Biosynthesis of Bone Sialoprotein by a Human Osteoclast-like Cell Line (FLG 29.1)

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ABSTRACT

Biosynthesis of bone sialoprotein (BSP) by a human osteoclastic cell line (FLG 29.1) during its differentiation induced by phorbol 12-myristate 13-acetate (TPA) was studied using metabolic radiolabeling experiments. The FLG 29.1 cells were metabolically radiolabeled with [3H]glucosamine and [35S]sulfate, and the labeled glycoproteins were analyzed by anion exchange chromatography, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoprecipitation experiments. One of the major glycoproteins synthesized by the TPA-treated FLG 29.1 cells was sulfated, had an identical electrophoretic mobility to purified BSP, and could be immunoprecipitated with a specific antibody against human BSP (LF 6). Thus, this glycoprotein was tentatively identified as the BSP. Furthermore, mRNA for BSP was also detected in TPA-treated FLG 29.1 cells by RNA-polymerase chain reaction. Most BSP synthesized by FLG 29.1 cells remained cell-associated, and this is in contrast with those synthesized by osteoblasts, where the protein is rapidly released into the extracellular matrix. Immunocytochemistry using an anti-BSP antibody showed a prominent paranuclear (suggestive of Golgi apparatus) localization of BSP in the TPA-treated FLG 29.1 cells after permeabilization, while untreated cells were not significantly immunostained. Localization of BSP at the plasma membrane was also demonstrated in the TPA-treated FLG 29.1 cells by the fluorescence-activated cell sorting analysis. Since TPA has been demonstrated to induce expression of various osteoclastic characteristics in FLG 29.1 cells, induction of BSP expression by TPA suggests that the protein may play a role during the differentiation process of osteoclasts or in functions of differentiated osteoclasts.

INTRODUCTION

OSTEOCLASTS ARE MULTINUCLEATED giant cells within bone and are the major cellular effectors of bone resorption. Several lines of evidence have shown that osteoclasts are derived from bone marrow cells that have reached the bone surface via a blood-borne mononuclear precursor.1,2 However, a clonal osteoclast cell line from bone tissue has not yet been established. Recently, a human leukemic cell line, designated FLG 29.1, with preosteoclastic characteristics has been cloned.3 These cells can be induced to differentiate in vitro and to exhibit various characteristics specific to osteoclasts when treated with a phorbol ester.3 Therefore, they provide a useful experimental system to study the differentiation and biological characteristics of osteoclasts. Specific cellular interactions with components of the extracellular matrix can influence cellular differentiation and development of several tissues. The protein components of the extracellular matrix of bone consist primarily of type I collagen,4 proteoglycans, and less abundant noncollag-

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MATERIALS AND METHODS

Materials

Sephadex G-50 (fine), Q-Sepharose, protein A-Sepharose and Superose-6 were purchased from Pharmacia/LKB Biotechnology, Inc. (Piscataway, NJ). Protein molecular weight standards for SDS-PAGE were from Bio-Rad (Richmond, CA). Precast polyacrylamide gels (4–20% gradient and 7.5% acrylamide concentrations) were obtained from Daiichi Fine Chemicals (Tokyo, Japan), through Integrated Separation Systems. [35S]Sulfuric acid (~40 Ci/mg) and [3H]glucosamine (40 Ci/mmol) were from Du Pont-New England Nuclear (Boston, MA). All culture media (including sulfate-free medium) were from Flow Laborato-

Abbreviations used arc: BSP, bone sialoprotein; TPA, phorbol 12-myristate 13-acetate; PAGE, polyacrylamide gel electrophoresis.

enous proteins, some of which appear to be unique to the bone tissue. One such bone-specific protein is a sialic acid-rich protein, referred to as bone sialoprotein (BSP), which was originally identified by Herring. Subsequently, Fisher and coworkers purified an intact, high molecular weight (70,000–80,000 D) sialoprotein from fetal calf bone using a nondegradative isolation procedure, and the primary amino acid sequence was determined by cDNA cloning. BSP has been shown to be a major glycoprotein product of osteoblasts. It is extensively glycosylated and a large proportion of its tyrosine residues are sulfated. The difference in molecular weights between the predicted protein (~33,000 D) and the isolated form is largely accounted for by these post-translational modifications.

BSP contains the Arg-Gly-Asp (RGD) peptide sequence, an integrin-recognition motif. It has been shown that BSP-coated plastic surfaces promote the attachment of both osteoblasts and cementoblasts when they are involved in the differentiation and unique sulfation of BSP.

BSP has been implicated in cell-matrix interactions in bone. BSP contains the Arg-Gly-Asp (RGD) peptide sequence, which recognizes BSP and its mRNA by immunohistochemistry and in vitro hybridization techniques by Chen and coworkers demonstrated that BSP is synthesized by osteoblasts, osteocytes, hypertrophic chondrocytes, odontoblasts, and cementoblasts when they are involved in active mineralization. A recent study by Bianco and coworkers has also indicated a highly specific localization of BSP in bone tissues (except in placental tissue). They detected mRNA encoding BSP in both osteoblasts and osteoclasts by in situ hybridization, suggesting the possibility that osteoclasts can also synthesize this protein.

In the present study we have investigated the biosynthesis of BSP by the FLG 29.1 preosteoclastic cell line and demonstrated that BSP is synthesized by FLG 29.1 cells. We show that BSP sulfation may be regulated during the phorbol ester-induced cell differentiation processes. Metabolic radiolabeling protocols using [3H]glucosamine and [35S]sulfate were chosen based on the extensive carbohydrate substitution and unique sulfation of BSP.

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Identification of BSP

After metabolic radiolabeling, cells were separated from the labeling medium by centrifugation. Cell pellets were extracted with 1 ml of 4 M guanidine HCl, 50 mM sodium acetate, pH 6.0, containing 2% (w/v) Triton X-100 and protease inhibitors (1 mM N-ethylmaleimide and 0.1 mM phenylmethylsulfonyl fluoride). Solid guanidine HCl and protease inhibitors as indicated above were added to the culture medium to make a final 4 M guanidine HCl solution. The extracts were chromatographed on Sephadex G-50 (bed volume 8 ml) equilibrated in 8 M urea, 50 mM Na acetate, 0.5% Triton X-100, pH 6.0, to remove unincorporated radioactive precursors. Aliquots of excluded fractions were measured for radioactivity, and the total radioactive incorporation was calculated. Macromolecular fractions from the Sephadex G-50 gel chromatography were applied to Q-Sepharose ion exchange columns (bed volume 2 ml) equilibrated with the same 8 M urea buffer. After sample application, the columns were washed with an additional 3 bed volumes of the loading buffer, and bound macromolecules were eluted with a linear NaCl gradient.

Abbreviations used arc: BSP, bone sialoprotein; TPA, phorbol 12-myristate 13-acetate; PAGE, polyacrylamide gel electrophoresis.
were fixed in 2% formaldehyde in PBS at 22°C for 15 min. Volumes of 4-fold concentrated SDS-PAGE sample buffer were added to samples prior to boiling, and fluorography (using Enlightning from Du Pont) were performed according to Bonner and Laskey. Autoradiography and fluorography (using Enlightning from Du Pont) were performed according to Bonner and Laskey.

Immunoprecipitation

Radiolabeled BSP was precipitated from the cell extracts, after fractionation by Q-Sepharose chromatography, using protein A–Sepharose conjugated with a rabbit antiserum against human BSP (LF 6). Details of conjugation procedures of the antibody to protein A–Sepharose have been reported. Pooled fractions from Q-Sepharose chromatography were dialyzed against immunoprecipitation buffer (0.2 M Tris HCl, 0.3 M NaCl, 2% Triton X-100, 0.2% Aprotinin, pH 7.2), and incubated first with protein A–Sepharose conjugated with normal rabbit serum and then with the resin conjugated to antibody against human BSP. Both nonimmune and immune precipitates were solubilized by diluting the sample 1:1 with SDS–PAGE sample buffer and boiling for 5 minutes. All samples were electrophoresed on polyacrylamide gradient (4–20%) gels as described by Fisher et al. After electrophoresis, the radioactive bands were visualized by autoradiography and fluorography.

Thrombin digestion

Digestion with thrombin was carried out on aliquots of cell extracts fractionated with Q-Sepharose ion-exchange chromatography. The digestion was performed at 37°C for 30 min in 1 ml of 10 mM Tris HCl buffer, pH 8.0, containing 10 mM CaCl₂ and 1 U of thrombin as described by Nagata et al. Digestion was terminated by the addition of 0.25 volumes of 4-fold concentrated SDS–PAGE sample buffer containing 60 mg DTT/ml and heating at 56°C for 25 minutes.

Immunocytochemistry of BSP

The FLG 29.1 cells cultured with or without 0.1 μM TPA were fixed in 2% formaldehyde in PBS at 22°C for 15 min. The immunocytochemical labeling was performed in situ by using the peroxidase-antiperoxidase technique with or without permeabilization of the cells with Triton X-100. A polyclonal rabbit antibody against human BSP (LF 6, 1:500 dilution) was incubated with the specimens for 2 h at 22°C. 3-Amino 9-ethyl carbazole was used as chromogen with hematoxylin (Mayer’s haemalum) counterstain.

Fluorescence flow cytometric analysis

FLG 29.1 cells were grown in tissue culture flasks (1 × 10⁶ cells/flask) in the presence or absence of 0.1 μM TPA. After 48 h of incubation, cells were separated from culture medium by centrifugation. The pellet was washed twice with PBS. Cells were immunolabeled successively with the rabbit polyclonal antibody against human BSP (1:500 dilution, LF 6) and then with the fluorescein-labeled goat anti-rabbit IgG (GAR-FITC: Kierkegaard & Perry laboratories). Following incubation, cells were washed twice and resuspended in PBS (1 × 10⁶ cells/ml). Flow cytometric analysis was performed on a FACSscan (Beckton Dickinson).

RNA-polymerase chain reaction

The presence of mRNA for BSP was demonstrated by RNA–polymerase chain reaction (PCR) (RNA–PCR kit, Perkin Elmer, Norwalk, CT). Briefly, total RNA was extracted from FLG 29.1 cells (1 × 10⁶ cells/flask) cultured in the presence or absence of 0.1 μM TPA, using RNA STAT-60 (TEL-TEST "B", Inc.) according to the manufacturer's instructions. Two micrograms of the total RNA from each preparation was subjected to reverse transcription using an antisense primer (5’-TTGCTCAGCATTITGGGAA-3’), followed by amplification by PCR with an additional primer (5’-ATCGTAGCC-3’), according to the manufacturer's instructions. Sequences of primers for rat UMR 106-01 cells were used as a reference for quantification of mRNA. PCR products were analyzed by agarose gel (1%) electrophoresis.

RESULTS

Fractionation of radiolabeled glycoproteins and proteoglycans

In the present study, expression of BSP has been investigated in the FLG 29.1 preosteoclastic cell line treated with

<table>
<thead>
<tr>
<th>TPA treatment</th>
<th>[3H]Glucosamine incorporation (cpm)/10⁶ cells</th>
<th>SD</th>
<th></th>
<th>[35S]Sulfate incorporation (cpm)/10⁶ cells</th>
<th>SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>-</td>
<td>97,000</td>
<td>15,000</td>
<td>-</td>
<td>193,000</td>
<td>8,000</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>194,000</td>
<td>6,000</td>
<td>&lt;0.01</td>
<td>480,000</td>
<td>20,000</td>
</tr>
<tr>
<td>Cell</td>
<td>-</td>
<td>370,000</td>
<td>35,000</td>
<td>-</td>
<td>104,000</td>
<td>47,000</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>415,000</td>
<td>105,000</td>
<td>0.08</td>
<td>216,000</td>
<td>14,000</td>
</tr>
</tbody>
</table>

* Compared with no TPA treatment.
FIG. 1. Q-Sepharose ion exchange chromatography analyses of FLG 29.1 cell cultures labeled with \([\text{H}^3\text{H}]\)glucosamine and \([\text{S}^{35}\text{S}]\)sulfate. Cell cultures were treated with or without TPA for 48 h and metabolically radiolabeled with \([\text{H}^3\text{H}]\)glucosamine and \([\text{S}^{35}\text{S}]\)sulfate for the last 24 h. Cells and media were separately extracted with 4 M guanidine buffer. After removing unincorporated radioisotopes by Sephadex G-50 gel chromatography, radiolabeled macromolecules were analyzed by Q-Sepharose ion exchange chromatography in 8 M urea buffer with a linear NaCl gradient (0–1.0 M). (A–D) Analyses of cell extracts from control cultures, cell extracts from TPA-treated cultures, medium extracts from control cultures, and medium extracts from TPA-treated cultures, respectively, are shown. Three major peaks were identified for both cells and media extracts (I, II, and III). Fractions from cell extract samples were pooled as indicated (1–5) for further analyses.

and without TPA, a phorbol ester known to induce expression of various osteoclastic phenotypes in this cell line. Based on the fact that BSP is a glycoprotein that can be sulfated, metabolic radiolabeling experiments were carried out using \([\text{S}^{35}\text{S}]\)sulfate and \([\text{H}^3\text{H}]\)glucosamine as precursors. After 24 h of metabolic radiolabeling, cells were separated from the labeling culture medium by centrifugation, and both compartments were extracted with 4 M guanidine HCl buffer containing detergent and protease inhibitors. The total radioactive incorporation was measured after removing unincorporated isotopes by Sephadex G-50 gel chromatography. The experiments were carried out in triplicate and the values of \([\text{H}^3\text{H}]\)glucosamine and \([\text{S}^{35}\text{S}]\)sulfate incorporation into macromolecules were corrected for cell number and expressed as cpm/10^6 cells (mean ± SD), as shown in Table 1.
Table 2. Percent Distribution of Radiolabeled Materials in Q-Sepharose Fractions

<table>
<thead>
<tr>
<th>Medium extract</th>
<th>Peak I</th>
<th>Peak II</th>
<th>Peak III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without TPA</td>
<td></td>
<td>85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14</td>
</tr>
<tr>
<td>With TPA</td>
<td>31 (18)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60 (19)</td>
<td>11 (62)</td>
</tr>
<tr>
<td>Cell extract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without TPA</td>
<td>30 (25)</td>
<td>69 (15)</td>
<td>2 (60)</td>
</tr>
<tr>
<td>With TPA</td>
<td>33 (9)</td>
<td>65 (32)</td>
<td>4 (60)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values outside parentheses indicate <sup>3</sup>H-activity (labeling medium contained 17 μCi/ml [<sup>3</sup>H]glucosamine).

<sup>b</sup> Values in parentheses indicate <sup>35</sup>S-activity (labeling medium contained 50 μCi/ml [<sup>35</sup>S]sulfate).

TPA treatment increased the accumulation of <sup>35</sup>S-labeled macromolecules in both culture medium and cell compartments by 2.5- and 2.1-fold (p < 0.05), respectively. A significant increase of [<sup>3</sup>H]glucosamine incorporation was observed only in the medium compartment (2-fold) after TPA treatment. The macromolecular fractions resulting from Sephadex G-50 analysis were further analyzed by Q-Sepharose ion exchange chromatography. Elution profiles of radiolabeled molecules in extracts of FLG 29.1 cells and medium are shown in Fig. 1. Three major radioactive peaks were identified (peaks I, II, and III) for both cell and medium extracts, and the distribution of radioactivity for each peak is shown in Table 2. In the cell extract, there was a 2-fold increase of <sup>35</sup>S-radioactivity in peak II after TPA treatment; however, the distribution of <sup>3</sup>H-radioactivity in the three peaks was not significantly affected. In culture medium, a significant radioactivity was observed in peak I after TPA treatment.<sup>5</sup> The total radioactivity recovered from Q-Sepharose chromatography ranged between 85–95%.

SDS–PAGE

Radioactive fractions from cell extracts that constituted the three peaks after Q-Sepharose ion exchange chromatography were pooled into five fractions as indicated in Fig. 1 and further analyzed by SDS–PAGE under reducing conditions. Radioactive bands were detected by autoradiography (detection of <sup>35</sup>S) and fluorography (detection of both <sup>3</sup>H and <sup>35</sup>S), and the results of cell extract analyses are shown in Fig. 2. Pool I showed a broad smear without prominent bands after long exposure. Peak II (pools 2, 3, and 4) showed two major radioactive bands at apparent molecular weights of ~70,000 and ~90,000 D. The most prominent band was observed at ~70 kD. For the cells without TPA treatment, this 70 kD band was present in pool 2 and was only detected by fluorography, indicating the presence of mostly <sup>3</sup>H-radioactivity. After TPA treatment, the 70 kD band was mostly present in pool 3 and was detected by both fluorography and autoradiography, indicating that the 70 kD material has a higher negative charge.

<sup>5</sup> This peak may be due to nonmetabolic labeling of serum proteins added to culture medium.<sup>221</sup>
TPA (+)

UMR FLG29.1

BSP →

**FIG. 3.** SDS–PAGE analysis of immunoprecipitation experiments using a specific antibody against human BSP (LF 6). Cell extracts from FLG 29.1 cultures metabolically labeled with [3H]glucosamine and [35S]sulfate in the presence or absence of TPA were fractionated by Q-Sepharose ion exchange chromatography. Pooled fractions as indicated in Fig. 1, were immunoprecipitated using a specific antibody against human BSP, and immunoprecipitates were analyzed by gradient (4–20%) polyacrylamide gel electrophoresis under reducing conditions. Radioactive bands were detected by fluorography.

and contains 35S-radioactivity. The migration position of the 70 kD bands was indistinguishable from that of the purified rat BSP analyzed in the same gel (Fig. 2A, arrow). The band at 90 kD was present in cell extracts before and after TPA treatment in the same fractions but its intensity was much reduced after TPA treatment. Pool 5 contained a large molecular weight, broad band containing 35S-activity, which was identified as mainly chondroitin sulfate proteoglycan by specific enzyme digestion and gel filtration analyses on Superose-6 (data not shown). SDS–PAGE analysis of medium extracts did not show discrete bands (data not shown).

**Immunoprecipitation**

The identity of the 70 kD band as BSP was demonstrated by immunoprecipitation experiments. Q-Sepharose fractions were pooled as described above and immunoprecipitated using a specific polyclonal antibody against human BSP (LF 6). The immunoprecipitate was analyzed by SDS–PAGE (Fig. 3). The 70 kD material present after TPA treatment was the only band specifically precipitated by the antibody, thus tentatively identified as BSP. The 70 kD material from the untreated FLG 29.1 cells was not detected by the present immunoprecipitation procedure (data not shown).

**Detection of mRNA for BSP**

The presence of BSP mRNA was examined by reverse transcription of the total RNA with an antisense primer for mRNA encoding BSP followed by PCR amplification as described in the Materials and Methods section. A PCR product of the expected size was detected from the FLG 29.1 cells treated with TPA, Fig. 4. The amount of BSP mRNA was approximately 1/50th that of UMR cells. BSP mRNA was not detected in untreated FLG 29.1 cells.

**FIG. 4.** Detection of mRNA for BSP by RNA-PCR. Total RNA extracted from FLG 29.1 cells with or without TPA treatment and that from rat osteoblastic cells (UMR) were reverse transcribed and amplified by PCR using sense- and antisense primers for BSP. PCR products were analyzed by agarose (1%) gel electrophoresis and stained with ethidium bromide. Amounts of RNA used for the reaction were indicated in micrograms for UMR cells. Two micrograms each of the total RNA was used for FLG cells treated (+) or untreated (–) with TPA. Expected size of the amplified product for BSP mRNA (918 bp) is indicated by an arrow.
FIG. 5. Time course of BSP expression induced by TPA treatment. FLG 29.1 cells were cultured in the presence of TPA for 24, 48, 72, and 96 h and labeled with [3H]glucosamine and [35S]sulfate for the last 24 h period. Unincorporated isotopes were removed from the cell extract by Sephadex G-50 gel chromatography, and the labeled macromolecules were directly analyzed by SDS-PAGE using 4–20% gradient polyacrylamide gels. Radioactive bands were detected by fluorography. Labeling time (h) and TPA treatment are indicated above the lanes. Migration position of purified BSP is also indicated.

**Thrombin digestion**

Aliquots of radiolabeled proteins were digested with 1 U of thrombin, and digestion products were analyzed by SDS-PAGE. The 70 kDa glycoprotein was not susceptible to thrombin digestion (data not shown).

**Time course and dose effects of TPA on BSP expression**

FLG 29.1 cells were cultured with or without TPA for 24, 48, 72, and 96 h and were metabolically labeled with [3H]glucosamine and [35S]sulfate for the last 24 h. SDS-PAGE analysis of cell extracts (Fig. 5) showed that the cells already expressed the sulfated form of BSP within 24 h incubation with TPA and that the expression of the sulfated form was relatively constant throughout the incubation period (result of autoradiography not shown). The expression of BSP was examined by incubating FLG 29.1 cells with varying concentrations (from 10^{-9} to 10^{-6} M) of TPA for 48 h and metabolically labeling the cells with [3H]glucosamine and [35S]sulfate for the last 24 h. A TPA concentration of 10^{-9} M was sufficient to induce the expression of sulfated BSP by FLG 29.1 cells (Fig. 6). Higher concentrations of TPA did not elicit further changes compared with the treatment using 10^{-9} M TPA.

**Immunocytochemical detection of BSP**

FLG 29.1 cells cultured for 48 h in the presence or absence of TPA were examined by immunocytochemistry using a specific polyclonal antibody raised against the human BSP (Fig. 7) before and after permeabilization of the cells. Control cells, either with or without permeabilization, showed little immunostaining (Figs. 7A and 7B). TPA-treated cells showed diffuse staining without permeabilization, consistent with the cell surface staining (Fig. 7C). A strong positive reaction was demonstrated, however, in the paranuclear area, corresponding to the Golgi apparatus, of almost all TPA-treated FLG 29.1 cells after permeabilization (Fig. 7D).

**Fluorescence flow cytometric analysis**

Fluorescence flow cytometric analysis of intact FLG 29.1 cells using specific antibodies against human BSP demonstrated that about 95% of the TPA-treated FLG 29.1 cells expressed BSP at the cell surface (Fig. 8, filled profile). Control cells showed little fluorescence (Fig. 8, unfilled profile).
FIG. 7. Immunocytochemical examination of FLG 29.1 cells using a polyclonal antibody against human BSP (LF 6) before (A and C) and after permeabilization (B and D). TPA-treated cells (C and D) show a positive reaction for BSP, while control cells (A and B) show very little reaction. Bar, 20 μm.

DISCUSSION

FLG 29.1 is a human monoblastic cell line cloned from the bone marrow of a patient with acute monoblastic leukemia. After exposure to 0.1 μM TPA, these cells have been found to express several osteoclastic features. These include surface antigens typical of fetal osteoclasts, tartrate-resistant acid phosphatase, receptors for calcitonin, and the ability to liberate calcium from prelabeled bone particles.

Noncollagenous proteins in the extracellular matrix are thought to be involved in bone remodeling and several of them have been isolated from skeletal and dental tissues. A major, well-characterized noncollagenous protein of the bone matrix is BSP, initially described as a 23,000 D, sialic acid-rich-protein by Herring. This small glycoprotein was later hypothesized to be a breakdown product of a Mr 75,000 sialoprotein. The nucleotide sequence of mRNA encoding BSP has been determined, and localization of the gene has been identified on human chromosome 4. The RGD peptide sequence found in BSP is a recognition motif for integrins, and BSP has been shown to promote in vitro cell attachment and spreading onto plastic petri dishes coated with BSP. Both osteoblasts and osteoclasts have been shown to attach to BSP-coated plastic petri dishes, and osteoclasts have been demonstrated to express appropriate integrins that can interact with BSP. Osteoclasts have also been shown to exhibit physiological responses when exposed to BSP. Thus, the protein may be involved in cell-matrix interactions or function as a signaling molecule in bone.

Several studies have demonstrated that BSP is a bone-specific protein and osteoblasts have been shown as the principal source of BSP in bone. However, recent in situ hybridization studies have shown the presence of BSP mRNA in osteoclasts. In the present study we have demonstrated by metabolic radiolabeling and immunoprecipitation experiments that BSP is also expressed in a human preosteoclastic cell line, suggesting a role for the protein in the differentiation of immature precursor cells to functioning osteoclasts or that it is required by differentiated osteoclasts. Undifferentiated FLG 29.1 cells also synthesized a prominent protein of ~70 kD, which exhibited little or no sulfation. Since this protein was not immunoprecipitated with the anti-BSP antibody and the mRNA for BSP was not detected in the FLG 29.1 cell without TPA treatment, its identity is presently unknown. However, it is possible that the 70 kD protein produced in untreated FLG 29.1 cells is a nonsulfated form of BSP and the TPA treatment induced increased levels of BSP mRNA and sulfation of the protein. Post-translational modifications of BSP may represent an important mechanism in the modulation of osteoclast progenitors. Indeed, the sulfation of proteins could be
BIOSYNTHESIS OF BSP IN AN OSTEOCLAST-LIKE CELL LINE

considered an important step in the induction of the adhesion properties of the molecule, along with its RGD sequence (12,26,34). Expression of other major sulfated glycoproteins synthesized by FLG 29.1 cells is also modulated during the differentiation induced by TPA. Their identity and functions remain to be elucidated.

It is of interest that BSP was detected with a specific antibody both on the surface as well as in the paranuclear area corresponding to the Golgi apparatus. (19,26) SDS-PAGE analysis confirmed that BSP in FLG 29.1 cells was cell-associated. Similarly, Bianco and coworkers (10,22) found BSP in mature osteoclasts with a distribution at the cell/bone interface and in multiple paranuclear areas. Paranuclear localization of BSP in FLG 29.1 cells is remarkably similar to that observed in osteoblasts. (10,22) Concentrated localization of BSP in the Golgi structure was interpreted as the slow traffic of the protein through Golgi structures. (22)

This interpretation is especially relevant for FLG 29.1 cells, since very little BSP is secreted by the cells. Another interesting finding of BSP immunolocalization in osteoblasts was that little BSP was detected in the rough endoplasmic reticulum. (22) This was interpreted as due to either a very weak cross-reactivity of the anti-BSP antibody to the protein before certain post-translational modifications or the presence of the protein below the detection limit. LF 6 anti-BSP antibody may, therefore, require proper post-translational modifications of BSP for its high affinity recognition.

The role of BSP in the bone remodeling process is not fully understood. Here, we demonstrated that preosteoclasts synthesized BSP coinciding with the cell differentiation process. This model will prove useful in evaluating the role of BSP, as well as of other bone matrix proteins, in the process of osteoclast differentiation.

REFERENCES

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