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Marker of cemento-periodontal ligament junction associated with periodontal regeneration

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Objective: The purpose of this study was to identify factors promoting formation of the cemento-periodontal ligament junction.

Background: Regeneration of the cemento-periodontal ligament junction is an important factor in recovery of the connective tissue attachment to the cementum and it is important to identify all specific substances that promote its formation. To clarify the substances involved in cemento-periodontal ligament junction formation, we produced a monoclonal antibody (mAb) to human cemento-periodontal ligament junction (designated as the anti-TAP mAb) and examined its immunostaining properties and reactive antigen.

Methods: Hybridomas producing monoclonal antibody against human cemento-periodontal ligament junction antigens were established by fusing P3U1 mouse myeloma cells with spleen cells from BALB/c mice immunized with homogenized human cemento-periodontal ligament junction. The mAb, the anti-TAP mAb for cemento-periodontal ligament junction, was then isolated. The immunoglobulin class and light chain of the mAb were examined using an isotyping kit. Before immunostaining, antigen determination using an enzymatic method or heating was conducted. Human teeth, hard tissue-forming lesions, and animal tissues were immunostained by the anti-TAP mAb.

Results: The anti-TAP mAb was positive in human cemento-periodontal ligament junction and predentin but negative in all other human and animal tissues examined. In the cemento-osseous lesions, the anti-TAP mAb was positive in the peripheral area of the cementum and cementum-like hard tissues and not in the bone and bone-like tissues. The anti-TAP mAb showed IgM (kappa) and recognized phosphoprotein.

Conclusion: The anti-TAP mAb is potentially useful for developing new agents promoting cementogenesis and periodontal regeneration.

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Cementum is a thin mineralized tissue covering the root surface of teeth that provides an interface through which the periodontal ligament anchors the tooth to the alveolar socket. Cementum can be cellular and acellular, and both types play important roles in the fibrous attachment. Loss of the fibrous attachment with cementum occurs when the periodontal ligament is destroyed by periodontitis. Regeneration of the periodontal ligament requires reattachment (1).

Among the structures that make up periodontal tissue, the periodontal ligament and cementum are the most important that contribute to the regeneration of the connective tissue attachment. Although bone morphogenetic protein (2–4) and Emdogain®

(enamel matrix protein: BIORA AB, Malmö, Sweden) (5–7) are used in periodontal therapy and contribute to regeneration of the fibrous attachment, their use does not guarantee good results. The newly formed cementum that they produce is almost always cellular. Acellular cementum forms a much stronger bond to dentin than does the cellular type. Formation of new acellular cementum is a key process in the regeneration of lost periodontium (1).

Toluidine blue staining shows a large accumulation of proteoglycans at the junction between dentin and cementum. Although the junction is positive for toluidine blue, it is not impregnated by silver stain. When the cementodentinal junction is digested with hyaluronidase, it shows no affinity for toluidine blue. On the other hand, the junction between bone and the periodontal ligament shows similar stainability with toluidine blue and silver-impregnated stain. The junction between bone and the periodontal ligament anchors the two tissues and is similar to the junction between cementum and the periodontal ligament. However, because the periodontal ligament does not stain with toluidine blue, the cemento-periodontal ligament junction does not stain the same as the cementodentinal junction or the junction between bone and the periodontal ligament (8). Although these junctions are highly interesting structures, the factors crucial to periodontal regeneration are not well understood (9). Thus, research on proteoglycans involving glycosaminoglycans (10), laminin-5 (11), and osteopontin (12) have been undertaken; however, research on phosphoprotein, which concerns calcification, has not been the focus of much attention.

To identify the factors that foster formation of the periodontal ligament and cementum, we must first learn what substances are present in the periodontal ligament and cementum. Little is known about the molecular mechanisms that regulate the periodontal ligament and cementum, because specific human anti-cementum monoclonal antibodies (mAbs) are unavailable (13). mAbs are very useful for isolating specific tissue proteins. In the oral cavity, mAbs have been developed to bovine cementum (14, 15), the mouse tooth germ (16, 17), and bovine enamel proteins (18, 19). However, because there are currently no mAbs to human cementum, we attempted to produce one and characterize its properties to identify the specific factors involved in periodontal regeneration.

Materials and methods

Preparation of antigen

After approximately 300 extracted teeth were fixed in 10% neutral buffered formalin for 24 h, they were decalcified in 10% EDTA or K-CX (chelating agent and hydrochloride; Falma, Tokyo, Japan). Following decalcification, the cemento-periodontal ligament junction from each specimen was collected as antigen. The specimens were sliced, minced into 1 mm^3 cubes, shattered with a 30 μm Medicon (automatically homogenizing system, DAKO Cytomation, Glostrup, Denmark) using a Medimachine (DAKO Cytomation), and triturated using a homogenizer and a mixing mill (MM30, Retsch GmbH & Co. KG, Haan, Germany). The obtained solution was dried and used as antigen.

An emulsion was made by mixing 100 mg/ml of the antigens with distilled water and an equal volume of Freund's Complete Adjuvant (TiterMax Gold: CytRx Corp., Norcross, GA, USA). Three 5-week-old female BALB/c mice were immunized intraabdominally once weekly for 4 weeks with 0.25 ml of emulsion (400 mg/ml antigen) as the antigen. The spleens were extracted under anesthesia 3 days after the last injection (20). The mice were treated according to the Guidelines for Animal Research at Osaka Dental University.

Cell fusion, screening, and cloning

Spleen cells ($1 \sim 2 \times 10^8$) and P3U1 myeloma cells (1×10^8) were mixed and fused with 1 ml of 50% polyethylene glycol 1500 (Roche Ltd, Mannheim, Germany) as described previously (20–23). Fused cells were centrifuged, and the pellet was gently re-suspended in RPMI containing 2% hypoxanthine–aminopterin–thymidine (ICN Biomedicals, Inc., Aurora, OH, USA) and 15 ml of Bri Clone (Archport Ltd, Dublin, Ireland). Thereafter, growing hybridomas were examined both macroscopically and with a phase contrast microscope 20 days after fusion. Yellow supernatant fluid in wells was screened by an indirect immunohistochemical method using formalin-fixed, paraffin-embedded sections of roots of extracted teeth, and also Tris-buffered saline was used instead of the first antibody or the secondary antibody as a negative control. Before immunostaining, antibody activities of supernatant fluids were examined by enzyme-linked immunosorbent assay (ELISA) using peroxidase goat anti-mouse immunoglobulins (DAKO Cytomation). Hybridomas producing supernatant fluids with positive staining for cemento-periodontal ligament junction were cloned by limiting dilution, and mAb was isolated. The mAb produced is referred to as the anti-TAP mAb (was merely derived from our professor, Tanaka Akio).

Determination of immunoglobulin class of monoclonal antibody

The immunoglobulin class of the anti-TAP mAb was determined by using a mouse mAb isotyping test kit (Serotec Ltd, Raleigh, NC, USA) (24).

Stainability of monoclonal antibody in various tissues

Periodontal tissues from rats, dogs, pigs, and cows, and including lesions of cemental dysplasia, cemento-ossifying fibroma, osteoma, and ossifying fibroma from humans were immunostained to examine cross-reactive immunostaining. The procedures used were approved by the Ethics Committee, Osaka Dental University.

Stability of antigen determinant

To examine the stability of antigen determinant, sections were treated with 0.4% pepsin for 10 min at 37°C, 0.1% trypsin for 20 min at 37°C, pronase for 10 min at 37°C or proteinase K for

10 min at 37°C, and then autoclaved for 20 min at 121°C, microwaved at 500 W for 5 min, heated for 40 min at 95°C or heated for 1 h at 75°C, and treated with 1% periodic acid for 30 min, and 10 U/ml collagenase in Tris-buffered saline for 12 h at 37°C, 10 U/ml neuraminidase in Tris-buffered saline for 12 h at 37°C, nitrous acid (1 : 1 mixed solution of 33% AcOH and 5% $NaNO_2$) for 5 min at room temperature, and 1.0% testicular hyaluronidase in Tris-buffered saline for 36 h at 37°C or toluidine blue staining (pH 6.0) (8, 25). In addition, to examine the stability of periodontal ligament cells from a human patient that were stocked in our laboratory, the cells were cultured over glass slides.

Extraction of antigen from human extracted teeth and immunoblotting

Cementum (100 μg) was collected from decalcified extracted teeth, minced, and homogenized in Tris-buffered saline. After centrifugation, the sediment was suspended in 30 ml of buffer with 2% Tween-20, and left overnight at room temperature. This was repeated for Tween-80, Triton X, sodium dodecyl sulfate, and Nonidet P-40. After centrifugation, supernatant fluids were filtered with a 20 μm Filcon N filter (DAKO Cytomation) and stored at 4°C until use. Membranes used were: Immobilon polyvinyl difluoride (PVDF) Transfer Membrane, 0.45 μm pore size (Nihon Millipore Ltd, Yonezawa, Japan); Immun-Blot PVDF membrane for protein blotting, 0.2 μm pore size (Bio-Rad, Hercules, CA, USA); and Protran BA 85 Cellulose-nitrat (E), 0.45 μm pore size (Schleicher & Schuell, Dassel, Germany). Membranes were cut to size, and the PVDF membranes were pre-wetted in methanol 10 min. All membranes were washed every 5 min in distilled water followed by Tris-buffered saline. After 5 μl of extracted antigen and 3.16 mg/ml of epithelial keratin (Upstate, Lake Placid, NY, USA) as negative controls were absorbed onto each membrane, 30 μl of the anti-TAP mAb was dropped onto each membrane (26). The membranes were washed in Tris-buffered saline three times every 15 min after 2 h at room temperature and incubated with 30 μl of a secondary antibody, EnVision (DAKO Cytomation), for 30 min at room temperature, or Tris-buffered saline was used instead of the anti-TAP mAb

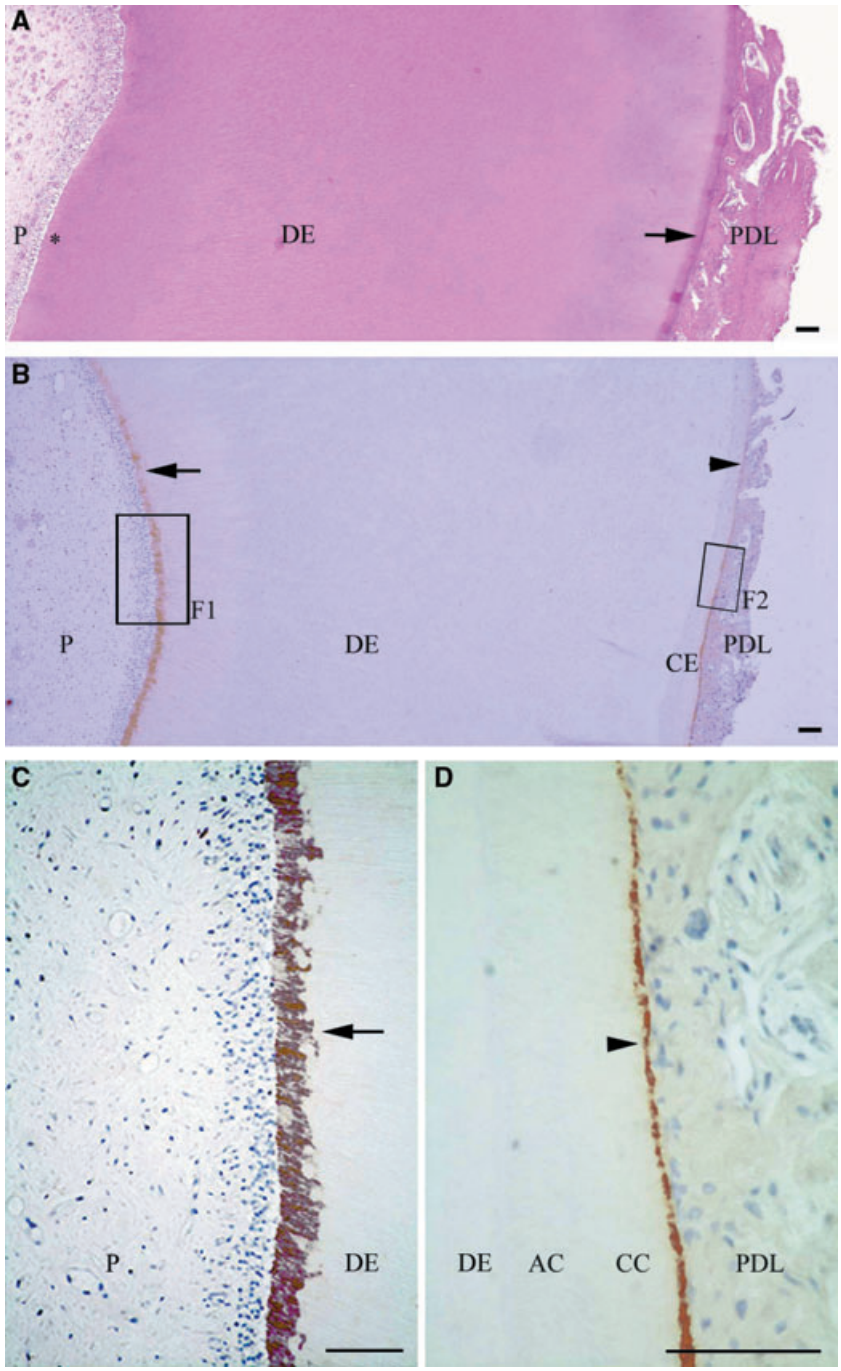

Fig. 1. (A) Transverse histologic sections of human tooth. Dental pulp (P), predentin (*), dentin (DE), acellular cementum (arrow), and periodontal ligament (PDL) are observed (hematoxylin and eosin stain). (B) Immunohistochemical staining of the anti-TAP mAb in a transverse section of a human tooth. Positive reaction is seen in the predentin (arrow) and the cemento-periodontal ligament junction (arrowhead) but not in the dental pulp, dentin, cementum (CE), or PDL. (C) High-power view of the framed area F1 from B. The anti-TAP mAb is positive only in the predentin (arrow). (D) High-power view of the framed area F2 from B. The anti-TAP mAb is positive only in the cemento-periodontal ligament junction (arrowhead). CC, cellular cementum. Bar = 100 μm.

or secondary antibody as the negative control. The membranes were then washed in Tris-buffered saline three times for 15 min and reacted with 3,3′-diaminobenzidine tetrahydrochloride (DAB) for observation (27).

Purification of antigens

For immunoaffinity purification of antigens against the anti-TAP mAb, an affinity column was prepared using 5 ml of Hi Trap NHS-activated HP (Amersham Biosciences, Corp., Piscataway, NJ, USA), which is prepacked ready to use and able to be administered with a 10 ml syringe. Water and chemicals used for buffer preparation were of high purity. All buffers were filtered by passing them through a 0.45 μm filter before use. Antigens according to the manufacturer's instructions for Hi Trap NHS-activated HP were eluted with elution buffer (0.1 M Glycine-HCl, pH 2.7). To confirm the antigen, these purified antigens were screened by sodium dodecyl sulfate–polyacrylamide gel electrophoresis using the anti-TAP mAb (28).

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and western blotting

Antigens extracted and epithelial keratin as a negative control were processed for electrophoresis in a 5–20% gradient gel. Antigens and epithelial keratin were separated using 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis under reducing conditions, visualized using a Coomassie Brilliant Blue R-350 (Bio-Rad), and then transferred electrophoretically to the PVDF membrane. The membranes were blocked with 1% balanced saline solution in Tris-buffered saline containing 0.1% Tween-20 for 1 h. Antigens and epithelial keratin were then probed with the anti-TAP mAb for 2 h and then with the horseradish peroxidase-linked secondary antibody and developed with an enhanced chemiluminescence (ECL) western blotting detection kit and chemiluminescence detection using an ECL minicamera (Amersham Biosciences) or DAB staining. Sample ECL DualVue western blotting markers and High-Range Rainbow molecular markers (Amersham Biosciences) were used as molecular weight standards (29–31). No blocking buffer other than the buffer mentioned above was used.

Analysis of antigen

The purified antigens were identified as phosphoprotein by western blotting with the anti-TAP mAb or the ECL Phosphorylation System (Amersham Biosciences) and ELISA with supernatants of phosphoserine peptide, phosphothreonine peptide, or phosphotyrosine peptide as antigen and using the anti-TAP mAb. The purified antigens were then screened by ELISA using anti-phosphoserine mAb, anti-phosphothreonine mAb, or anti-phosphotyrosine mAb.

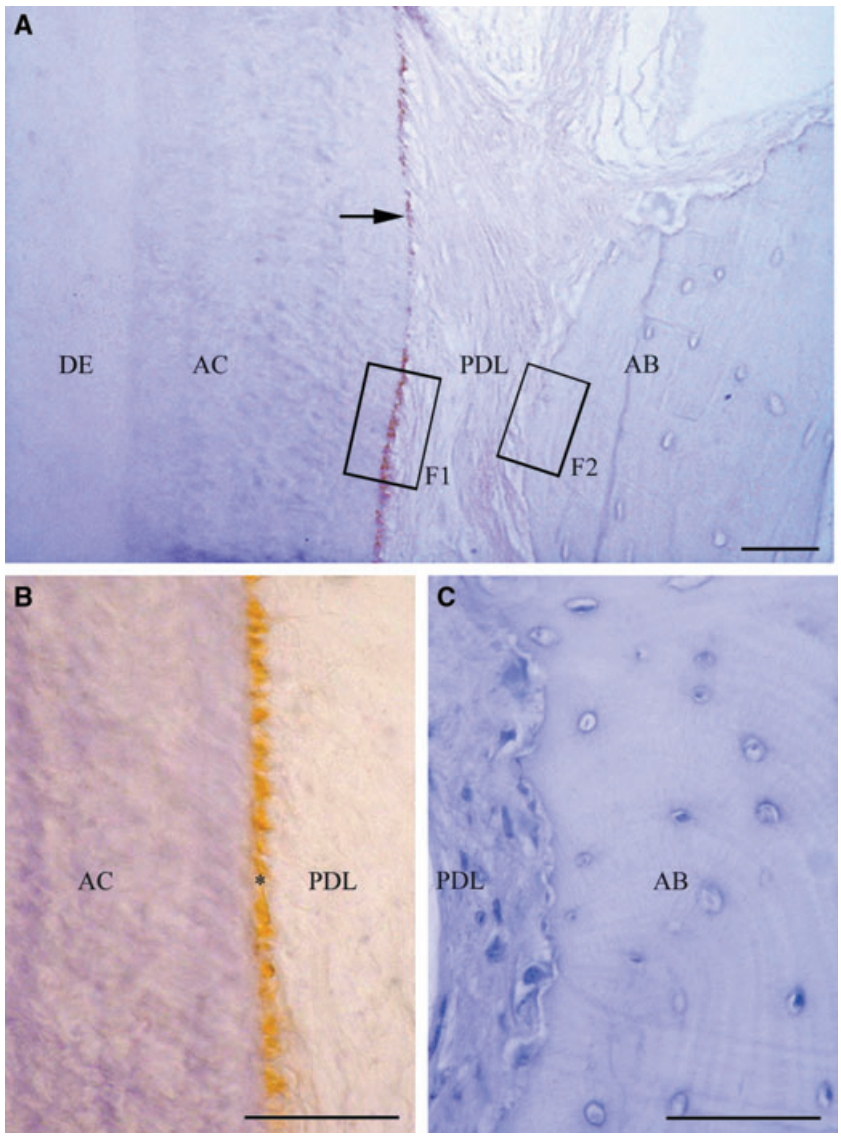

Fig. 2. (A) Immunohistochemical staining of the anti-TAP mAb around a periodontal ligament (PDL) including acellular cementum (AC) and alveolar bone (AB) of the vertical section. A positive reaction is visible only in the cemento-periodontal ligament junction (arrow) and not in the dentin (DE), acellular cementum, PDL, or alveolar bone. (B) High-power view of the framed area F1 from A. The anti-TAP mAb is positive only in the cemento-periodontal ligament junction (*). (C) High power view of the framed area F2 from A. The anti-TAP mAb is negative in the PDL, interface between PDL and alveolar bone, and alveolar bone. Bar = 100 μm.

Immunoprecipitation using anti-phosphoserine-threonine monoclonal antibody

We verified that the antigens were purified by immunoprecipitation using anti-phosphoserine-threonine mAb (Anti-pSer/Thr PKA substrate, Daiichi Pure Chemicals Co., Ltd, Tokyo, Japan) (25, 28, 32).

Two milliliters of purified antigens by affinity column chromatography were added into a 2 ml Eppendorf tube (Eppendorf Co., Ltd, Tokyo, Japan) involving NaF as a phosphatase inhibiter adjusted to final concentrations of 10 mM. Then 5 μl of protein-*A* beads (Upstate) and 5 μl of normal IgG saturated protein-*A* beads (Upstate) were added to the tube, which was then rotated for 1 h at 4°C. The supernatant was decanted to high-speed ultracentrifugation at 23 000 ***g*** for 1 min at 4°C. Anti-phosphoserine-threonine mAb was added to the tube, which was then rotated for 1 h at 4°C. Then 5 μl of protein-*A* beads was added and the tube was rotated again for 1 h at 4°C. The gel was harvested by centrifugation at 700 ***g*** for 1 min and washed with Tris-buffered saline with Tween-20 [10 mM Tris (pH 7.4), 100 mM NaCl 0.1% (v/v), Tween-20]. The gel was purified by immunoprecipitation using anti-phosphoserine-threonine mAb and screened by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, western blotting, or ELISA with the anti-TAP mAb or immuno-DAB staining.

Results

Monoclonal antibody to cemento-periodontal ligament junction

Thirty-eight hybridoma cell lines were established after cell fusion using spleens from mice immunized with human cemento-periodontal ligament junction three times for four consecutive weeks. Antibodies produced by these hybridomas were used to immunohistochemically stain formalin-fixed, paraffin-embedded sections of extracted human teeth. Only one of the antibodies isolated, the anti-TAP mAb, immunostained cemento-periodontal ligament junction and predentin (Fig. 1).

The anti-TAP mAb showed IgM immunoglobulin class and a kappa light chain with the mouse mAb isotyping test kit.

Immunohistochemical staining with the anti-TAP monoclonal antibody in various tissues

In both transverse (Fig. 1A) and vertical (Fig. 2A) formalin-fixed, paraffin-embedded sections of teeth, immunoreactivity with the anti-TAP mAb was restricted to predentin (Figs 1B and C) and the cemento-periodontal ligament junction (Figs 1D and 2B). No staining was detected in the dental pulp (Figs 1B and C), gingival connective tissue, gingival epithelium, sulcular epithelium, periodontal ligament (Figs 1D and 2B), or alveolar bone (Fig. 2C), nor was any staining observed in any rat, canine, porcine, or bovine tissues (data not shown). Exactly in the same manner as indicated for cemento-periodontal ligament junction, immunohistochemical staining was detected by light microscopy in the periphery of the cementum-like tissues (Fig. 3A), but not in the peripheral margin of the bone, bone tissues, connective tissues or bone-like tissues from cemental dysplasia (Fig. 3B), cemento-ossifying fibroma, ossifying fibroma, or osteoma. In addition, in the negative control of each section, Tris-buffered saline instead of the anti-TAP mAb or secondary antibody was used as a negative control, no positive reactions were observed.

Characterization of antigen reactive with the anti-TAP monoclonal antibody

The immunohistochemical reactivity with the anti-TAP mAb was not affected by treatment of histologic human tooth sections with pepsin, trypsin, proteinase K, or pronase. For proteoglycan demonstration, periodic acid-Schiff and toluidine blue (Fig. 4A) stained sections treated with periodic acid, nitrous acid, neuraminidase, or hyaluronidase were also positive for the anti-TAP mAb and did not reduce the anti-TAP mAb staining (Figs 4B and C); thus, the antigen apparently did not include glycogen, sialic acids, heparin, heparan sulfate, or hyaluronic acid. The activity of the antigen protein reactive with the anti-TAP mAb was not inhibited by autoclaving, microwaving, heating, organic solvents, or hydrolysis with various enzymes. In addition, cultured periodontal ligament cells from a patient that were stocked in our laboratory were negative for the anti-TAP mAb and sections of human tooth treated with collagenase were also positive for the anti-TAP mAb and did not reduce the anti-TAP mAb staining; thus, the antigen apparently did not include collagen.

Unpurified (Fig. 5, lane 1) or purified antigen (Fig. 5, lanes 2 and 3) reactive with the anti-TAP mAb was extracted using various surfactants and detected by dot blotting with Immun-Blot PVDF membrane for protein blotting (data not shown). Strong positive intensity was observed. Even though sections were oxidized with periodate, the anti-TAP mAb immu-

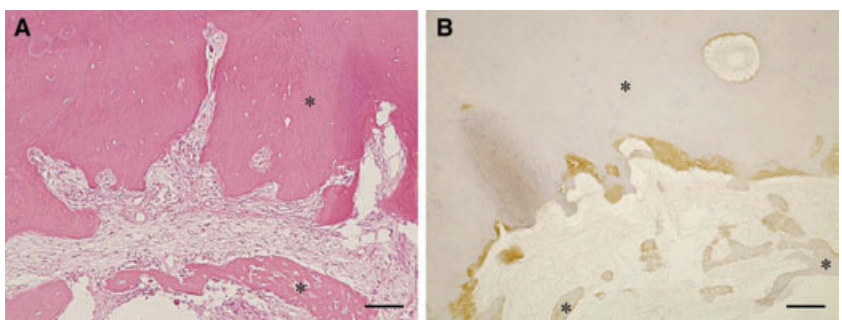

Fig. 3. Histological section of cemental dysplasia. (A) Cementum-like hard tissue (*) is visible (hematoxylin and eosin stain). (B) The anti-TAP mAb immunostaining. A positive reaction is seen in the periphery of the cementum-like hard tissue (*). Bar = 100 μm.

nostaining was observed, indicating that its antigen determinant apparently was not a glycochain. The anti-TAP mAb recognizes a strong acidic serine phosphorylated protein based on examinations of phosphorylated protein by western blot (Fig. 5, lane 4), protein absorption testing (Fig. 5, lanes 5–7), and two types of ELISA. In one type of ELISA, phosphoserine peptide, phosphothreonine peptide, and phosphotyrosine peptide were used separately as the individual antigen, and the anti-TAP mAb was used as the first antibody. The ELISA reactions for phosphoserine were strong, but for phosphothreonine were weak, and no ELISA reactions for phosphotyrosine were observed. In the other type of ELISA, antigen purified from cementum and antigen by the anti-TAP mAb affinity column chromatography, and anti-phosphoserine mAb, anti-phosphothreonine mAb, and anti-phosphotyrosine mAb were used as the first antibody. Again, we demonstrated that the ELISA reactions for anti-phosphoserine were strong, but for anti-phosphothreonine were weak, and no ELISA reactions for anti-phosphotyrosine were observed (33–35).

The antigen had two molecular mass bands of about 40 kDa each separated by sodium dodecyl sulfate (Fig. 5, lanes 2, 3, 5).

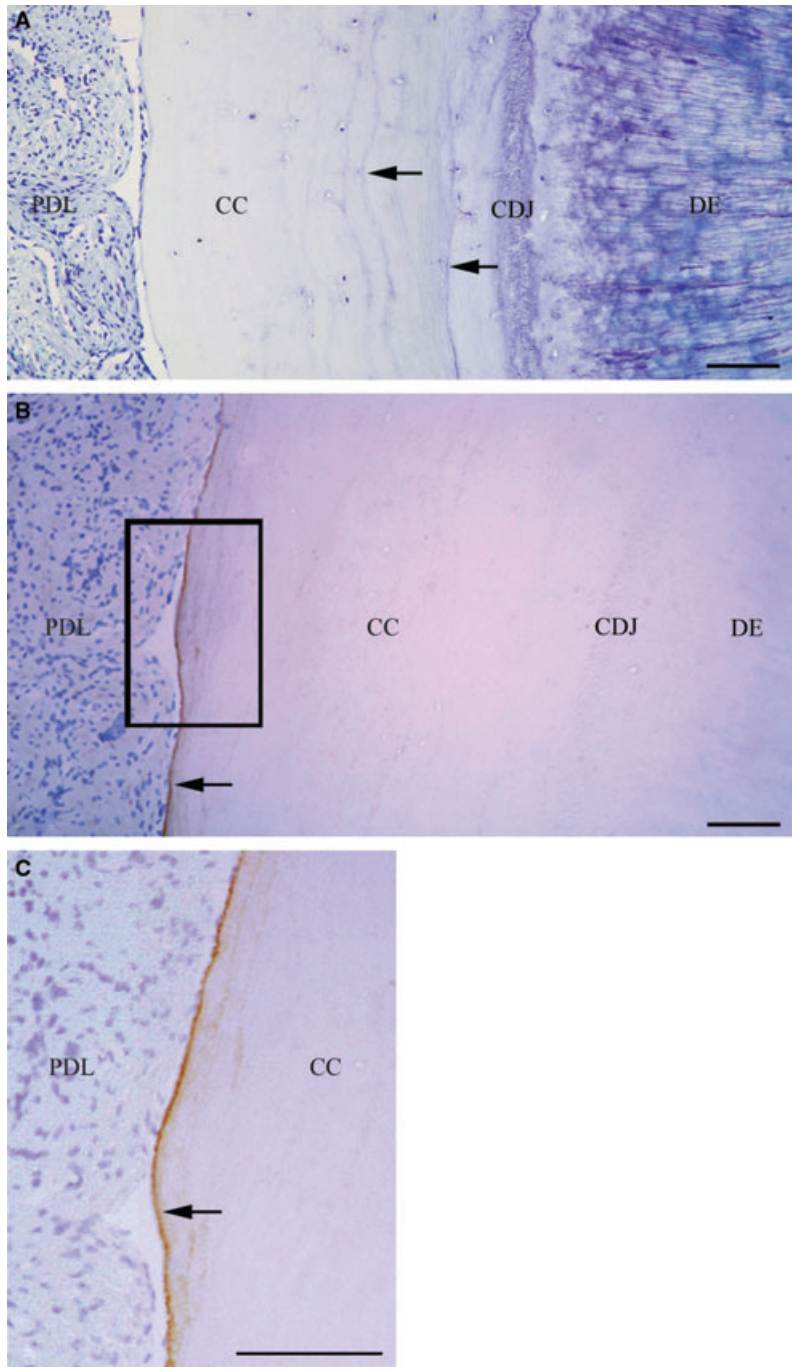

Fig. 4. (A) Transverse histological sections of human tooth stained with toluidine blue staining (pH 6.0). Metachromatic reaction is seen in dentin (DE), cemento-dentino junction (CDJ), and cementum interlamellar layer (arrows). PDL, periodontal ligament; CC, cellular cementum. (B) Immunohistochemical staining with the anti-TAP mAb in a serial section of A. The anti-TAP mAb is positive only in cemento-periodontal ligament junction (arrow). (C) High-power view of the framed area from B. The anti-TAP mAb is positive only in cemento-periodontal ligament junction (arrow). Bar = 100 μm.

Discussion

Calcium-binding, non-collagenous common proteins in predentin and cemento-periodontal ligament junction are classified into four groups, of which the calcification-related proteins include phosphoproteins, glycoproteins, and osteocalcins (Gla proteins). Phosphophoryn, or dentin phosphoprotein, is a phosphoprotein that is specific to dentin and a primary component of the non-collagenous protein uniformly distributed in dentin. This protein shows strong acidity, because its composition includes 80% aspartic acid and phosphoserine. Therefore, this protein is prone to strong calcification. However, the molecular mass has not yet been precisely defined, although it ranges from 30 to 150 kDa (36). The mass varies with the diffusion artifact when measured by gel filtration and sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and its measurement also is hampered by the difficulty in establishing its mAb because of its very low antigen activity.

The glycoproteins include osteonectin, osteopontin (bone sialoprotein)

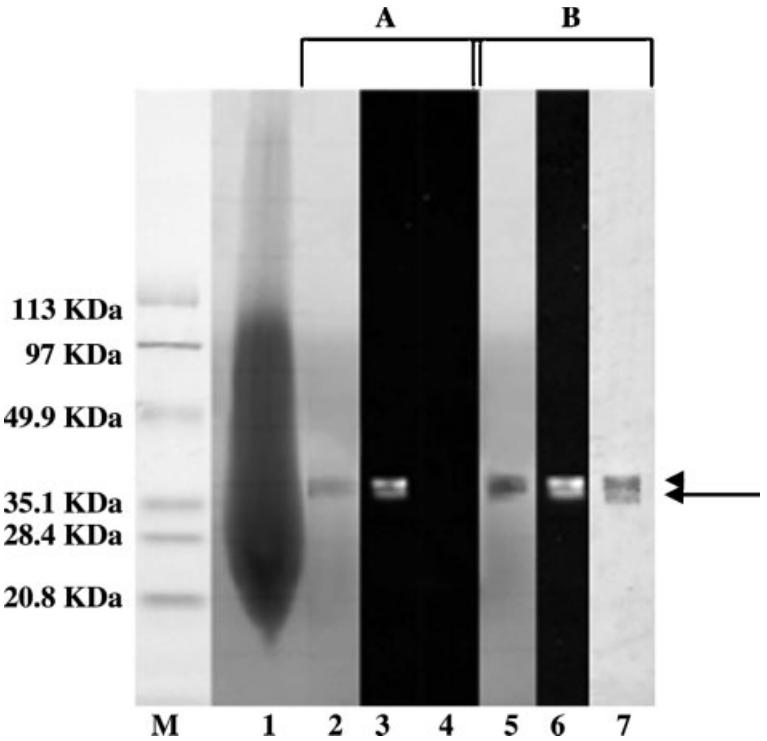

Fig. 5. Immunoelectrophoresis. Unpurified antigens show no clear bands in sodium dodecyl sulfate–polyacrylamide gel electrophoresis with Coomassie Brilliant Blue (CBB) staining (lane 1). Antigens purified by affinity-column chromatography using the anti-TAP mAb (A). The antigens separated with 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and visualized by CBB staining (lane 2), immunoblotting (western blotting) with the anti-TAP mAb (lane 3) and the same fractions do not bind to anti-phosphotyrosine mAb (lane 4). The antigens purified a second time by immunoprecipitation using anti-phosphoserine-threonine mAb (B). The antigens separated with 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and visualized with CBB staining (lane 5), western blotting (lane 6) and immuno-DAB staining (lane 7) with the anti-TAP mAb. Lane M is molecular weight standards with sizes marked on the left (in kDa). Two bands (arrow and arrowhead) indicate the antigens separated by sodium dodecyl sulfate with a molecular mass of about 40 kDa each.

(37), and dentin sialoprotein. Osteonectin, which contains abundant glutamic acid and aspartic acid and small amounts of phosphoric acid, glucosamine, galactosamine, and sialic acid, seems to play a role in cell differentiation, specifically in the initial stage of development, cell migration, morphogenesis, and repair. Osteopontin, or bone sialoprotein, a protein that forms the connection between the osteocyte and the matrix, is an acidic protein containing abundant glutamic acid, aspartic acid, and sialic acid. In addition, osteopontin readily binds with calcium ion and rare earth ions. However, this protein is not specific to cementum and bone, and is present even in the kidney and placental tissues (37, 38).

Gla proteins contain osteocalcin and matrix Gla protein, and the former is related to calcification. Osteocalcin, or bone Gla protein, which is specific to bone and dentin and a component of hydroxyapatite, plays a role in the control of calcification (38).

As mentioned previously, decalcified teeth contain various elements, and the substances extracted depend on the methods used to decalcify the hard tissue. Although EDTA decalcification requires a long time, it is suitable for the extraction of unstable proteins. However, mineral/matrix-binding proteins and proteins covalently bound to the matrix are also extracted. Therefore, it is difficult to extract osteonectin and bone sialoprotein. However, hydrochloric acid decalcification does not require a long time and allows extraction of such non-collagenous proteins as osteocalcin, osteopontin, and phosphophoryn. Consequently, the anti-TAP mAb identified in the present study is strongly suspected to be a phosphophoryn because of its strong acidity and confirmed serine phosphoprotein content. However, as the anti-TAP mAb isolated reacts only with cemento-periodontal ligament junction and predentin and does not react with bone, gingiva, pulp, or periodontal ligament, it might be a new subtype of phosphophoryn (13, 39, 40).

The anti-TAP mAb we isolated showed no immunohistochemical cross reaction in any animal tissue examined and seems to be specific to human tissue. Only cemento-periodontal ligament junction and predentin were positive for the anti-TAP mAb in the normal tissue. In addition, in cemento-osseous dysplasia and fibro-osseous lesions, the peripheral area of the cementum-like tissues was positive for the anti-TAP mAb, whereas bone and bone-like tissues were negative. On histological section (Fig. 3A) of cemental dysplasia, the positive area might be larger and clearer than that of the cemento-periodontal ligament junction (Fig. 3B). As cemental dysplasia is a tumor-like lesion, the quantity of the antigen positive for anti-TAP mAb is increased. In addition, the slice direction of the section is tangential, and thus the positive area might be large and clearly different from the result for cemento-periodontal ligament junction. In summary, we generated an anti-cementum mAb, the anti-TAP mAb, that recognizes cemento-periodontal ligament junction and predentin specifically and has two molecular mass bands of about 40 kDa each separated by sodium dodecyl sulfate because of modification on phosphorylation protein. Its corresponding antigen seems to be associated with the connection between the cementum and periodontal ligament. The anti-TAP mAb has potential applications in the development of useful materials for periodontal regeneration and can be used to identify

cementum-like tissues distinct from bone and bone-like tissues.

Acknowledgements

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