometric method (21).

Detection of a point mutation of $G_S\alpha$ protein. DNA was isolated from the fibrous cells of the two MAS patients with a standard method. DNA fragment encoding exon 8 was amplified by a PCR method using the reported primers except deletion of GC repeats (4). The primer pair was 5′-CTCTGAGCCCTCTTTCCA AACTAC-3′ (MAS 1) and 5′-GGTTATTCCAGAGGGACTGGGGTGAA-3′ (MAS 2). After the digestion of the PCR products by NlaIII, they were visualized in 3% agarose gel. In addition, DNA sequence of the PCR products was analyzed by automated fluorescent method (Applied Biosystems, Inc., Foster City, CA), after the PCR products were subcloned in a pT7 Blue-T vector (Novagen, Inc., Madison, WI).

Statistical analysis. The results were shown as the mean±SD. The data were analyzed by two-way ANOVA.

Results

Bone histology. The number of osteoclasts was remarkably increased in both patients, compared with normal controls (Table I). The numbers of the nuclei in osteoclasts from MAS patients A and B were 10.5±2.5 (n = 10) and 5.5±2.1 (n = 10), respectively. By contrast, those were approximately three in number in normal controls (Fig. 1).

cAMP contents. In both patients, there were fibrous cells in which cAMP contents were significantly increased com-

Table I. The Number of Osteoclasts in the Bone Specimen of MAS Patients

	Patient A	Patient B	Normal controls (n = 3)
Osteoclasts (number/mm)	29	30	0.8±0.4

In patient A, bone specimen was stained with Villanueva bone stain. In patient B, decalcified bone specimen was stained with hematoxylin and eosin. Then, the number of osteocflasts surrounding the fibrous bone dysplasia tissues on the bone surface was measured under the light microscope.

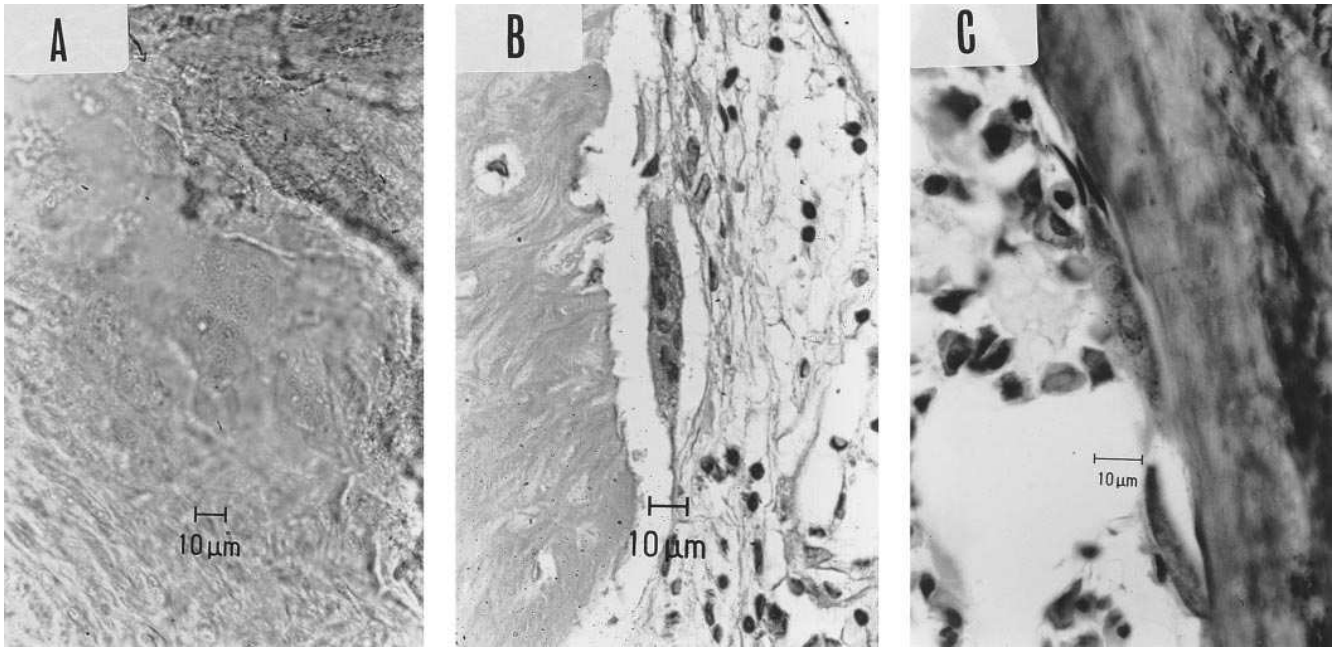

Figure 1. Osteoclasts in the patient with MAS. Osteoclasts in the bone specimen were stained with Villanueva bone stain in patient A and normal control. In patient B, hematoxylin and eosin staining was used. (*A*) MAS in patient A. (*B*) MAS in patient B. (*C*) Normal control. Note that the number of the nuclei in the osteoclasts from the patients with MAS is increased.

pared with normal controls (Fig. 2). In response to 10^{-7} M hPTH-(1-34), the increase in cAMP synthesis was blunted in the cells from MAS patient A compared with a normal control (MAS patient A, 4.07±0.54 vs. 11.9±1.08 pmol/μg DNA, 2.92-fold induction; normal control, 1.57±0.04 vs. 18.3±0.50 pmol/μg DNA, 11.7-fold induction). The cells with the elevated cAMP contents were used in the following experiments.

Characterization of the patient fibrous cells. Alkaline phosphatase activities of the fibrous cells with the increased cAMP contents were 12.3 and 11.9 nmol PNP/μg DNA/30 min in pa-

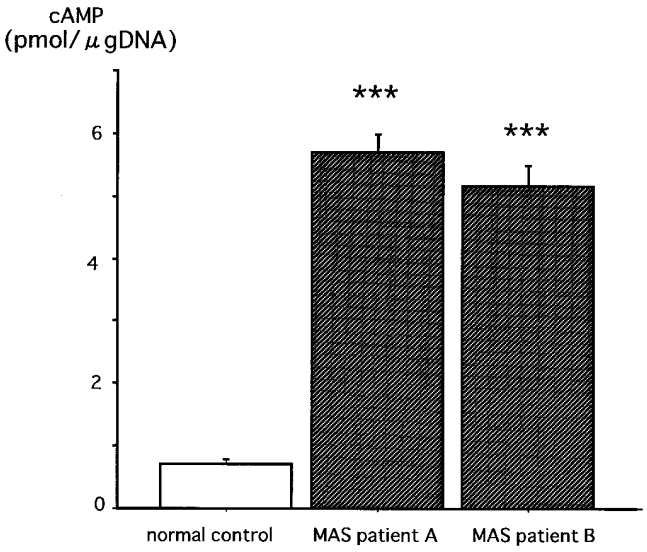

Figure 2. cAMP contents in the fibrous cells of the patients with MAS. cAMP in the confluent cells was extracted by 0.1 N HCl and then assayed by RIA. Values are the mean±SD of triplicate determinations. ***$P < 0.001$ vs. control.

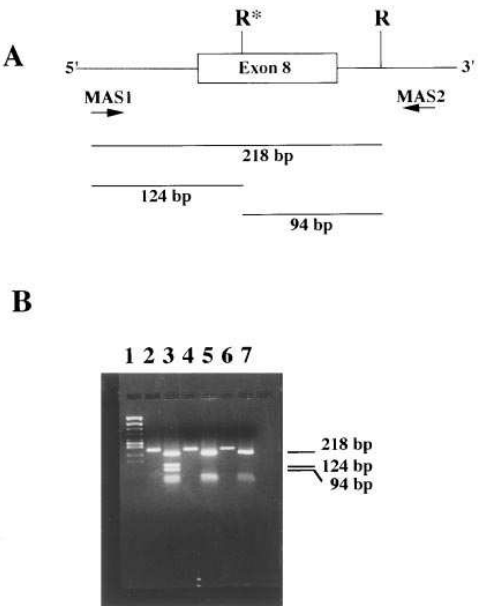

Figure 3. $G_S\alpha$ protein mutation in patients with MAS. (*A*) The positions of PCR primers (MAS 1 and MAS 2) and the expected size of PCR products with or without NlaIII digestion. DNA fragment encoding exon 8 was amplified by a PCR method, then digested by a restriction enzyme, NlaIII. *R*, the position of NlaIII restriction site; *R**, the position of a second NlaIII restriction site introduced by the mutation in the codon for Arg^{201}. (*B*) Agarose gel electrophoresis of PCR products with ethidium bromide staining. Note that $G_S\alpha$ mutation ($Arg^{201}\rightarrow$His) was observed in patient A. In contrast, the same mutation was not clear in patient B by this method. Lane *1*, markers; lanes *2* and *3*, patient A; lanes *4* and *5*, patient B; lanes *6* and *7*, normal control; lanes *3*, *5*, and *7*, with NlaIII digestion; lane *2*, *4*, and *6*, without NlaIII digestion.

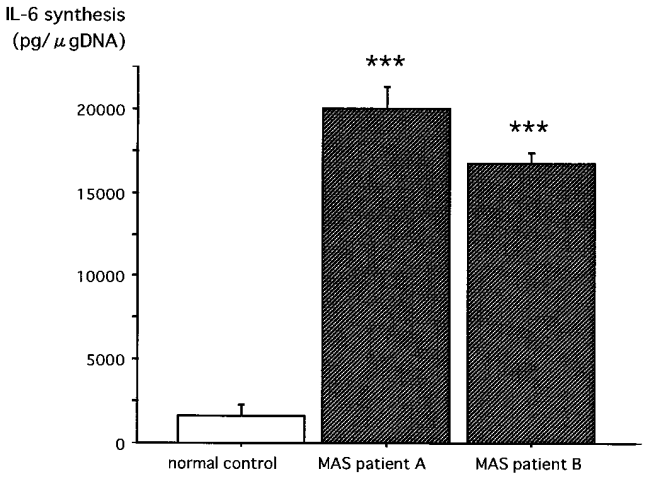

Figure 4. IL-6 synthesis by the fibrous cells of the patients with MAS. IL-6 concentrations in the culture media were assayed by an ELISA after the incubation of 48 h. IL-6 concentration in the media (0.1% BSA-DMEM) was undetectable. Values are the mean±SD of triplicate determinations. ***$P < 0.001$ vs. control.

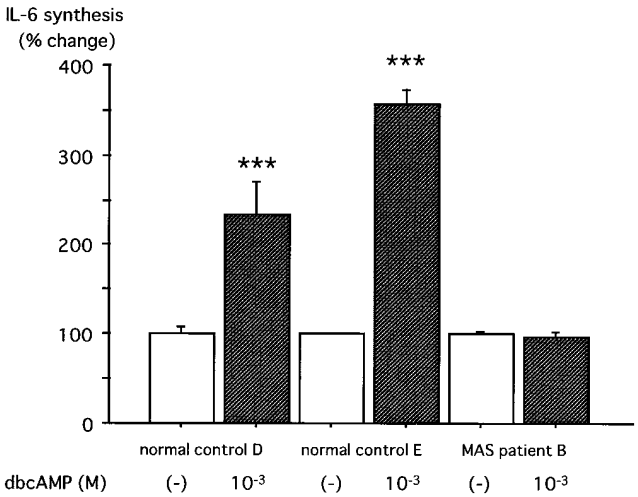

Figure 6. Effect of dbcAMP on IL-6 synthesis by fibrous cells from the patient with MAS (patient B). Confluent cells were incubated with 0.1% BSA-DMEM in the presence or absence of 10^{-3} M dbcAMP for 48 h. IL-6 in the culture media was assayed by an ELISA. Values are mean±SD of triplicate determinations. ***$P <$ 0.001 vs. no treatment in the normal control.

tients A and B, respectively. Osteocalcin was not detected in the culture media of the fibrous cells from both patients.

Point mutation of $G_S\alpha$ protein. In patient A, the cells that contain high levels of cAMP had a point mutation of $G_S\alpha$ protein (Arg^{201}→His), detected by the digestion of the PCR-amplified products by NlaIII (Fig. 3). This mutation was confirmed by DNA sequence analysis (data not shown).

In patient B, the same mutation in the $G_S\alpha$ protein was not detected by digesting the PCR products by NlaIII. However, the DNA sequence analysis of the PCR products revealed the same mutation of $G_S\alpha$ protein with low frequency (data not shown).

IL-6 synthesis. In both patients, IL-6 synthesis by the fibrous cells with the increased cAMP contents was significantly increased compared with normal controls (Fig. 4). The increased IL-6 synthesis in both patients was blocked by the treatment with Rp-8Br-cAMP. In contrast, Rp-8Br-cAMP had no effect on the control cells (Fig. 5). The addition of dbcAMP had no effect on IL-6 synthesis in patient B, while it significantly increased IL-6 synthesis in normal control (Fig. 6).

Cytokine syntheses other than IL-6. IL-1α and β were undetectable (< 1.0 pg/ml) in the culture media from both normal control and MAS patients. IL-11 synthesis was increased in the media of MAS patients compared with normal controls (Table II).

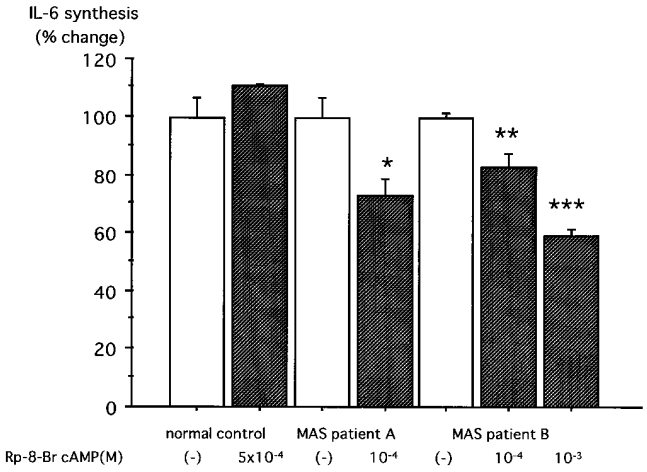

Figure 5. Effect of Rp-8Br-cAMP on IL-6 synthesis by the fibrous cells from the patients with MAS. Confluent cells were incubated with 0.1% BSA-DMEM in the presence or absence of Rp-8Br-cAMP (10^{-3}–10^{-4} M) for 48 h. In the experiments, the cells were preincubated with Rp-8Br-cAMP for 30 min, and then the new media containing the same concentration of Rp-8Br-cAMP were applied. IL-6 in the media was assayed by an ELISA. Values are mean±SD of triplicate determinations. *$P < 0.05$ vs. no treatment in patient A; **$P <$ 0.01, ***$P < 0.005$ vs. no treatment in patient B.

Table II. IL-11 Synthesis by Fibrous Cells from Patients with MAS

	IL-11 synthesis (pg/μg DNA)
Normal control	
D	64±16
E	< 4
F	30±2.5
MAS	
Patient A	211±33*
Patient B	337±91‡

Culture media were obtained from the confluent cells after incubation for 48 h in 24-well multiplates. Then, IL-11 was measured by ELISA. IL-11 concentration in the media (0.1% BSA-DMEM) was undetectable. Values are mean±SD of triplicate determinations. *$P < 0.01$ vs. D, E, or F. ‡$P < 0.001$ vs. D, E, or F.

Discussion

MAS is characterized by precocious puberty, café-au-lait spot, and fibrous dysplasia of bone (1). A postzygotic missense mutation in $G_S\alpha$ gene was discovered to be responsible for this disease (3, 4), leading to an activated $G_S\alpha$ function in various tissues, including ovary (5), thyroid gland (5), skin (5), and bone (7, 8). Bone resorption in MAS was known to be increased (9), but osteoclast number in the bone biopsy specimen has not been investigated. We describe that the osteoclast numbers of the patients were significantly increased, and this observation is consistent with the elevated bone resorption markers, such as the urinary excretion of pyridinoline and deoxypyridinoline (22). In addition, most osteoclasts were associated with the increased numbers of the nuclei. The increased numbers of nuclei in osteoclasts are often observed in Paget's disease (16), whose pathogenesis is explained by increased IL-6 secretion (15). Since IL-6 is known to increase the number of osteoclasts (12, 13), it is reasonable that an increase in the number of osteoclasts in MAS is due to the increased synthesis of IL-6 by the fibrous cells isolated from the fibrous bone dysplasia tissues. However, serum IL-6 levels were not increased in MAS patient A (data not shown), suggesting that the increased IL-6 synthesis is restricted to the local bone lesions where a $G_S\alpha$ mutation exists.

It is plausible that the increased IL-6 synthesis by the patient cells results from the elevated cAMP contents, because the cAMP responsive element exists in the promoter region of IL-6 gene (17). In fact, the addition of 10^{-3} M dbcAMP markedly increased IL-6 synthesis in normal control cells, whereas the same experimental condition had no effect in the patient cells (patient B) (Fig. 6). These data suggest that the pathway from the increased cAMP levels to enhance IL-6 production is continuously activated in the MAS patient due to the $G_S\alpha$ mutation. This speculation is also supported by our observation that Rp-8Br-cAMP, a specific inhibitor of protein kinase A (23), was effective in suppressing IL-6 synthesis in the patient cells (Fig. 5).

AP-1 and NF-κB binding sites were reported to be found in the promoter regions of IL-6 gene (17). The expression of c-*fos* gene was reported to be increased in a study of experimental $G_S\alpha$ mutation (24). Since c-*fos* is linked to form the transcription factor AP-1 (25), it is possible that AP-1 is another important factor to increase IL-6 secretion by the fibrous cells of MAS patients. In fact, Candeliere et al. (26) reported the increased c-*fos* expression in bone tissues of MAS patients. In addition, a recent study (27) indicated that NF-κB is activated by dbcAMP or forskolin. Thus, it is also possible that NF-κB is another important factor in increasing the IL-6 production.

Recently, it was reported that thyroid hormone stimulates IL-6 production (28). However, it is unlikely that thyroid hormone is responsible for the elevated IL-6 production in these patients, since patient A was in subclinical hyperthyroid state, and patient B was in euthyroid state by the treatment.

The data suggesting that IL-6 plays an essential role in the pathogenesis of MAS do not exclude the possibility that other cytokines are also involved in the bone lesions of MAS. In our case, the synthesis of IL-11, which is known to increase the number of osteoclasts (29), was also significantly increased in the fibrous cells from the MAS patients although its levels were $\sim$ 100 times less than those of IL-6 in the cultured media. This observation might be related to the report that the analogous sequence of AP-1 binding site is found in the 5′ region of the human IL-11 gene (30) and 12-*O*-tetra-decanoylphorbol 13-acetate (PMA) is capable of inducing IL-11 mRNA in the human osteosarcoma cell line (31).

In conclusion, increased IL-6 secretion by the fibrous cells in the fibrous bone dysplasia tissues of MAS patients was proven. It is likely that this phenomenon is derived from the elevated cAMP contents due to a point mutation of the $G_S\alpha$ gene and may explain the increased osteoclast numbers in the bone lesions of MAS patients.

Acknowledgments

We thank Dr. Yasuko Naya at Osaka Prefecture Hospital for giving us the clinical data on MAS patient B. We also thank Miss Kaori Ohshima and Miss Ok-Soon Park for preparing the manuscript.

References

1. Spiegel, A.M., A. Shenker, and L.S. Weinstein. 1992. Receptor effector coupling by G proteins: implications for normal and abnormal signal transduction. *Endocr. Rev.* 13:536–565.

2. Les, P.A., C. Van Dop, and C.J. Migeon. 1986. McCune-Albright syndrome: long term follow-up. *J. Am. Med. Assoc.* 256:2980–2984.

3. Weinstein, L.S., A. Shenker, P.V. Gejman, M.J. Merino, E. Friedman, and A.M. Spiegel. 1991. Activating mutations of the stimulatory G protein in the McCune-Albright syndrome. *N. Engl. J. Med.* 325:1688–1695.

4. Schwindinger, W.F., C.A. Francomano, and M.A. Levine. 1992. Identification of a mutation in the gene encoding the α subunit of the stimulatory G protein of adenylyl cyclase in McCune-Albright syndrome. *Proc. Natl. Acad. Sci. USA.* 89:5152–5156.

5. Shenker, A., L.S. Weinstein, A. Moran, O.H. Pescovitz, N.J. Charest, C.M. Boney, J.J. Van Wyk, M.J. Merino, P.P. Feullan, and A.M. Spiegel. 1993. Severe endocrine and non endocrine manifestations of the McCune-Albright syndrome associated with activating mutations of stimulatory G protein Gs. *J. Pediatr.* 123:509–518.

6. Landis, C.A., S.B. Masters, A. Spada, A.M. Pace, H.R. Bourne, and L. Vallar. 1989. GTPase inhibiting mutations activate the α chain of Gs and stimulate adenylyl cyclase in human pituitary tumors. *Nature (Lond.).* 340:692–696.

7. Shenker, A., L.S. Weinstein, D.E. Swest, and A.M. Spiegel. 1994. An activating $G_S\alpha$ mutation is present in fibrous dysplasia of bone in the McCune-Albright syndrome. *J. Clin. Endocrinol. & Metab.* 79:750–755.

8. Shenker, A., P. Chanson, L.S. Weinstein, P. Chi, A.M. Spiegel, A. Lomri, and P.J. Marie. 1995. Osteoblastic cells derived from isolated lesions of fibrous dysplasia contain activating somatic mutations of the $G_S\alpha$ gene. *Hum. Mol. Genet.* 4:1675–1676.

9. Liens, D., P.D. Delmas, and P.J. Meunier. 1994. Long term effects of intravenous pamidronate in fibrous dysplasia of bone. *Lancet.* 343:953–954.

10. O'Doherty, D.P., D.R. Bickerstaff, E.V. McCloskey, N.A.T. Handy, M.N.C. Beneton, S. Harris, M. Mian, and J.A. Kanis. 1990. The treatment of Paget's disease of bone with aminohydroxybutylidene bisphosphonate. *J. Bone Miner. Res.* 5:483–491.

11. Fleisch, H. 1993. Bisphosphonates in osteoporosis: an introduction. *Osteoporosis Int.* 3:53–55.

12. Yamamoto, T., N. Kurihara, K. Yamaoka, K. Ozono, M. Okada, K. Yamamoto, S. Matsumoto, T. Michigami, J. Ono, and S. Okada. 1993. Bone marrow-derived osteoclast-like cells from a patient with craniometaphyseal dysplasia lack expression of osteoclast reactive vacuolar proton pump. *J. Clin. Invest.* 91:362–367.

13. Yoneda, T., M. Nakai, K. Moriyama, L. Scott, N. Ida, T. Kishimoto, and G.R. Mundy. 1993. Neutralizing antibodies to human interleukin-6 reverse hypercalcemia associated with a human squamous carcinoma. *Cancer Res.* 53:737–740.

14. Jilka, R.L., G. Hangoc, G. Girasole, G. Passeri, D.C. Williams, J.S. Abrams, B. Boyce, H. Broxmeyer, and S.C. Manolagas. 1992. Increased osteoclast development after estrogen loss: mediation by interleukin-6. *Science (Wash. DC).* 257:88–91.

15. Roodman, G.D., N. Kurihama, Y. Ohsaki, A. Kukita, D. Hosking, A. Demulder, F.J. Smith, and F.R. Singer. 1992. Interleukin-6: a potential autocrine/ paracrine factor in Paget's disease of bone. *J. Clin. Invest.* 89:46–52.

16. Since, E.S. 1993. Paget's disease of bone. *In* Primers on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. 2nd ed. M.J. Fravus, editor. Raven Press, New York. 375–384.

17. Kishimoto, T. 1989. The biology of interleukin-6. *Blood.* 74:39–48.

18. Utiger, R.D. 1994. Subclinical hyperthyroidism-just a low serum thyrotropin concentration, or something more? *N. Engl. J. Med.* 331:1302–1303.

19. Yamaoka, K., H. Tanaka, H. Kurose, M. Shima, K. Ozono, S. Nakajima, and Y. Seino. 1989. Effect of single oral phosphate loading on vitamin D metabolites in normal subjects and in X-linked hypophosphatemic rickets. *Bone Miner.* 7:159–169.

20. Frost, H.M. 1958. Preparation of thin decalcified bone sections by rapid manual method. *Stain Technol.* 33:273–277.

21. Yamamoto, T., B. Ecarot, and F.H. Glorieux. 1991. In vivo osteogenic activity of isolated human bone cells. *J. Bone Miner. Res.* 6:45–51.

22. Delmas, P.D. 1993. Markers of bone formation and resorption. *In* Primers on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. 2nd ed. M.J. Fravus, editor. Raven Press, New York. 108–112.

23. Chao, A.C., F.J. de Sauvage, Y.J. Dong, J.A. Wagner, D.V. Goeddel, and P. Gardner. 1994. Activation of intestinal CFTR Cl^- channel by heat-stable enterotoxin and guanylin via cAMP-dependent protein kinase. *EMBO (Eur. Mol. Biol. Organ.) J.* 13:1065–1072.

24. Gaiddon, C., A.L. Boutillier, D. Momior, L. Mercken, and J.P. Loeffler. 1994. Genomic effects of the putative oncogene $G_s\alpha$: chronic transcriptional activation of the c-fos proto-oncogene in endocrine cells. *J. Biol. Chem.* 269: 22663–22671.

25. Abate, C., and T. Curran. 1990. Encounters with Fos and Jun on the road to AP-1. *Semin. Cancer Biol.* 1:19–26.

26. Candeliere, G.A., F.H. Glorieux, J. Preud'homme, and R.S. Arnaund. 1995. Increased expression of the c-fos proto-oncogene in bone from patients with fibrous dysplasia. *N. Engl. J. Med.* 332:1546–1551.

27. Pendorfer, V., P. Oettgen, and T.A. Libermann. 1994. Multiple regulatory elements in the interleukin-6 gene mediate induction by prostaglandins, cyclic AMP, and lipopolysaccharide. *Mol. Cell. Biol.* 14:4443–4454.

28. Passeri, M., G. Girasole, N. Giuliani, M. Pedrazzoni, E. Roti, M. Salvi, R. Minelli, C. Gatti, and C. Campanini. 1995. Serum soluble interleukin-6 receptors and interleukin-6 are increased in Grave's disease and correlated with thyroid hormones levels. *J. Bone Miner. Res.* 10(Suppl. 1):S143*a*. (Abstr.)

29. Girasole, G., G. Passeri, R.L. Jilka, and S.C. Manolagas. 1994. Interleukin-11: a new cytokine critical for osteoclast development. *J. Clin. Invest.* 93: 1516–1524.

30. McKinley, D., Q. Wu, T. Yang-Feng, and Y.C. Yang. 1992. Genomic sequence and chromosomal location of human interleukin-11 gene (IL-11). *Genomics.* 13:814–819.

31. Ellias, J.A., W. Tang, and M.C. Horowitz. 1995. Cytokine and hormonal stimulation of human osteosarcoma interleukin-11 production. *Endocrinology.* 136:489–498.