ORIGINAL ARTICLE

Immunohistochemical localization of αvβ3 integrin receptor during experimental tooth movement

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During orthodontic treatment, multinucleated clast cells carry out the resorption of mineralized tissues. Adhesion of clast cells to the mineralized tissues is mediated by transmembrane cell-surface glycoproteins called *integrins*, specifically by the αvβ3 integrin, which plays an important role in the process of bone resorption. The role of the αvβ3 integrin in bone resorption leading to osteoporosis has been demonstrated, but its role in alveolar bone and root resorption during orthodontic tooth movement is unknown. This study examined the expression of the αvβ3 integrin during experimental tooth movement. Tooth movement was achieved in 16 male Sprague-Dawley rats (each weighing 120-200 g) with elastic bands between their maxillary first and second molars. The molar-bearing segments were dissected and processed for histologic and immunohistochemical examination. The expression of αvβ3 integrin was examined with 2 primary antibodies: a polyclonal anti-αv integrin subunit antibody and a polyclonal anti-β3 integrin subunit antibody. Negative controls were similarly processed but without incubation with primary antibodies. The αvβ3 integrin was expressed both by osteoclasts associated with alveolar bone resorption and by odontoclasts associated with root resorption during experimental tooth movement. Furthermore, the β3 integrin subunit was expressed by the epithelial rests of Malassez in the periodontal ligament. Negative controls did not show immunolabeling. The αvβ3 integrin adhesion receptor is expressed during experimental tooth movement and might be involved in the process of mineralized tissue resorption and the functions of the epithelial rests of Malassez. (Am J Orthod Dentofacial Orthop 2004;125:178-84)

Orthodontic forces used during treatment of malaligned teeth trigger various cellular reactions in the surrounding supporting tissues, including resorption and apposition of the alveolar bone and remodeling of periodontal ligament (PDL). The process of mineralized tissue (bone, dentin, and cementum) resorption involves several tightly regulated steps: (1) proliferation of hemopoietic clast cell precursors, (2) differentiation into mononuclear clast cells, (3) fusion of precursor cells to form multinucleated clast cells, (4) clast cell migration to the sites to be resorbed, (5) adhesion of the clast cells to the mineralized surface and formation of the cytoplasmic tight seal, and (6) actual resorption by a low pH in the resorption lacunae produced by cell membrane–associated proton translocating pumps and the release of certain proteases to degrade the organic component of the mineralized tissue.[1] Multinucleated odontoclasts involved in root resorption are structurally similar to osteoclasts, the bone resorbing cells. It can be further hypothesized that they are functionally similar, even though they resorb different mineralized tissue.[1] Mineralized tissue recognition and attachment by clast cells to the mineralized tissue surface is made possible by special cell-surface transmembrane glycoproteins, called *integrins*. Integrins are a superfamily of glycoprotein transmembrane cell-surface receptors composed of noncovalently linked heterodimers containing α and β subunits. Integrins serve as transmembrane linkers and mediate cell-matrix adhesion and cell-cell heterophilic adhesion. They mediate many cellular functions, such as cell growth, differentiation, migration, and survival.[2] It has been shown that a specific integrin, αvβ3, is essential for the formation of the clear zone and the transmission of bone matrix-derived signals necessary for cytoskeletal organization to form the ruffled border in clast cells.[3]

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The αvβ3 integrin was first identified as a vitronectin adhesion glycoprotein receptor. Clast cells express αvβ3, α2β1, and αvβ5 integrin heterodimers.[4] The αvβ3 integrin seems to enable clast cells to adhere to the mineralized tissue through binding to specific adhesion glycoproteins, such as osteopontin and bone sialoprotein.[5] The amino acid sequence necessary for integrin binding consists of 3 amino acids: arginine, glycine, and aspartic acid (RGD).[5] Immunocytochemical studies of the distribution of integrin αvβ3 in rat tissues showed strong labeling on the osteoclast domains facing the bone surface corresponding to the areas of the clear zone around the ruffled borders.[6] Other integrins showed very low-intensity labeling throughout all parts of the osteoclasts. These findings suggest that the αvβ3 integrin is the most abundant type involved in the cellular attachment of osteoclasts to bone during bone resorption.[6] The role of αvβ3 in skeletal development was investigated in vivo with β3 knockout mice, and it was found that these rats develop histologically and radiographically evident osteosclerosis with age. Their mutant osteoclasts were, however, dysfunctional, as evidenced by their reduced ability to resorb whale dentin in vitro and the significant hypocalcemia seen in the knockout mice.[7]

The epithelial rests of Malassez (ERM) are remnants of Hertwig's root sheath. After the fragmentation of the root sheath during dental development, these cells become part of the PDL and remain near the root surface.[8] The functions of the ERM in the PDL are not fully understood. Because of reports that they are involved in the formation of radicular cysts, it has been speculated that they have no physiologic significance but only a pathologic role in the PDL.[9] However, more recent in vitro investigations showed that the ERM secrete collagenase and might have a role in PDL remodeling.[10,11] It has also been shown that the ERM express the β1 subunit of integrin cell-surface adhesion receptor in vitro.[12]

The expression of the αvβ3 integrin during bone resorption has been shown. However, its involvement in root resorption needs to be demonstrated. Such information is a prerequisite to formulating strategies to inhibit root and bone resorption and has obvious implications for orthodontic therapy. If integrins can be targeted through the use of antagonists in the form of peptides containing the RGD sequence to inhibit bone or root resorption,[13,14] this blockage could enhance orthodontic anchorage and possibly inhibit root resorption in high-risk patients. The aim of this study was to examine immunohistochemically the expression of cell-surface αvβ3 integrin in clast cells and PDL cells during experimental tooth movement.

MATERIAL AND METHODS

Tooth movement was achieved in 16 male Sprague-Dawley rats (each weighing 120-200 g) by placing elastic bands between the maxillary first and second molars.[15] The elastic bands produced a wedging effect that caused the maxillary first molars to migrate mesially, whereas the second molars moved distally. This created areas of compression and tension around the roots of the first and second molars. The elastic bands were left in place for 6, 12, 18, 24, 60, and 72 hours. The rats were killed by intracardial perfusion with a fixative solution of 4% paraformaldehyde, pH 7.2. All procedures were approved by the University of Illinois at Chicago Animal Care Committee and were performed under anesthesia by a mixture of ketamine (90 mg/kg) (Abbott Laboratories, North Chicago, Ill) and Xylazine (5 mg/kg) (Burns Veterinary Supply, Rockville, NY). After perfusion, the maxillae were dissected free. Molar-bearing segments of alveolar bone were cut from each side and further fixed in separate jars containing 50 mL of 4% paraformaldehyde overnight at 4°C.

The specimens were decalcified in 10% ethylenediaminetetraacetic acid (Sigma, St. Louis, Mo), pH 7.2, for 6 to 8 weeks. The end point of decalcification was determined by periodic (every 2 weeks) radiographic examination. The decalcified specimens were dehydrated in ascending concentrations of ethanol. Subsequently, the specimens were marked and embedded in paraffin. Oblique, serial 4- to 6-μm-thick sections including the crestal areas mesial and distal to the maxillary molars were cut with a microtome. The sections were mounted on L-poly-lysine–coated glass slides (Corning, Acton, Mass), and every other slide was stained with hematoxylin and eosin.

The sections were deparaffinized with 2 changes of xylene for 5 minutes and were rehydrated in graded solutions of ethanol: 2 changes at 100% for 5 minutes each, 95% for 1 minute, and 70% for 1 minute. The sections were washed in distilled water for 1 minute, and then the slides were placed in phosphate-buffered saline (PBS) solution, pH 7.4. The sections were placed in 3% hydrogen peroxide to quench the endogenous peroxidase for 5 minutes. The slides were washed in 2 changes of PBS, 5 minutes each; then the slides were removed and blotted to remove the excess fluid around the sections. The sections were incubated in a humid chamber with 5% blocking goat serum for 20 minutes. Then the sections were incubated with a primary polyclonal anti-mouse anti-β3 antibody and anti-αv antibody (Chemicon International, Temecula, Calif) for 1 hour. The slides were washed in 3 changes of PBS for

2 minutes each. The sections were incubated with goat anti-rabbit immunoglobulin G horseradish peroxidase–conjugated secondary antibody (Chemicon International) in PBS for 1 hour, followed by 3 changes of PBS for 2 minutes each. Diaminobenzidine (Vector Laboratories, Burlingame, Calif) was applied to the sections until brown labeling appeared under light microscopy. The slides were washed under running tap water for 5 minutes, counterstained with Mayer's hematoxylin for 3 minutes, and again washed under running tap water for 5 minutes. The sections were dehydrated in ascending grades of ethanol and mounted for light microscopy examination. Negative controls were labeled with the same procedures but omitting the incubation with primary antibodies.

RESULTS

Forces delivered to the clinical crown with elastic bands placed between the maxillary first and second molars created areas of compression and tension. Areas of compression were characterized by narrowing of the PDL. Areas of tension were characterized by widening of the PDL. Hyalinization of the PDL could be seen in areas of compression. Root resorption lacunae were seen on the root surface as early as 6 hours after force application, associated with areas of compression and hyalinization. In hematoxylin and eosin–stained sections, multinucleated odontoclasts were seen adhering to resorption lacunae along the root surface. Numerous mononucleated cells were also observed in root resorption lacunae, often associated with a few multinucleated odontoclasts. Multinucleated giant cells were observed in the middle of the PDL in intimate association with hyalinized tissues (Fig 1). Use of an antibody against the αv subunit of the integrin showed labeling on the root surface both laterally and apically in areas of compression and tension (Fig 2). The expression of the αv subunit could be seen in both odontoclasts and osteoclasts (Fig 3, *a* and *b*). Furthermore, use of an antibody against the β3 integrin subunit showed immunolabeling of both odontoclasts and osteoclasts (Fig 4, *a*). Negative controls showed no immunolabeling (Figs 3, *c*, and 4, *b*). Expression of αv integrin by osteoclasts could be seen in both areas of frontal and undermining bone resorption (Fig 5, *a* and *b*, respectively). The β3 integrin subunit was expressed by the ERM within the PDL (Fig 6, *a* and *b*), whereas the control specimens showed no labeling (Fig 6, *c*). Expression of the αv subunit by the ERM was not observed.

DISCUSSION

Findings of tissue reaction to orthodontic forces, such as areas of compression and tension in the PDL in this study, agree with many previous histologic studies.[15-20] We saw large root resorption lacunae associated with areas of compression and hyalinization, as has been previously reported.[21] Also, root resorption was observed on the tension side. It is not known

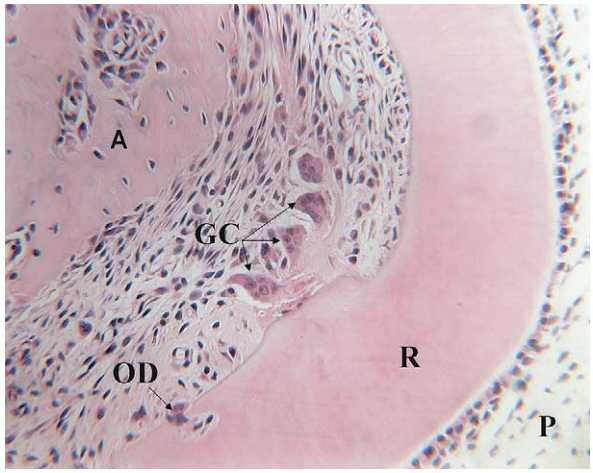

Fig 1. Photomicrograph showing multinucleated giant cells (*GC*) in contact with hyalinized area of periodontal ligament and multinucleated odontoclasts (*OD*) associated with root (*R*) surface resorption. *P*, Pulp; *A*, alveolar bone. Original magnification, 60×.

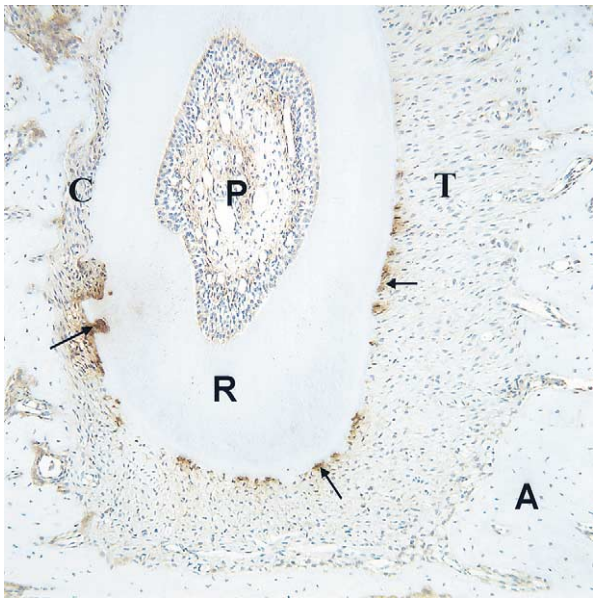

Fig 2. Photomicrograph showing low-power view with positive immunolabeling (*arrows*) with anti-αv antibody on mesial root (*R*) surfaces of maxillary right first molar facing both compression (*C*) and tension (*T*) regions of periodontal ligament. *P*, Pulp; *A*, alveolar bone. Original magnification, 60×.

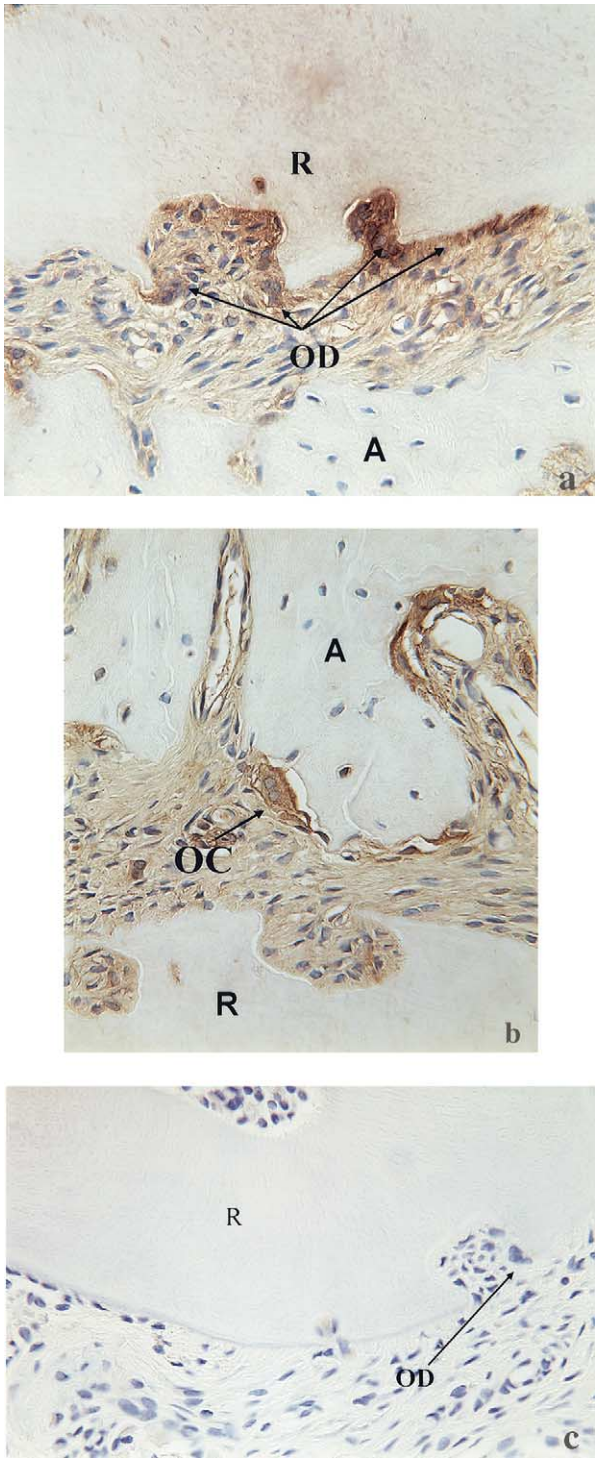

Fig 3. a, Photomicrograph showing positive immunolabeling with anti-αv antibody in odontoclasts (*OD*) associated with root (*R*) resorption. *A*, Alveolar bone. Original magnification, 400×. **b**, Photomicrograph showing immunolabeling with anti-αv antibody in osteoclasts (*OC*) at alveolar bone (*A*) resorption lacuna. *R*, root. Original magnification, 400×. **c**, Negative control showing no immunolabeling of odontoclasts (*OD*) associated with root (*R*) resorption. Original magnification, 400×.

whether this resorption can be attributed to orthodontic forces or whether it resulted from physiologic root resorption.

Our observations of multinucleated giant cells in the PDL were also described in previous studies and are assumed to be responsible for eliminating the hyalinized areas of the PDL.[22] The cells lack ruffled borders, a characteristic feature of clast cells. Furthermore, it is not known whether these cells, despite their different morphology, constitute a distinct population, and their fate is unknown. Previous studies reported low numbers of multinucleated odontoclasts and the involvement of mononucleated odontoclasts in the process of root resorption.[22] Our study confirms these earlier results. From our observations, it could not be determined whether these mononucleated cells are odontoclasts or cementoblasts involved in the process of repair.

Many attempts have been made by clinicians and researchers to prevent or minimize root resorption by identifying the predisposing risk factors. These risk factors include prolonged treatment time, amount of root movement, root shape, and amount of overjet.[23-25] However, few attempts to prevent root resorption at the molecular and cellular levels have been made. The use of bisphosphonates, which putatively interfere with the function of clast cells and hence inhibit both bone and root resorption, has been reported.[26] It was found that topical administration of bisphosphonates (risedronate) before orthodontic tooth movement reduces root resorption. This inhibitory effect, which was dose dependent, was mainly due to inhibition of the odontoclasts' function rather than a decrease in their number.[26] The expression of αvβ3 integrin by osteoclasts is well established.[4] However, the in vivo expression of αvβ3 by odontoclasts during experimental tooth movement has not been shown previously. It is assumed that odontoclasts and osteoclasts use the same mechanism of mineralized tissue resorption, and our findings are consistent with this assumption. The expression of the αvβ3 integrin receptor by odontoclasts might indicate the involvement of this integrin in the process of root resorption during experimental tooth movement. The level of αvβ3 integrin expression by osteoclasts and odontoclasts could not be accurately correlated with the intensity of immunolabeling observed, because of inherent limitations of the method used.

The process of alveolar bone resorption that occurs during tooth movement can take 2 forms: frontal bone

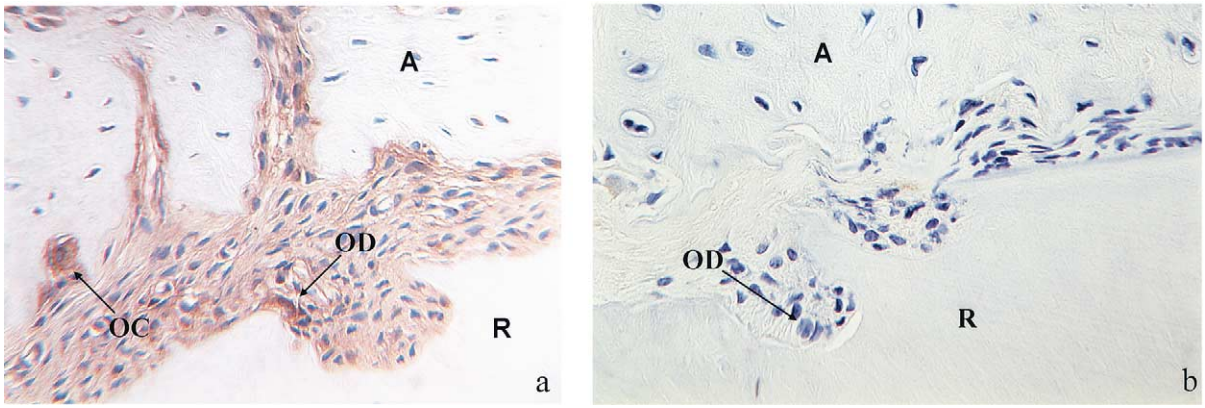

Fig 4. Photomicrograph showing immunolabeling with anti-β3 antibodies in odontoclasts (*OD*) and osteoclasts (*OC*). *A*, Alveolar bone; *R*, root. Original magnification, 160×. **b**, Negative control showing no immunolabeling of odontoclasts (*OD*) associated with root (*R*) resorption. *A*, Alveolar bone. Original magnification, 400×.

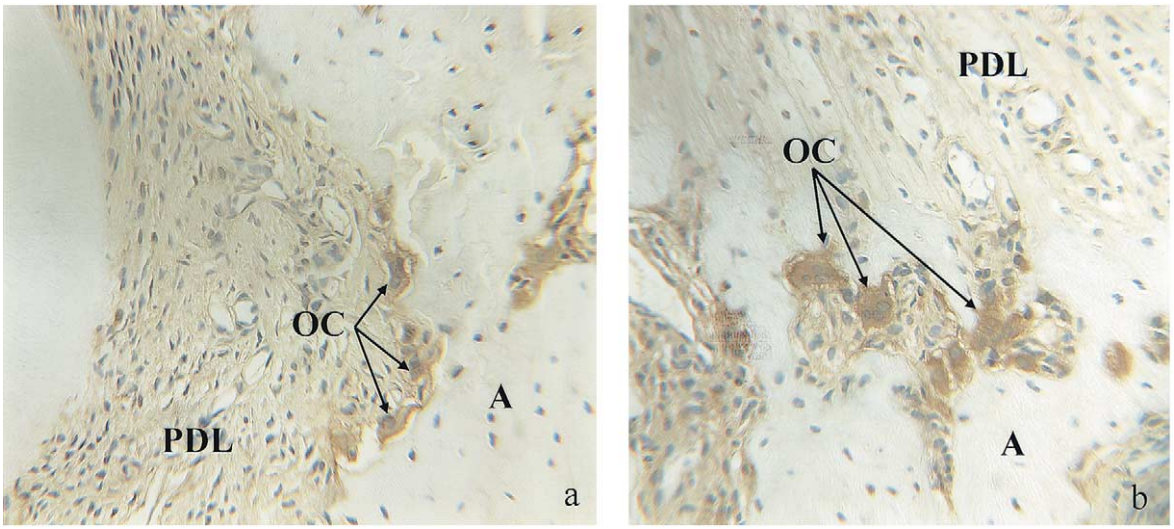

Fig 5. **a**, Photomicrograph showing positive immunolabeling with anti-αv antibody in osteoclasts (*OC*) involved in frontal bone resorption (*A*). *PDL*, Periodontal ligament. Original magnification, 160×. **b**, Photomicrograph showing positive immunolabeling with anti-αv antibody in osteoclasts (*OC*) involved in undermining alveolar bone (*A*) resorption. *PDL*, Periodontal ligament. Original magnification, 160×.

resorption or undermining bone resorption. Frontal bone resorption is carried out by osteoclasts lining the alveolar bone from the PDL surface, whereas undermining bone resorption takes place from the medullary surfaces and leads to the resorption of bone spicules. Immunohistochemical findings in our study demonstrate a similar expression of αvβ3 integrin in osteoclasts involved in both types of alveolar bone resorption. Identification of the cell-surface receptors involved in the process of bone and root resorption is essential for the development of future treatment modalities targeting these receptors. Targeting αvβ3 with an antagonist might inhibit bone and root resorption. The inhibition of bone resorption might enhance anchorage needs during orthodontic treatment. Possible inhibition of root resorption might help prevent this problem in high-risk orthodontic patients.

The functions of the ERM in the PDL are not fully understood. The in vitro expression of β1 integrin in these cells has been previously reported, and it was

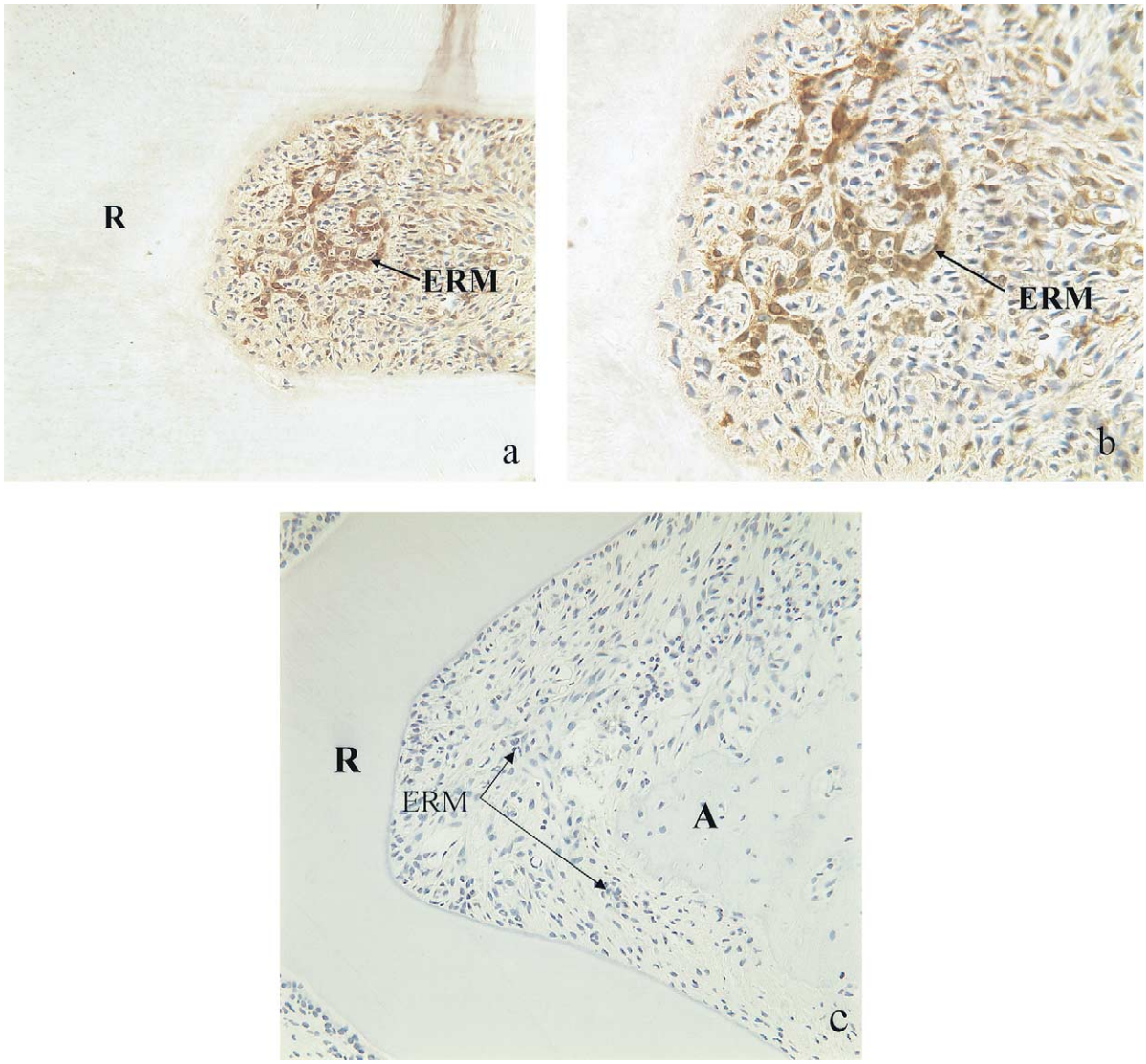

Fig 6. **a**, Low-power photomicrograph showing positive immunolabeling with anti-β3 antibody in epithelial rests of Malassez (*ERM*). *R*, root. Original magnification, 60×. **b**, Higher magnification of ERM immunolabeled with anti-β3 antibody. Original magnification, 400×. **c**, Negative control showing no immunolabeling of ERM. *R*, Root; *A*, alveolar bone. Original magnification, 100×.

suggested that β1 integrin is involved in mediating cell-matrix adhesion.[12] Our in vivo study demonstrated the expression of β3 integrin subunit by the ERM, but its function is not known. However, αv integrin subunit expression was not observed. But because integrins are present on the cell surface as heterodimers, one expects αv to also be expressed by the ERM. It is conceivable that the epitopes of the αv subunit were not readily available for interaction with the antibody during incubation, and that hence no labeling was detected. Integrins, in general, trigger the intracellular signaling pathways for cell survival, migration, and even cell division. Previous studies have shown that the ERM respond to mechanical stimulation during experimental tooth movement by cell division.[27] A recent study showed that ERM cells increased their proliferative activity after 10 days of mechanical injury to the PDL with antiproliferative cell nuclear antigen antibody.[28] Also, ERM can secrete matrix proteins, such as osteopontin and ameloblastin; this suggests a role for these cells in cementum repair after induced root resorption.[28] Furthermore, these cells can synthesize collagenase, the collagen-degrading enzyme.[10] Thus, the ERM might play a critical role in the remodeling of

the PDL or cementum repair during orthodontic tooth movement, potentially by β1 and β3 integrin cell-surface receptors.

CONCLUSIONS

The αvβ3 integrin is expressed by both odontoclasts associated with root resorption and osteoclasts associated with bone resorption during experimental tooth movement. Future drugs targeting these cell-surface receptors might help enhance anchorage during orthodontic treatment or inhibit root resorption. Future studies are needed to determine the role of the ERM in PDL remodeling and the specific role of the β3 integrin in the function of these cells.

REFERENCES

1. Shigeyama Y, Grove T, Strayhorn C, Somerman M. Expression of adhesion molecules during tooth resorption in feline teeth: a model system for aggressive osteoclastic activity. J Dent Res 1996;75:1650-7.
2. Rodan S, Rodan G. Integrin function in osteoclasts. J Endocrinol 1997;154:s47-56.
3. Teitelbaum S. Bone resorption by osteoclasts. Science 2000;289: 1504-8.
4. Nesbitt S, Nesbit A, Helfrich M, Horto M. Biochemical characterization of human osteoclasts integrins: osteoclasts express αvβ3 α2β1 and αvβ1 integrins. J Biol Chem 1993;268:16737-45.
5. Miyauchi A, Alvarez J, Greenfield E, Teti A, Grano M, Colucci S, et al. Recognition of osteopontin and related peptides by an αvβ3 integrin stimulates immediate cell signals in osteoclasts. J Biol Chem 1991;266:20369-74.
6. Hultenby K, Reinholt F, Heinegard D. Distribution of integrin subunits on rat metaphyseal osteoclasts and osteoblasts. Eur J Cell Biol 1993;62:86-93.
7. McHugh K, Hodivala-Dilke K, Zheng M, Namba N, Lam J, Novack D, et al. Mice lacking β3 integrins are osteosclerotic because of dysfunctional osteoclasts. J Clin Invest 2000;105: 433-40.
8. Spouge JD. The rests of Malassez and chronic marginal periodontitis. J Clin Periodontol 1984;11:340-7.
9. Ten Cate AR. The epithelial cell rests of Malassez and the genesis of the dental cysts. Oral Surg 1972;34:956-64.
10. Ragnarsson B. Isolation and characterization of human periodontal ligament cells in vitro. Chicago: University of Illinois at Chicago; 1984.
11. Ragnarsson B, Carr G, Daniel J. Isolation and growth of human periodontal ligament cells in vitro. J Dent Res 1985;64:1026-30.
12. Uitto V, Larjava H, Peltonen J, Brunette D. Expression of fibronectin and integrins in cultured periodontal ligament epithelial cells. J Dent Res 1992;71:1203-11.
13. Sato M, Sardana M, Grasser W, Garsky V, Murray J, Gould R. Echistatin is a potent inhibitor of bone resorption in culture. J Cell Biol 1990;111:1713-23.
14. Sato M, Garsky V, Majeska R, Einhorn T, Murry J, Tashjian A, et al. Structure-activity studies of the s-echistatin inhibition of bone resorption. J Bone Miner Res 1994;9:1441-9.
15. Oppenheim A. Tissue changes, particularly of the bone, incident to tooth movement. Trans Eur Orthodont Soc 1911;8:11.
16. Reitan K. The initial tissue reaction incident to orthodontic tooth movement as related to influence of function. Acta Odont Scand 1951;9(suppl 6):1-240.
17. Waldo C, Rothblatt J. Histological response to tooth movement in the laboratory rat. J Dent Res 1954;33:481-6.
18. Zaki AE, Van Huysen G. Histology of the periodontium following tooth movement. J Dent Res 1963;43:1373-9.
19. Reitan K, Kvam E. Comparative behavior of human and animal tissue during experimental tooth movement. Angle Orthod 1971; 41:1-14.
20. Reitan K. Initial tissue response behavior during apical tooth resorption. Angle Orthod 1974;44:68-82.
21. Brudvik P, Rygh P. Root resorption beneath the main hyalinized zone. Eur J Orthod 1994;16:249-63.
22. Brudvik P, Rygh P. Multinucleated cells remove the main hyalinized tissue and start resorption of the adjacent root surface. Eur J Orthod 1994;16:265-73.
23. Linge B, Linge L. Apical root resorption in upper anterior teeth. Eur J Orthod 1983;5:173-83.
24. Linge L, Linge B. Patient characteristics and treatment variables associated with apical root resorption during orthodontic treatment. Am J Orthod Dentofacial Orthop 1991;99:35-43.
25. Mirabella A, Artun J. Risk factors for apical root resorption of maxillary anterior teeth in adult orthodontic patients. Am J Orthod Dentofacial Orthop 1995;108:48-55.
26. Igarashi K, Adachi H, Mitani H, Shinoda H. Inhibitory effect of the topical administration of bisphosphonate (risedronate) on root resorption incident to orthodontic tooth movement in rats. J Dent Res 1996;75:1644-9.
27. Talic N, Evans C, Daniel J, Zaki AE. Proliferation of epithelial rests of Malassez during experimental tooth movement. Am J Orthod Dentofacial Orthop 2003;123:527-33.
28. Hasegawa N, Kawaguchi H, Ogawa T, Uchida T, Kurihara H. Immunohistochemical characteristics of epithelial rests of Malassez during cementum repair. J Periodontal Res 2003;38: 51-6.