β₁ Integrin/Focal Adhesion Kinase-mediated Signaling Induces Intercellular Adhesion Molecule 1 and Receptor Activator of Nuclear Factor κB Ligand on Osteoblasts and Osteoclast Maturation*

We have assessed characteristics of primary human osteoblasts, shedding light on signaling mediated by β₁ integrin. β₁ integrins are major receptors for these matrix glycoproteins. 1) Integrins β₁, α₂, α₅, α₆, α₉, and α₆ were highly expressed on primary osteoblasts. 2) Engagement of β₁ integrins on osteoblasts by cross-linking with specific antibody or ligand matrices, such as fibronectin or collagen, augmented expression of intercellular adhesion molecule 1 (ICAM-1) and receptor activator of nuclear factor κB ligand (RANKL) on the surface. 3) Up-regulation of ICAM-1 and RANKL on osteoblasts by β₁ stimulation was completely abrogated by pretreatment with herbinycin A and genistein, tyrosine kinase inhibitors, or transfection of dominant negative truncations of FAK. 4) Engagement of β₁ integrins on osteoblasts induced tartrate-resistant acid phosphatase-positive multinuclear cell formation in the coculture system of osteoblasts and peripheral monocytes. 5) Up-regulation of tartrate-resistant acid phosphatase-positive multinuclear cell formation by β₁ stimulation was completely abrogated by transfection of dominant negative truncations of FAK. Our results indicate that β₁ integrin-dependent adhesion of osteoblasts to bone matrices induces ICAM-1 and RANKL expression and osteoclast formation via tyrosine kinase, especially FAK. We here propose that β₁ integrin/FAK-mediated signaling on osteoblasts could be involved in ICAM-1- and RANKL-dependent osteoclast maturation.

Bone metabolism in health and disease is based on a self-regulating cellular event. The two major processes of bone remodeling, bone formation and resorption, are closely regulated by intercellular signaling involving soluble factors, systemic hormones, and cellular adhesion (1–5). Osteoblasts play a central role in bone formation by synthesizing multiple bone matrix proteins and by differentiation into osteocytes. However, osteoblasts also regulate osteoclast maturation by producing bone-resorbing cytokines and by direct cell attachment, resulting in bone resorption (6–8). Cell adhesion of osteoblasts and osteoclastic precursors of hematopoietic origin is a prerequisite for osteoclast maturation. Several studies have demonstrated that interaction of receptor activator of nuclear factor κB ligand (RANKL) on osteoblasts and RANK on osteoclast precursors provides an essential signal to osteoclast precursors for their maturation into resorbing cells (9–11). We have previously reported that human osteoblasts express intercellular adhesion molecule 1 (ICAM-1) and that interaction between ICAM-1, expressed on osteoblasts, and leukocyte function-associated antigen (LFA)-1, expressed on monocytes, is required for osteoclast maturation by RANKL on osteoblasts (12).

Adhesion molecules play a fundamental role in cell-to-cell and cell-to-extracellular matrix (ECM) interactions. However, recent findings have indicated that certain adhesion molecules not only function as glue but also regulate several cellular functions by transducing signaling. We have reported that ICAM-1 on rheumatoid synovial cells induced transcription of interleukin-1β by activation of a nuclear factor, AP-1, and that stimulation of β₁ integrin up-regulated ICAM-1 and Fas, and Fas mediated apoptosis of rheumatoid synovial cells through focal adhesion kinase (FAK) (13, 14). These results prompted us to investigate the adhesion molecules involved in regulating the expression of other adhesion molecules, such as ICAM-1 and RANKL, on human osteoblasts. Cell adhesion to matrices is primarily mediated by integrins, cell surface receptors that comprise an expanding family of transmembrane heterodimers of α and β subunits (15–18). Interaction of integrins with their protein ligands increases tyrosine phosphorylation and triggers the assembly of cytoskeletal proteins, signaling complexes including FAKs, and their substrates into membrane-substratum junctions referred to as focal adhesions (19–23).

Although osteoblasts are always surrounded by and encounter ECMs including type I collagen and fibronectin mainly through β₁ integrin, the relevance of β₁ integrin to the intracellular signaling and functions in osteoblasts remains unclear. It is well established that osteoblast differentiation and maturation are regulated by their interaction with ECMs such as type I collagen (24, 25). However, such an adhesive interaction may act on osteoblasts to modulate bone metabolism, not only bone formation by activating osteoblasts to proliferate and...
synthesize bone matrix protein but also bone resorption by indirectly activating osteoclast function and differentiation mediated through osteoblasts. The aim of the present study was to determine the role of \( \beta_1 \) integrin-mediated signaling in the regulation of cell surface adhesion molecules on osteoblasts. Our results demonstrate that engagement of \( \beta_1 \) integrin by a specific antibody or ligand matrices up-regulated ICAM-1 and RANKL expression on osteoblasts and induced osteoblast formation via tyrosine kinase, especially FAK.

EXPERIMENTAL PROCEDURES

The study protocol was approved by the Human Ethics Review Committee of the University of Occupational and Environmental Health, Japan, and a signed consent form was obtained from each subject prior to taking tissue samples used in the present study.

**Purification of Human Osteoblastic Cells**—Osteoblast-like cells were purified from metaphyseal trabecular bone in the proximal femur of four osteoarthritis patients during total hip arthroplasty by the established procedures of Russell and colleagues (26, 27). After removing pieces of cortical bone, articular cartilage, and soft connective tissue, the fragments were cut into small pieces and washed extensively. The bone explants were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen) containing 10% fetal calf serum (FCS, Invitrogen) in 25-cm² culture flasks (Falcon, Lincoln Park, NJ) in a humidified 5% CO₂ atmosphere. When cell monolayers were confluent after the 6–8 week culture, the explants were removed, and the cells were replated and incubated, resulting in new cellular outgrowth and eventually a confluent monolayer of cells. At confluence, the cells were trypsinized, passaged at a 1:3 split ratio, and recultured. The medium was changed twice each week, and the cells were used after three to seven passages. The obtained cells showed a flattened polygonal shape with multiple spindle legs and possessed characteristics of osteoblast-like phenotype including osteocalcin, bone sialoprotein, type I collagen, and bone alkaline phosphatase as described previously (27).

**Antibodies and Other Reagents**—The following monoclonal antibodies (mAbs) were used as purified IgG in preparation of staining and analysis of cell surface or cytoplasmic molecules: control mAb thyl.2 (BD Biosciences), human CD29 (\( \beta_1 \) integrin) mAb MAB13, human CD18 (\( \beta_2 \) integrin) mAb TS1/18 (kindly provided by Dr. K. Y. Yamada, National Institutes of Health, Bethesda, MD), human CD61 (platelet integrin) mAb, human CD49a (\( \alpha_1 \) integrin) mAb TS2/7, human CD49b (\( \alpha_2 \) integrin) mAb, human CD49c (\( \alpha_3 \) integrin) mAb, human CD49d (\( \alpha_4 \) integrin) mAb NIH49E-1, human CD49e (\( \alpha_5 \) integrin) mAb MAB16, human CD49f (\( \alpha_6 \) integrin) mAb NIH49F-1, human CD51 (\( \alpha_5 \) integrin) mAb 2C6, human CD54 (ICAM-1) mAb 84H10 (kindly provided by Dr. S. Shaw, National Institutes of Health, Bethesda, MD), human anti-RANKL mAb (Sigma), human CD106 (VCAM-1) mAb 2G7 (kindly provided by Dr. S. Shaw, National Institutes of Health, Bethesda, MD), 0.5% human serum albumin (Yoshitomi, Osaka, Japan), and 0.2% Na₂S (Sigma) for 30 min at 4 °C. After washing the cells three times with FACS medium, they were further incubated with fluorescein isothiocyanate-conjugated goat anti-mouse IgG Ab, goat anti-rabbit IgG Ab, or rabbit anti-goat IgG Ab for 30 min at 4 °C. The staining of cells with mAbs was detected using FACSkan. Quantification of cell surface antigens on single cells was calculated using standard beads (QIFIKT, Dako Japan, Kyoto, Japan) as already described (30, 31). The data were used for the construction of the calibration curve (mean fluorescence intensity (MFI)) against antibody binding capacity (ABC). The cell specimen was analyzed on the FACSkan, and ABC was calculated by interpolation on the calibration curve. When the green fluorescence laser detector was set at the 525 level, the FACSkan analysis was performed by the following equation: ABC = (MFI of specimen - MFI of control Ab) / (MFI of optimal Ab - MFI of control Ab) × 100. The optimal Ab was defined as the anti-VSV Ab (VSV-FRNK) dissolved in 100 μl of serum-free medium (OPTI-MEM, Invitrogen) containing 10% fetal bovine serum (FBS, Hyclone, Logan, UT) and incubated at room temperature. The plasmids and liposome complex were added to osteoblasts plated in a 6-well culture dish, incubated for 3 h in OPTI-MEM, and then replaced with DMEM containing 10% FBS for 24 h. The expression of VSV-FAK, VSV-FAT, and VSV-FRNK in osteoblasts was confirmed by flow cytometric analysis using anti-VSV Ab after their transfection. High expression of the VSV was observed, and 50–80% of the cells were transfected by intracellular flow cytometric analysis. A marked difference in the transfaction efficiency among all three vectors was not observed in osteoblasts, and none of these vectors produced cytotoxic effects on osteoblasts as confirmed by trypan blue staining (data not shown).

**Flow Microfluorometry**—Staining and flow cytometric analysis of osteoblastic cells were conducted by standard procedures as described previously (18) using a FACSkan (BD Biosciences). In brief, 2 × 10⁵ cells were incubated with negative control mAb thyl.2, integrin \( \beta_1 \) mAb, \( \beta_2 \) mAb, \( \beta_3 \) mAb, \( \alpha_1 \) mAb, \( \alpha_2 \) mAb, \( \alpha_3 \) mAb, \( \alpha_4 \) mAb, \( \alpha_5 \) mAb, \( \alpha_6 \) mAb, CD54 (ICAM-1) mAb, RANKL mAb, or CD106 (VCAM-1) mAb in FACS medium consisting of Hanks’ balanced salt solution (Nissui, Tokyo, Japan), 0.5% human serum albumin (Yoshitomi, Osaka, Japan), and 0.2% Na₂S (Sigma) for 30 min at 4 °C. After washing the cells three times with FACS medium, they were further incubated with fluorescein isothiocyanate-conjugated goat anti-mouse IgG Ab, goat anti-rabbit IgG Ab, or rabbit anti-goat IgG Ab for 30 min at 4 °C. The staining of cells with mAbs was detected using FACSkan. Quantification of cell surface antigens on single cells was calculated using standard beads (QIFIKT, Dako Japan, Kyoto, Japan) as already described (30, 31). The data were used for the construction of the calibration curve (mean fluorescence intensity (MFI)) against antibody binding capacity (ABC). The cell specimen was analyzed on the FACSkan, and ABC was calculated by interpolation on the calibration curve. When the green fluorescence laser detector was set at the 525 level, the FACSkan analysis was performed by the following equation: ABC = (MFI of specimen - MFI of control Ab) / (MFI of optimal Ab - MFI of control Ab) × 100. The optimal Ab was defined as the anti-VSV Ab (VSV-FRNK) dissolved in 100 μl of serum-free medium (OPTI-MEM, Invitrogen) containing 10% fetal bovine serum (FBS, Hyclone, Logan, UT) and incubated at room temperature. The plasmids and liposome complex were added to osteoblasts plated in a 6-well culture dish, incubated for 3 h in OPTI-MEM, and then replaced with DMEM containing 10% FBS for 24 h. The expression of VSV-FAK, VSV-FAT, and VSV-FRNK in osteoblasts was confirmed by flow cytometric analysis using anti-VSV Ab after their transfection. High expression of the VSV was observed, and 50–80% of the cells were transfected by intracellular flow cytometric analysis. A marked difference in the transfaction efficiency among all three vectors was not observed in osteoblasts, and none of these vectors produced cytotoxic effects on osteoblasts as confirmed by trypan blue staining (data not shown).

**Statistical Analysis**—Data were expressed as mean ± S.D. of the number of indicated patients. Differences from the control were examined for statistical significance by analysis of variance followed by posthoc Scheffe’s F-test. A p value less than 0.05 denoted the presence of a statistically significant difference.

**RESULTS**

**\( \beta_1 \) Integrin Was Highly Expressed on Primary Human Osteoblastic Cells**—Initially we assessed the expression of various cell surface functional molecules on primary human osteoblastic cells using FACSkan. Fig. 1 shows the number of 12 representative molecules, including integrins, ICAM-1, and RANKL, on osteoblasts. Among the screened molecules, \( \beta_1 \) integrin was...
highly expressed on osteoblasts. Among α subunits of integrins, α5, α6, αv, αα, and αβ6 were expressed on osteoblasts. A histogram implied that the vast majority of osteoblasts expressed β1 integrin. We therefore assumed that β1 integrin, which is consistently highly expressed on osteoblasts, might play a functional role in primary osteoblasts. ICAM-1 and RANKL were moderately expressed on osteoblasts. Estimation from histograms of multiple donors showed that one-third to two-thirds of osteoblasts expressed ICAM-1 and RANKL without stimulation.

Cross-linking of β1 Integrin Up-regulated ICAM-1 and RANKL Expression on Osteoblasts—To characterize the function of β1 integrin on osteoblasts, we assayed the cell surface molecule expression by β1 cross-linking using a specific mAb and a second cross-linker Ab. Flow cytometry showed that expression of ICAM-1 and RANKL on the surface was markedly augmented by the β1 cross-linking on osteoblasts. As shown in Fig. 2, ICAM-1 and RANKL were moderately expressed on unstimulated osteoblasts. However, β1 cross-linking significantly up-regulated ICAM-1 and RANKL expression, whereas cross-linking of major histocompatibility complex class I using their specific mAbs and second cross-linker Ab had no effect. In contrast, cross-linking of β1 integrin on osteoblasts did not induce VCAM-1 expression (Fig. 2). The results were consistent in osteoblasts derived from four donors. Time course experiments showed that ICAM-1 and RANKL expression reached maximum levels within 3 h after β1 cross-linking (Fig. 2). These results suggest that β1 integrin appears to play a pivotal role in ICAM-1 and RANKL up-regulation on osteoblasts.

Engagement of β1 Integrin by Ligand Matrix Glycoproteins Augmented ICAM-1 and RANKL Expression on Osteoblasts—Fibronectin and type I collagen are major ligands for cell surface β1 integrin. We next assessed the biological activities of fibronectin and type I collagen on osteoblasts. Expression of both ICAM-1 and RANKL were markedly induced by incubation of these cells on fibronectin- or collagen-coated plastic plates (Fig. 3). In contrast, no change was noted when these cells were incubated on bovine serum albumin-coated plates (Fig. 3). Furthermore ICAM-1 and RANKL expression induced by fibronectin and type I collagen was completely inhibited by pretreatment of cells with anti-β1 mAb (Fig. 3). These data suggest that fibronectin and type I collagen are possible ligands involved in β1 integrin-induced ICAM-1 and RANKL expression on osteoblasts.

Involvement of Tyrosine Kinases in β1 Integrin-mediated Up-regulation of ICAM-1 and RANKL on Osteoblasts—Next, to determine signaling pathways involved in β1 integrin-induced induction of ICAM-1 and RANKL expression on osteoblasts, we pretreated the cells with or without different concentrations of various inhibitors of intracellular signaling, and then ICAM-1 and RANKL expression by β1 cross-linking was determined by FACScan (Fig. 4). Pretreatment of cells with the tyrosine kinase inhibitors herbimycin A or genistein completely
inhibited \( \beta_1 \)-integrin up-regulation of ICAM-1 and RANKL expression on osteoblasts. In contrast, H7 or staurosporine (protein kinase C inhibitors) and wortmannin (a PI 3-K inhibitor) did not influence \( \beta_1 \)-integrin-induced ICAM-1 and RANKL expression. These results suggest that \( \beta_1 \)-integrin-induced ICAM-1 and RANKL expression on osteoblasts is mediated mainly through tyrosine kinases, consistent with previous reports from our laboratories and those of others (14, 33, 34).

**Fig. 2.** Cross-linking of \( \beta_1 \)-integrin up-regulated ICAM-1 and RANKL expression on osteoblasts. Osteoblasts were cross-linked with (solid bars) or without (open bars) anti-CD29 (\( \beta_1 \)) mAb MAB13 and control major histocompatibility complex class I (MHC-I) mAb W6/32 at a concentration of 10 mg/ml for 6 h, and then ICAM-1 (A), RANKL (B), and VCAM-1 (C) expressions were analyzed by FACSscan. Time course of \( \beta_1 \)-triggered ICAM-1 (D) and RANKL (E) expression on human osteoblasts was also evaluated. Osteoblasts were stimulated with 10 mg/ml anti-CD29 mAb for the indicated duration. Each value represents the number of molecules expressed per one cell calculated using standard QIIFKIT beads from four similar experiments as described under "Experimental Procedures." Data are presented as mean ± S.D. *, \( p < 0.05 \) compared with controls.

**Fig. 3.** Engagement of \( \beta_1 \)-integrin by ligand matrix glycoproteins augmented ICAM-1 and RANKL expression on human osteoblasts. Osteoblasts with or without pretreatment with anti-\( \beta_1 \)-integrin blocking mAb were incubated on plastic plates that were precoated with bovine serum albumin (BSA), fibronectin (FN), or type I collagen (COLL) (10 mg/ml) at 37 °C for 6 h. ICAM-1 (A) and RANKL (B) expression was determined by FACSscan. Each value represents the number of molecules expressed per one cell calculated using standard QIIFKIT beads from four similar experiments. Data are presented as mean ± S.D. *, \( p < 0.05 \) compared with controls.

\( \beta_1 \)-mediated Signaling Increased TRAP<sup>+</sup> MNC Formation in Osteoblasts from Peripheral Monocytes—In a coculture system using osteoblasts with peripheral monocytes, \( \beta_1 \)-stimulated osteoblasts that did or did not express VSV-FAK significantly induced the formation of TRAP<sup>+</sup> MNCs from the monocytes compared with untreated osteoblasts. However, on the cells expressing VSV-FAT or VSV-FRNK, such induction of TRAP<sup>+</sup> MNCs by stimulation of \( \beta_1 \) was markedly inhibited (Fig. 6). These results also indicate that FAK is involved in \( \beta_1 \) stimulation, and this helps commit hematopoietic precursors toward osteoclast development.

**DISCUSSION**

Integrins are a superfamily of cell surface receptors involved in cell-cell and cell-matrix adhesion. Signals from matrices transduced by integrins play critical roles in regulating gene expression, tissue-specific differentiation, and survival of primary osteoblasts and fibroblasts (6, 35, 36). It has been reported that human osteoblasts express a diverse range of integrins, particularly of the \( \beta_1 \) integrin, including \( \alpha_1 \) through \( \alpha_5 \) subunits. While our and others’ studies show different patterns...
of integrin expression, this may reflect the heterogeneity of osteoblast-like populations and the different stages of osteoblasts from fetal or adult bone (37–40). Although osteoblasts express $\alpha_5\beta_1$ integrins, they differ from osteoclasts in that $\beta_1$ integrins appear to have the major functional role, which has been underscored by in vivo data (41). $\beta_1$ integrins are major adhesion receptors mediating interactions between osteoblasts and ECMs found in bone, such as collagen, fibronectin, osteopontin, thrombospondin, and vitronectin. The $\alpha_5\beta_1$ integrins bind to type I collagen, which is the dominant bone matrix.

Fig. 4. Involvement of tyrosine kinases in $\beta_1$ integrin-mediated up-regulation of ICAM-1 and RANKL on human osteoblasts. Osteoblasts were pretreated with or without the indicated concentration of various inhibitors of intracytoplasmic signaling for 30 min. Osteoblasts were then cross-linked with $10\, \mu$g/ml anti-$\beta_1$ mAb for 6 h. ICAM-1 (A) and RANKL (B) expression was determined by FACScan. Each value represents the number of molecules expressed per one cell calculated using standard QIFKIT beads from four similar experiments. *, $p < 0.05$ compared with controls.

Fig. 5. Involvement of FAK in $\beta_1$-mediated signaling inducing ICAM-1 and RANKL on human osteoblasts. Osteoblasts transfected with or without control vectors encoding VSV-FAK, VSV-FAT, or VSV-FRNK were cross-linked with $10\, \mu$g/ml anti-$\beta_1$ mAb for 6 h and analyzed for expression of ICAM-1 (A) and RANKL (B) using FACScan. Each value represents the number of molecules expressed per one cell calculated using standard QIFKIT beads from four similar experiments. *, $p < 0.05$ compared with controls.
As RANKL, a member of the tumor necrosis factor family expressed on the cell surface membrane of COS cells and osteoblasts/stromal cells, induces osteoclast formation from its precursor through cognate interaction between osteoblasts and osteoclast precursors, it is thereby required for RANKL-induced osteoclastogenesis (9–11). Thus, higher affinity adhesion between osteoblasts and osteoclast precursors is emerging as a prerequisite for interaction of membrane-bound RANKL to be efficiently presented to its receptor, RANK. However, the binding affinity between tumor necrosis factor family molecules and tumor necrosis factor receptor family molecules, including CD40/CD40L, CD30/CD30L, Fas/FasL, and RANKL/RANK binding, is not sufficient to support static or firm cell adhesion (43). Furthermore we reported that anti-RANKL antibody did not inhibit the adhesion of osteoblasts to osteoclast precursors, whereas anti-LFA-1 antibody completely blocked the adhesion in a human cell culture system (12). Thus, based on our in vitro study, it can be assumed that up-regulation of ICAM-1 and RANKL expression on osteoblasts by β1 integrin-mediated signaling could affect cellular adhesion between osteoblasts and osteoclast precursors through the ICAM-1/LFA-1 and RANKL/RANK pathways and lead to differentiation of osteoclast progenitors to osteoclasts in vivo.

During bone remodeling processes, adhesion-dependent interaction among osteoblasts and osteoclasts causes an imbalance in bone metabolism by favoring bone resorption through the expression of RANKL, ICAM-1, and other factors involved in cellular interaction. Although several studies have reported that β1 integrin-mediated adhesion to bone matrix induces proliferation, differentiation, and bone matrix synthesis of osteoblasts, our novel findings suggest that β1 integrin/FAK-mediated signaling on osteoblasts could be involved in ICAM-1- and RANKL-dependent osteoclast maturation. Thus, it can be assumed that β1 integrin-mediated signaling on osteoblasts could be involved in high turnover on bone metabolism through two paradoxical features of bone formation and bone resorption. However, it is as yet unclear how the same signaling pathway controls such diverse cellular events. After ligation of β1 integrins with surrounding ECMs, the integrins are found in focal adhesion plaques where various cytoskeletal proteins accumulate. Engagement of β1 integrins leads to initiation of intracellular signal transduction through accumulated cytoskeletal signaling kinases, resulting in the activation, differentiation, development, and mobility of various cell types (15, 16). Several studies have established that among various cytoskeletal proteins, FAK, a cytoplasmic protein-tyrosine kinase that localizes focal adhesions, is an important mediator of integrin-mediated signaling and that the initial events triggered by the stimulation of β1 integrin are tyrosine phosphorylation and activation of FAK. In the present study, we observed that β1 integrin-mediated induction of ICAM-1 and RANKL expression was completely inhibited when the cells were pretreated with tyrosine kinase inhibitors. Furthermore β1 integrin-induced up-regulation of ICAM-1 and RANKL on osteoblasts expressing FRNK or FAT (dominant negative truncations of FAK) was completely inhibited. These findings suggest that β1 integrin-induced up-regulation of both ICAM-1 and RANKL expression was brought about by the signaling pathway of tyrosine kinases, specifically involving FAK. Phosphorylated FAK activates several transduction molecules including Src and Grb2, which may cause the activation of mitogen-activated protein kinase or PI 3-K via Ras (22). Recent evidence indicates that small guanine nucleotide-binding regulatory proteins (G-proteins) control signaling pathways critical for diverse cellular functions. Among several small G-proteins, Ras proteins are molecular switches that act as a “hub,” which radiates multiple signaling pathways critical for diverse cellular functions, including Raf-1/mitogen-activated protein kinase and PI 3-K (30, 44, 45). We have reported the relevance of H-Ras and its downstream effectors to functions of osteoblasts and proposed that H-Ras signals, especially those followed by the Raf-1/mitogen-activated protein kinase pathway, but not PI 3-K, induce cell cycle arrest and subsequent apoptosis via Fas up-regulation and Bcl-2 down-regulation (46). Although further evidence is required, there is a possibility that such diverse cellular regulation by the β1 integrin-bone matrix interaction may be mediated by these G-protein signaling cascades and that FAK is...
the immediate transducer of \(\beta_1\) integrin-mediated signaling. In conclusion, our results suggest a novel mechanism of \(\beta_1\) integrin-bone matrix cross-talk and a pivotal role in osteoclasto-gensis. Further studies will be required to understand \(\beta_1\) integrin function in bone metabolism.

Acknowledgments—We thank T. Adachi for the excellent technical assistance. We also thank Drs. K. M. Yamada, W. Newman, and S. Shaw for kindly providing mAbs and reagents used in this study.

REFERENCES
1. Manolagas, S. C., and Jilka, R. L. (1995) Nature 377, 305–311
2. Manolagas, S. C. (2000) Science 289, 2457–2506
3. Duong, L. T., and Rodan, G. A. (2001) J. Cell. Biochem. 81, suppl. 31–61
4. Mundy, G. R., Chen, D., Zhao, M., Dallas, S., Xu, C., and Harris, S. (2001) Rev. Endocr. Metab. Disord. 2, 95–104
5. Rodan, G. A. (1998) J. Cell. Biochem. 71, suppl. 55–61
6. Bennett, J. H., Moffatt, S., and Horton, M. (2000) Histol. Histopathol. 15, 603–611
7. Barreau, J. D., Schinke, T., and Karsenty, G. (2000) Science 289, 1501–1504
8. Anderson, D. M., Maraskovsky, E., Billingsley, W. L., Dougall, W. C., Tometsko, M. E., Roux, E. R., Teepen, M. C., Dulboe, R. F., Cosman, D., and Gallibert, L. (1997) Nature 390, 175–179
9. Wong, B. R., Rho, J., Arron, J., Robinson, E., Orlinick, J., Chao, M., Sr, R., Arron, J., and Simpson, A. (1996) J. Bone Miner. Res. 11, 1633–1640
10. Tanaka, Y., Nakamura, T., and Minami, Y. (2002) J. Biol. Chem. 277, 32994–32999
11. Takeuchi, Y., Suzawa, M., Kikuchi, T., Nishida, E., Fujita, T., and Matsumoto, T. (1997) J. Biol. Chem. 272, 29309–29316
12. Beresford, J. N., Poser, A. W., and Russell, R. G. (1984) Metab. Bone Dis. Relat. Res. 5, 229–234
13. Tanaka, Y., Morimoto, I., Nakano, Y., Okada, Y., Hirota, S., Nomura, S., Nakamura, T., and Eto, S. (1995) J. Bone Miner. Res. 10, 1462–1469
14. Frantz, R., and Schlaepfer, D. D. (2000) J. Biol. Chem. 275, 21511–21515
15. Brakebusch, C., Bouvard, D., Stanchi, F., Sakai, T., and Fassler, R. (2002) J. Clin. Investig. 109, 999–1006
16. Schwartz, M. A. (2000) Trends. Cell Biol. 11, 466–470
17. Schaller, M. D. (2001) Biochim. Biophys. Acta 1540, 1–21
18. Parsons, J. T. (2000) J. Cell Sci. 116, 1409–1416
19. Tanaka, M., Osajima, A., Nakayamada, S., Anai, H., Kabushima, N., Kamegai, K., Oka, T., Tanaka, Y., and Nakashima, Y. (2003) Kidney Int. 63, 722–731
20. Kato, S., Sato, K., Kim, K. J., Takahashi, N., Udagawa, N., Nakamura, I., Yamaguchi, A., Kishimoto, T., Suda, T., and Kashikawa, S. (1996) J. Bone Miner. Res. 11, 88–95
21. Martin, K. H., Slack, J. K., Boerner, S. A., Martin, C. C., and Parsons, J. T. (2002) Science 296, 1652–1653
22. Schwartz, M. A., Schaller, M. D., and Ginsberg, M. H. (1995) Ann. Rev. Cell Dev. Biol. 11, 549–589
23. Raiss, L. G. (1999) Clin. Chem. 45, 1353–1355
24. Damsky, C. H. (1999) Bone (N.Y.) 25, 95–96
25. Clover, J., Dobbs, R. A., and Gowen, M. (1992) J. Cell Sci. 103, 267–271
26. Toyama, Y., Sato, K., Kim, K. J., Takahashi, N., Udagawa, N., Nakamura, I., Yamaguchi, A., Kishimoto, T., Suda, T., and Kashikawa, S. (1996) J. Bone Miner. Res. 11, 88–95
27. Martin, K. H., Slack, J. K., Boerner, S. A., Martin, C. C., and Parsons, J. T. (2002) Science 296, 1652–1653
28. Schwartz, M. A., Schaller, M. D., and Ginsberg, M. H. (1995) Annu. Rev. Cell Dev. Biol. 11, 549–589
29. Raiss, L. G. (1999) Clin. Chem. 45, 1353–1355
30. Damsky, C. H. (1999) Bone (N.Y.) 25, 95–96
31. Clover, J., Dobbs, R. A., and Gowen, M. (1992) J. Cell Sci. 103, 267–271
32. Toyama, Y., Sato, K., Kim, K. J., Takahashi, N., Udagawa, N., Nakamura, I., Yamaguchi, A., Kishimoto, T., Suda, T., and Kashikawa, S. (1996) J. Bone Miner. Res. 11, 88–95
33. Martin, K. H., Slack, J. K., Boerner, S. A., Martin, C. C., and Parsons, J. T. (2002) Science 296, 1652–1653
34. Schwartz, M. A., Schaller, M. D., and Ginsberg, M. H. (1995) Ann. Rev. Cell Dev. Biol. 11, 549–589
35. Raiss, L. G. (1999) Clin. Chem. 45, 1353–1355
36. Damsky, C. H. (1999) Bone (N.Y.) 25, 95–96
37. Clover, J., Dobbs, R. A., and Gowen, M. (1992) J. Cell Sci. 103, 267–271
38. Toyama, Y., Sato, K., Kim, K. J., Takahashi, N., Udagawa, N., Nakamura, I., Yamaguchi, A., Kishimoto, T., Suda, T., and Kashikawa, S. (1996) J. Bone Miner. Res. 11, 88–95
39. Martin, K. H., Slack, J. K., Boerner, S. A., Martin, C. C., and Parsons, J. T. (2002) Science 296, 1652–1653
40. Schwartz, M. A., Schaller, M. D., and Ginsberg, M. H. (1995) Annu. Rev. Cell Dev. Biol. 11, 549–589
41. Raiss, L. G. (1999) Clin. Chem. 45, 1353–1355
42. Damsky, C. H. (1999) Bone (N.Y.) 25, 95–96
43. Clover, J., Dobbs, R. A., and Gowen, M. (1992) J. Cell Sci. 103, 267–271
44. Toyama, Y., Sato, K., Kim, K. J., Takahashi, N., Udagawa, N., Nakamura, I., Yamaguchi, A., Kishimoto, T., Suda, T., and Kashikawa, S. (1996) J. Bone Miner. Res. 11, 88–95
45. Martin, K. H., Slack, J. K., Boerner, S. A., Martin, C. C., and Parsons, J. T. (2002) Science 296, 1652–1653
46. Schwartz, M. A., Schaller, M. D., and Ginsberg, M. H. (1995) Annu. Rev. Cell Dev. Biol. 11, 549–589
47. Raiss, L. G. (1999) Clin. Chem. 45, 1353–1355
48. Damsky, C. H. (1999) Bone (N.Y.) 25, 95–96
49. Clover, J., Dobbs, R. A., and Gowen, M. (1992) J. Cell Sci. 103, 267–271
50. Toyama, Y., Sato, K., Kim, K. J., Takahashi, N., Udagawa, N., Nakamura, I., Yamaguchi, A., Kishimoto, T., Suda, T., and Kashikawa, S. (1996) J. Bone Miner. Res. 11, 88–95
51. Martin, K. H., Slack, J. K., Boerner, S. A., Martin, C. C., and Parsons, J. T. (2002) Science 296, 1652–1653
52. Schwartz, M. A., Schaller, M. D., and Ginsberg, M. H. (1995) Annu. Rev. Cell Dev. Biol. 11, 549–589
β1 Integrin/Focal Adhesion Kinase-mediated Signaling Induces Intercellular Adhesion Molecule 1 and Receptor Activator of Nuclear Factor κB Ligand on Osteoblasts and Osteoclast Maturation
Shingo Nakayamada, Yosuke Okada, Kazuyoshi Saito, Masahito Tamura and Yoshiya Tanaka

J. Biol. Chem. 2003, 278:45368-45374.
doi: 10.1074/jbc.M308786200 originally published online September 3, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M308786200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 44 references, 19 of which can be accessed free at http://www.jbc.org/content/278/46/45368.full.html#ref-list-1